Different effects of the Ca^{2+}-binding protein, KChIP1, on two Kv4 subfamily members, Kv4.1 and Kv4.2

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Abstract The Ca^{2+}-binding protein, K^+ channel-interacting protein 1 (KChIP1), modulates Kv4 channels. We show here that KChIP1 affects Kv4.1 and Kv4.2 currents differently. KChIP1 slows Kv4.2 inactivation but accelerates the Kv4.1 inactivation time course. Kv4.2 activation is shifted in a hyperpolarizing direction, whereas a depolarizing shift occurs for Kv4.1. On the other hand, KChIP1 increases the current amplitudes and accelerates recovery from inactivation of both currents. An involvement of the Kv4 N-terminus in these differential effects is demonstrated using chimeras of Kv4.2 and Kv4.1. These results reveal a novel interaction of KChIP1 with these two Kv4 members. This represents a mechanism to further increase the functional diversity of K^+ channels. **© 2001 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.**

Key words: Kv4-subunit; K^+ channel-interacting protein 1; Regulatory subunit; Molecular diversity; Potassium channel

1. Introduction

The molecular and functional diversity of K^+ channels is vast [1]. This is due partly to the large number of principal (α) K^+ channel subunits but also to other processes such as heteromultimerization, alternative splicing and phosphorylation [2]. This diversity is magnified several fold by regulatory auxiliary (β)-subunits, which can alter the function and/or expression levels of a variety of K^+ channel principal subunits. For example, Kvβ-subunits enhance the trafficking of Kvα-subunits to the surface membrane [3,4] and participate in N-type inactivation [5]. Similarly, KChAP proteins act as chaperone-type proteins to increase expression levels of Kvα-subunits, including Kv2 channels [6,7]. Auxiliary proteins that specifically interact with Kv4-subunits exist, as suggested from mRNA fractionation experiments [8,9]. However, their existence remained elusive until the recent description of a family of Ca^{2+}-binding proteins (K^+ channel-interacting protein (KChIP) 1 to KChIP3) that specifically alter Kv4.2 and Kv4.3 current expression levels and kinetics [10]. We have tested the effects of KChIP1 on the remaining member of the Kv4 family, Kv4.1. Our data surprisingly show that although Kv4.1 and Kv4.2 share high sequence similarity, KChIP1 has opposite effects on their voltage dependence of activation and the inactivation time courses. Depending on the relative levels and expression patterns, the implication of our results is that the already vast functional diversity of K^+ channels can be increased even further by an auxiliary subunit when they have opposite effects on Kv members belonging to the same subfamily. This allows cells to tailor their complement of K^+ channels to suit their individual roles in excitability.

2. Materials and methods

2.1. Molecular biology

Full-length human KChIP1 cDNA (1.7 kb) was obtained as an expressed sequence tag (EST) from IMAGE Consortium and sequenced to confirm its integrity and identity with published sequences (GenBank: AF199597). Kv4 chimeric constructs were generated by cassette mutagenesis. *Ecl*XI and *Apa*I restriction sites were respectively incorporated 5′ and 3′ of the coding regions of Kv4.1 and Kv4.2, followed by subcloning into pSGEM. A cassette construct was developed by incorporating a *Spe*I and an *Xba*I site in such a manner that the *Ecl*XI–*Spe*I and *Xba*I–*Apa*I fragments respectively correspond to their N-and C-termini. For Kv4.1 or Kv4.2 respectively, the amino acid positions at the *Spe*I subcloning junction were T181 and T180 (four amino acids before the S1 transmembrane domain) and S412 or S410 (just after the S6 domain) for the *Xba*I subcloning junction. Chimeric constructs were generated using standard subcloning techniques.

2.2. In vitro transcription and oocyte injection

cDNAs were linearized and cRNAs were synthesized using run-off in vitro transcription (Ambion Inc). The integrity and sizes of all synthesized cRNAs were verified by denaturing agarose gel electrophoresis. Their concentrations were quantified by densitometry and by comparison to a known RNA size marker. *Xenopus laevis* frogs were anaesthetized with 0.17% 3-aminobenzoic acid ethyl ester and unfertilized stage V–VI oocytes were harvested, enzymatically defolliculated and injected with 50 nl cRNA (2–8 ng).

2.3. Electrophysiology

Oocytes were voltage-clamped (20 ± 2°C) 2–3 days after the injection [11] using a Geneclamp 500 amplifier (Axon Instruments, Inc.) with data sampled at 5 kHz and filtered at 1 kHz. We used a low Cl^- recording solution (in mM; Na-glutamate 96, K-glutamate 2, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 5; pH 7.5 adjusted with NaOH) to avoid activation of endogenous Cl^- currents. Data were analyzed using the pClamp suite of software and Origin for Windows (Microcal). Leak subtraction was performed for each trace.

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Abbreviations: KChIP, K^+ channel-interacting protein

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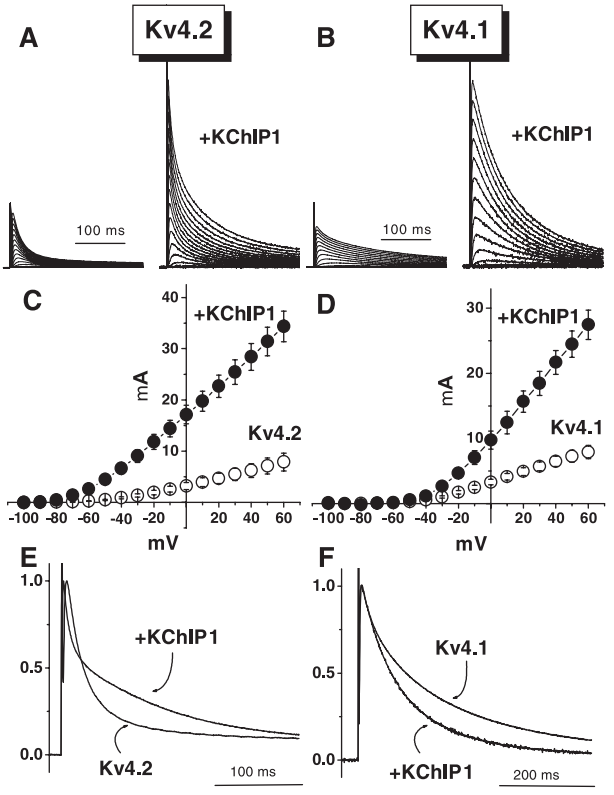

Fig. 1. Differential effects of KChIP1 on Kv4.2 and Kv4.1 currents expressed in *Xenopus* oocytes. Depolarizing voltage steps were applied from −100 to +60 mV (1000 ms duration) in 10-mV increments (at 15-s intervals, to allow full recovery from inactivation) from a holding potential of −120 mV. A, B: Representative traces of Kv4.2 (A) and Kv4.1 (B) currents in the absence (left) or presence (right) of KChIP1. C, D: Averaged *I–V* relationship of peak currents with (●) or without (○) KChIP1 (means ± S.E.M. of six to eight experiments). E, F: Normalized Kv4.2 or Kv4.1 currents (at +50 mV; with or without KChIP1) illustrating the effects of KChIP 1 on the inactivation time course.

3. Results

The Ca^{2+}-binding proteins (KChIP1, KChIP2 and KChIP3) modulate the expression levels and inactivation kinetics of Kv4.2 and Kv4.3 currents [10]. We compared the effects of KChIP1 on Kv4.2 and Kv4.1 currents in a *Xenopus* oocyte expression system. Co-injection with KChIP1 led to greatly enhanced expression levels of Kv4.2 currents (Fig. 1A; see also [10]). KChIP1 similarly increased Kv4.1 current amplitudes (Fig. 1B). This occurred over the entire range of voltages tested, as demonstrated by the current–voltage (*I–V*) relationships (Fig. 1C,D). KChIP1 may possess a chaperone-like function to facilitate membrane trafficking and expression of Kv4 proteins, as suggested by immunocytochemistry data showing a facilitation of membrane-bound KChIP1 and Kv4.2 proteins when they are co-expressed [10].

An important and functionally relevant difference was observed in the way in which KChIP1 affected Kv4 inactivation properties. KChIP1 slowed the overall Kv4.2 inactivation time course (Fig. 1E; see also [10]), as indicated by the increased median inactivation time (Table 1). Surprisingly, however, Kv4.1 currents inactivated significantly faster when co-expressed with KChIP1 (Fig. 1F), as demonstrated by the reduced median inactivation time (Table 1). Using a sum of two exponential functions (see also [11]), we performed exponential curve fitting to the inactivating portion of Kv4.2 currents. Interestingly, although KChIP1 slightly reduced the inactivation time constants, its main effect was to reverse the relative contributions of the fast and slow components of inactivation (Table 1). For example, the contribution of the slow inactivation component is 13% in its absence, but increases to 57% when KChIP1 is present. This is the reason for the overall slower time course of Kv4.2 inactivation despite the small reduction in inactivation time constants. We performed a similar exponential analysis for Kv4.1 currents, which were best described as the sum of three exponential functions (see also [12]). As with Kv4.2 currents, KChIP1 had relatively minor effects on the individual Kv4.1 inactiva-

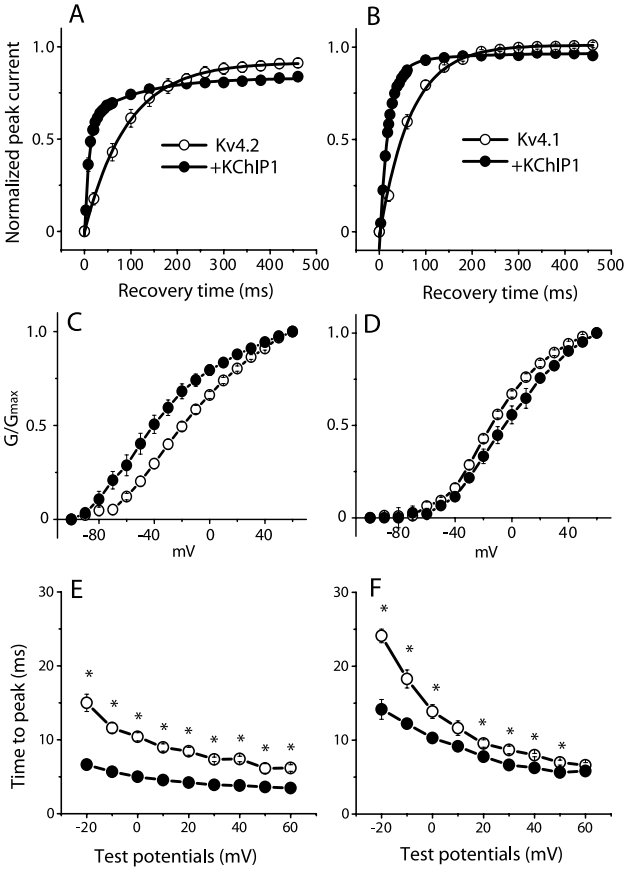

Fig. 2. Effects of KChIP1 on the activation and the inactivation parameters. A, B: Recovery from inactivation of Kv4.2 (A) or Kv4.1 (B) currents in the absence (○) and presence (●) of KChIP1. Two depolarizing pulses to +20 mV were separated by an interval at −120 mV of increasing duration (from 3 to 460 ms). The peak current amplitude during the test pulse (*I*) was normalized to that recorded during the first pulse (I_o) and plotted as a function of the inter-pulse duration. Curve fitting of the data points was performed to a single exponential function under control conditions and a sum of two exponentials for currents in the presence of KChIP1. C, D: Conductance–voltage relationships of Kv4.2 (C) and Kv4.1 (D) currents. Conductance (*G*) was calculated as: $G = I_{peak}/(E - E_K)$, where I_{peak} is the peak current amplitude, *E* is the test potential and E_K is the K^+ equilibrium potential (−99 mV under these experimental conditions). The conductance (*G*) was normalized to the maximal conductance (G_{max}) at +60 mV and plotted as a function of the test potential. E, F: Time to peak of Kv4.2 (E) and Kv4.1 (F) currents without (○) or with KChIP1 (●). Voltage steps were to −20 to +60 mV (in 10-mV increments) from a holding potential of −120 mV. $^*P < 0.05$.

tion time constants, but significantly altered the relative contributions of the fast and slower components of inactivation (Table 1). The increased contribution of fast inactivation is responsible for the overall acceleration of the Kv4.1 time course.

KChIP1 also affected other inactivation parameters. Recovery from inactivation of both Kv4.1 and Kv4.2 currents was accelerated (Fig. 2A,B; Table 1) whereas the voltage dependence of steady-state inactivation was not significantly affected by KChIP1 (Table 1). Activation parameters were also affected. KChIP1 induced a hyperpolarizing shift of about −20 mV in the voltage dependence of Kv4.2 activation (Fig. 2C; Table 1). In contrast, KChIP1 caused a small but significant depolarizing shift in Kv4.1 activation gating (Fig. 2D and Table 1). The time to peak of Kv4.1 and Kv4.2 currents were both accelerated when co-expressed with KChIP1 (Fig. 2E,F).

To examine the molecular basis of the differential effects of KChIP1 on the two Kv4 currents, we generated chimeras in which the divergent N- or C-termini of Kv4.2 and Kv4.1 were swapped. We refer to the construct in which Kv4.2 contains the Kv4.1 N-terminus as 1-2-2. Similarly, chimera 2-1-1 contains the Kv4.2 N-terminus but has the Kv4.1 core (S1-S6) and Kv4.1 C-terminus. The use of these chimeras identified the N-terminus as conferring the effect of KChIP1 on the inactivation time course. For example, when replacing the Kv4.2 N-terminus with that of Kv4.1 (chimera 1-2-2), the action of KChIP1 was converted from a slowing effect to an acceleration of inactivation (Fig. 3A). The median 1-2-2 inactivation time was 98 ± 3.9 ms ($n = 5$) but decreased to 69 ± 2.7 ms ($n = 5$; $P < 0.05$) when co-expressed with KChIP1. The involvement of the N-terminus was confirmed in the reciprocal experiment, in which the Kv4.2 N-terminus promoted slow inactivation in the presence of KChIP1. The median 2-1-1 current inactivation time was 46 ± 1.4 ms, but slowed to 64 ± 3.8 ($n = 5$; $P < 0.05$) when co-expressed with KChIP1 (Fig. 3B). In contrast, swapping the C-termini did not significantly alter the effects of KChIP1. As with Kv4.1, the inactivation time course of 1-1-2 current was accelerated by KChIP1 (the median inactivation was 109 ± 9.8 ms but 75 ± 2.7 ms with KChIP1, $n = 4$, $P < 0.05$), whereas (like Kv4.2) the 2-2-1 inactivation was slowed (the median inactivation time was 46 ± 6.3 and 62 ± 2.2 ms without and with KChIP1, $n = 4$, $P < 0.05$). KChIP1 retained its ability to increase the current amplitude of each of these chimeras (Fig. 3). These results provide strong support for the notion that structural elements within the Kv4 N-terminus are responsible for the observed differences that KChIP1 has on the inactivation time course of the individual Kv4 subfamily members and that this effect occurs irrespective of which C-terminus is present.

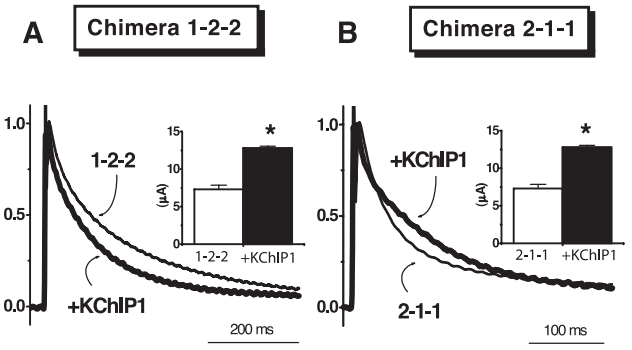

Fig. 3. Structural elements within the Kv4 N-terminus are involved in mediating the effects of KChIP1 on the Kv4 inactivation time course. Chimeras of Kv4.1 and Kv4.2, in which their N-termini were swapped, were co-expressed with KChIP1. Normalized currents and peak current amplitudes (at +50 mV; inset) of chimeras 1-2-2 (A) and 2-1-1 (B). Data in column format were obtained in the presence (filled columns) or absence (open columns) of KChIP1.

4. Discussion

Rapidly inactivating voltage-gated K^+ currents play an important role in electrical excitability. In neuronal cells, A-type

Table 1
Effects of KChIP1 on the activation and inactivation parameters of Kv4 currents

	Inactivation					Voltage dependence	
	median inactivation time (ms)	kinetics of inactivation		kinetics of recovery		activation	inactivation
		τ (ms)	contribution (%)	τ (ms)	contribution (%)	$V_{1/2}$ (mV), (k)	$V_{1/2}$ (mV), (k)
Kv4.2	26 ± 0.8	21 ± 0.6 188 ± 2	80 ± 0 13 ± 0	102 ± 5.7	100	−17.4 ± 2.5 (23 ± 0.3)	−110 ± 2.9 (8.4 ± 0.4)
Kv4.2+KChIP1	37 ± 5.5*	16 ± 1.9 122 ± 5*	37 ± 7* 57 ± 7*	9.1 ± 1.2* 120 ± 12	63 ± 2 37 ± 2	−37.9 ± 6.0* (23 ± 0.5)	−107 ± 6.2 (8.6 ± 0.6)
Kv4.1	80 ± 4.2	18 ± 0.9 85 ± 2.8 254 ± 5.1	19 ± 2 38 ± 2 41 ± 2	77 ± 5.5	100	−12.7 ± 1.6 (18 ± 1.0)	−77 ± 2.8 (13 ± 0.8)
Kv4.1+KChIP1	56 ± 4.0*	29 ± 6.3 99 ± 8.0 435 ± 74	35 ± 4* 46 ± 2* 17 ± 6*	19.7 ± 0.3* 160 ± 32*	95 ± 2 5 ± 1	−3.8 ± 3.2* (19 ± 0.4)	−72 ± 5 (10 ± 0.7)

The median inactivation times were obtained by measuring the time until half-maximal inactivation has occurred. The inactivation time constants (τ) were obtained by curve fitting of the inactivating current to a sum of two or three exponential functions. Recovery kinetics was analyzed by curve fitting to a single (in control) or double exponential functions (with KChIP1). The relative contribution of each component was calculated. Voltage dependence of activation and steady-state inactivation were, respectively, analyzed from normalized G–V relationships (in Fig. 1) and from steady-state inactivation curves by curve fitting using a Boltzmann equation. $V_{1/2}$ is the voltage (ms) at which channels are open half-maximally (for activation) or at which half-maximal steady-state inactivation occurred (for steady-state inactivation). k is the slope factor. *Student's t-test with Bonferroni correction ($P < 0.05$) (or Mann–Whitney Rank Sum test if the t-test failed) vs. the values without KChIP1. $n = 6$–13 from three different batches of oocytes.

K^+ channels operate in the subthreshold voltage range to control the timing, duration and frequency of action potentials. In heart muscle, the functional correlate of neuronal A-type currents is the transient outward K^+ current (I_{to}) that contributes to repolarization of the cardiac action potential. The principal molecular components of neuronal A-type K^+ currents and cardiac I_{to} are thought to be predominantly Kv4-subunits [2]. Three Kv4 subfamily members have been described to date (Kv4.1, Kv4.2 and Kv4.3) [13–15]. Kv4.2 and Kv4.3 are more closely related to each other (75% identity at the amino acid level) than to Kv4.1 (65–68% identity). Given these differences, we investigated whether Kv4.1 and Kv4.2 are differentially regulated by the Kv4 auxiliary subunit, KChIP1.

KChIP1 shifted the voltage dependence of Kv4.2 activation in a hyperpolarized direction (see also [10]) but interestingly, caused a small but significant depolarizing shift in Kv4.1 activation. Prevailing evidence suggest that S4 movement occurs upon membrane depolarization and the S4 segment thus acts as a 'voltage sensor' to trigger channel opening [16,17]. Accumulating evidence demonstrates that other structural elements may also be involved in gating. The T1 domain (the assembly domain within the proximal N-terminus), for example, also participates in Kv1 channel gating [18,19]. Since the T1-domain crystallizes as a tetramer and is thought to be situated directly below the channel pore [18,20], activation gating may be altered by channel structures not directly associated with the pore. Similar to the disruptive effects of mutations of the Kv1 T1 domain on activation gating [18], our data show that KChIP1 may decrease the stability of the Kv4.2 closed state, as assessed by a −20-mV shift in the activation voltage. Since KChIP1 shifts the Kv4.1 activation voltage in a depolarized direction, it may affect the stability of the Kv4.1 channel in an opposite way.

We found the inactivation time course of Kv4.2 currents to be substantially slowed by KChIP1 (this was also observed for Kv4.3 currents [10]). In contrast, KChIP1 markedly accelerated Kv4.1 inactivation. These changes occurred without major effects on the voltage dependence of steady-state inactivation of either Kv4.1 or Kv4.2 currents. Curve fitting revealed that these effects were not due to changes in the exponential time constants, but to alterations in the relative contributions of the fast and slow components of inactivation (Table 1). It is difficult to understand these effects of KChIP1 because the mechanisms of inactivation of Kv4 channels are not well understood. The two well established molecular mechanisms of inactivation, known as N-type [21] and C-type inactivation [22,23] do not seem to mediate most of Kv4 channel inactivation [12]. Evidence for a concerted action of the N- and C-termini in mediating the fast part of the inactivation process of Kv4 currents has been described [12] and mutations in the cytosolic Kv4 core region have been implicated in slow inactivation of Kv4.1 currents [24]. Molecular mechanisms that could account for these results have not yet been generated. Using a chimeric approach, we reported a unique role for the Kv4.2 N-terminus in promoting rapid inactivation [25]. Interestingly, KChIP1 also interacts with the Kv4.2 N-terminus [10]. It is possible, therefore, that KChIP1 may affect the inactivation process by virtue of its association with the cytosolic N-terminus of Kv4 channels. The differential effects of KChIP1 on Kv4.1 and Kv4.2 inactivation may reflect differences in the interaction of the accessory subunit to the N-termini. Alternatively, it is possible that the differential effects are merely reflective of differences that exist in the inactivation processes of the two channels (as suggested by the differences in their inactivation kinetics in the absence of KChIP1). For example, it is possible that upon binding of KChIP1 to the pore-forming subunit, the time course at which a particular inactivation conformation is attained may be slower for Kv4.2 and faster for Kv4.1 channels, thus resulting in apparently inverted effects. The differential effect of KChIP1 on Kv4 inactivation may provide a valuable future tool to facilitate our understanding of the mechanisms involved in the Kv4 inactivation process.

Kv4 channels operate in a voltage range where other voltage-activated K^+ channels are closed. Subtle differences in the voltage range of activation or the rate of activation and inactivation (as exist when comparing Kv4.1 and Kv4.2 currents) will alter the overall excitability of cells expressing these channels. Further alterations by KChIP1 may drastically modify subthreshold excitability by selectively altering kinetics or available number of Kv4.2 or Kv4.1 channels at certain potentials in cells that express these α and regulatory subunits. Our data point to a novel mechanism by which the molecular and functional diversity of K^+ channels can be increased, over and above the mechanisms that have already been described [2]. The interactions and functional effects observed here are likely to be of physiological significance since it is likely that the α and regulatory subunits are co-expressed in the same cells [10,26,27].

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