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The modulation of the rate of inactivation of the mKv1.1 K^+ channel by the β subunit, Kvβ1 and lack of effect of a Kvβ1 N-terminal peptide

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Abstract The coexpression of the rat Kvβ1 subunit with the mouse Kv1.1 (mKv1.1) K^+ channel in Chinese hamster ovary cells caused an increase in the rate of inactivation of whole-cell current. Current decayed in a bi-exponential fashion with a fast voltage-dependent and a slower voltage-independent component. The inactivating current component accounted for around 40% of the total outward current. In contrast to previous studies using K^+ channel α subunits, peptides based on the N-terminal of the Kvβ1 subunit were unable to mimic the action of the entire subunit. The findings indicate differences between the inactivation induced by the Kvβ1 subunit and the N-type inactivation mechanism associated with certain rapidly-inactivating cloned K^+ channel α subunits.

Key words: Cloned K^+ channel; β Subunit; Inactivation; Patch clamp; CHO cell

1. Introduction

Voltage-dependent K^+ channels, in common with channels selectively permeable to Ca^{2+} and Na^+, possess accessory subunits that modulate their function. A number of subtypes of the K^+ channel accessory β subunit, designated Kvβ1–3, have recently been cloned from mammalian brain and heart tissue and functionally expressed [1–5]. The Kvβ family are putative intracellular proteins consisting of around 400 amino acids. Coexpression of Kvβ1 with Kv1.1 (RCK1) has been shown to promote a rapid inactivation of current [2]; this was prevented by an amino terminal deletion, which may suggest comparisons with the N-type inactivation of K^+ channels by a ball and chain-type mechanism. For a number of channels that display N-type inactivation, it has been possible to apply exogenously short peptides based on their amino terminal and to accelerate inactivation [6,7]. Such peptides have been used to predict which structural motifs are important for inactivation and led to the hypothesis that such regions may be conserved between different amino terminal inactivating domains. We show here that whilst coexpression of the entire Kvβ1 subunit with mKv1.1 was able to increase the rate of inactivation, peptides based on the Kvβ1 N-terminal alone were without effect. A preliminary report of some of these findings has been published [8].

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2. Materials and methods

2.1. Cell transfection and culture

Chinese hamster ovary (CHO) cells, stably expressing mKv1.1 channels (supplied by Dr. Bruce Tempel, University of Washington, USA) were cultured as previously described [9]. After 48 h, subconfluent CHO-mKv1.1 cells in individual 35 mm Petri dishes were incubated in Roswell Park Memorial Institute (RPMI) 1640 media to which 10 μl of 'transfection solution' (containing 10 μg rat Kvβ1 subunit and 10 μl Lipofectin Reagent (GibcoBRL) made up to 100 μl with serum-free RPMI 1640 media) was added.

2.2. Peptide synthesis

Peptides were based on the amino-terminal of Kvβ1 (sequence MQVSIACTEHNLKSRNGEDRLLSKQSSTAPMVV) and corresponded to either the first 24 or first 33 amino acids. Peptides were synthesized by Mike Pisano and Herb MacGregor (Wyeth-Ayerst, USA) using solid-phase peptide synthesis and were confirmed as >95% pure by mass spectrometry.

2.3. Electrophysiological recording and data analysis

Cells were investigated using the whole-cell patch clamp technique between 2 and 4 days post-transfection. Cells were mounted in a recording chamber and perfused at 3 ml·min^{-1} with an 'extracellular solution' which comprised (in mM): NaCl 135, $MgCl_2$ 4, EGTA 1, KCl 5, $CaCl_2$ 2, glucose 25 and Hepes 10; pH 7.4 adjusted with NaOH. Patch electrodes were made from thin-walled borosilicate glass (GC150TF-10, Clark Electromedical Instruments). Electrodes were pulled and fire-polished using a DMZ Universal Puller (Zeitz Instruments) to give electrodes with resistance of 3–5 MΩ. Electrodes were back-filled with an 'intracellular solution' that comprised (in mM): K aspartate 120, KCl 20, $MgCl_2$ 1, MgATP 2, ethylene glycol-bis (b-aminoethyl ether) *N,N,N′N′*-tetra acetic acid (EGTA) 10, Hepes (*N*-[2-hydroxyethyl] piperazine-*N′*-[2-ethanesulfonic acid]) 10, pH 7.4 adjusted with NaOH. Peptides (320 μM) were tested on untransfected control cells and were added directly to the intracellular environment via the patch electrode. Whole-cell currents were acquired and stored on a Macintosh computer using an EPC9 amplifier (HEKA Electronik GmbH). Currents were sampled at rates between 500 μs–1.0 ms per point, filtered at 1/3 of the sampling rate using the PULSE (v7.4) program. Cells were held at a potential of −90 mV and currents were recorded with the cell capacitance compensated and leak currents subtracted using a p/4 subtraction protocol. Series resistance was compensated up to 70%. All experiments were conducted at room temperature (21–24°C).

Steady-state activation data were fitted with a Boltzmann function of the form:

$$g = g_{max}/[1+\exp((E_{1/2}-E_M)/k)]$$

where g is the conductance, g_{max} is maximum limiting conductance, $E_{1/2}$ is the mid-point for voltage dependence and k is the slope factor. Steady-state inactivation data were fitted with an extra component to describe the non-inactivating component. All data are expressed as means ± S.E.M.

3. Results

The transient transfection of the Kvβ1 subunit into a CHO cell line stably expressing mKv1.1 K^+ channels was able to

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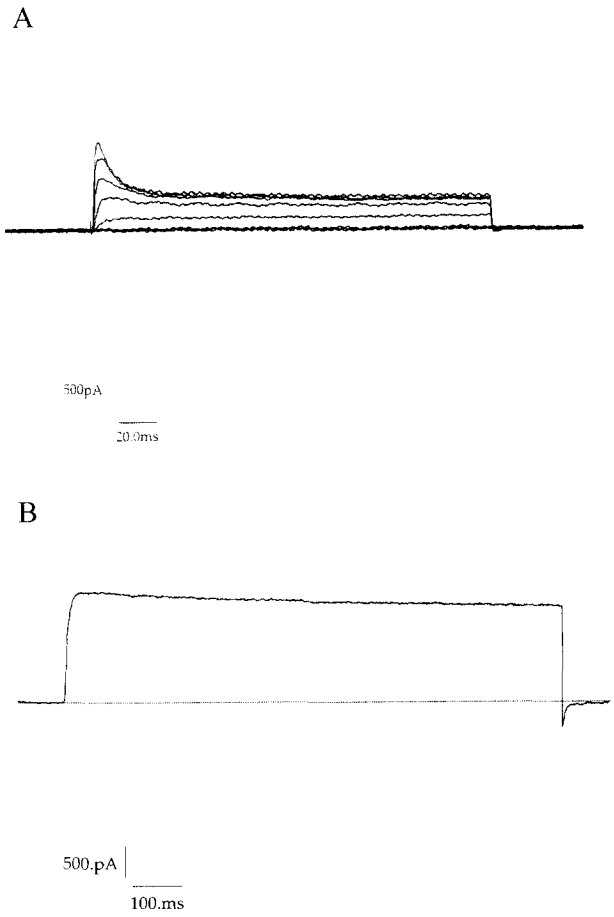

Fig. 1. Effect of Kvβ1 subunit and amino-terminal peptide on mKv1.1. (A) Family of outward currents of mKv1.1 coexpressed with the entire Kvβ1 subunit. 250 ms voltage pulses were applied from a holding potential of −90 mV in 20 mV increments. (B) Outward current for intracellular application of 320 μM Kvβ1–24 peptide to mKv1.1. A 1 s voltage pulse was applied to +70 mV from a holding potential of −90 mV.

increase the rate of inactivation of mKv1.1, a channel that normally shows essentially no inactivation over this time course (Fig. 1A); this effect was seen in 11 out of 23 cells tested. Current decay became more pronounced at increasing depolarized potentials and was best fitted with a double exponential function with a fast (12 ± 1 ms at +70 mV) and slow (390 ± 50 ms at +70 mV) component over a 1 s voltage step (n = 11). In contrast, inclusion of a peptide based on just the first 24 amino acids of Kvβ1 (Kvβ1 1–24) in the patch pipette had no similar effect on the rate of inactivation in 10 separate cells (Fig. 1B), even at depolarized potentials and over long time courses. Rettig et al. [2] have shown that a deletion of the first 34 amino acids of Kvβ1 prevented the induction of inactivation of Kv1.1(RCK1) channels when coexpressed in *Xenopus* oocytes. In the present study, a Kvβ1 1–33 peptide had no effect on the rate of inactivation of mKv1.1 (n = 5, results not shown).

Fig. 2 shows some of the electrophysiological characteristics of mKv1.1 when co-expressed with Kvβ1 (mKv1.1/Kvβ1). The voltage-dependence of steady-state activation of mKv1.1 was not shifted by Kvβ1 (Fig. 2A). Steady state activation of mKv1.1/Kvβ1 was fitted with a single Boltzmann function which gave an $E_{1/2}$ of −3 ± 4 mV and k = 14 ± 1 mV (n = 6); in control cells, mKv1.1 alone had an $E_{1/2}$ of −3 ± 1 mV and k = 14 ± 1 mV (n = 5). The fast component of current decay for mKv1.1/Kvβ1 was voltage-dependent, speeding up with depolarization (Fig. 2B). In contrast, the slow component of inactivation was independent of voltage. Steady-state inactivation of

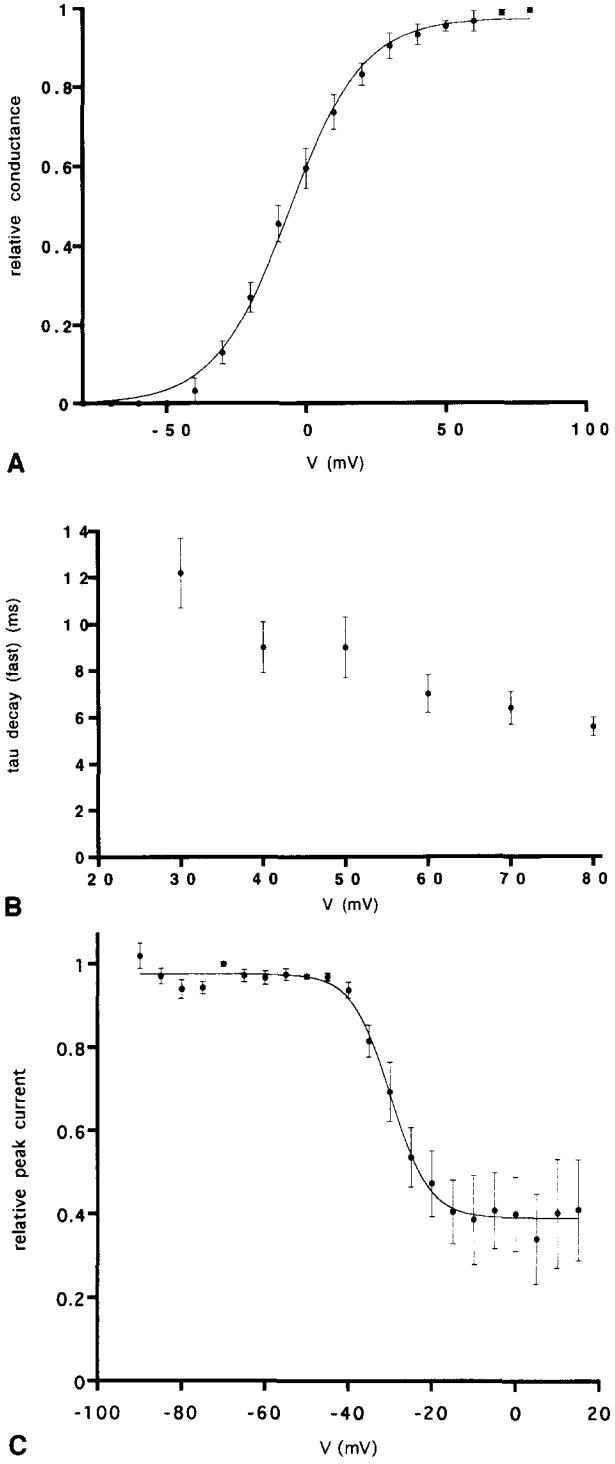

Fig. 2. Electrophysiological characteristics of mKv1.1/Kvβ1. (A) Steady state activation of mKv1.1/Kvβ1. Group data from 6 separate cells was well fitted with a single Boltzmann function with $E_{1/2}$ = −6 mV and k = 13 mV. (B) Voltage-dependence of fast τ_d component. (C) Steady-state inactivation of mKv1.1/Kvβ1. Group data from 5 separate cells was well fitted with a single Boltzmann function with $E_{1/2}$ = −30 mV and k = −5 mV.

mKv1.1/Kvβ1 was fitted by a modified Boltzmann function with an extra component to described the non-inactivating plateau current; this gave an $E_{1/2}$ of -28 ± 2 mV and $k = -5 \pm 1$ mV ($n = 5$). In these cells, the inactivating component accounted for $62 \pm 8\%$ ($n = 5$) of the total outward current.

4. Discussion

We report here that the Kvβ1 subunit is able to increase the rate of inactivation of mKv1.1 in a mammalian cell expression system. In the presence of Kvβ1, current decayed bi-exponentially and the inactivation was typically only partial. Removal of the amino terminal of Kvβ1 removes the inactivation induced in Kv1.4 channels lacking an endogenous ball [2], which may suggest similarities with the N-type inactivation mechanism displayed by α subunits. In the present study, a voltage-dependent increase in the fast τ_d component was seen for Kvβ1 acting on mKv1.1, as reported for inactivation peptides based on the amino terminal of the α subunit of *Shaker* B [9] and Kv3.4 [7] acting on mKv1.1. However, a number of differences between the inactivation associated with K^+ channel α and β subunits were noted. Firstly, inactivation was only apparent at more depolarized (>+20 mV) potentials; secondly, a large non-inactivating component (around 40% of current) was retained; and thirdly, an amino terminal peptide was unable to induce an increase in inactivation rate.

The partial inactivation suggests a looser association between the channel and the proposed 'β-ball' than the association with the corresponding region in α subunits. Similar characteristics have been reported for two β subunits that possess an extended amino terminal, Kvβ1 [2] and Kvβ3 [3–5]. Differences between Kvβ subunits also exist. Whilst we saw no effect of Kvβ1 on the voltage-dependence of mKv1.1 activation, the action of Kvβ3 on Kv1.5 was associated with an 18 mV hyperpolarizing shift in activation curves [5]; this study also showed a slowing of tail currents in the presence of Kvβ3. A major difference between Kvβ subunits with a proposed inactivation domain is in their specificity. Kv1.1 channels can be modified by Kvβ1, but not by Kvβ3 [3,4]; indeed Kvβ3 is also without effect on Kv1.2 and Kv2.1, but does cause an increase in the rate of inactivation in ShBΔ6–46, Kv1.4 and Kv1.5 [3–5]. Such differences in the permutations of subunit association may help to explain the functional diversity of K^+ channels.

We were unable to mimic the action of Kvβ1 on mKv1.1 with a peptide based on the Kvβ1 amino terminal, this was in contrast to previous reports showing that peptides based on the amino terminal of the α subunit of *Shaker* B [9], Kv3.4 [7] and Kv1.4 [8] were able to modify mKv1.1. The net charge of K^+ channel α subunit peptides has been correlated to the binding on-rates for *Shaker* B [10], Ca-dependent K^+ channels [11] and mKv1.1 (G. Stephens, unpublished results). The Kvβ1 1–24 peptide has been reported to cause a modest increase in the rate of inactivation of Kv1.4 channels lacking an amino terminal [2]; the rate of inactivation was increased to that seen with the entire Kvβ1 subunit by the substitution of residues to raise the net positive charge of the peptide from +1 to +3. Therefore, it is possible that lack of sufficient positive charge may contribute to the lack of effect exhibited by the Kvβ1 peptides here. A further possibility is that the peptide forms only a very loose association with the channel as suggested by the large non-inactivating component seen when Kvβ1 was co-expressed with mKv1.1. The findings do not contradict the hypothesis that the N-terminal of Kvβ1 forms an inactivation ball that occludes the channel pore, but rather that other regions of the subunit may also be required for this action to occur within the cell. In K^+ channel α subunits, structures such as an extended amino acid chain adjacent to the ball sequence has been shown to affect the rate of channel inactivation [12]. The identification of such regions in Kvβ1 will require further work, but these preliminary findings are consistent with differences in N-type inactivation mechanisms between K^+ channel α and β subunits.

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