Molecular cloning and functional expression of a novel potassium channel β-subunit from human atrium

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Abstract We report the cloning and functional expression of a novel K^+ channel β-subunit from human atrium, hKvβ3. hKvβ3 is highly homologous to the two β-subunits cloned from rat brain, Kvβ1 and Kvβ2, but has an essentially unique stretch of 79 N-terminal residues. Upon expression in *Xenopus* oocytes, hKvβ3 accelerates the inactivation of co-injected hKv1.4 currents and induces fast inactivation of non-inactivating co-injected hKv1.5 currents. By contrast, hKvβ3 had no effect on hKv1.1, hKv1.2, or hKv2.1 currents. Thus, hKvβ3 represents a third type of K^+ channel β-subunit which modulates the kinetics of a unique subset of channels in the Kv1 subfamily.

Key words: Potassium channel; β-Subunit; Human heart; cDNA cloning; *Xenopus* oocyte

1. Introduction

Voltage-gated ion channels are formed by a pore-forming α-subunit the functional characteristics of which may be modified by one or more accessory proteins [1]. In the case of Na^+ channels, the kinetic properties of the α-subunit may be altered by one or more β-subunits [2]. For Ca^{2+} channels, a variety of accessory proteins, including α_2-δ, γ, and four different β-subunits have been described [1]. K^+ channels have been shown to associate with accessory subunits as well [3,4], and two members of a new family of β-subunits which interact with K^+ channels have recently been cloned [5,6]. Rat brain Kvβ1 introduces inactivation into a non-inactivating delayed rectifier K^+ channel, Kv1.1, and accelerates the inactivation of a fast-inactivating K^+ channel, Kv1.4, while no effect on K^+ channel kinetics has been described for Kvβ2 [6]. K^+ channels constitute the most diverse group of ion channels [7,8], and modulatory β-subunits might serve to increase the functional diversity of K^+ currents. Thus, characterizing the interaction of β-subunits with K^+ channel gene products is a critical step in determining the molecular composition of individual native K^+ currents.

Cardiac K^+ currents are crucial to the maintenance of the complex electrical activity of the heart [9]. A variety of K^+ channel genes are expressed in the heart [10], yet little is known about the molecular basis of individual cardiac K^+ currents. Here we have examined human atrium for the presence of K^+ channel β-subunit sequences, and report the cloning of a novel member of this family, hKvβ3. Through heterologous expression in *Xenopus* oocytes, the functional effects of this accessory subunit on a number of human K^+ channels, including the voltage-gated channels hKv1.1, hKv1.2, hKv1.4, hKv1.5, and hKv2.1, have been examined. We find that hKvβ3 specifically modulates the kinetics of hKv1.5 and hKv1.4. The existence of multiple β-subunits with specific functional effects on K^+ channel subsets brings a new perspective to the search for the link between K^+ channel gene products and native currents.

2. Materials and methods

2.1. Cloning of hKvβ3 from human atrium

Specimens of atrial appendages were obtained from adult patients undergoing coronary bypass surgery. Total RNA was extracted using RNA-Stat 60 (Tel-Test Inc., Houston, TX) according to the manufacturer's protocol. Poly(A)$^+$ RNA was isolated from total RNA with the oligo-dT-based Messenger RNA Isolation Kit (Stratagene). A mixture of oligo(dT)$_{12-18}$ primers and random hexamers [pd(N)$_6$] was used to prime first strand cDNA synthesis from 10 μg poly(A)$^+$ RNA using the TimeSaver cDNA Synthesis Kit (Pharmacia). Construction of the cDNA library was done with the cDNA Synthesis Kit from Stratagene. 120 ng of double-stranded cDNA (>700 bp), following fractionation on Sephacryl S-400, was ligated to 1 μg *Eco*RI-CIAP treated λ-ZAPII vector (Stratagene). The ligation product was packaged in Phagemaker In Vitro Packaging System (Novagen), and resulted in 1×10^6 independent recombinant clones. The unamplified library was screened at low stringency for sequences homologous to bovine Kvβ2. The probe, bovine Kvβ2 cDNA [1], was obtained by RT-PCR from bovine brain poly(A)$^+$ RNA (Clontech), and ^{32}P random prime labelled (Multiprime DNA Labeling System; Amersham). Hybridization was for 16–18 h at 45°C in RapidHyb buffer (Amersham) at a probe concentration of 3×10^6 cpm/ml, and the filters were washed at 45°C with 1 × SSC, 0.1% SDS (1 × SSC contains 150 mM NaCl, 15 mM sodium citrate, pH 7.0). Three positive clones were isolated, and the pBluescript SK(−) plasmids excised from λ-ZAPII. The clone with the longest insert, 2.6 kb, was sequenced in its entirety in both directions, and found to contain a single open reading frame encoding 408 amino acids with homology to rat and bovine Kvβ subunits.

2.2. Expression of hKvβ3 cRNA in Xenopus oocytes

To boost the expression of hKvβ3 in oocytes, the hKvβ3 cDNA was subcloned into an expression vector which contains a poly(A)$^+$-tail, A$^+$-pCRII [14]. The coding region of hKvβ3 was amplified by PCR to facilitate cloning into the *Apa*I–*Eco*RI sites of A$^+$-pCRII. The hKvβ3 PCR product was sequenced completely and found to be identical to the original clone.

Complementary RNA (cRNA) for injection into oocytes was prepared as previously described following linearization of the plasmid with *Bam*HI [10]. The origin of the cDNAs co-injected with hKvβ3 is as follows. hKv1.5 was previously cloned in our laboratory [11], and subcloned into A$^+$-pCRII for oocyte expression. hKv1.4 was obtained by RT-PCR of human heart total RNA using oligonucleotides derived

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Abbreviations: SDS, sodium dodecyl sulfate; NMDG, *N*-methyl-D-glucamine; MES, 2(*N*-morpholino)ethanesulphonic acid; HEPES, *N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfoni c acid; SSC, saline-sodium citrate; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase polymerase chain reaction; kb, kilobase pairs; bp, base pairs; N- terminal, amino-terminal.

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from the published sequence [12], sequenced, and subcloned into A^+-pCRII. hKv1.1 and hKv1.2 were kindly provided by O. Pongs. For oocyte expression, the coding region of hKv1.1 was amplified by PCR, sequenced, and subcloned into A^+-pCRII. We cloned hKv2.1 from a human brain cDNA library, and found the predicted amino acid sequence to be consistent with the published sequence [13]. Oocytes were injected with 46 nl of cRNA at concentrations ranging from 50 to 150 ng/ml. Weight ratios of hK$v\beta3$: K^+ channel cRNAs of 1 : 1. 2 : 1, and 4 : 1 were tested with comparable results.

Macroscopic currents were recorded in oocytes voltage-clamped with two intracellular microelectrodes as previously described [14]. No linear leakage and capacity current subtraction was performed unless specified. The external bathing solution consisted of either (in mM): NMDG, 122.5; MES, 122.5; $MgCl_2$-H_2O, 2.5; HEPES, 10 pH 7.4: or NMDG, 22.5; KOH, 100; MES, 122.5; $MgCl_2$-H_2O, 2.5, HEPES pH 7.4.

3. Results and discussion

3.1. cDNA isolation and sequence analysis

To probe for the expression of K^+ channel β-subunit sequences in human heart, we screened a human atrial cDNA library with a fragment derived from the coding region of bovine brain K$v\beta2$. Under conditions of low stringency we isolated three positive clones, one of which contained a 2.6 kb insert. This clone, hK$v\beta3$, when sequenced in its entirety in both directions, was shown to encompass a complete opening reading frame of 1224 bp (Fig. 1A). The clone contains 68 bases of putative 5′ untranslated sequence, as well as approximately 1.3 kb of untranslated sequence at the 3′ end (only a small portion is shown). There is an in-frame stop codon (TGA) just 3 bases upstream of the presumed start ATG, indicating that the 2.6 kb clone contains the entire coding sequence.

The open reading frame of hK$v\beta3$ predicts a protein of 408 amino acids with significant homology to bovine K$v\beta2$ as well as rat K$v\beta1$ and K$v\beta2$ (Fig. 1B). Over the C-terminal 329 amino acids, hK$v\beta3$ is 100% identical to rat brain K$v\beta1$, and 85% identical to both bovine and rat brain K$v\beta2$. The three types of β-subunits differ, however, in the length and sequence of their N-terminal regions. hK$v\beta3$ has an N-terminal region of 79 amino acids, slightly longer than the N-terminus of $\beta1$ (72 amino acids) and twice the length of $\beta2$ (38 residues). Virtually no homology exists between the N-terminal regions of $\beta2$ and $\beta3$, but two regions of the N-terminus of hK$v\beta3$ can be aligned with the putative ball peptide sequence [15,16] of $\beta1$, as shown in Fig. 1C. Both alignments have been made relative to a conserved cysteine residue (marked with an arrow). Rat K$v\beta1$ has a cluster of positively charged residues (K and R) following the cysteine residue, as do both regions of hK$v\beta3$. Thus, hK$v\beta3$ may contain two ball peptide-like sequences whereas rat $\beta1$ has only one.

3.2. Functional expression of hKvβ3 in Xenopus oocytes

To investigate the functional role of hK$v\beta3$ in human heart,

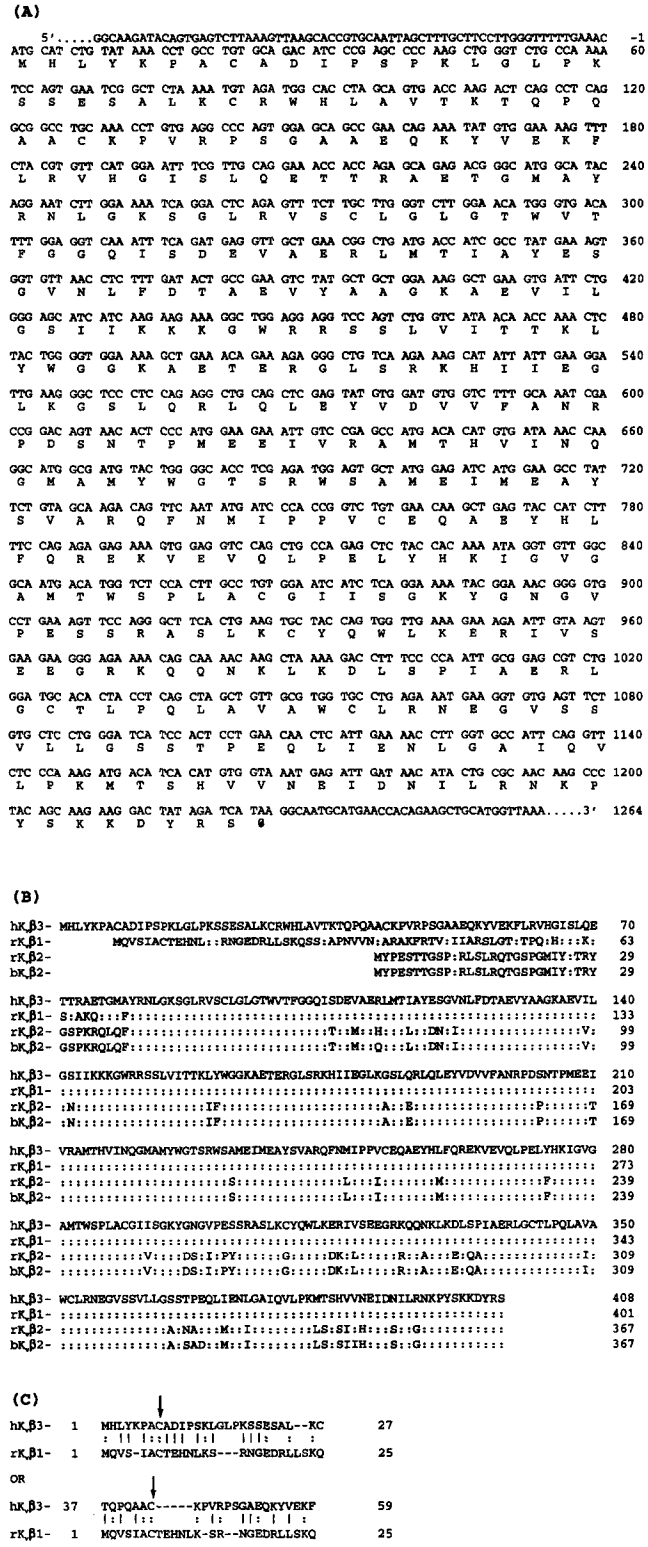

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Fig. 1. Primary Structure of hK$v\beta3$. (A) Nucleotide and predicted amino acid sequence of hK$v\beta3$ cDNA. Nucleotides are numbered beginning with the first base of the initiating methionine codon, ATG. Only a small stretch of the 3′ untranslated sequence is shown. There is one potential phosphorylation site for cAMP-dependent protein kinase (R/K-X-X-S/T) at S150, and ten potential sites for protein kinase C (S/T-X-R/K) at S13, T71, T157, T168, S173, T228, S293, S303, S307 and S402. The hK$v\beta3$ sequence has been submitted to the Genbank (accession number U16953). (B) Alignment of the deduced amino acid sequence of hK$v\beta3$ with rat K$v\beta1$, rat K$v\beta2$ and bovine K$v\beta2$. Residues identical to hK$v\beta3$ are denoted with a colon (:). (C) Alignment of the two putative ball peptide sequences of hK$v\beta3$ with the reported ball sequence of K$v\beta1$. Two stretches in the N-terminus of hK$v\beta3$ (amino acids 1–27 and amino acids 37–59) have been aligned separately with the ball peptide sequence of K$v\beta1$ (residues 1–25). Identical residues are marked with a colon (:) and conservative substitutions, based on charge, polarity and hydrophobicity, are indicated with a vertical line (|). The conserved C (cysteine) residue is marked with an arrow.

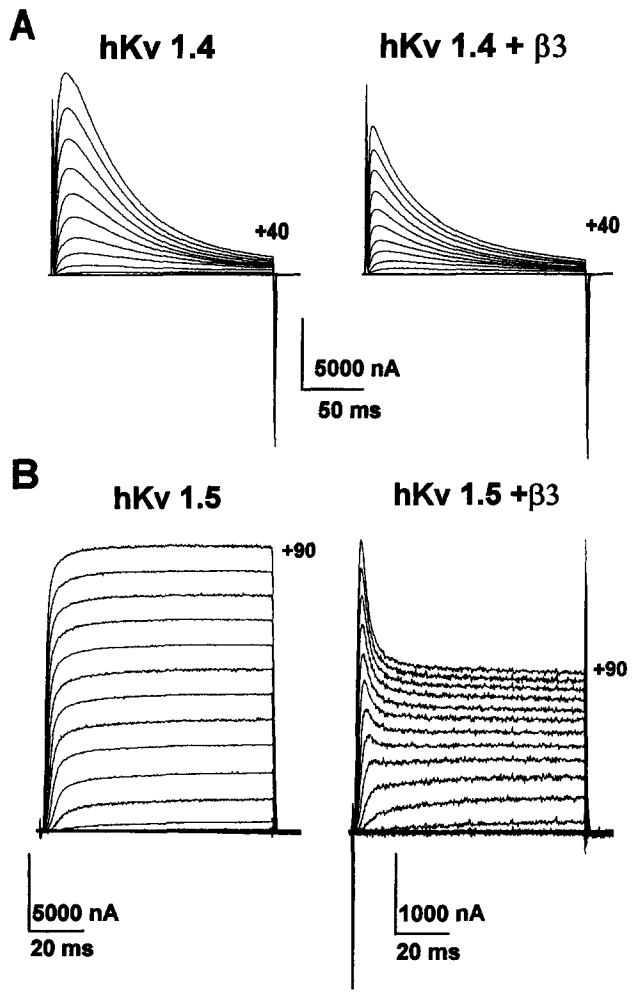

Fig. 2. Effect of hKvβ3 on human K^+ channels hKv1.4 and hKv1.5. (A) Families of currents recorded from hKv1.4 (left) and hKv1.4 co-expressed with hKvβ3 (right), stepping from −60 mV to +40 mV with 10 mV steps from a holding potential of −80 mV. (B) K^+ currents recorded from oocytes injected with hKv1.5 (left) and hKv1.5 plus hKvβ3 (right). The recordings were obtained by stepping from a holding potential of −80 mV with 10 mV steps from −60 mV to +90 mV. A P/4 subtraction was used to correct for leakage and capacitance currents only in the record in the left panel.

we heterologously expressed hKvβ3 in *Xenopus* oocytes in the presence of a variety of K^+ channel subunits. Initially we examined the interaction of hKvβ3 with a fast inactivating channel, hKv1.4, which expresses an A-type current when expressed in *Xenopus* oocytes (Fig. 2A, left). When co-injected with hKvβ3, inactivation of hKv1.4 currents was accelerated at potentials ranging from +20 to +80 mV (Fig. 2A, right). The currents were best fitted to a double exponential function. The faster time constant was 2–3 times smaller than that calculated for hKv1.4 alone (at +70 mV, $\tau 1 = 13.5 \pm 1.9$ ms for hKv1.4 plus hKvβ3 compared to 39.8 ± 10.2 ms for hKv1.4 alone, $P < 0.01$; at +40 mV, $\tau 1 = 22.8 \pm 3.6$ ms for hKv1.4 plus hKvβ3 compared to 39.9 ± 5.8 ms for hKv1.4, $P < 0.05$; $n = 5$–8). The slower time constant, $\tau 2$, was also decreased in the presence of hKvβ3 (at +70 mV, $\tau 2 = 62.1 \pm 3.0$ ms for hKv1.4 plus hKvβ3 compared to 117.4 ± 31.8 ms for hKv1.4 alone, $P > 0.05$; at +40 mV, $\tau 2 = 77.8 \pm 5.8$ for hKv1.4 plus hKvβ3 compared to 119.5 ± 27.5 for hKv1.4 alone, $P > 0.05$; $n = 5$–8). In comparison, when fitted to a single exponential, Kvβ1 decreases the τ_i of Kv1.4 approximately six-fold at +50 mV [6].

We also examined the effects of hKvβ3 on the delayed rectifier hKv1.5, which exhibits little inactivation during a 200 ms pulse (Fig. 2B, left). Upon co-expression with hKvβ3, fast inactivation of the whole-cell currents appeared (Fig. 2B, right; $n = 16$) at very depolarized potentials (+30 to +90 mV): $13.0 \pm 0.2\%$ and $36.0 \pm 0.2\%$ of the whole-cell currents were inactivated at +40 mV and +90 mV, respectively ($n = 25$). The current decay was best fitted with a double exponential function. The faster time constant ($\tau 1$) (3.83 ± 0.1 ms at +90 mV; 3.50 ± 0.2 ms at +40 mV; $n = 11$) was not voltage-dependent ($P > 0.05$), whereas the slower time constant ($\tau 2$) was voltage-dependent with values of 39.7 ± 4.3 ms at +90 mV and 94.0 ± 10.7 ms at +40 mV ($P < 0.001$; $n = 11$). In addition, unlike hKv1.4, the level of hKv1.5 currents appeared to be five- to ten-fold lower in the presence of hKvβ3. The reason for this effect is presently unknown.

Of the channels examined in the Kv1 subfamily, hKvβ3 was specific for hKv1.4 and hKv1.5. Two other human channels, hKv1.1 and hKv1.2, generate non-inactivating delayed rectifier currents when expressed in oocytes, but, as illustrated in Fig. 3A and B, no inactivation was observed at potentials up to +80 mV ($n = 31$ and 20, respectively) when either channel was co-expressed with hKvβ3. Furthermore, hKvβ3 had no observable effect on the expression levels of either Kv1.1 or Kv1.2 currents.

The specificity of hKvβ3 was further confirmed in co-injection experiments with the voltage-gated delayed rectifier, hKv2.1. No effects on inactivation or level of expression of whole-cell currents were observed when hKvβ3 was co-injected with equal amounts of hKv2.1 cRNA (Fig. 3C; $n = 8$). We also examined two inward rectifiers which share homology in the H5 or putative pore region with voltage-gated K^+ channels, IRK1 [17] and hIRK, cloned from human atrium [18]. No kinetic changes in the presence of hKvβ3 at potentials ranging from −120 mV to +50 mV were observed (data not shown). Finally, currents from oocytes injected with hKvβ3 resembled those of uninjected oocytes (Fig. 3D).

The human heart clone, hKvβ3, constitutes the third member of a newly described β-subunit gene family. hKvβ3 affects the kinetic properties of hKv1.5 and hKv1.4, both of which are expressed in heart [19]. Kv1.2 and Kv2.1 are also expressed in heart [19], yet, interestingly, hKvβ3 has no apparent effect on the inactivation properties of these two channels or on hKv1.1, which is abundantly expressed in the brain [20]. Thus, hKvβ3 is able to differentially affect members of the Kv1 subfamily.

The specificity of hKvβ3 is different from that observed for rat brain Kvβ1, which introduces fast inactivation into Kv1.1 and accelerates the inactivation of Kv1.4 [6]. The proposed mechanism for Kvβ1 induced inactivation is through a ball peptide-like sequence in the N-terminus. hKvβ3 is identical to Kvβ1 except in the N-terminal region, however, sequence alignments within this region indicate that hKvβ3 may actually possess two ball peptide-like sequences. Mutational analysis in this region may bring insight into the mechanism and specificity of hKvβ3.

For heart, one of the unanswered questions is the molecular nature of the channels that underlie the transient outward current, I_{to} [21,22]. Heteromultimeric assembly of specific K^+ channel subunits has been shown to generate inactivating currents

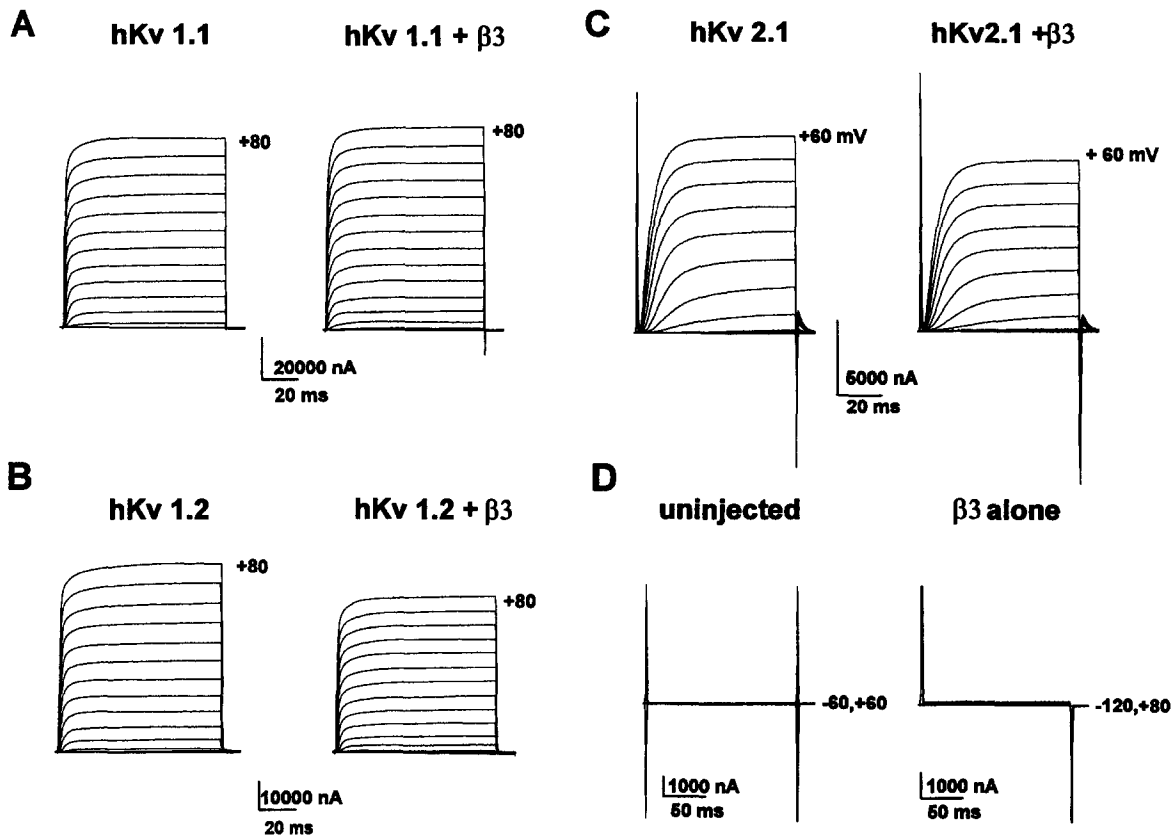

Fig. 3. Effects of hKvβ3 on hKv1.1, hKv1.2 and hKv2.1. (A) hKv1.1 (left) and hKv1.1 plus hKvβ3 currents recorded with 10 mV steps from −60 mV to +80 mV from a holding potential of −60 mV. P/4 substraction was performed to correct for leakage and capacitance currents. (B) hKv1.2 (left) and hKv1.2 plus hKvβ3 (right) currents elicited by the same pulse protocol described in A. A P/4 subtraction protocol was also applied to these records. (C) Whole-oocyte currents recorded from hKv2.1 alone (left) or in the presence of hKvβ3 (right) with 10 mV steps from −60 mV to +60 mV from a holding potential of −60 mV. (D) Currents recorded from either uninjected oocytes (left) or oocytes injected with cRNA encoding hKvβ3 alone.

which resemble, but do not reproduce entirely, the properties of native I_{to} [23]. The interaction of endogenous cardiac β-subunits with K^+ channel gene products might provide the missing molecular component of I_{to} currents.

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