

# Alternative splicing of the human *Shaker* K<sup>+</sup> channel $\beta 1$ gene and functional expression of the $\beta 2$ gene product

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**Abstract** Mammalian voltage-activated *Shaker* K<sup>+</sup> channels associate with at least three cytoplasmic proteins: Kv $\beta 1$ , Kv $\beta 2$  and Kv $\beta 3$ . These  $\beta$  subunits contain variable N-termini, which can modulate the inactivation of *Shaker*  $\alpha$  subunits, but are homologous throughout an aldolase core. Human and ferret  $\beta 3$  proteins are identical with rat  $\beta 1$  throughout the core while  $\beta 2$  proteins are not;  $\beta 2$  also contains a shorter N-terminus and has no reported physiological role. We report that human  $\beta 1$  and  $\beta 3$  are derived from the same gene and that  $\beta 2$  modulates the inactivation properties of Kv1.4  $\alpha$  subunits.

**Key words:** Potassium channel;  $\beta$  subunit; Aldo-keto reductase; Hippocampus; NADPH-dependent oxidoreductase

## 1. Introduction

*Shaker* and other voltage-dependent K<sup>+</sup> channel proteins help to determine the electrical properties of excitable cells and play additional physiological roles in non-excitable cell types [1]. Purification of K<sup>+</sup> channel proteins indicated that they were associated with additional subunits [2,3] while expression studies reported that a putative  $\beta$  subunit modulated the inactivation and amplitudes of K<sup>+</sup> channel currents from rat brain [4]. Scott et al. [5] isolated and cloned the first associated subunit,  $\beta 2$ , from bovine brain; its physiological role was not established. Subsequently, a novel subunit,  $\beta 1$ , was isolated [6] and found to increase the inactivation rate of *Shaker* K<sup>+</sup> channels through its N-terminal domain. The isolated N-terminal domain of  $\beta 1$  proteins is sufficient to induce inactivation in at least some non-inactivating *Shaker* K<sup>+</sup> channels [6]. Novel  $\beta 3$  subunit cDNAs isolated from both ferret [7] and human [8] heart tissue, vary from  $\beta 1$  in their N-termini but are identical to the rat  $\beta 1$  protein over the C-terminal 329 residues.  $\beta 3$  gene products have also been shown to alter the inactivation properties of some *Shaker*  $\alpha$  subunits [7,8] although the mechanism of its inactivation has not been established.

Because *Shaker* K<sup>+</sup> channel  $\beta$  subunits belong to a superfamily of NAD(P)H-dependent enzymes [9], which suggests that they may play other physiological roles, we were interested in determining whether, like  $\beta 1$  and  $\beta 3$ , the  $\beta 2$  subunit is also capable of modulating  $\alpha$  subunit activity. Moreover, we were interested in developing an assay for the expression of the  $\beta 2$  subunit. Here, we report the highly conserved sequences of the

human  $\beta 1$  and  $\beta 2$  gene products and that human  $\beta 1$  and  $\beta 3$  are alternative products of the  $\beta 1$  gene. In addition, we report that  $\beta 2$  modulates the inactivation properties of the *Shaker* K<sup>+</sup> channel Kv1.4 although it does not induce inactivation in non-inactivating Kv1.4 channel constructs or Kv1.1 channels. Moreover, both  $\beta 1$  and  $\beta 2$  are able to increase the amplitude of Kv1.4 currents expressed in *Xenopus* oocytes. Although apparently not evolutionarily related to the accessory proteins of other voltage-gated channels [10], the  $\beta$  subunits do appear to regulate a number of similar properties in K<sup>+</sup> channel complexes.

## 2. Materials and methods

### 2.1. Generation of human $\beta 1$ and $\beta 2$ PCR fragments

Degenerate oligonucleotides were designed for nested PCR; in the first round, the outer sense and antisense oligonucleotides (Fig. 1) were added to a standard PCR reaction mixture [11] using a 1- $\mu$ l aliquot of a human hippocampal cDNA library. Annealing temperature was stepped from 55 to 57 and 60°C for 90 s for the first 8, second 8 and last 16 cycles, respectively. Extension and denaturation was constant at 72°C for 120 s and 94°C for 90 s. The reaction products were diluted 400-fold and 2- $\mu$ l aliquots were used in identical secondary reactions, using the inner sense and antisense oligonucleotides. Sense oligonucleotide sequences were (5' to 3') GGNYTNGGNACNTGG and GAYACNGCNGARGTNTA, corresponding to GLGTW and DTAENV, respectively. Antisense oligonucleotides TCNACRTAYTCNARYTG and CANGCNAGNGGNGACCA, correspond to the amino acid sequences QLEYVD and WSPLAC. N = A,C,G,T, Y = C,T, R = A,G. PCR-generated fragments of the predicted size (207 bp) were cloned into a T-overhang vector (Amersham) and 16 independent clone fragments were sequenced; only  $\beta 1$  and  $\beta 2$  human homologs were found.

### 2.2. Isolation of human $\beta 1$ and $\beta 2$ cDNA and genomic clones

$\beta 1$  and  $\beta 2$  cDNA fragments were used to screen the same library for full-length clones. Nitrocellulose filters (Schleicher and Schuell) were hybridized in 50% formamide, 5  $\times$  SSPE, 50 mM Hepes, pH 7.5, 0.25% SDS and 100  $\mu$ g/ml salmon sperm DNA overnight at 42°C with <sup>32</sup>P-random primed probe, washed in 2  $\times$  SSC and 0.1% SDS three times at 22°C for 10 min followed by a 15-min wash in 0.1  $\times$  SSC, 0.1% SDS at 60°C. cDNA clones were isolated, both strands sequenced and 3' untranslated sequences deleted before they were used as probes for screening an EMBL3 human genomic library (gift of M. Ramaswami) under similar conditions.

### 2.3. Expression of cRNA in *Xenopus* oocytes

Prior to RNA transcription,  $\beta 1$  and  $\beta 2$  cDNAs were subcloned into a vector containing 40 adenosine nucleotides downstream of the 3' cloning site and linearized with Not I. The  $\beta 1$  cDNA was truncated 10 nucleotides 3' of the stop codon while the  $\beta 2$  cDNA contained over 2.5 kb of 3' untranslated sequence. K<sup>+</sup>  $\alpha$  subunit constructs; Kv1.4, an N-terminal truncated and non-inactivating Kv1.4 and Kv1.1 [12] were transcribed from the same vector. For  $\beta 2$  expression, the 5' untranslated sequence was changed from GGCTGGCTCC to CGCCGCCAAG. Oocytes were injected with 40 nl of cRNA at various dilutions. All

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electrophysiology experiments were carried out at 16°C. Two micro-electrode recordings were obtained with an OC-725 voltage clamp (Warner Instruments) and the pClamp program (Axon Instruments). Electrodes (0.4–0.8 MΩ) were filled with 1 M KCl. Recordings were obtained in ND-96 solution [13]. Data were filtered at 2 kHz and leak subtracted on-line using hyperpolarizing p/4 subtraction pulses from a holding potential of –80 mV.

### 3. Results and discussion

#### 3.1. Human $\beta 1$ and $\beta 2$ cDNAs

Human  $\beta 1$  and  $\beta 2$  fragments were generated from a human hippocampal cDNA library using polymerase reaction (PCR) with degenerate oligonucleotides (Fig. 1) to amino acid sequences conserved in rat  $\beta 1$  and  $\beta 2$  [6] and used to screen for full-length cDNAs from the same library. Deduced amino acid sequences of the full-length human  $\beta 1$  and  $\beta 2$  gene products are aligned in Fig. 1 with previously reported  $\beta$  subunit sequences. The human and rat  $\beta 1$  brain homologs show 100% identity over their entire length of 401 residues and 93% identity at the nucleotide level while the  $\beta 2$  gene product shows 99% homology to the bovine and rat proteins and 91–93% at the nucleotide level. Both the *Shaker* K<sup>+</sup> channel  $\beta$  subunits and channel forming  $\alpha$  subunits [12] are highly conserved in mammals, indicating highly conserved physiological functions among the various species.

#### 3.2. $\beta 1$ and $\beta 3$ are alternative splice forms of the $\beta 1$ gene

$\beta 1$  and  $\beta 3$  proteins from the various mammalian species vary throughout their N-termini but are identical over their remaining 329 C-terminal residues (Fig. 1). Homologies with both the  $\beta 2$  subunits (Fig. 1) and other members of the aldo-keto reductase superfamily occurs over the same C-terminal region [9]. However, identity between the amino acid sequences of  $\beta 1$  and

$\beta 3$  proteins indicates that they could be alternative splice forms of the same gene. Alignment of the human  $\beta 1$  and  $\beta 3$  cDNA nucleotide sequences (Fig. 2A) indeed indicates that at the beginning of the aldo-keto core region the nucleotide sequences of these cDNAs become identical. In order to further establish that  $\beta 1$  and  $\beta 3$  represent alternative splice products we isolated and sequenced a genomic clone containing the human  $\beta 1$  N-terminus (Fig. 2B) and found that it is encoded by a single exon containing a consensus splice site at the predicted location: the first potential –2, –1 AG sequence downstream of the last nucleotide difference between human  $\beta 1$  and  $\beta 3$  cDNAs (nucleotide 219 of  $\beta 1$ ). This indicates that  $\beta 1$  and  $\beta 3$  are alternative forms of the  $\beta 1$  gene (Fig. 2C). In keeping with the terminology submitted by Rettig et al. [6], we suggest that  $\beta 1$  be called Kv $\beta 1a$  and  $\beta 3$  [7,8] be called Kv $\beta 1b$ . In addition to determining the alternative N-terminal splice junction site, we have determined from several incompletely processed cDNAs and additional genomic clones that the aldo-keto core is composed of multiple exons (data not shown).

#### 3.3. Functional expression of $\alpha_{1.4}\beta_2K^+$ channel complexes

We obtained inactivating currents (Fig. 3A) from human Kv1.4 cRNA-injected oocytes similar to those previously described for rat and human channels [12,14]. As found previously [6], co-injection of  $\beta 1a$  (0.25 ng) with 1.4 cRNA (0.25 ng) substantially increased current inactivation (Fig. 3C). In contrast, our initial co-expression experiments with Kv1.4 and  $\beta 2$  elicited currents that were difficult to distinguish from those of Kv1.4 alone. To establish whether the  $\beta 2$  cRNA was being translated, we altered the immediate 5' untranslated sequence of the  $\beta 2$  cDNA (section 2) to that of the 29-4 *Drosophila Shaker* cDNA which expresses large currents in oocytes [13]. Co-expression of this  $\beta 2$  cRNA substantially in-

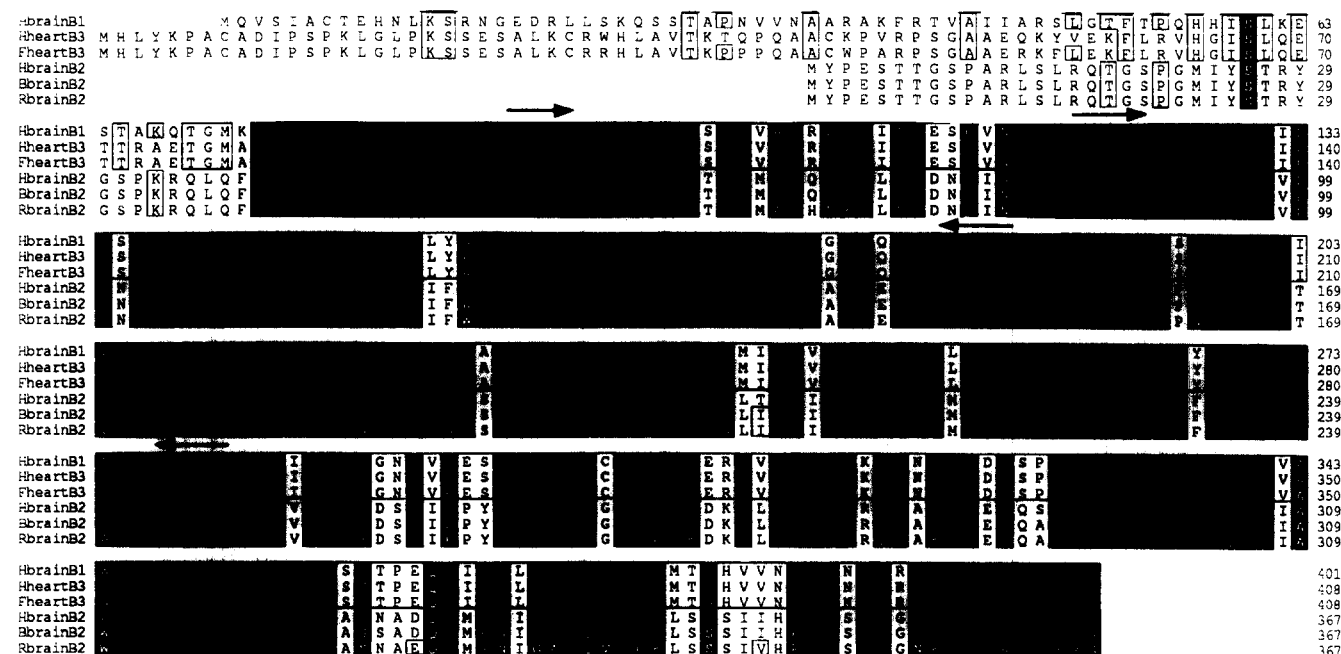


Fig. 1. Primary sequence of human  $\beta 1$  and  $\beta 2$  proteins. Alignments of the deduced amino acid sequences of  $\beta 1$  and  $\beta 2$  from human brain with those reported for  $\beta 3$  from human and ferret heart, and  $\beta 2$  from rat and bovine brain. The human brain  $\beta 1$  gene product is identical with that reported for rat brain  $\beta 1$  (not shown). Residues conserved among all  $\beta$  subunits are shaded while other identities to the  $\beta 1$  sequence are boxed. A core region conserved among all the  $\beta$  subunits begins near residue Y73 of  $\beta 1$ .

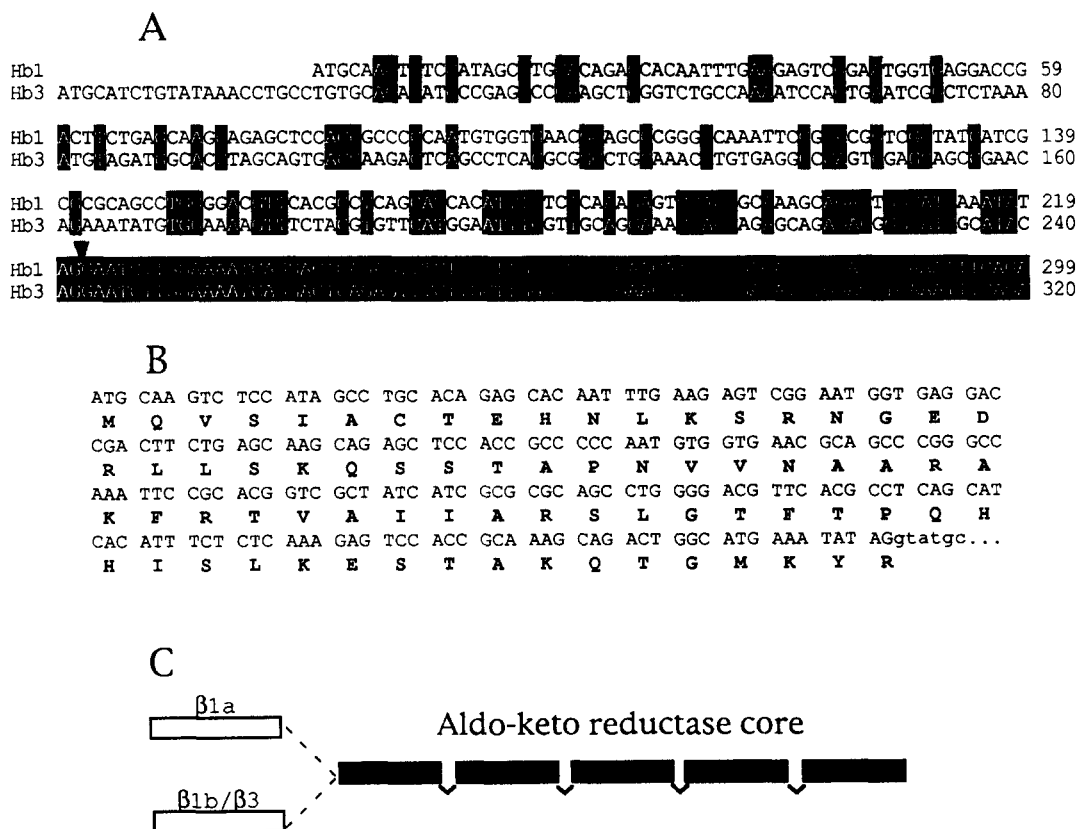


Fig. 2. Alternative splice forms of the  $\beta 1$  gene. (A) cDNA sequences encoding alternative N-terminal amino acid products of human  $\beta 1$  and  $\beta 3$  (genbank no. L39833) proteins. Nucleotide identities are shaded. Downstream of nucleotide 219 (encoding Y73 in  $\beta 1$ ) throughout the remainder of the coding sequence and over 1500 nucleotides of 3' untranslated sequence (with the exception of at least one polymorphism of 4 nucleotides - not shown) the nucleotide sequences of  $\beta 1$  and  $\beta 3$  are identical. The arrowhead indicates the proposed splice junction of N-terminal exons. (B) Nucleotide sequence and deduced amino acid sequence for the  $\beta 1$  N-terminal exon and 5' acceptor splice site derived from human genomic DNA. (C) Schematic diagram of alternative splice forms of the  $\beta 1$  gene; alternative N-terminal segments a and b are spliced onto a constant aldo-keto reductase related core region composed of multiple exons.

creased the inactivation rate of the expressed currents (Fig. 3B). Thus, we conclude that  $\beta 2$  does increase the inactivation rate of Kv1.4 channels and that the 5' untranslated sequence of the  $\beta 2$  cDNA is not efficiently translated in *Xenopus* oocytes; downstream methionines may be used as start codons in the native  $\beta 2$  cDNA and these translation products may not modulate 1.4 inactivation.

Maximal increases in the inactivation rate occurred when using at least equal weight of  $\beta 1a$  to 1.4 cRNA (0.25 ng) or about twice as much  $\beta 2$  to 1.4 cRNA (0.5 ng); both give molar ratios of about 1.7:1 for  $\beta$  subunit to 1.4 cRNA. Due to potential differences in translation efficiency, RNA stability and other factors we do not know what ratios exist at the protein level. The relative effects of the  $\beta$  subunits is illustrated in overlapping traces of the Kv1.4 currents with saturating amounts  $\beta 1a$  and  $\beta 2$  (Fig. 3D). The time constants of inactivation from the currents obtained with +60 mV pulses were fit to a single exponential decay. Inactivation rates were increased 3.7-fold in the presence of  $\beta 1a$  and 2.3-fold in the presence of  $\beta 2$  ( $\tau_{60} = 55.4 \pm 3.4$  ms for 1.4,  $15.0 \pm 1.44$  ms with  $\beta 1a$  and  $24.1 \pm 1.48$  ms with  $\beta 2$ ;  $n = 9-11$ ). In addition,  $\beta$  subunit co-expression increased the amplitudes of the elicited currents; the amplitudes were  $1.9 \pm 0.28$   $\mu A$  for 1.4 alone,  $3.43 \pm 1.16$   $\mu A$  for 1.4 and  $\beta 1a$  and  $4.28 \pm 1.01$   $\mu A$  for 1.4 with  $\beta 2$  ( $n = 9-11$ ). Thus, both  $\beta 1a$  and  $\beta 2$  appear to speed up the inactivation rates

and increase the amplitude of 1.4 currents in *Xenopus* oocytes. Increased current amplitudes have also been reported for the co-expression of a putative  $\beta$  subunit with  $K^+$  channels from rat brain [4].

$\beta 1a$  is thought to modulate channel inactivation through an intrinsic N-terminal inactivation 'ball' [5]. The N-terminus of  $\beta 2$  is significantly shorter than  $\beta 1a$  or  $\beta 1b$  and we were interested in determining whether  $\beta 2$  was capable of inducing its own inactivation mechanism or whether it increased the rate of the endogenous 1.4 inactivation process. We therefore expressed the  $\beta$  subunits with an N-terminal truncated and non-inactivating human 1.4 channel (Fig. 4) similar to that of Rettig et al. [6]. While  $\beta 1a$  induced similar fast inactivation in the truncated channel,  $\beta 2$  did not. This indicates that  $\beta 2$  increases the rate of the endogenous 1.4 inactivation process and that it does not contain a similar inactivation ball. Similar results were obtained when we co-expressed the  $\beta$  subunits with the non-inactivating Kv1.1 channel (Fig. 4B). However,  $\beta 1a$ -induced inactivation of this channel was incomplete and only observed at higher potentials.

### 3.4. Functional expression of $\alpha_{1.4}\beta_{1a}\beta_2K^+$ channel complexes

Because mammalian *Shaker*  $\alpha$  subunits form heteromultimeric complexes in heterologous systems [15,16] and in vivo [17,18] and  $\alpha\beta K^+$  channel complexes are thought to be com-

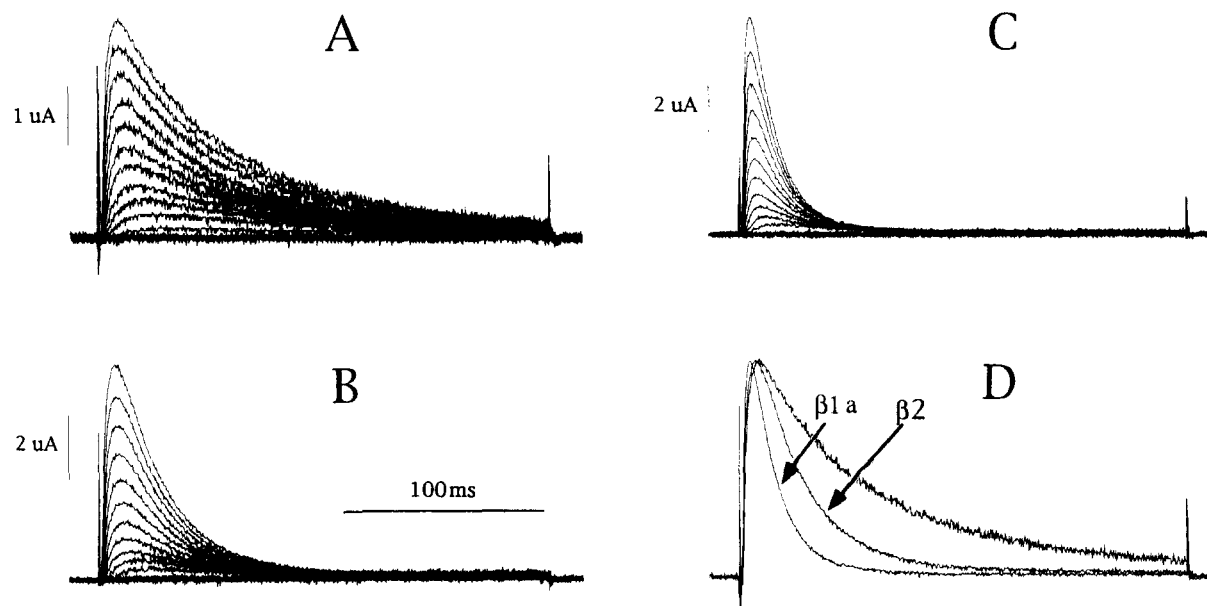
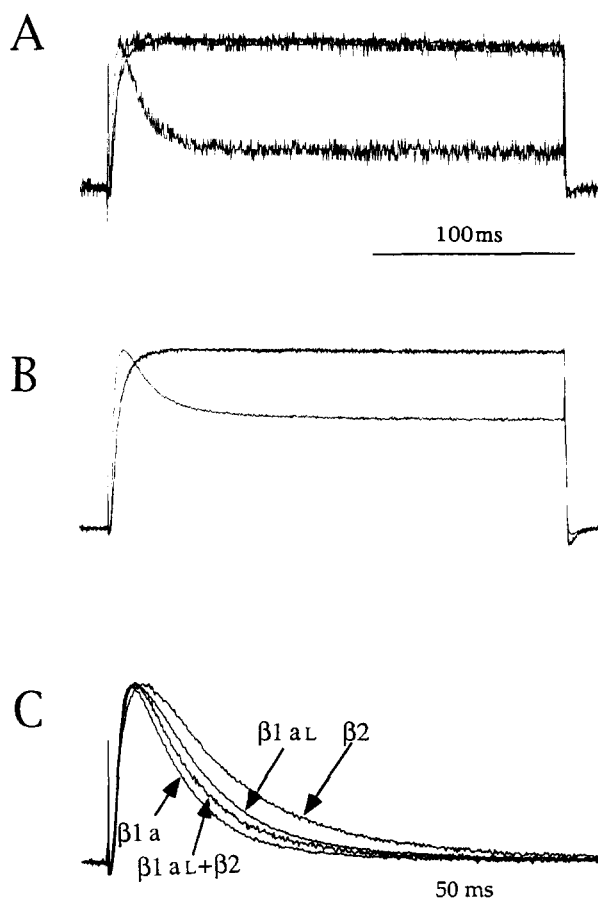


Fig. 3. Functional modulation of endogenous Kv1.4 inactivation by  $\beta 2$ . Whole oocyte currents generated from  $-70$  mV to  $+60$  mV from a holding potential of  $-90$  mV in  $10$ -mV steps. (A) Oocytes injected with cRNA from (A) Kv1.4 (B) Kv1.4 +  $\beta 2$ , (C) Kv1.4 +  $\beta 1a$  and (D) overlap of  $+60$  mV traces, scaled for amplitude. The horizontal bar indicates the time scale for all traces.



posed of four  $\alpha$  and four  $\beta$  subunits [6], we investigated the effects of the combined expression of  $\beta 1a$  and  $\beta 2$  on the inactivation of 1.4 currents. Less  $\beta 1a$  cRNA ( $0.125$  ng.) was injected – below that sufficient to induce the fastest  $\beta 1a$ -induced inactivation rates – in combination with the same amounts of 1.4 and  $\beta 2$  cRNA ( $0.25$  and  $0.50$  ng, respectively). Inactivation rates in the presence of non-saturating amounts of  $\beta 1a$  plus saturating amounts of  $\beta 2$  ( $\tau_{60} = 16.4 \pm 0.47$  ms,  $n = 7$ ) were faster than those when only non-saturating amounts of  $\beta 1a$  were used ( $t_{60} = 19.1 \pm 0.28$  ms,  $n = 6$ ). The added  $\beta 2$  cRNA increased the inactivation rate in a faster range than that observed when only  $\beta 2$  are 1.4 are co-expressed ( $\tau_{60} = 24$  ms), indicating that the observed currents are derived from heteromultimeric  $\alpha_{1.4}\beta_{1a}\beta_2$  Shaker channel complexes. Furthermore, because the observed currents inactivate at a rate closer to that of the saturating  $\beta 1a$  ( $\tau_{60} = 15$  ms) it appears that the  $\beta 1a$  inactivation properties dominate in heteromultimeric complexes. The heteromultimeric dominance of a fast-inactivating  $\beta$  subunit is similar to that found for fast-inactivating *Drosophila* Shaker  $\alpha$  subunits [19]. It will be of further interest to determine the properties of other  $\alpha\beta$  complexes and the composition of in vivo  $K^+$  channels, such as those responsible for the presynaptic A current [18].

Fig. 4.  $\beta 2$  does not induce inactivation of non-inactivating  $K^+$  channels and  $\beta 1a$  dominates heteromultimeric  $\beta$  complexes. (A) Overlap of scaled  $+60$  mV current traces obtained from oocytes injected with truncated 1.4, with or without  $\beta 2$  or  $\beta 1a$  cRNAs. While  $\beta 2$  co-expression did not alter inactivation,  $\beta 1a$ -co-expressed currents show fast inactivation. (B) Scaled  $+60$  mV traces from human Kv1.1 channels in the absence or presence of  $\beta 2$  and  $\beta 1a$  subunits.  $\beta 1a$  induced inactivation of some of the 1.1 currents while currents obtained in the presence of  $\beta 2$  superimposed with those of 1.1 alone. (C) Scaled traces of currents obtained at  $+60$  mV from oocytes injected with combinations of Kv1.4,  $\beta 2$  and non-saturating (L) or saturating amounts of  $\beta 1a$  cRNA. Horizontal bars indicate time scales for A and B or C.

Here, we show that products of the human  $\beta 1$  and  $\beta 2$  genes are expressed in human hippocampus and that the  $\beta 1$  gene encodes at least two products which are differentially expressed in brain, heart and other tissues [6,7]. Although the  $\beta$  subunits vary in their N-terminal domains, they contain a core region related to the aldo-keto reductase superfamily [9]; this relation is unique among voltage-gated ion channels accessory proteins. However, both  $\beta 1$  and  $\beta 2$  gene products are capable of playing modulatory roles similar to the accessory proteins of voltage-gated  $\text{Na}^+$  and  $\text{Ca}^{2+}$  channels [10]. Thus, the primary physiological role of the  $\text{K}^+$  channel  $\beta$  subunits may be to modulate the inactivation and amplitudes (and perhaps mediate localization) of specific  $\text{K}^+$  channel currents in vivo.

It remains of significant interest to determine the mechanism of  $\beta$  subunit modulation as well as other non-traditional roles that these subunits might play, e.g., the effects that cofactor binding and redox state may have on  $\text{K}^+$  channel activity as well as any enzymatic activity of the  $\alpha\beta$  complex [9]. Like frog lens crystallin [20], the enzymatic function of an aldo-keto reductase superfamily member have been lost to perform a structural role. However, given that ancestral  $\text{K}^+$  channels evolved into a large number of channel proteins [21,22], they may play a role in other physiological functions as found for the voltage-sensing role of  $\text{Ca}^{2+}$  channel subunits in excitation–contraction coupling. Determination of the physiological roles of the  $\beta 1$  and  $\beta 2$  subunits and related proteins, including their biochemical and evolutionary relationships with channel proteins, will provide greater insight into the extent and diversity of  $\text{K}^+$  channel function.

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