FEBS Letters 370 (1995) 32–36

FEBS 15857

Alternative splicing of the human *Shaker* K^+ channel $\beta 1$ gene and functional expression of the $\beta 2$ gene product

Ken McCormack[a,b,*], Tom McCormack[c], Mark Tanouye[b], Bernardo Rudy[c], Walter Stühmer[a]

[a]*Max-Planck-Institüt für experimentelle Medizin, Abteilung 11, 37075 Göttingen, Germany*
[b]*Department of Entomology and Parisitology, University of California at Berkeley, Berkeley, CA 94720, USA*
[c]*Department of Physiology and Neuroscience, New York University Medical Center, New York, NY 10016, USA*

Received 22 June 1995

Abstract Mammalian voltage-activated *Shaker* K^+ channels associate with at least three cytoplasmic proteins: Kvβ1, Kvβ2 and Kvβ3. These β subnunits contain variable N-termini, which can modulate the inactivation of *Shaker* α subunits, but are homologous throughout an aldo-keto reductase core. Human and ferret β3 proteins are identical with rat β1 throughout the core while β2 proteins are not; β2 also contains a shorter N-terminus and has no reported physiological role. We report that human β1 and β3 are derived from the same gene and that β2 modulates the inactivation properties of Kv1.4 α subunits.

Key words: Potassium channel; β subunit; Aldo-keto reductase; Hippocampus; NADPH-dependent oxidoreductase

1. Introduction

Shaker and other voltage-dependent K^+ 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^+ channel proteins indicated that they were associated with additional subunits [2,3] while expression studies reported that a putative β subunit modulated the inactivation and amplitudes of K^+ channel currents from rat brain [4]. Scott et al. [5] isolated and cloned the first associated subunit, β2, from bovine brain; its physiological role was not established. Subsequently, a novel subunit, β1, was isolated [6] and found to increase the inactivation rate of *Shaker* K^+ channels through its N-terminal domain. The isolated N-terminal domain of β1 proteins is sufficient to induce inactivation in at least some non-inactivating *Shaker* K^+ channels [6]. Novel β3 subunit cDNAs isolated from both ferret [7] and human [8] heart tissue, vary from β1 in their N-termini but are identical to the rat β1 protein over the C-terminal 329 residues. β3 gene products have also been shown to alter the inactivation properties of some *Shaker* α subunits [7,8] although the mechanism of its inactivation has not been established.

Because *Shaker* K^+ channel β 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 β1 and β3, the β2 subunit is also capable of modulating α subunit activity. Moreover, we were interested in developing an assay for the expression of the β2 subunit. Here, we report the highly conserved sequences of the human β1 and β2 gene products and that human β1 and β3 are alternative products of the β1 gene. In addition, we report that β2 modulates the inactivation properties of the *Shaker* K^+ channel Kv1.4 although it does not induce inactivation in non-inactivating Kv1.4 channel constructs or Kv1.1 channels. Moreover, both β1 and β2 are able to increase the amplitude of Kv1.4 currents expressed in *Xenopus* ooctyes. Although apparently not evolutionarily related to the accessory proteins of other voltage-gated channels [10], the β subunits do appear to regulate a number of similar properties in K^+ channel complexes.

2. Materials and methods

2.1. Generation of human β1 and β2 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-μl 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-μl 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 DTAEVY, respectively. Antisense oligonucleotides TCNACRTAYTCNARYTG and CANGCNAGNGGNGACCA, correspond to the 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 β1 and β2 human homologs were found.

2.2. Isolation of human β1 and β2 cDNA and genomic clones

β1 and β2 cDNA fragments were used to screen the same library for full-length clones. Nitrocellulose filters (Schleicher and Schuell) were hybridized in 50% formamide, 5 × SSPE, 50 mM Hepes, pH 7.5, 0.25% SDS and 100 μg/ml salmon sperm DNA overnight at 42°C with ^{32}P-random primed probe, washed in 2 × SSC and 0.1% SDS three times at 22°C for 10 min followed by a 15-min wash in 0.1 × 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, β1 and β2 cDNAs were subcloned into a vector containing 40 adenosine nucleotides downstream of the 3′ cloning site and linearized with Not I. The β1 cDNA was truncated 10 nucleotides 3′ of the stop codon while the β2 cDNA contained over 2.5 kb of 3′ untranslated sequence. $K^+\alpha$ subunit constructs; Kv1.4, an N-terminal truncated and non-inactivating Kv1.4 and Kv1.1 [12] were transcribed from the same vector. For β2 expression, the 5′ untranslated sequence was changed from GGCTGGCTCC to CGCCGCCAAG. Oocytes were injected with 40 nl of cRNA at various dilutions. All

*Corresponding author. Department of Genetics, 445 Henry Hall, University of Wisconsin, Madison, WI 53706, USA.
Fax: (1) (608) 262 2976. E-mail: kjmccorm@neurophys.wisc.edu

SSDI 0014-5793(95)00785-7

electrophysiology experiments were carried out at 16°C. Two microelectrode 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* β1 and β2 cDNAs

Human β1 and β2 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 β1 and β2 [6] and used to screen for full-length cDNAs from the same library. Deduced amino acid sequences of the full-length human β1 and β2 gene products are aligned in Fig. 1 with previously reported β subunit sequences. The human and rat β1 brain homologs show 100% identity over their entire length of 401 residues and 93% identity at the nucleotide level while the β2 gene product shows 99% homology to the bovine and rat proteins and 91–93% at the nucleotide level. Both the *Shaker* K^+ channel β subunits and channel forming α subunits [12] are highly conserved in mammals, indicating highly conserved physiological functions among the various species.

3.2. β1 and β3 are alternative splice forms of the β1 gene

β1 and β3 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 β2 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 β1 and β3 proteins indicates that they could be alternative splice forms of the same gene. Alignment of the human β1 and β3 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 β1 and β3 represent alternative splice products we isolated and sequenced a genomic clone containing the human β1 N-terminus (Fig. 2B) and found that it is encoded by a single exon containing a consenus splice site at the predicted location: the first potential −2, −1 AG sequence downstream of the last nucleotide difference between human β1 and β3 cDNAs (nucleotide 219 of β1). This indicates that β1 and β3 are alternative forms of the β1 gene (Fig. 2C). In keeping with the terminology submitted by Rettig et al. [6], we suggest that β1 be called Kvβ1a and β3 [7,8] be called Kvβ1b. 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* $\alpha_{1.4}\beta_2$,K^+ 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 β1a (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 β2 elicited currents that were difficult to distinguish from those of Kv1.4 alone. To establish whether the β2 cRNA was being translated, we altered the immediate 5′ untranslated sequence of the β2 cDNA (section 2) to that of the 29-4 *Drosophila Shaker* cDNA which expresses large currents in oocytes [13]. Co-expression of this β2 cRNA substantially in-

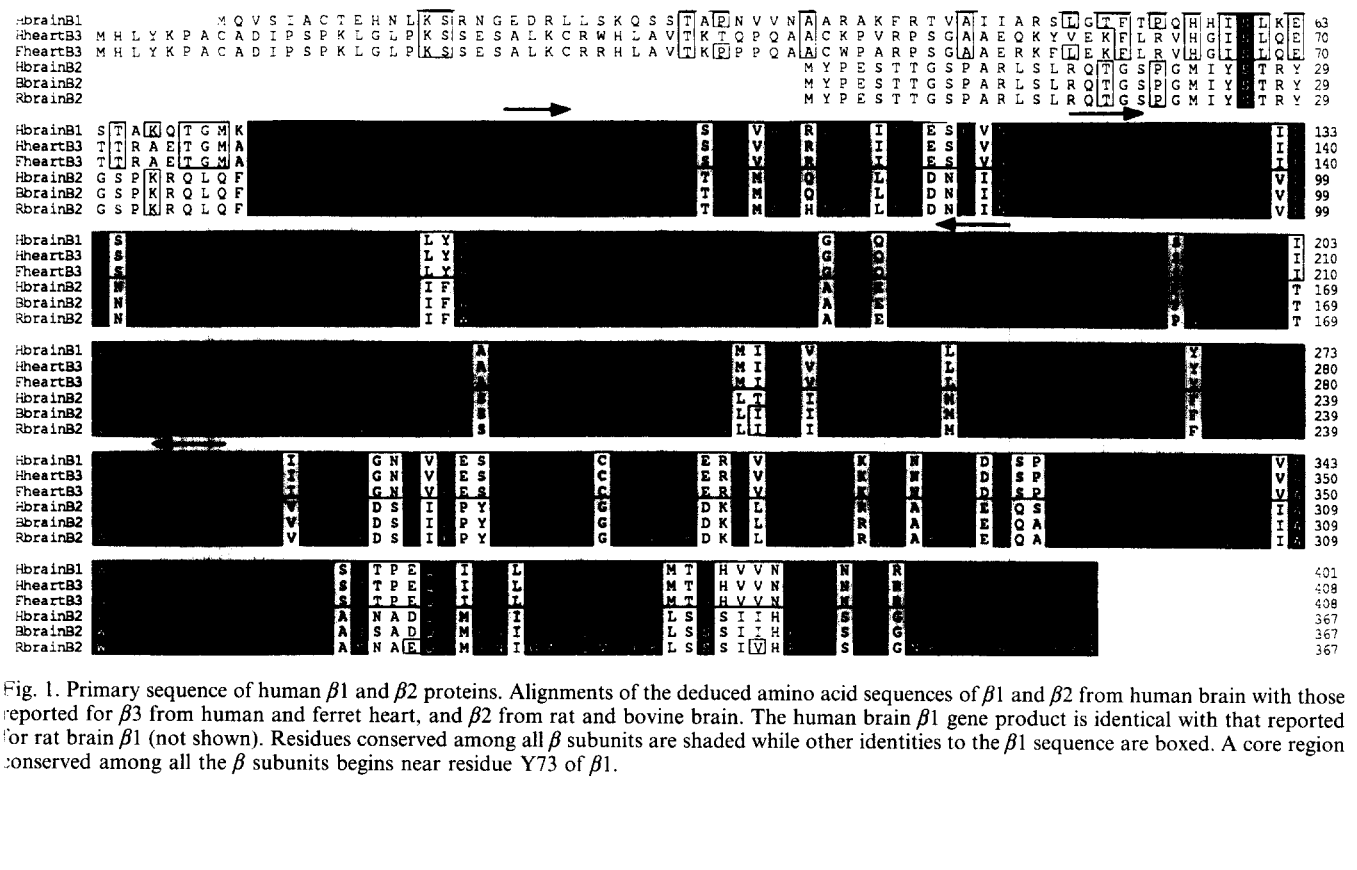


Fig. 1. Primary sequence of human β1 and β2 proteins. Alignments of the deduced amino acid sequences of β1 and β2 from human brain with those reported for β3 from human and ferret heart, and β2 from rat and bovine brain. The human brain β1 gene product is identical with that reported for rat brain β1 (not shown). Residues conserved among all β subunits are shaded while other identities to the β1 sequence are boxed. A core region conserved among all the β subunits begins near residue Y73 of β1.

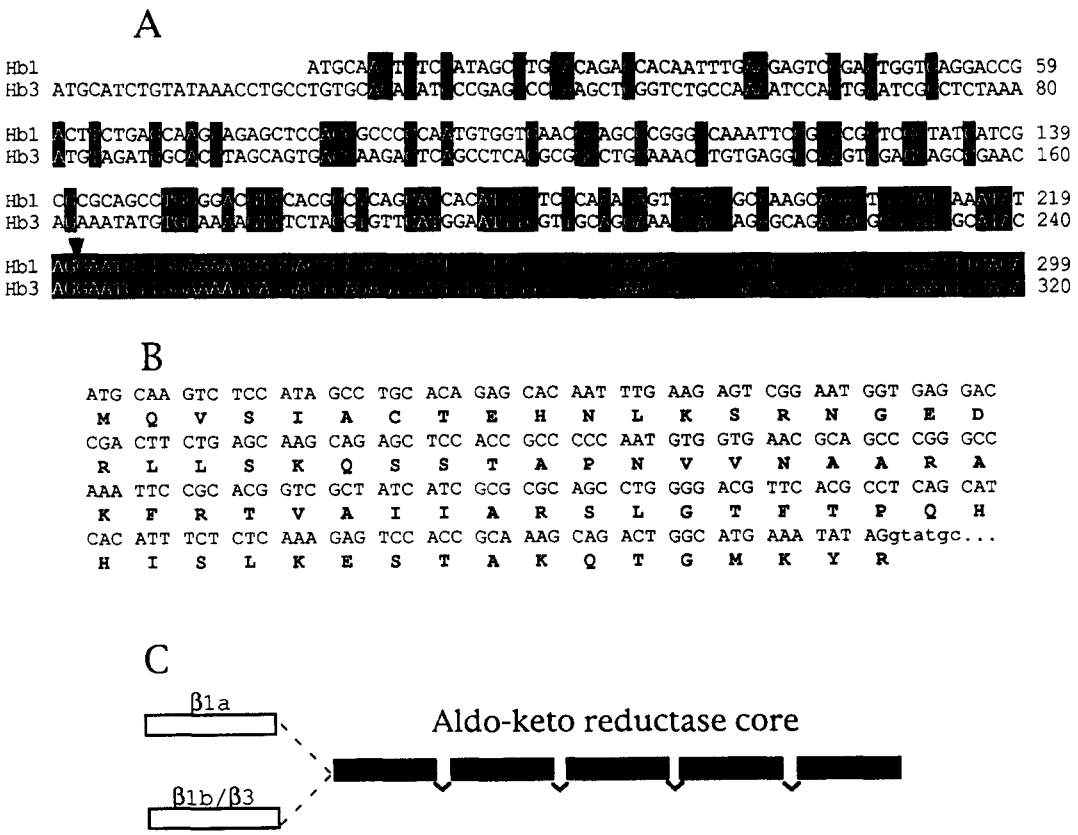


Fig. 2. Alternative splice forms of the β1 gene. (A) cDNA sequences encoding alternative N-terminal amino acid products of human β1 and β3 (genbank no. L39833) proteins. Nucleotide identities are shaded. Downstream of nucleotide 219 (encoding Y73 in β1) throughout the remainder of the coding sequence 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 β1 and β3 are identical. The arrowhead indicates the proposed splice junction of N-terminal exons. (B) Nucleotide sequence and deduced amino acid sequence for the β1 N-terminal exon and 5′ acceptor splice site derived from human genomic DNA. (C) Schematic diagram of alternative splice forms of the β1 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 β2 does increase the inactivation rate of Kv1.4 channels and that the 5′ untranslated sequence of the β2 cDNA is not efficiently translated in *Xenopus* oocytes; downstream methionines may be used as start codons in the native β2 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 β1a to 1.4 cRNA (0.25 ng) or about twice as much β2 to 1.4 cRNA (0.5 ng); both give molar ratios of about 1.7:1 for β 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 β subunits is illustrated in overlapping traces of the Kv1.4 currents with saturating amounts β1a and β2 (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 β1a and 2.3-fold in the presence of β2 (τ_{60} = 55.4 ± 3.4 ms for 1.4, 15.0 ± 1.44 ms with β1a and 24.1 ± 1.48 ms with β2: n = 9–11). In addition, β subunit co-expression increased the amplitudes of the elicited currents; the amplitudes were 1.9 ± 0.28 μA for 1.4 alone, 3.43 ± 1.16 μA for 1.4 and β1a and 4.28 ± 1.01 μA for 1.4 with β2 (n = 9–11). Thus, both β1a and β2 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 β subunit with K^+ channels from rat brain [4].

β1a is thought to modulate channel inactivation through an intrinsic N-terminal inactivation 'ball' [5]. The N-terminus of β2 is significantly shorter than β1a or β1b and we were interested in determining whether β2 was capable of inducing its own inactivation mechanism or whether it increased the rate of the endogenous 1.4 inactivationprocess. We therefore expressed the β subunits with an N-terminal truncated and non-inactivating human 1.4 channel (Fig. 4) similar to that of Rettig et al. [6]. While β1a induced similar fast inactivation in the truncated channel, β2 did not. This indicates that β2 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 β subunits with the non-inactivating Kv1.1 channel (Fig. 4B). However, β1a-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* α 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-

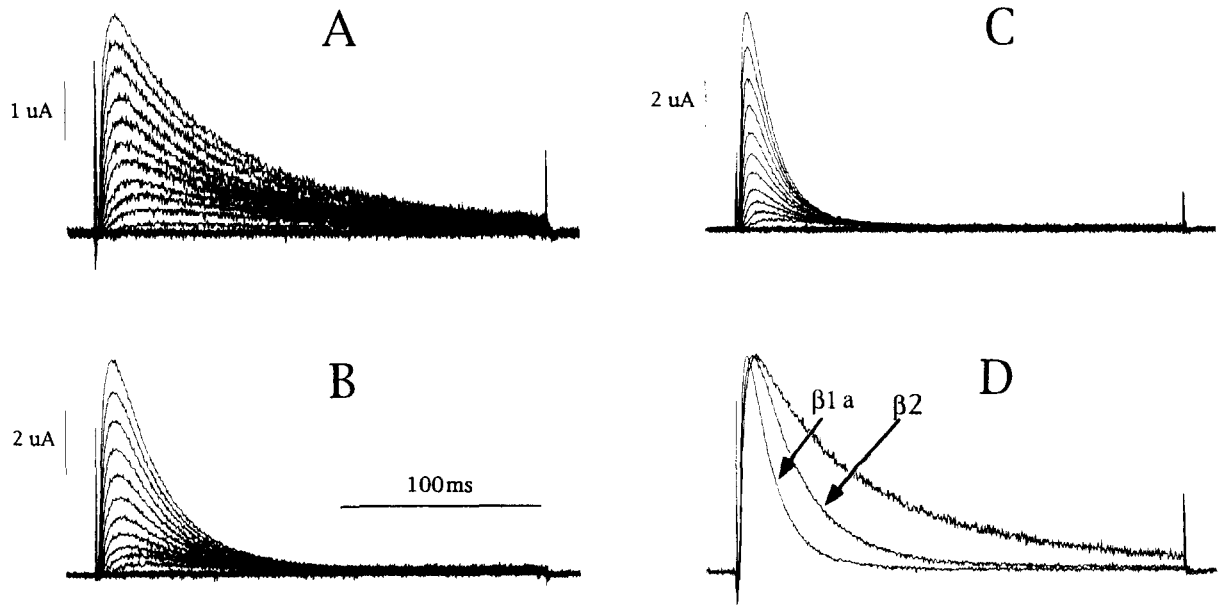
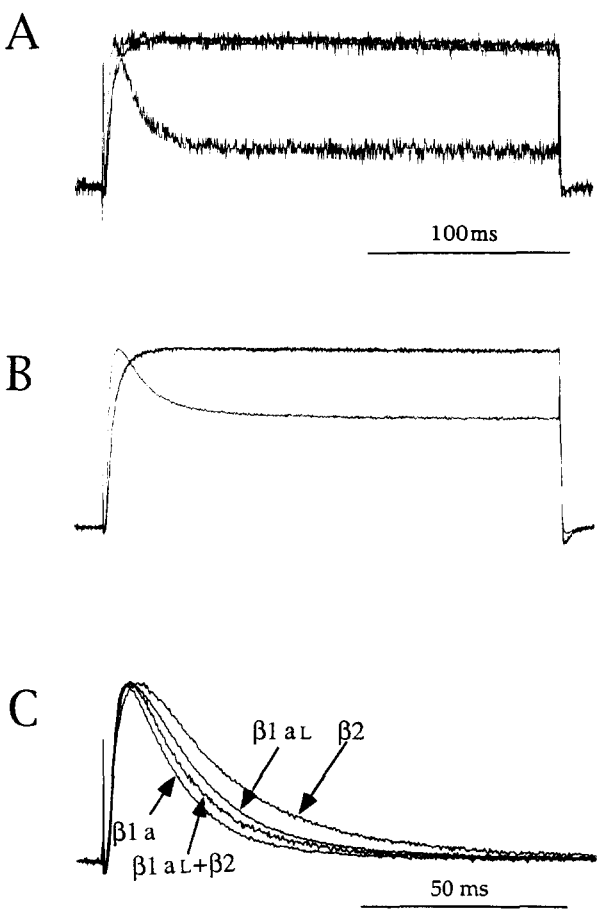


Fig. 3. Functional modulation of endogenous Kv1.4 inactivation by $\beta 2$. Whole oocyte currents generated from −70 mV to +60 mV from a holding potential of −90 mV in 10-mV steps. (A) Oocytes injected with cRNA from (A) Kv1.4 (B) Kv1.4+$\beta 2$, (C) Kv1.4+$\beta 1$a and (D) overlap of +60 mV traces,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



posed of four α and four β subunits [6], we investigated the effects of the combined expression of $\beta 1$a and $\beta 2$ on the inactivation of 1.4 currents. Less $\beta 1$a cRNA (0.125 ng.) was injected – below that sufficient to induce the fastest $\beta 1$a-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 $\beta 1$a plus saturating amounts of $\beta 2$ (τ_{60} = 16.4 ± 0.47 ms, n = 7) were faster than those when only non-saturating amounts of $\beta 1$a were used (t_{60} = 19.1 ± 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 (τ_{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 $\beta 1$a (τ_{60} = 15 ms) it appears that the $\beta 1$a inactivation properties dominate in heteromultimeric complexes. The heteromultimeric dominance of a fast-inactivating β subunit is similar to that found for fast-inactivating *Drosophila Shaker* α 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 $\beta 1$a dominates heteromultimeric β complexes. (A) Overlap of scaled +60 mV current traces obtained from oocytes injected with truncated 1.4. with or without $\beta 2$ or $\beta 1$a cRNAs. While $\beta 2$ co-expression did not alter inactivation, $\beta 1$a-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 $\beta 1$a subunits. $\beta 1$a 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 $\beta 1$a 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 β 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 Na^+ and Ca^{2+} channels [10]. Thus, the primary physiological role of the K^+ channel β subunits may be to modulate the inactivation and amplitudes (and perhaps mediate localization) of specific K^+ channel currents in vivo.

It remains of significant interest to determine the mechanism of β 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 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 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 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 K^+ channel function.

Acknowledgements: We thank M. Garcia-Guzman, H. Menke, L. Iverson, Gordon, A. Karschin, M. Ramaswami, C. Oh, H. Moreno, E. Vega, G.F. Wilson and B. Chiu for assistance. K. McCormack was supported for part of this work by an HFSP long-term fellowship. B. Rudy was supported by NIH Grants NS30989 and IBN9209523.

References

[1] HIlle, B. (1992) Ionic Channels of Excitable Membranes, 2nd Ed., Sinauer Associates, Sunderland, MA.
[2] Rehm, H. and Lazdunski, M. (1988) Proc. Natl. Acad Sci USA 85, 4919–4923.
[3] Parcej, D.N. and Dolly, J.O. (1989) Biochem. J. 257, 899–903.
[4] Rudy, B., Hoger, J.H., Lester, H.A. and Davidson, N. (1988) Neuron 1, 649–658.
[5] Scott, V.E.S., Rettig, J., Parcej, D.N., Keen J.N., Findlay, J.B.C., Pongs, O. and Dolly, J.O. (1994) Proc. Natl. Acad. Sci USA 91, 1637–1641.
[6] Rettig, J., Heinemann, S.H., Wunder, F., Lorra, C., Parcej, D.N., Dolly, J.O. and Pongs, O. (1994) Nature 369, 299–294.
[7] Morales, M.J., Castellino, R.C., Crews., A.L., Rasmussen, R.L. and Strauss, H.C. (1995) J. Biol. Chem. 270, 6272–6277.
[8] Majumder K., De Biasi, M., Wang, Z. and Wible, B.A. (1995) FEBS Lett. 361, 13–16.
[9] McCormack, T. and McCormack, K. (1994) Cell.
[10] Isom, J.L., DeJongh, K.S. and Catterall, W.A. (1994) Neuron 12, 1183–1194.
[11] Kamb, A., Weir, M., Rudy, B., Varmus, H. and Kenyon, C. (1989) Proc. Natl. Acad. Sci. USA 86, 4372–4376.
[12] Ramaswami, M., Gautam, M., Kamb, A., Rudy, B., Tanouye, M.A. and Mathew, M.K. (1990) Mol. Cell. Neurosci. 1, 214–223.
[13] McCormack, K., Joiner, W.J. and Heinemann, S.H. (1994) Neuron 12, 301–315.
[14] Stühmer, W., Ruppersburg, J.P., Schroter, K.H., Sakmann, B., Stocker, M., Giese, K.P., Perschke, A., Baumann, A. and Pongs, O. (1989) EMBO J. 8, 3235–3244.
[15] Christie, M.J., North, R.A., Osborne, P.B., Douglass, J. and Adelman, J.P. (1990) Neuron 4, 405–411.
[16] Ruppersburg, J.P., Schroter, K.H., Sakmann, B., Stocker, M., Sewing, S. and Pongs, O. (1990) Nature 345, 535–537.
[17] Wang, H., Kunkel, D.D., Martin, T.M., Schwartzkroin, P.A. and Tempel, B.L. (1993) Nature 365, 75–79.
[18] Sheng, M., Liao, Y.J., Jan, L.Y. and Jan, Y.N. (1993) Nature 365, 72–75.
[19] McCormack, K., Lin, J.W., Iverson, L.E. and Rudy, B. (1990) Biochem. Biophys. Res. Commun. 171,1361–1371.
[20] Fujii, Y., Watanabe, K., Hayashi, H., Urade, Y., Kuramitsu, S., Kagamiyama, H. and Hayaishi, O. (1990) J. Biol. Chem. 265, 9914–9923.
[21] Milkman R. (1994) Proc. Natl. Acad. Sci. USA 91, 3510–3514.
[22] Wood, M.W., Van Dongen, H.M.A. and Van Dongen, A.M.J. (1995) Proc. Natl. Acad. Sci. USA 92, 4882–4886.