Coexpression of the KCNA3B Gene Product with Kv1.5 Leads to a Novel A-type Potassium Channel*

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Shaker-related voltage-gated potassium (Kv) channels may be heterooligomers consisting of membrane-integral α-subunits associated with auxiliary cytoplasmic β-subunits. In this study we have cloned the human Kvβ3.1 subunit and the corresponding KCNA3B gene. Identification of sequence-tagged sites in the gene mapped KCNA3B to band p13.1 of human chromosome 17. Comparison of the KCNA1B, KCNA2B, and KCNA3B gene structures showed that the three Kvβ genes have very disparate lengths varying from ≥350 kb (KCNA1B) to ~7 kb (KCNA3B). Yet, the exon patterns of the three genes, which code for the seven known mammalian Kvβ subunits, are very similar. The Kvβ1 and Kvβ2 splice variants are generated by alternative use of 5′-exons. Mouse Kvβ4, a potential splice variant of Kvβ3, is a read-through product where the open reading frame starts within the sequence intervening between Kvβ3 exons 7 and 8. The human KCNA3B sequence does not contain a mouse Kvβ4-like open reading frame. Human Kvβ3 mRNA is specifically expressed in the brain, where it is predominantly detected in the cerebellum. The heterologous coexpression of human Kv1.5 and Kvβ3.1 subunits in Chinese hamster ovary cells yielded a novel Kv channel mediating very fast inactivating (A-type) outward currents upon depolarization. Thus, the expression of Kvβ3.1 subunits potentially extends the possibilities to express diverse A-type Kv channels in the human brain.

Voltage-gated potassium (Kv) channel diversity has probably evolved to match the various roles of Kv channels in the regulation and control of the electrical potential across the plasma membrane of excitable and nonexcitable cells (1). Different mechanisms have been discovered in recent years that may contribute to the expression of diverse Kv channels in the mammalian nervous system (6–8). Kvα subunits as well as heterotetramers may increase the plasma membrane expression (14, 15) and modulate the gating behavior of Kv channels (16–25). Most remarkably, heterologous coexpression of Kvβ1.1 from rat (16, 26) and human brain (23) confers rapid inactivation on otherwise noninactivating delayed rectifier channels. It could be shown that Kvβ1.1 contains a distinct N-terminal sequence similar to the N-terminal structures of rapidly inactivating (A-type) Shaker channels (16). This structure functions as an inactivating “ball” domain (27), which, upon depolarization, can occlude the channel pore by binding to a receptor near or at the cytoplasmic side of the pore (28). It has been shown that several splice variants of Kvβ1 (Kvβ1.1, Kvβ1.2, and Kvβ1.3) exist, which have variant N-terminal sequences and accordingly variant inactivating domains (18–23) and yet have similar functional properties.

We previously cloned another Kvβ subunit from rat brain, Kvβ3 (17). It also contains an N-terminal inactivating domain that is similar in structure and function to the ones found in some Shaker Kvα and in Kvβ1 subunits (17, 26). Coexpression of rat Kvβ3 in Xenopus oocytes conferred rapid inactivation on Kv1.4Δ1–110 channels but not on other Kv1α channels (17). In this study, we present structural and functional properties of the human KCNA3B gene and the encoded Kvβ3.1 subunit, respectively. In contrast to Xenopus oocytes, coexpression of Kvβ3.1 from human brain in Chinese hamster ovary (CHO) cells conferred rapid inactivation on human Kv1.5 channels. Apparently, Kvβ3.1 and Kv1.5 assemble to a novel type of heteromultimeric A-type channel, which inactivates completely and significantly faster than previously in vitro expressed A-type Kv channels. Thus, Kvβ3.1 subunits potentially extend the possibilities to express diverse A-type Kv channels in the human brain.

EXPERIMENTAL PROCEDURES

Isolation and Analysis of hKvβ3.1 cDNA

A human brain cDNA library in agt10 (CLONTECH) was hybridized in 5× SET (75 mM NaCl, 0.5 mM EDTA, 15 mM, Tris-HCl, pH 7.4, 1% SDS, 50% formamide, 5× Denhardt’s solution, and 0.1 mg/ml dena-

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tured herring testis DNA at 42 °C with a rat Kvβ3.1 cDNA probe (nucleotides 658–1198; accession no. X76723) labeled by random priming (29) with \([\alpha-\beta^4P]dCTP.\) Filters were washed in 1 × SET, 0.1% SDS at 65 °C. Exposure to x-ray films (Kodak BioMax MS) was overnight with an amplifying screen (Cronex Hi3, DuPont) at ~70 °C. Eight positive phage plaques were isolated with hKvβ3.1 cDNA insert varying in length between 0.9 and 1.9 kb. cDNA inserts were cloned into the EcoRI site of pBluescript KS+ (Stratagene). Both strands were sequenced using the dyeoxide chain termination method (30) and a T7 sequencing kit from Pharmacia Biotech. Genetics Computer Group Inc. (GCG, Madison, WI) software was used for sequence analysis. The sequence of the 5′-flanking reading (ORF) has been submitted to the National Center for Biotechnology Information (NCBI) data bank (accession no. AF016411).

**Isolation and Sequence Analysis of Genomic DNA**

**KCNA2B Gene—**hKvβ2-specific probes were generated using specific PCR fragments of the coding and noncoding region. The KCNA2B-specific primer sequences are available on request (31). A human genomic library in AEMBL3 SP6/T7 (CLONTECH) was hybridized with \([\alpha-\beta^4P]dCTP-labeled hKvβ2 probes as described (see above and Ref. 23). Twenty-five positive phages were isolated with KCNA2B DNA inserts varying in length between 14 and 20 kb. Restriction site mapping of phage inserts was carried out using standard protocols. Subcloned restriction fragments containing exon sequences were sequenced for genomic and cDNA sequence alignments. The 5′-terminal of phage inserts 3, 18, 21, 24, 26, 62, 69, and the 3′-terminal of phage insert 18, 21, 23, 24, 62, 64, and 69 were used as probes to construct the contiguous KCNA2B map shown in Fig. 1. A detailed map is available on request (31). Genomic and cDNA sequence alignments were done with GCG software.

**KCNA3B Gene—**The AEMBL3 SP6/T7 genomic DNA library was hybridized with a \([\alpha-\beta^4P]dCTP-labeled hKvβ3 probe (nucleotides 1–1215). Hybridization conditions were as above. One positive phage was isolated containing the complete KCNA3B gene. The insert DNA (11.85 kb) was subcloned and completely sequenced using the dyeoxide chain termination method (31). The sequence was analyzed employing GCG software and the electronic PCR program of the NCBI data bank.

**Chromosomal Localization**

DNA panels from human/rodent hybrid somatic cell lines were purchased from BIOS Laboratories (Scotlab GmbH, Wiesloch, Germany). PCR and chromosomal localization studies were performed using primers B3–107 (ACCCAAAGTGAGGGGATATG) and B3–208 (CTGCTTTGAGAGAGAGAGAGAGA). The primers amplify a 620-bp product from human genomic DNA, corresponding to nucleotides 3237–3857 of the KCNA2B gene. The inserts were subcloned and sequenced. One insert of 1857 bp contained the complete KCNA3B probe (nucleotides 8327–8947 of the KCNA3B gene).

**Northern Blot Analysis**

Northern blots of human brain poly(A+) mRNA were obtained from CLONTECH. The blots were hybridized according to the manufacturer’s protocol with a \([\alpha-\beta^4P]dCTP-labeled hKvβ3.1 cDNA probe (nucleotides 1–550 or 912–1162). After three washes with 0.5% SDS, the blots were autoradiographed. Exposure to x-ray films (Kodak BioMax MS) was for 72 h with an amplifying screen (Cronex Hi3, DuPont) at ~70 °C.

**Channel Expression and Functional Characterization**

Both hKv1.5 and hKvβ3.1 cDNAs were cloned into the eukaryotic expression vector pcDNA3 (Invergent). Recombinant Kvo1.5 cDNA (10 ng/ml) alone or together with Kvβ3.1 cDNA (200 ng/ml) was microinjected into CHO cells with green fluorescent protein cDNA (5 ng/ml) as a reporter gene to allow the identification of injected cells during electrophysiological experiments. A 20-fold excess of β-subunit cDNA was used to favor high expression, which should allow maximal ββ interaction. Currents activated by depolarizing voltage pulses in the outside-out patch configuration were obtained 12 h after DNA injection. Patch pipettes were pulled from borosilicate capillaries using an DMZ puller (Zeitz, Augsburg, Germany) and had bath resistances between 3 and 4 MΩ when filled with an intracellular solution containing 115 mM KCl, 1 mM CaCl2, 1 mM MgCl2, 11 mM EGTA, 10 mM HEPES, 2 mM Na3ATP, and 2 mM glutathione, pH 7.2, KOH. The bath solution contained 135 mM NaCl, 5 mM KCl, 2 mM CaCl2, 2 mM MgCl2, 5 mM HEPES, 10 mM glucose, 20 mM sucrose, pH 7.4, NaOH. Currents were recorded with an EPAC patch clamp amplifier (HEKA Elektronik, Landbrueck, Germany), and the program package PULSE + PULSEFIT (HEKA Elektronik) was used for data acquisition and analysis. Activation and inactivation kinetics were fitted simultaneously with a Hodgkin-Huxley-related formalism (PULSEFIT) as described previously (32). Voltage-dependent processes were fitted with Boltzmann functions of the form \(I_{\text{max}} = \frac{I_{\text{max}}}{1 + \exp((-V_{m} - V_{\text{m}})/k)}\). For a first power Boltzmann function \((n = 1)\), which was used to fit steady-state inactivation, \(V_{\text{m}}\) is the potential of half-maximal inactivation. For a fourth power Boltzmann function \((n = 4)\), which was used to fit channel activation, \(V_{\text{m}}\) is the potential at which each of the four subunits is activated half of the time. The latter \(V_{\text{m}}\) value yielded an estimate of the activation threshold \((0.5^4 = 6.25\% of the maximal activation). The steepness of voltage dependences is described by the slope factor \(k.\) Data are given as mean ± S.E. All experiments were performed at room temperature.

**RESULTS**

**Cloning of Human Voltage-gated Potassium Channel Kvβ3.1 cDNA—**To isolate human Kvβ3.1 cDNA, we prepared a \([\beta^3P]dCTP-labeled rat Kvβ3 cDNA probe for screening a human brain cDNA library (see “Experimental Procedures”). Eight phages were isolated, and the inserts were subcloned and sequenced. One insert of 1857 bp contained the complete Kvβ3.1 ORF. The derived human Kvβ3.1 protein sequence has a length of 404 amino acids (Fig. 1).

The hKvβ3.1 sequence shares 92.3% identity with the previously published rat Kvβ3 protein sequence (17). This contrasts with an identity of 74.6% to the human Kvβ2.1 and 72.4% to the human Kvβ1.1 protein sequence, respectively. Alignment of hKvβ1.1, hKvβ2.1, and hKvβ3.1 sequences showed that the amino termini are highly divergent (Fig. 1). The remaining carboxyl-terminal Kvβ sequences are conserved and share a sequence identity of 85%. Accordingly, several consensus sequences for the possible phosphorylation by protein kinases have been conserved between the different hKvβ protein sequences (Fig. 1). Remarkably, only one of the 10 consensus sites is a protein kinase A phosphorylation site.

**Similar Exon/Intron Structure for Kvβ1, Kvβ2, and Kvβ3 Genes—**Previously, we have cloned the human Kvβ1 (KCNA1B) gene (23). A comparison of the genomic DNA sequence with human Kvβ1 cDNA showed that the KCNA1B gene has an exceptionally large size of >380 kb. The Kvβ1 ORFs are composed of 14 exons, and the first exon(s) (1.1, 1.2, and 1.3 in Fig. 2A) encode the alternative Kvβ1 amino termini. Now, we have cloned the human Kvβ2 (KCNA2B) and Kvβ3 (KCNA3B) genes. A \([\beta^3P]labeled human hKvβ2.1 cDNA (21) was used to probe a human genomic λ- phage library. In order to construct a physical map of 91-kb DNA containing the KCNA2B gene, 25 positive partially overlapping phages were isolated (Fig. 2B). A comparison of the genomic sequence with the hKvβ2.1 cDNA revealed 15 exons (spread out over ~70-kb DNA). The last exon of the KCNA2B ORF contained an additional 1800 bp of 3′-untranslated sequence. The size of the intervening introns varied between 236 bp and 33 kb. All exon/intron borders followed the GT/AG rule (33). In contrast to Kvβ1 amino termini, the corresponding hKvβ2 amino terminus is encoded in two exons (1a and 1b in Fig. 2A). Skipping of exon 1b may give rise to the hKvβ2.2 splice variant, which has been discovered recently in human lens epithelium (accession no. AF044253).

To characterize the KCNA3B gene we probed a human genomic λ-library with \([\beta^3P]labeled hKvβ3.1 cDNA. One phage was isolated, containing an 11.8-kb insert. It encoded apparently the complete KCNA3B gene (Fig. 2B). In comparison, the KCNA3B gene is approximately 50 times smaller than the
**Human Kvβ3 Subunit**

**Fig. 1.** Alignment of derived hKvβ1.1, hKvβ2.1, and hKvβ3.1 subunit sequences. Sequence of hKvβ3.1 was aligned with the ones of hKvβ1.1 (23) and hKvβ2.1 (43) using BEST FIT. Amino acids are given in the standard single-letter code. Numbers on the right refer to the last amino acid of the corresponding sequence. Dots indicate gaps introduced for optimal sequence alignment. Identical amino acids are indicated by dashes. Exons along the derived polypeptide sequences are indicated by alternatively shaded boxes. For orientation, exons 2 and 14 have been labeled. Potential phosphorylation sites for protein kinase A (□), for protein kinase C (□), and casein kinase II (□) have been marked. Filled symbols indicate conserved sites found in all three Kvβ subunits.

**KCNA1B** gene and approximately 10 times smaller than the **KCNA2B** gene. Alignment of the genomic **KCNA3B** DNA and hKvβ3.1 cDNA sequences showed that the **KCNA3B** gene comprised 14 exons (Fig. 1), comparable in size and structure with those of the **KCNA1B** and **KCNA2B** genes (Fig. 2A). All exon/intron borders followed the GT/AG rule (33). The last exon contained a 3′-untranslated sequence with two consensus polyadenylation signals (AATAAA) 752 and 760 bp downstream of the last amino acid of the corresponding sequence. They have been mapped to band p13.1 on the short arm of human chromosome 17. Thus, the **KCNA3B** gene was localized between the microsatellite markers D17S786 and D17S960 (Ref. 34; Fig. 3). All exon/intron borders followed the GT/AG rule (33). The last exon contained a 3′-untranslated sequence with two consensus polyadenylation signals (AATAAA) 752 and 760 bp downstream of the last amino acid of the corresponding sequence. They have been mapped to band p13.1 on the short arm of human chromosome 17. Thus, the **KCNA3B** gene was localized between the microsatellite markers D17S786 and D17S960 (Ref. 34; Fig. 3).

The microsatellite markers are located near the genes for the postsynaptic density protein 95 (34) and retinal guanylate cyclase (35). Mutations in the retinal guanylate cyclase gene cause a form of Leber congenital amaurosis (36) and an autosomal inherited dominant form of progressive cone dystrophy (CORD6; Ref. 37). Two-point analyses in a five-generation Swedish family carrying the CORD5 syndrome demonstrated linkage to the markers D17S786 and D17S960 with maximum logarithmic odds ratio scores of 6.5 and 4.4, respectively (39). Since these markers flanked the **KCNA3B** gene, we analyzed DNA obtained from Swedish families with CORD5-affected members. The results did not reveal mutations in the **KCNA3B** exon sequences. Apparently, CORD5 is not linked to the **KCNA3B** gene.

**Tissue-specific Expression of hKvβ3.1 mRNA**—The distribution of Kvβ3.1 mRNA in human tissue and in various areas of human brain was investigated in Northern blot analysis. With a Kvβ3.1 cDNA-specific probe, we detected two mRNAs of 2.8 and 4.4 kb in brain but not in heart, lung, liver, kidney, pancreas, placenta, and skeletal muscle (Fig. 4A). The exon structure of the **KCNA3B** gene predicts a ~4.4-kb-long transcript. The 2.8-kb transcript may correspond to an as yet undetected **KCNA3B** splice variant comparable with mouse Kvβ4 (Ref. 14; see "Discussion").

The expression of hKvβ3.1 mRNA in human brain was analyzed in more detail using Northern blots containing mRNA derived from several brain areas. We obtained a strong hybridization signal for Kvβ3.1 mRNA with poly(A)⁺ RNA of human cerebellum, weaker signals with the ones of cortex, occipital lobe, frontal lobe, and temporal lobe (Fig. 4B). No significant hybridization signals were obtained with poly(A)⁻ RNA of medulla and spinal cord (Fig. 4B) as well as amygdala, caudate nucleus, corpus callosum, hippocampus, hypothalamus, substantia nigra, subthalamic nucleus, and thalamus (data not shown). The results suggest similar mRNA expression patterns...
of Kvβ3.1 in human and rat brain (17) except for thalamic nuclei.

Functional Expression of hKββ3.1—We have previously shown that hKββ1 subunits confer rapid inactivation to hKv1.5 channels when coexpressed in mammalian tissue culture cells (23). In the present study, we tested a possible interaction between hKv1.5 and hKvβ3.1 subunits. The injection of hKv1.5 cDNA alone in CHO cells led to the expression of channels mediating typical outward currents with fast activation in response to depolarizing voltage pulses (Ref. 40; Fig. 5A). Kv1.5-mediated currents inactivated slowly, resulting in 70.9 ± 1.6% (n = 6) of the peak current at the end of a 1-s pulse to +60 mV. Inactivation kinetics were best described by two exponentials with time constants \( t_1 = 70.0 \pm 14.0 \ m\) s (28.1 ± 4.3% of the total decay) and \( t_2 = 1.5 \pm 0.3 \ m\) s (n = 4).

When we coexpressed hKvβ3.1 with hKv1.5, rapidly inactivating potassium currents were elicited upon depolarization (Fig. 5A). With equimolar injection of Kvae and Kvβ subunit cDNA (10 ng/µl of each), we obtained currents with rapid but largely incomplete decay (data not shown). Apparently, in these experiments the steady-state current component represented a distinct population of noninactivating channels rather than equilibrium conditions of a single rapidly inactivating channel population. The current decay at +60 mV was virtually complete (2.3 ± 0.3% of the peak current at the end of a 1-s pulse) in 25 out of 29 patches when the amount of injected Kvβ cDNA was increased 20-fold (Fig. 5A). The inactivation kinetics of hKv1.5 channels in combination with hKvβ3.1 subunits were best described by two exponentials with \( t_1 = 5.8 \pm 0.4 \ m\) s (87.6 ± 2.1% of the total decay) and \( t_2 = 32.2 \pm 4.2 \ m\) s (n = 25). The steady-state inactivation due to the presence of hKvβ3.1 showed a steep voltage dependence with \( V_{1/2} = -29.0 \pm 1.1 \ m\) V and \( k = 2.7 \pm 0.2 \ m\) V (n = 5; Fig. 5B). The recovery from inactivation measured at −80 mV showed a fast component with \( t_1 = 0.8 \pm 0.2 \ s\), accounting for 48.9 ± 11.7% of the complete recovery followed by a slower component with \( t_2 = 4.6 \pm 0.9 \ s\) (n = 4; Fig. 5C).

Many investigators have described a hyperpolarizing shift in the voltage dependence of channel activation, when Kvae subunits were coexpressed with Kβ subunits (24-26, 41, 42). We tested the voltage dependence of hKv1.5 channel activation using a pulse protocol with a holding potential of −80 mV and test potentials between −50 and +100 mV. For the expression of hKv1.5 alone, a fourth power Boltzmann-function (see “Experimental Procedures”) was fit to the conductance-voltage relationship (Fig. 5D; open circles) with a \( V_{1/2} = -32.3 \pm 0.8 \ m\) V and a slope factor \( k = 21.0 \pm 0.6 \ m\) V (n = 6). The value for \( V_{1/2}\) obtained by Heinemann et al. (26) from a similar Boltzmann analysis of Kv1.5 channel activation in Xenopus oocytes is slightly more negative, and their slope factor \( k\) suggests a steeper voltage dependence of gating. For the coexpression of hKv1.5 and hKvβ3.1 in CHO cells, Boltzmann analysis yielded
**Kvβ Gene Structures**—The Kvβ genes map to different chromosomes. **KCNA1B** is located on chromosome 3q25.1 (23), **KCNA2B** on 1p36 (43), and **KCNA3B** on 17p13.1 (this paper). Despite their different chromosomal locations, the **KCNA** genes have a similar exon/intron pattern. This pattern reflects the conserved structure of Kvβ proteins. They have differing amino termini, varying in length from 26 to 91 amino acids and a highly conserved carboxyl terminus of ~325 amino acids. The variant Kvβ1.1, -1.2, -1.3, and -3.1 amino termini are encoded each in a distinct exon. Thus, the different Kvβ subunits are produced by alternative splicing of exons 1. By contrast, the alternative Kvβ2 amino termini may be generated by an exon-skipping mechanism, leaving out exon 1b (Fig. 2A). The 5’-terminal exons are spliced to a Kvβ “core” region. It invariably consists of 13 exons that are highly homologous among the three genes. In contrast to the exon pattern, the **KCNA1B**, **KCNA2B**, and **KCNA3B** genes contain intervening sequences, which vary markedly in length. Accordingly, the **KCNA1B** gene is ~50 times larger and the **KCNA2B** gene ~10 times larger than the **KCNA3B** gene. The similarity of the exon/intron patterns, despite the greatly varying sizes of the different genes, suggests that the three human Kvβ genes were derived from an ancestral precursor gene. The data indicate that unlike Kvα subunit genes (44, 45) Kvβ subunit genes are not clustered in the human genome. Most likely, Kvβ subunit diversity has evolved from chromosomal rearrangements rather than from local gene duplications.

Another Kvβ genomic structure has been reported for **Drosophila** Hk. The derived Hk protein sequence of 546 amino acids indicates that this Kvβ subunit is considerably larger than the human Kvβ subunits. Yet, the Hk ORF comprises six instead of 14 exons (12). Due to the lack of detailed sequence information, it is not clear how Hk exons relate to hKβ exons. Nevertheless, the nonconserved exon/intron structures between **Drosophila** and humans suggest that they have arisen in evolution after the separation of vertebrates and invertebrates. Interestingly, the exon/intron structures of **Drosophila Shaker** and human Kvα subunit genes (2, 3) are not similar either, despite the highly conserved protein sequences of Shaker-related Kvα subunits (2).

**Kvβ3.1 mRNA Expression**—The hKvβ3.1 cDNA probes hybridized to two mRNA species (4.4 and 2.8 kb in length) in the Northern blot experiments. The longer transcript is consistent with our genomic sequence analysis of the **KCNA3B** gene, which predicts a hKvβ3.1 mRNA length of 3868 nucleotides without the poly(A) tail. The shorter transcript may be a cross-hybridizing mRNA species or an alternatively spliced **KCNA3B** transcript. This notion may be supported by a recent report describing a Kvβ4 subunit in mice (14). A comparison of the derived hKvβ3.1 and mouse Kvβ4 protein sequences revealed that both differ in their amino termini, whereas the carboxyl termini consisting of 225 amino acid residues are nearly identical, sharing an identity of 95%. The hKvβ3.1/mKvβ4 sequence identity starts exactly with the first amino acid of **KCNA3B** exon 8. Analysis of the mouse Kvβ3 gene showed that the mouse Kvβ4 amino terminus is encoded in the intron 7 sequence preceding mouse exon 8 (Fig. 6). This indicates that mKvβ4 is translated from a mouse Kvβ3 mRNA variant where intron 7 is not spliced out. The mouse and human intron 7 sequences have a similarity of 70%. However, an Alu element is inserted into human intron 7 DNA (Fig. 6). Therefore, the **KCNA3B** cannot code for a Kvβ3 variant that is analogous to mKvβ4.

**Rapid Kv Channel Inactivation Confirmed by Kvβ3.1—Kvβ1 and Kvβ2 subunits can assemble with Shaker-related Kvα subunits both in vitro (9, 10, 16) and in vivo (46). This assembly**
obtained in our experiments are in excellent agreement with both steady-state inactivation and recovery from inactivation the completeness of inactivation as well as the parameters for have been heterologously expressed so far. The time course and shows the fastest inactivation of all cloned Kv channels that conductance values obtained by using a calculated \( I_{\text{max}} \) function. Mean values for \( 2 \) between \( 50 \) and \( 10 \) mV in 5-mV steps from a holding potential of \(-80 \) mV. The curve fitted to the data points represents a first power Boltzmann function. Mean values for \( V_{1/2} \) and \( k \) as indicated. C, recovery from inactivation measured with a set of twin pulses to +60 mV separated by intervals of different length at \(-80 \) mV. The line fitted to the data points represents a double-exponential function with the mean values for \( \tau_1 \) and \( \tau_2 \) as indicated. D, voltage-dependent activation of hKv1.5 channels in the absence (open circles) and presence of hKv3.1 (filled triangles). Conductance-voltage plots were obtained from the peak amplitudes measured during 200-ms pulses to potentials between \(-50 \) and \(+100 \) mV in 10-mV increments from a holding potential of \(-80 \) mV. Conductance values were normalized to the value obtained at \(+100 \) mV. Open circles represent conductance values obtained by using a calculated \( I_{\text{max}} \) not accounting for inactivation. The inset illustrates fitting of a Hodgkin-Huxley-related formalism to a recorded A-type current trace (only 25% of acquired data points are shown for clarity). \( I_{\text{max}} \) was with the obtained parameters for activation calculated by setting \( m = 0 \). This eliminates inactivation from the model. Curves fitted to the conductance-voltage plots represent fourth power Boltzmann-functions. Mean values for the respective parameters were as follows: \( V_{1/2} = -32.5 \) mV and \( k = 21.0 \) (hKv1.5); \( V_{1/2} = -31.7 \) mV and \( k = 28.3 \) (hKv1.5 + hKv3.1); \( V_{1/2} = -31.9 \) mV and \( k = 20.0 \) (from simulated \( I_{\text{max}} \) values). Note that no apparent shift in activation threshold was observed irrespective of the method of analysis.

may enhance the surface expression of Kv1.1 subunits, leading to an increased number of Kv channels in the plasma membrane (15). In addition, Kv1.3 but not Kv1.2 subunits may affect the inactivating properties of Kv1.1 subunits (18–20, 47). Kv3.1 contains an N-terminal inactivating domain, which confers rapid N-type inactivation to otherwise noninactivating channels of the Kv1 family (16). The Kv3.1 N-terminal inactivating domain is similar in structure and function to the ones of Shaker channels (16). They inactivate the channel by binding to a receptor at or near the inner pore entrance (28), resembling a tethered ball and chain type mechanism (27). The Kv3.1 amino terminus also has the characteristic features of N-terminal inactivating domains (17). Coexpression of rat Kvβ3 in Xenopus oocytes imposed rapid inactivation on Kv1.4Δ1–110 channels, and the sensitivity of this inactivation to intracellular redox potential and its kinetics were comparable with the inactivation conferred on Kv1.4Δ1–110 channels by Kvβ1.1 (17). However, it was not possible to detect in the Xenopus oocyte expression system functional assemblies of Kvβ3 with Kv1.1 or Kv1.5 channels (26). In the present study, we show that hKvβ3 subunits confer rapid inactivation to hKv1.5 channels upon coexpression in CHO cells. From a kinetic point of view, the Kv1.5/hKvβ3.1-mediated A-type channel shows the fastest inactivation of all cloned Kv channels that have been heterologously expressed so far. The time course and the completeness of inactivation as well as the parameters for both steady-state inactivation and recovery from inactivation obtained in our experiments are in excellent agreement with

![Diagram](https://example.com/diagram.png)
previously published data for Kv1.5 channels coexpressed with an N(Kvβ3)-(C-Kvβ2) chimeric subunit in Xenopus oocytes (26). These findings indicate comparable mechanisms for the Kvβ3.1 ball-mediated N-type inactivation observed in the CHO cell and the Xenopus oocyte expression system. The opposing results obtained for Kv1.4A1–110 (17) and Kv1.5 (26) suggest an intracellular environment in the Xenopus oocyte nonpermissive for the functional interaction between Kvβ3.1 and Kv1.5.

The binding of Kvβ1 and Kvβ2 subunits is highly specific for members of the Kv1-subfamily (9, 10). This is consistent with immunoprecipitation experiments of Shamotienko et al. (48), who investigated the subunit composition of native Kv channels containing Kvα subunits of the Shaker-related family. Interestingly, mKvβ4, the splice variant of the Kvβ3 gene in mice, can be communoprecipitated with both Kv1.5 and Kv2.2 subunits when coexpressed in Sf9 cells (14). However, coexpression with Kvβ3.1 in CHO cells does not confer rapid inactivation to Kv2.2 channels. Therefore, future experiments should be focused on the question of which Kvα subunits are associated with Kvβ3.1 in native Kv channels.

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3 R. Bähring, unpublished observation.
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