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A Novel β Subunit Increases Rate of Inactivation of Specific Voltage-gated Potassium Channel α Subunits*

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Voltage-gated potassium channel β subunits are cytoplasmic proteins that co-purify with the pore-forming α subunits. One of these subunits, Kvβ1 from rat brain, was previously demonstrated to increase the rate of inactivation of Kv1.1 and Kv1.4 when co-expressed in *Xenopus* oocytes. We have cloned and characterized a novel voltage-gated K^+ channel β subunit. The cDNA, designated Kvβ3, has a 408-amino acid open reading frame. It possesses a unique 79-amino acid N-terminal leader, but is identical with rat Kvβ1 over the 329 C-terminal amino acids. The Kvβ3 transcript was found in many tissues, but was most abundant in aorta and left ventricle of the heart. Co-expression of Kvβ3 with K^+ channel α subunits shows that this β subunit can increase the rate of inactivation from 4- to 7-fold in a Kv1.4 or *Shaker* B channel. Kvβ3 had no effect on Kv1.1, unlike Kvβ1 which can increase rate of inactivation of this α subunit more than 100-fold. Other kinetic parameters were unaffected. This study shows that voltage-gated K^+ channel β subunits are present outside the central nervous system, and that at least one member of this family selectively modulates inactivation of K^+ channel α subunits.

Voltage-gated potassium channels are present in all excitable and most nonexcitable eukaryotic cell types. They are involved in a diverse array of functions, including electrogenesis, secretion, and cell motility. In mammals, a diverse group of more than 50 members of an extended gene family homologous to the *Shaker* gene of *Drosophila* have been identified that are responsible for generating many voltage-gated K^+ currents (1–5).

Shaker-type K^+ channel proteins, expressed in *Xenopus* oocytes or mammalian cell lines, form tetramers of α subunits that selectively conduct K^+ ions and reproduce many properties of native channels. However, there are inconsistencies between native and cloned K^+ channel currents, suggesting a failure to reconstitute the native channels fully in heterologous expression systems. These inconsistencies have several possible explanations. Some native K^+ channels are heteromultimeric associations of more than one type of α subunit. These associations can modify the current phenotypes, making them distinct from currents produced by either α subunit alone (6–8). It has also been suggested that differences between cloned and native channels are due to differences in post-translational processing or intracellular environment between heterologous expression systems and the channel's native environment (9–11). Finally, K^+ channel structure in heterologous expression systems may be incomplete (12, 13). Two voltage-gated K^+ channels recently purified from mammalian brain (14, 15) were shown to consist of an α subunit, identical with previously described *Shaker*-like K^+ channel proteins, and a smaller β subunit (M_r = 40,000). This suggests that complete reconstitution of K^+ channels in heterologous expression systems requires the presence of appropriate β subunits.

Partial amino acid sequencing of the bovine K^+ channel β subunit led to the cloning of one bovine brain (14) and two rat brain (16) β subunit cDNAs. They were divided into two classes based on differences in sequence: Kvβ1, found in rat, and Kvβ2, found in both rat and bovine. These β subunits consisted of 401 and 367 amino acids, respectively, and had 85% identity in their 329 C-terminal amino acids. The respective N termini of 72 and 38 amino acids exhibit no conservation. Co-expression of rat Kvβ1 with a delayed rectifier type K^+ channel (RCK1; Kv1.1) increased the rate of inactivation more than 100-fold. Site-directed mutagenesis showed that the increase in inactivation rate was mediated through the N-terminal region of the β subunit. The Kvβ2 subunit did not affect the properties of RCK1 or of a fast-inactivating Kv1.4 channel, RCK4 (16).

Kvβ1 mRNA was detected exclusively in brain (16). To determine if novel K^+ channel β subunits might be found in other tissues, we attempted to clone these proteins from heart. The large array of K^+ currents in heart (17, 18) made it a logical source of unknown β subunits. A cDNA was isolated from a ferret ventricular cDNA library. It contained a single open reading frame highly conserved with previously described voltage-gated K^+ channel β subunits (16, 19). Its deduced amino acid sequence contained a unique leader sequence, followed by 329 amino acids identical with rat Kvβ1. The Kvβ3 transcript was most abundant in aorta and left ventricle, although it was also found in other locations in the heart as well as brain, skeletal muscle, and kidney. Kvβ3 is conserved in humans and rats. Kvβ3 was co-expressed in *Xenopus* oocytes with K^+ channel α subunits from ferret (Kv1.4 (20)), rat (Kv1.1 (21)), and *Drosophila* (*Shaker* BΔ6–46 (22)). It accelerated inactivation in Kv1.4 in a manner analogous to that of Kvβ1 (16). However, unlike Kvβ1, which increased the inactivation rate of Kv1.1 ≈100-fold, Kvβ3 had no effect on this α subunit. As Kvβ3 was also capable of modulating inactivation of *Shaker* B, the failure of Kvβ3 to alter inactivation of Kv1.1 was not due to strict specificity for Kv1.4 α subunits.

MATERIALS AND METHODS

Unless otherwise specified, standard molecular biological methods were used (23, 24). Enzymes were used according to manufacturers'

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directions.

Cloning of Kvβ3—Ferret heart RNA was isolated as described (25); 5 μg were reverse-transcribed using Superscript reverse transcriptase (Life Technologies, Inc.) with oligo(dT) primers as described by the manufacturer. Oligonucleotides were designed based on the amino acid sequences NIIKKKGW and NQGMAMYW from amino acids 101–108 and 178–185, respectively, of the Kvβ2 subunit from bovine brain ((19); Fig. 1). The oligonucleotide sequences were: 5′-TCGAATTCAAY-CAGGGMATGGCIATGTAYTGG-3′ and 5′-GACCTCGAGCCYTCGT-TICKIARRCACCA-3′ (R = A/G; M = A/C; I = inosine; K = G/T; Y = C/T). PCR[1] was performed as described (26), with 1/20 of the cDNA from the reverse transcription reaction. The cycling parameters utilized "Touchdown PCR" (27), with a starting annealing temperature of 55 °C, and a concluding annealing temperature of 42 °C for the final 10 cycles. A probe was made by random-priming the PCR product with digoxigenin-11-dUTP to allow chemiluminescent detection (Boehringer Mannheim). The random-primed PCR product was used to probe a ferret ventricle cDNA library (20) constructed in λZAPII (Stratagene). Plaque lifts, hybridization, and detection were performed as described by Boehringer Mannheim. The positive plaque, λFHBK1, was rescued by *in vivo* excision; the resulting plasmid, pFHBK1, contained the full length of the β subunit open reading frame and about 1.5 kilobase pairs of 3′-untranslated region, including the poly(A)$^+$ tail. Single-stranded phagemid DNA (28) was utilized for sequencing with Sequenase 2.0 (U. S. Biochemicals) using custom oligonucleotides. Each nucleotide on both strands was unambiguously identified at least once. Sequence similarity searches were performed through the NCBI BLAST server (29).

Human and rat Kvβ3 cDNAs were isolated by standard PCR of cDNA synthesized as above. Human heart whole cell RNA was purchased from Clontech. The primers used were FKβ3/58 (5′-GTATAAACCTGCCT-GTGC-3′; covering amino acids 4–9 in ferret Kvβ3) and FKβ3/37 (5′-AGCCTTTCCAGCAGCATAGACTTC-3′; covering amino acids 129–135; Fig. 1). PCR was performed as described (30). After a 5-min 94 °C denaturation step, the reactions were cycled 35 times at 94 °C for 30 s, 53 °C for 45 s, and 72 °C for 1 min. The procedure was completed with a 5-min incubation at 72 °C. The PCR products were cloned into pBS-SK$^+$, and two independent clones of each were sequenced. Partial cDNA sequences of the rat and human Kvβ3 have been deposited in GenBank™ under the accession numbers U17967 and U17968, respectively.

Competitive PCR—Whole cell RNA was prepared from ferret heart, brain, liver, kidney, skeletal muscle (25), aorta, and aortic endothelial cells (31). Endothelial cells were isolated and cultured as described (20). Complementary DNA was made from 5 μg of RNA using SuperScript reverse transcriptase with a random hexamer primer (Life Technologies, Inc.). Competitive PCR was performed essentially as described (32). One-twentieth of each reverse transcriptase reaction (along with a sham cDNA control lacking reverse transcriptase) was amplified by PCR. Each reaction contained 2 μM primer FKβ3/37, 2 μM primer FKβ3/56 (5′-TCTCAGAGCTAAAGACTGTGAAATGAGC-3′; identical with nucleotides 4–31 upstream of the translational start site), and 0.2 fmol internal standard. The cycling parameters were the same as for isolation of rat and human Kvβ3, except the annealing temperature was 58 °C. The reaction products were separated on a 5% polyacrylamide, 1 × TBE gel, stained with ethidium bromide, and photographed under ultraviolet light. The 272-bp internal standard was synthesized as described (33) using primers FKβ3/56 and FKβ3/37 (5′-AGCCTTTC-CAGCAGCATAGACTTCATTCCTGTAAGCCATG-3′; the first 24 nucleotides are identical with FKβ3/37, the last 16 nucleotides are complementary to nucleotides 286–301; see Fig. 2*A*).

K^+ Current Measurement—*Xenopus laevis* oocytes (stage V-VI) were defolliculated by gently shaking for 3 h in Ca^{2+}-free OR2 solution (in mM: 82.5 NaCl, 2 KCl, 1 $MgCl_2$, 5 HEPES-NaOH, pH 7.40) with 1–2 mg/ml collagenase (20). Oocytes were injected with 50 nl of cRNA solution containing up to 50 ng of RNA with and without β subunit at ratios varying from 1:1 (α:β) to 1:16, as described previously (20). Voltage-clamp experiments were conducted on oocytes within 3–6 days of injection using a two electrode "bath clamp" amplifier (OC-725A, Warner Instruments) as described previously. Briefly, bath potential was sensed using an agar bridge electrode, and bath potential was maintained using a separate current passing agar bridge electrode. The amplifier was used with the "DC gain feature" which reduced errors due to finite open loop gain. Average peak current size at +50 mV for all analyzed currents was 7.4 ± 0.9 μA (n = 118). No correlation between current size and inactivation rate was detected for the range of currents measured in this study. Extracellular solution was ND-96 (in mM: 96 NaCl, 2 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, 5 HEPES-NaOH, pH 7.4). Electrodes were filled with 3 M KCl (resistances 0.6–1.5 megaohms) and shielded to reduce capacitive coupling. All measurements were carried out at room temperature. Data were not leakage- or capacitance-subtracted, unless otherwise specified. Activation was measured using the cut-open oocyte technique (34), as described previously (20). Data from two-electrode voltage clamp were filtered at 2 kHz or 5 kHz for cut-open oocyte and analyzed using an Axon Instruments TL-1 interface and pClamp software. Confidence levels were calculated using an unpaired t test.

[1] The abbreviations used are: PCR, polymerase chain reaction; bp, base pair(s).

RESULTS AND DISCUSSION

Fig. 1 shows an alignment of the deduced amino acid sequence of ferret Kvβ3 to K^+ channel β subunits from rat (Kvβ1 and Kvβ2) and bovine (Kvβ2). Strikingly, Kvβ3 and rat Kvβ1 are identical over the last 329 amino acids, a region where Kvβ1 and Kvβ2 share 85% identity. However, there is no similarity among the first 79 amino acids of the ferret β subunit and the first 72 or 38 amino acids of Kvβ1 and Kvβ2, respectively. Similarity searches of both the entire β subunit and the N-terminal 79 amino acids revealed no significant similarity between ferret Kvβ3 and any other protein except the previously described β subunits. No similarity was found between the N terminus of ferret Kvβ3 and any other protein sequence. Despite their apparent lack of similarity, the N termini of Kvβ1 and Kvβ3 share one potentially important structural feature: a cysteine residue near the N terminus. Oxidation of this amino acid, also found near the N termini of other fast inactivating channels, can dramatically decrease inactivation rates (11, 16). Hydropathy and secondary structural analyses of the Kvβ1 and Kvβ3 N termini fail to show any apparent similarity in predicted secondary structure (35–40). Hydropathy analysis also suggested Kvβ3 is a cytoplasmic protein, as has been predicted for Kvβ1 and Kvβ2 (16, 19). Comparison of the deduced Kvβ3 amino acid sequence with protein motif data bases (41, 42) showed the presence of 33 consensus phosphorylation sites.

To determine the tissue localization of Kvβ3, competitive PCR (32) was performed on cDNA made from several tissues. Oligonucleotides were chosen at the 5′ end of the clone to ensure specific amplification of Kvβ3 (Fig. 2*A*). Ferret Kvβ3 transcript was most abundant in aorta followed by left ventricle (Fig. 2*B*). Transcripts were also detected in right ventricle, atrium, brain, skeletal muscle, and kidney. None were detectable in liver or cultured aortic endothelial cells. Aorta is rich in smooth muscle, fibrous tissue, and endothelium; as the transcript was not present in endothelial cells, it is likely that this transcript is present in smooth muscle or fibroblasts, although its presence in other cell types cannot be ruled out.

Kvβ3 is not unique to ferret. Cross-species PCR using oligonucleotides FKβ3/37 and FKβ3/58 (which is complementary to the unique portion of the ferret cDNA) was performed on rat and human heart cDNA. The reactions yielded the expected 400-nucleotide DNA species, which was cloned and sequenced. There was extensive similarity in the nucleotide sequence among ferret, human, and rat, showing that Kvβ3 is present in all three species. The amino acid sequence conservation in the N-terminal region was 88% between ferret and human and 80% between ferret and rat (data not shown).

Kvβ1 can increase the rate of inactivation of both a delayed rectifier and a fast-inactivating (A-type) K^+ channel α subunit (16). Conservation between Kvβ3 and Kvβ1 suggested that Kvβ3 might have a similar influence. To test the effect of Kvβ3 on inactivation and other channel properties, it was co-expressed in *Xenopus* oocytes with FK1, a ferret Kv1.4 channel (20), and RCK1 (rat Kv1.1 (21)). Co-injection of Kvβ3 mRNA with FK1 mRNA caused subtle changes in inactivation kinetics (Fig. 3*A*). Inactivation of FK1 channels has been previously

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Ferret Kvβ3 MHLYKPACADIPSPKLGLPKSSESALKCRRHLAVTKPPPQAACWPARPSGAAERKFLEKFLRVHGISLQETT 72
Rat Kvβ1           MQVSIACTEHNL--RNGEDRLLSKQSS-A-NVVN-ARAKFRTV-IIARS-GT-TPQ-H---K-S- 65
Rat Kvβ2                                              MYPESTTGSPARLSLRQTGSPGMIY-TRYGS 31
Bovine Kvβ2                                           MYPESTTGSPARLSLRQTGSPGMIY-TRYGS 31

Ferret Kvβ3 RAETGMAYRNLGKSGLRVSCLGLGTWVTFGGQISDEVAERLMTIAYESGVNLFDTAEVYAAGKAEVILGSII 144
Rat Kvβ1    AKQ---K------------------------------------------------------------- 137
Rat Kvβ2    PKRQLQF-------------------------T--M--H---L--DN-I---------------V--N-- 103
Bovine Kvβ2 PKRQLQF-------------------------T--M--Q---L--DN-I---------------V--N-- 103

Ferret Kvβ3 KKKGWRRSSLVITTKLYWGGKAETERGLSRKHIIEGLKGSLQRLQLEYVDVVFANRPDSNTPMEEIVRAMTH 216
Rat Kvβ1    ------------------------------------------------------------------- 209
Rat Kvβ2    --------------IF--------------------A--E---------------P------T------ 175
Bovine Kvβ2 --------------IF--------------------A--E---------------P------T------ 175

Ferret Kvβ3 VINQGMAMYWGTSRWSAMEIMEAYSVARQFNMIPPVCEQAEYHLFQREKVEVQLPELYHKIGVGAMTWSPLA 288
Rat Kvβ1    ------------------------------------------------------------------- 281
Rat Kvβ2    ---------------S-------------L---I-------M------------F------------- 247
Bovine Kvβ2 ---------------S-------------L---I-------M------------F------------- 247

Ferret Kvβ3 CGIISGKYGNGVPESSRASLKCYQWLKERIVSEEGRKQQNKLKDLSPIAERLGCTLPQLAVAWCLRNEGVSS 360
Rat Kvβ1    ------------------------------------------------------------------- 353
Rat Kvβ2    ---V----DS-I-PY------G-----DK-L-----R--A---E-QA------------I---------- 319
Bovine Kvβ2 ---V----DS-I-PY------G-----DK-L-----R--A---E-QA------------I---------- 319

Ferret Kvβ3 VLLGSSTPEQLIENLGAIQVLPKMTSHVVNEIDNILRNKPYSKKDYRS                       408
Rat Kvβ1    ------------------------------------------------                       401
Rat Kvβ2    ----A-NA---M--I--------LS-SI-H---S--G----------                       367
Bovine Kvβ2 ----A-SAD--M--I--------LS-S-IH---S--G----------                       367
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FIG. 1. **Deduced amino acid sequence of ferret Kvβ3.** The sequence is shown aligned with the other known K^+ channel β subunit sequences: rat Kvβ1, rat Kvβ2, and bovine Kvβ2 (16, 19). Amino acids identical with those in Kvβ3 are designated with a *hyphen* in the Kvβ1 and -2 sequences. The nucleotide sequence of ferret Kvβ3 has been deposited in GenBank under the accession number U17966.

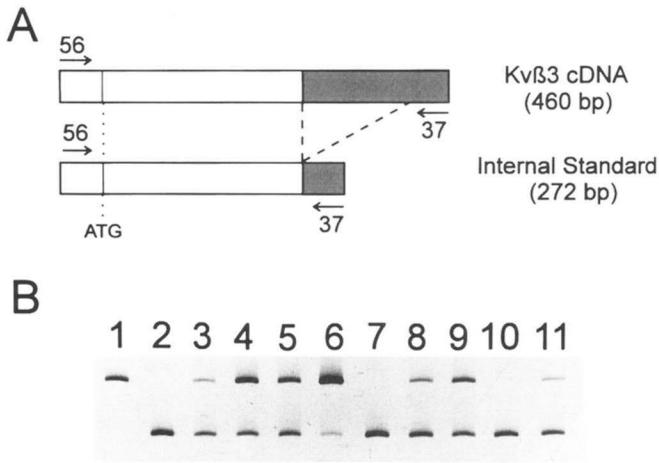

FIG. 2. *A*, strategy for detection of Kvβ3 transcripts by competitive PCR. Ferret transcripts were detected using oligonucleotides Kvβ3/56 and Kvβ3/37, which give a 460-bp DNA molecule (*B*). The internal standard, added to PCR reactions in *B*, was a 183-bp deletion of Kvβ3 that preserved the nucleotide sequence complementary to the oligonucleotides; its product was 272 bp. The *open boxes* represent unconserved regions, and the *shaded boxes* represent the conserved region of Kvβ3. *B*, competitive PCR of cDNAs from various ferret tissues. Five μg of whole cell RNA were reverse-transcribed and amplified by PCR in the presence of 0.2 fmol internal standard. *Lanes 1*, cDNA alone; *2*, internal standard alone; *3*, right ventricle; *4*, left ventricle; *5*, atrium; *6*, aorta; *7*, cultured endothelial cells; *8*, brain; *9*, skeletal muscle; *10*, liver; and *11*, kidney. Competitive PCR of sham reverse transcription controls showed no detectable signal (data not shown).

shown to be biexponential, with two closely spaced time constants at +50 mV (20). The time constant of the fast component of inactivation decreased from 42 ± 3 (n = 20) for FK1 alone to 6.2 ± 0.2 ms (n = 28); the slow component decreased from 313 ± 94 to 101 ± 2 ms. The decrease of the fast component of inactivation was comparable to the overall decrease induced by Kvβ1 expressed with rat Kv1.4. Co-expression of Kvβ3 with FK1 also changed the relative contribution of each component of inactivation; the ratio of the amplitude of fast to slow time constants decreased from 5.0 ± 1.7 to 0.67 ± 0.03. Because of the greater participation of the slow component, the net result was a minor alteration in the overall rate of inactivation.

In contrast to the results obtained with FK1, co-injection of RCK1 (Kv1.1) with Kvβ3 failed to show any effect on channel inactivation (Fig. 3*B*). This was true despite a 16:1 (by weight) co-injection of Kvβ3:RCK1 mRNA; parallel experiments using FK1 and FK1Δ2–146 (see below) showed that the Kvβ3 subunit was active (data not shown). These results are strikingly different from those obtained by Rettig *et al.* (16) with Kvβ1. They showed an approximately 100-fold increase in the rate of inactivation of RCK1. Since Kvβ3 was co-injected with the identical RCK1 α subunit, and conservation of RCK4 and FK1 is 97% (20), it is likely that the difference in inactivation is due to the different N termini of Kvβ3 and Kvβ1.

It is possible that the change in rate of inactivation caused by Kvβ3 may be dependent on the ratio of expressed α subunit to β subunit. If the β subunits failed to saturate all available binding sites on the α subunits, the overall rate and degree of β subunit-mediated inactivation would likely be decreased. A second explanation is that the maximal association of β subunits to α subunits was achieved, but that Kvβ3 simply induced slower inactivation, or perhaps caused inactivation by a different mechanism. To discriminate between these possibilities, a mutant form of FK1, FK1Δ2–146, in which the first 146 N-terminal amino acids have been deleted to remove fast N-type inactivation ((20); referred to as FK1ΔNco), was co-expressed with Kvβ3. As shown in Fig. 3*C*, co-expression with Kvβ3 greatly increased the rate at which FK1Δ2–146 inactivated. The time constant of inactivation for FK1Δ2–146 was 1795 ± 81 ms (at +50 mV), decreasing to 409 ± 27 ms with co-injection of Kvβ3 mRNA. The effects of co-expression of Kvβ3 mRNA on the inactivation rate of FK1Δ2–146 were dependent upon the ratio of injected Kvβ3 to FK1Δ2–146 mRNA for ratios less than 2 to 1 but were insensitive to ratios higher than 2 to 1 (Fig. 4), suggesting that the β subunits were saturating at the mRNA concentrations used in these experiments.

These data showed that ferret Kvβ3 can associate with ferret

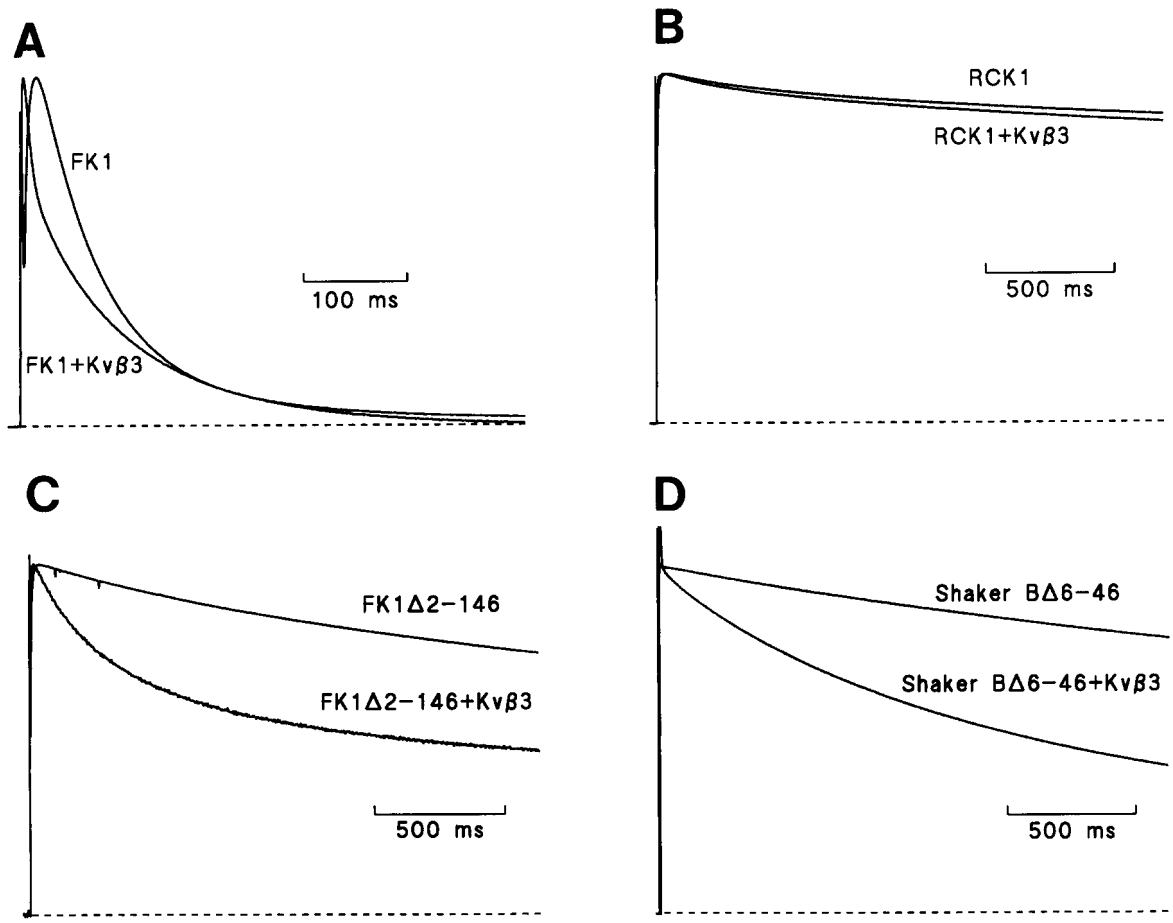

FIG. 3. **Outward K^+ currents elicited by depolarizing pulses to +50 mV from a holding potential of −90 mV using a two-electrode voltage clamp.** Currents were normalized relative to the same peak current to emphasize the kinetic changes induced by co-expression of Kvβ3. Currents resulted from depolarizations to +50 mV from a holding potential of −90 mV, stimulation rate of 0.1 Hz, and perfused with ND-96. Oocytes were injected with mRNA encoding various K^+ channel subunits types. *A*, FK1 alone (Kv1.4, 8 ng of mRNA/oocyte, peak current 12.7 μA) or FK1 + Kvβ3 (8 and 32 ng of mRNA/oocyte, peak current 9.7 μA). Note that expression of FK1 alone results in a smooth inactivation with a small but slow late component of inactivation. Co-expression of FK1 with Kvβ3 results in an initial small rapid component followed by a much larger slow component of inactivation. *B*, RCK1 alone (Kv1.1, 2 ng of mRNA/oocyte, peak current 14.4 μA) or RCK1 + Kvβ3 (2 and 32 ng of mRNA/oocyte, respectively, peak current 10.2 μA). Co-injection of Kvβ3 with RCK1 did not increase the rate of inactivation. *C*, FK1Δ2–146 (8 ng of mRNA/oocyte, peak current 5.7 μA), an N-terminal deletion mutant (see Ref. 20) or FK1Δ2–146 + Kvβ3 (2 and 32 ng of mRNA/oocyte, respectively, peak current 1.7 μA). Co-injection of FK1Δ2–146 with Kvβ3 partially, but not completely, restored inactivation. *D*, *Shaker* BΔ6–46 alone (2 ng of mRNA/oocyte, 9.1 μA) or *Shaker* BΔ6–46 + Kvβ3 (8 and 32 ng of mRNA/oocyte, respectively, peak current 11.1 μA). The increase in inactivation rate was similar to the results with FK1Δ2–146. Kvβ3 partially restored inactivation in the mutant *Shaker* channel and induced a very small additional fast component that was not observed with FK1Δ2–146.

Kv1.4 (FK1), but did not rule out the possibility that this β subunit's effects might be specific for only a limited class of α subunits. Therefore, we co-injected Kvβ3 with the distantly related channel, *Shaker* B from *Drosophila*. Since this channel is only 50% conserved with FK1, it appeared to be an unlikely candidate to associate with a mammalian β subunit. We used *Shaker* BΔ6–46 (22), a mutant lacking fast N-type inactivation, to be able to clearly detect the effects of β subunit association. Co-expression of *Shaker* BΔ6–46 with Kvβ3 resulted in an increase in the degree and rate of inactivation similar to that observed for FK1Δ2–146 (Fig. 3*D*). Association with *Shaker* B suggested that Kvβ3 is probably binding to the much more highly conserved RCK1, but for some reason is unable to induce inactivation.

Other gating characteristics of FK1 and FK1Δ2–146 were not strongly altered by co-expression of Kvβ3. Fig. 5 shows data obtained using the cut-open oocyte clamp (34) for FK1Δ2–146 in the presence and absence of β subunit. FK1Δ2–146 was used to minimize the overlap of fast inactivation with activation. There was no obvious difference in the sigmoid onset of the current during the first 2 ms at +50 mV; however, the late rising phase of current was terminated prematurely by what appears to be the onset of an inactivation-like process. Although the time to peak current appears to have decreased with co-expression of Kvβ3, this is most likely due to the increased inactivation rate. Similarly, β subunit expression had only modest effects on recovery from inactivation when co-expressed either with full-length FK1 ($t_{½}$ = 4.88 ± 0.13 s (n = 4) with β subunit; $t_{½}$ = 2.95 ± 0.18 s (n = 4) without β subunit) or FK1Δ2–146 ($t_{½}$ = 2.94 ± 0.12 s (n = 5) with β subunit; $t_{½}$ = 2.20 ± 0.11 s (n = 4) without β subunit).

We have cloned, sequenced, and characterized a novel K^+ channel β subunit, Kvβ3. The deduced protein sequence is identical with Kvβ1 in its 329 C-terminal amino acids, but has a 79-amino acid N terminus with no sequence identity with Kvβ1 or Kvβ2. Conservation of C termini between Kvβ1 and Kvβ3, and their sharp transition to a nonconserved region, suggested that one mechanism for generating different β subunits might be alternative splicing of precursor transcripts.

The general structural similarity between Kvβ1 and Kvβ3 implied that Kvβ3 might induce inactivation in K^+ channel α subunits. This was true for mutant channels that have had

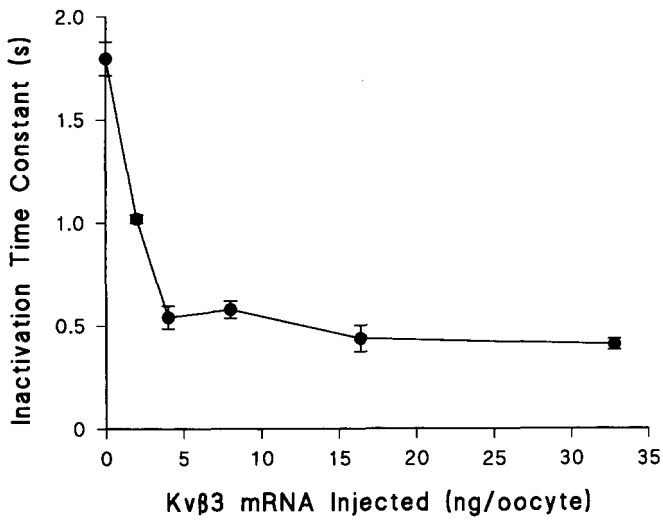

FIG. 4. **Saturation of FK1Δ2–146 with Kvβ3.** The alteration of inactivation time constants was sensitive to the ratio of β subunit to α subunit for low ratios but saturated for high ratios of β to α subunit, indicating that the rate of inactivation reflected the maximal association of Kvβ3 to FK1Δ2–146 channels. Two ng/oocyte of FK1Δ2–146 was injected into each oocyte followed by a second injection of 0, 2, 4, 8, 16, or 32 ng/oocyte Kvβ3 mRNA. Inactivation was measured with a single exponential time constant fit to the rate of current decay. The data for each particular level of injected β subunit mRNA is given as the mean ± S.E. (n = 11, 4, 8, 11, 6, 10 oocytes for each point, average peak currents were 4.9 ± 0.5, 3.7 ± 0.8, 3.1 ± 0.4, 2.3 ± 0.2, 2.4 ± 0.2, 1.5 ± 0.1 μA, respectively).

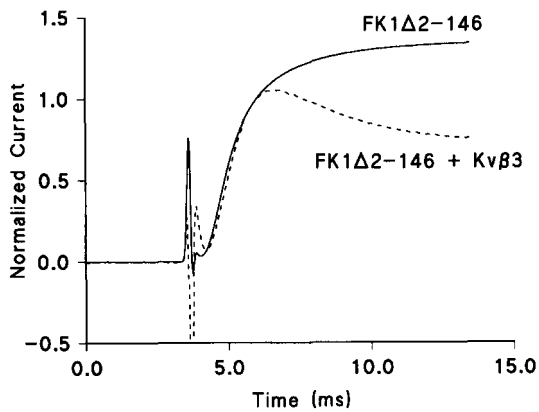

FIG. 5. **Early onset of current activation is unchanged by the addition of β subunit.** Currents recorded from FK1Δ2–146 (*solid line*) and FK1Δ2–146 co-expressed with Kvβ3 (*dashed line*) using the cut-open oocyte voltage clamp technique as described (20, 34). The currents were normalized for the purpose of comparison. The peak current occurred much earlier due to the overlap with β subunit-mediated inactivation, but was otherwise apparently unaffected. Current amplitudes were 15 and 2.1 μA for FK1Δ2–146 and FK1Δ2–146 + β subunit, respectively. Both currents were recorded in response to a depolarizing pulse to +50 mV from a holding potential of −90 mV and were capacitance and leakage compensated. No off-line subtraction procedures were employed.

N-type inactivation disrupted (FK1Δ2–146 and *Shaker* BΔ6–46) and in wild-type Kv1.4. However, unlike Kvβ1, it did not cause inactivation of a Kv1.1 α subunit. The failure of Kvβ3 to modify RCK1 inactivation kinetics may reflect a failure of this α and β subunit to associate, although association with *Shaker* argues against this. Alternatively, Kvβ3 and RCK1 may associate, but specific domains required for inactivation cannot interact.

Acceleration of inactivation by Kvβ1 is mediated by a "tethered ball" (16). If Kvβ3 operates through a similar mechanism, the implication is that the inactivation domain of Kvβ3 is specific for certain α subunits. This seems unlikely, given the recent demonstration that a variety of inactivation domains can induce inactivation in fast inactivating and non-inactivating α subunits (43, 44). Another possibility is that Kvβ3 alters inactivation through another mechanism. In either case, it seems clear that the nonconserved N-terminal region in β subunits confer some degree of specificity of interaction.

Kvβ3 is the third member of a family of ancillary subunits that associate with voltage-gated K^+ channels. Co-expression of Kvβ3 causes alterations in K^+ channel function that are distinct from the effects Kvβ1 or Kvβ2. This suggests that the diversity of *in vivo* K^+ channel function may result from expression of different K^+ channel β subunits, as well as diversity of expression and assembly of α subunits. This mechanism is similar to that of Na^+ and Ca^{2+} channels, which are known to generate diversity through assembly of different subunits (45). Given the wide variety of associated subunits for these other channels, it seems likely that Kvβ1, Kvβ2, and Kvβ3 are the first members of a large group of ancillary K^+ channel subunits that differ in their N termini. The novel differences in specificity of channel interaction and electrophysiological properties of Kvβ3 from Kvβ1 and Kvβ2 provide an additional basis for understanding K^+ channel function and diversity.

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