Coupling of Voltage-dependent Potassium Channel Inactivation and Oxidoreductase Active Site of Kvβ Subunits*

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The accessory β subunits of voltage-dependent potassium (Kv) channels form tetramers arranged with 4-fold rotational symmetry like the membrane-integral and pore-forming α subunits (Gulbis, J. M., Mann, S., and MacKinnon, R. (1999) Cell. 90, 943–952). The crystal structure of the Kvβ2 subunit shows that Kvβ subunits are oxidoreductase enzymes containing an active site composed of conserved catalytic residues, a nicotinamide (NADPH)-cofactor, and a substrate binding site. Also, Kvβ subunits with an N-terminal inactivating domain like Kvβ1.1 (Rettig, J., Heinemann, S. H., Wunder, F., Lorra, C., Parecej, D. N., Dolly, O., and Pongs, O. (1984) Nature 310, 289–294) and Kvβ3.1 (Heinemann, S. H., Rettig, J., Graack, H. R., and Pongs, O. (1996) J. Physiol. (Lond.) 493, 625–633) confer rapid N-type inactivation to otherwise non-inactivating channels. Here we show by a combination of structural modeling and electrophysiological characterization of structure-based mutations that changes in Kvβ oxidoreductase activity may markedly influence the gating mode of Kv channels. Amino acid substitutions of the putative catalytic residues in the Kvβ1.1 oxidoreductase active site attenuate the inactivating activity of Kvβ1.1 in Xenopus oocytes. Conversely, mutating the substrate binding domain and/or the cofactor binding domain rescues the failure of Kvβ3.1 to confer rapid inactivation to Kv1.5 channels in Xenopus oocytes. We propose that Kvβ oxidoreductase activity couples Kv channel inactivation to cellular redox regulation.

Shaker-related voltage-gated potassium (Kv) channels are assembled from membrane-integral pore-forming Kvα subunits with auxiliary cytoplasmic Kvβ subunits (4, 5, 6). The membrane topology of Kvα subunits shows six membrane-spanning segments, S1–S6, a pore-forming loop structure between S5 and S6, and cytoplasmic N and C termini. A tetramerization domain resides in the N terminus and directs assembly of Kvα subunits (7, 8). The tetramerization domain also associates with the auxiliary Kvβ subunits (9, 10). Cystallographic analysis of Kvβ2 tetramers showed that each subunit contains an interface for association with Kvα subunits (11) and an oxidoreductase active site (1), but the specific substrate is unknown.

Three Kvβ genes have been identified: Kvβ1, Kvβ2, and Kvβ3 (12). Kvβ1.1 and Kvβ3.1 subunits possess an N-terminal inactivating domain that confers rapid N-type inactivation to Kv channels (2, 3). Thus, the association of Kvβ1.1 and Kvβ3.1 subunits with certain Kvα subunits leads to the expression of rapidly inactivating A-type channels (2, 3). Interestingly, Kvβ3.1 confers rapid inactivation to Kv1.5 channels only when coexpressed in mammalian cells (12) but not in Xenopus oocytes (13). By contrast, Kv1.5/Kvβ1.1 channels mediate rapidly inactivating currents both in mammalian cells (14, 15) and in Xenopus oocytes (2).

We constructed chimeras between Kvβ1.1 and Kvβ1.1 to identify possible domains correlated with the apparent lack of function of Kvβ3.1 in particular with reference to the N-terminal inactivating domain (2, 3), the interface for association with Kvα subunits (11), and the oxidoreductase active site (Ref. 1; see Fig. 1, B and C). The results of our structure-function analysis demonstrate that the failure of Kvβ3.1 to confer rapid inactivation to Kv1.5 channels in the Xenopus oocyte expression system is associated with two C-terminal domains of Kvβ3.1, which contain structural elements of the oxidoreductase active site. According to the crystal structure of Kvβ2, the two Kvβ3.1 domains occur in Kvβ protein regions, which are part of the NADPH cofactor binding pocket and the substrate binding site, respectively. Chimeric replacement of Kvβ1.1 residues by Kvβ1.1 residues within these domains rescued the Kvβ3.1 inactivating activity, and point mutations of Kvβ1.1 active site residues attenuated the Kvβ1.1 inactivating activity. We propose that Kvβ oxidoreductase enzymatic activity and the biophysics of Kvβ inactivating activity are coupled.

EXPERIMENTAL PROCEDURES

In Vitro Mutagenesis and cRNA Synthesis—Kvβ1.1pAKS (2) and Kvβ3.1pAKS (3) were used for Kvβ1.1 and Kvβ3.1 cRNA synthesis as described previously (16). The cDNAs for the Kvβ3 subtypes between rat Kv1.1 (GenBank™ accession number X70662) and Kvβ3.1 (GenBank™ accession number X76273) were obtained exploiting appropriate restriction enzyme sites and/or using an overlap polymerase chain reaction (17). The chimeric cDNAs were cloned into the pAKS vector (16). For the construction of the different chimeras the following DNA fragments were joined together (note that numbers in parentheses refer to Kvβ-cDNA nucleotides): Kvβ1α, Kvβ1.1-(1–1080) and Kvβ1.1-(1000–1546); Kvβ1chAvv, Kvβ1.1-(1–997) and Kvβ1.1-(1075–1599); Kvβ1βB, Kvβ1.1-(390–1260) and Kvβ1.1-(1180–1534); Kvβ1chC, Kvβ1.1-(390–1260) and Kvβ1.1-(1180–1534); Kvβ1chD, Kvβ1.1-(390–1380) and 32 Kvβ1.1-(1300–1554); Kvβ1chE, Kvβ1.1-(390–1534) and Kvβ1.1-(1453–1534); Kvβ1chF, Kvβ1.1-(390–1450), Kvβ1.1-(1370–1453), and Kvβ1.1-(1535–1606); Kvβ1chG, Kvβ1.1-(390–1376), Kvβ1.1-(1304–1385), and Kvβ1.1-(1457–1606); Kvβ1chH, Kvβ1.1-(390–1222), Kvβ1.1-(1142–1237), Kvβ1.1-(1318–1440), Kvβ1.1-(1370–1453),
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Kvβ3.1-(1535–1606), Kvβδ1, Kvβ1.1-(390–1222), Kvβ1.1-(1142–1270), Kvβ3.1-(1285–1440), Kvβ1.1-(1370–1453), Kvβ3.1-(1535–1606).

DNA sequences amplified by the polymerase chain reaction were verified by sequencing using BigDye terminator cycle sequencing kit (PerkinElmer Life Sciences). The sequencing reactions were analyzed on an ABI 377 automated sequencer (PerkinElmer Life Sciences).

Point mutations in rat Kvβ1.1 were introduced by site-directed mutagenesis using appropriate mutation primers (17). Polymerase chain reaction products were cloned into Kvβ1.1pGEM using a DraIII and a Neol restriction site. DNA constructs were sequenced prior to use. RNA synthesis was done using the Message Machine in vitro transcription kit according to the manufacturer’s protocol (Novagen Inc.).

Recording Techniques and Data Analysis—Xenopus oocytes were prepared and injected with cRNA, and electrophysiological recordings were made as previously described (18). Briefly, oocytes were injected with 50 nl of a solution containing equal amounts (25 ng) of cRNA for rKv1.5a and rKvβ subunits (wild-type or Kvβδ1). Deviations from these cRNA concentrations are indicated in the figure legends. Oocytes were then incubated at 20 °C for 24–48 h in multiwell tissue culture plates (one oocyte/well) containing standard Barth’s solution (88 mM NaCl, 1.8 mM CaCl2, 0.82 mM MgSO4, 0.33 mM Ca(NO3)2, 0.4 mM CaCl2, 7.5 mM Tris-HCl, pH 7.6, 10,000 units/l penicillin, 100 mg/l streptomycin). To record expressed membrane currents, the oocytes were held in a recording chamber (50 μl volume) and continually perfused with Ringer’s solution (115 mM NaCl, 2.5 mM CaCl2, 10 mM HEPES adjusted to pH 7.2 with NaOH). Membrane currents were recorded with the two-microelectrode voltage-clamp technique (microelectrodes filled with 3 M KCl) using a Geneclamp 500 amplifier (Axon Instruments), and signals were filtered at 2 kHz. Twenty mV hyperpolarizing steps (1 s duration, 0.5 Hz) were applied and used to remove leak and capacitance currents. To construct current-voltage (I-V) relationships, the cell was held at −80 mV, 1-s duration test depolarizations to 0.1 Hz were applied in 10 mV increments from −70 to +120 mV, and peak and end current amplitudes were measured. I-V curves were fitted with Boltzmann functions of the form I = (I∞ − I0) × Gmax/(1 + exp(Vtail − Vth)), where Vtail = −80 mV. The inactivation time course was fit by a sum of two exponentials by the least squares technique. Student’s t test was used to test for statistical significance. In some experiments cDNA (vector pcDNAs) for Kv1.5 (25 ng/μl) together with Kvβδ cDNA (500 ng/μl) were microinjected into Chinese hamster ovary (CHO) cells. Whole-cell currents were measured with the patch clamp technique on the following day using an EPC9 amplifier and PULSE software (HEKA, Lambrecht, Germany). The extracellular solution contained (in mM) NaCl 135, KCl 5, CaCl2 2, MgCl2 1, HEPES 5, sucrose 10 (pH 7.4, NaOH). All experiments were conducted at room temperature (20–22 °C).

RESULTS

Kvβ1.1 but Not Kvβ3.1 Confers Rapid Inactivation to Kv1.5 Channels in Xenopus Oocytes—Kvβ1.1 and Kvβ3.1 subunits have functional inactivating domains, but Kvβ3.1 confers rapid inactivation to Kv1.5 channels only when coexpressed in mammalian cells (12). In agreement with previous results (13), wild-type Kvβ3.1 subunits did not cause rapid inactivation when we coexpressed it with Kv1.5 channels in Xenopus oocytes (Fig. 1A; Table I). Upon depolarization of the oocyte membrane, slowly inactivating outward currents were recorded, and 84% of the initial peak amplitude remained at the end of a 1-s test pulse to +80 mV (Fig. 1, A and D; Table I). By contrast, Kv1.5/Kvβ1.1 channels mediate rapidly inactivating currents both in mammalian cells (14, 15) and in Xenopus oocytes (2). In our experiments they decayed to ~13% of the initial maximum current amplitude at the end of a 1-s test pulse to +80 mV (Fig. 1, A and D; Table I). The inactivation time course (Fig. 1A) was fitted with two time constants, τ1 (13.0 ± 0.9 ms) and τ2 (75.0 ± 5 ms; n = 14; Table I). The fast time constant τ1 accounted for 90 ± 10% of the total current decay. We examined the structural motifs in Kvβ3.1 responsible for its inactivation failure in Xenopus oocytes by constructing chimeras between Kvβ1.1 and Kvβ3.1. Possible structural determinants correlated with the apparent lack of function of Kvβ3.1 may be located in the N-terminal inactivating domain (2, 3), the interface for association with Kvα subunits (11) and/or the oxidoreductase active site (Ref. 1; Fig. 1, B and C).

Failure of Kvβ3.1 in N-type Inactivation Linked to C-terminal Domains—We connected in the first chimera the Kvβ3.1 N terminus (residues 1–229), which contains the N-terminal inactivating domain and the conserved catalytic residues (1) of the Kvβ3 oxidoreductase (Fig. 1B), with the Kvβ1 C terminus corresponding to Kvβ1.1 residues 223–401 (Kvβδ1A; Fig. 1C). The N-terminal Kvβ3.1 inactivating domain became fully functional when connected to the Kvβ1 C terminus (Fig. 1, A and D), in agreement with the previous observation that the Kvβ3.1 inactivating domain becomes functional when connected to Kvβ2.1 (3). The results obtained with Kvβδ1A indicated that C-terminal Kvβ3.1 domain(s) must be responsible for the lack of functional interaction with Kv1.5 channels. To test this hypothesis, we constructed a reverse chimera (Kvβδ1Aev), in which the Kvβ1 N-terminal half was linked to the Kvβ3 C-terminal half (Kvβ1 residues 1–222 and Kvβ3 residues 230–404; Fig. 1B). The Kv1.5/Kvβδ1Aev-mediated currents exhibited inactivation kinetics similar to the ones observed for Kv1.5 with wild-type Kvβ3.1, with 80 ± 1% of the maximal current amplitude remaining at the end of a 1-s depolarizing pulse (Fig. 1A; Table I). These results confirmed the idea that the ability of Kvβδ1A to inactivate Kv1.5 channels was due to the presence of the Kvβ1.1 C terminus.

To identify the C-terminal domains responsible for the failure of Kvβ3.1 in N-type inactivation we constructed further Kvβ chimeras. For this purpose we expanded the Kvβ3 portion in Kvβδ1A in a stepwise fashion. Additional replacement of Kvβ1.1 residues 223–282 by those of Kvβ3.1 (Kvβδ1B) included the Kvδ1 interface domain for assembly with Kvα subunits (Ref. 11; Fig. 1, B and C). The respective Kv1.5/Kvβδ1B channels mediated outward currents with a relatively small reduction in the extent of inactivation (Fig. 1, A and D; Table I). The small reduction would be compatible with a subtle difference in the affinities of Kvβ1.1 and Kvβ3.1 subunits for the Kv1.5α subunits (11). Nevertheless, the results demonstrated that the Kvβ3.1 domains for N-type inactivation and the subunit interface for complex formation with Kv1.5α subunits were not responsible for the observed inactivation failure of Kvβ3.1. The exchange of Kvβ1.1 residues by those of Kvβ3.1 was extended to residues 283–346 (Kvβδ1C, D), which may cover the entire substrate binding site of the Kvβ oxidoreductase (Ref. 1; Fig. 1, B and C). The respective Kv1.5/Kvβδ1C and Kv1.5/Kvβδ1D currents showed a significant reduction in the extent of inactivation (Fig. 1, A and D) because of an increase in τ1 as well as an alteration in the relative weights of the fast (τ2) and slow (τ3) components (Table I). We extended the exchange of Kvβ1.1 by Kvβ3.1 residues further to residues 347–374 (Kvβδ1E), which encompassed a part of the Kvβ oxidoreductase active site that is essential for binding the cofactor NADPH (Ref. 1; Fig. 1, B and C). The resulting Kv1.5/Kvβδ1E currents showed an ineffective and slow inactivation (Fig. 1, A and D) due to an increase in both τ2 and τ3 (Table I). The results suggested that C-terminal Kvβ3.1 domains encompassing substrate and cofactor binding sites were responsible for the failure of Kvβ3.1 to inactivate Kv1.5 channels.

Inactivating Activity of Kvβ3.1 Rescued by Swapping with Kvβ1.1 Domains—With chimeras Kvβδ1F - 1, we tried to rescue the inactivation failure of Kvβ3.1 by exchanging Kvβ3.1 domains with appropriate Kvβ1.1 domains (Fig. 1, B and C). Exchanging Kvβ3.1 residues 354–381 for those of Kvβ1.1 produced Kvβδ1F, which conferred to the slowly inactivating Kv1.5 currents a rapid inactivation (Fig. 1, A and D; Table I). By contrast, replacement of Kvβ3.1 residues 328–353 by those
of Kvβ1.1 (KvβchiI) had no significant effect on inactivation (Fig. 1, A and D; Table I). Supplementation of KvβchiF with an additional Kvβ1.1 sequence replacing Kvβ3.1 residues 278–309 (KvβchiH) did not markedly alter the activity of KvβchiF toward Kv1.5 channels. However, extending the replacement in KvβchiH by an additional 21 amino acids (KvβchiI) generated Kv1.5/KvβchiI currents with rapid and nearly complete inactivation (Fig. 1, A and D; Table I). At the end of the 1-s test pulse to +80 mV the current was reduced to ~6% of the initial maximum current amplitude similar to Kv1.5/Kvβ1.1 currents (Fig. 1D). The data demonstrated that the exchange of two C-terminal Kvβ3.1 domains (domains I and II in Fig. 1C) by those of Kvβ1.1 sufficed to rescue the inactivation failure of Kvβ3.1 in Xenopus oocytes. Kvβ domains I and II provide amino acid residues to the Kvβ oxidoreductase active site. Domain I contributes to the Kvβ NADPH cofactor binding site (Ser-325, Gln-329, Glu-332, and Asn-333 in Kvβ2.1; Ref. 1). Residues in domain II (Kvβ1.1 amino acids 303–323; Fig. 1B and C; see also Fig. 4A) have been proposed to participate in substrate binding in Kvβ2 (1).

Voltage Dependence of Kv1.5 Current Activation Affected by Kvβ Chimeras—The voltage-gating properties of Kv1.5 channels were affected by the presence of different Kvβ1.1/Kvβ3.1 chimeras (Table I). This is typically observed upon assembly of Kvs with Kvβ subunits (3, 14, 19–21). Voltages of half-maximal current activation (V0.5 = +17 to +22 mV) and slope...
The decay phase of outward currents during a 1-s pulse to +80 mV was fit by a double-exponential function, which yielded \( \tau_1 \), \( \tau_2 \), and the percentage of the total decay accounted for by \( \tau_1 \). The amount of inactivation was accessed by the fractional current remaining after 1 s (\( I_{1s}/I_{peak} \)). \( V_{0.5} \) values and respective slope factors (\( k \)) for steady-state activation were obtained as described under "Experimental Procedures." Numbers of oocytes (\( n \)) are given for both kinetic analysis and voltage dependence of activation. In all cases 25 ng of Kv1.5 and Kv\( \beta \) cRNA was injected per oocyte.

| Subunits | \( \tau_1 \) | \% total decay | \( \tau_2 \) | \( I_{1s}/I_{peak} \) | \( n \) | \( V_{0.5} \) | \( k \) | \( n \) |
|----------|-------------|----------------|-------------|----------------|--------|------------|--------|--------|
| Kv1.5 + Kv\( \beta \)3.1 | 306 ± 55 | 39 ± 5 | 1341 ± 44 | 84 ± 2 | 11 | 21.6 ± 2.7 | 18.9 ± 1.3 | 11 |
| Kv1.5 + Kv\( \beta \)A | 22 ± 1* | 90 ± 4 | 45 ± 6* | 7 ± 1 | 17 | 17.4 ± 2.0 | 19.7 ± 0.6 | 17 |
| Kv1.5 + Kv\( \beta \)A3.1 | 260 ± 30 | 18 ± 2 | 1063 ± 40* | 80 ± 1 | 9 | 17.9 ± 0.9 | 17.6 ± 0.1 | 9 |
| Kv1.5 + Kv\( \beta \)B | 14 ± 1* | 72 ± 11 | 386 ± 16* | 22 ± 2* | 19 | 36.3 ± 1.7 | 20.2 ± 0.5 | 25 |
| Kv1.5 + Kv\( \beta \)C | 24 ± 2* | 44 ± 7 | 362 ± 19* | 46 ± 3* | 18 | 38.1 ± 2.1 | 19.7 ± 0.5 | 11 |
| Kv1.5 + Kv\( \beta \)D | 23 ± 3* | 75 ± 16 | 647 ± 70* | 41 ± 3* | 20 | 20.1 ± 0.9 | 21.2 ± 0.7 | 9 |
| Kv1.5 + Kv\( \beta \)E | 210 ± 49 | 37 ± 6 | 590 ± 100* | 81 ± 3 | 20 | 17.1 ± 1.2 | 24.1 ± 0.8 | 20 |
| Kv1.5 + Kv\( \beta \)F | 17 ± 1* | 22 ± 7 | 644 ± 20* | 53 ± 4* | 21 | 27.4 ± 1.4 | 27.3 ± 0.8 | 10 |
| Kv1.5 + Kv\( \beta \)G | 398 ± 31 | 46 ± 7 | 1346 ± 90 | 77 ± 1 | 34 | 17.1 ± 1.1 | 19.8 ± 0.7 | 13 |
| Kv1.5 + Kv\( \beta \)H | 25 ± 4* | 40 ± 6 | 667 ± 29* | 52 ± 3* | 10 | 22.1 ± 1.6 | 27.2 ± 1.1 | 10 |
| Kv1.5 + Kv\( \beta \)I | 30 ± 4* | 67 ± 16 | 291 ± 27* | 61 ± 1* | 25 | 17.4 ± 1.1 | 18.0 ± 0.5 | 9 |
| Kv1.5 + Kv\( \beta \)I1 | 13.0 ± 0.9* | 90 ± 10 | 75.0 ± 0.5* | 13 ± 1° | 14 | 17.2 ± 1.5 | 21.9 ± 0.3 | 14 |

*Significantly different from Kv1.5 + Kv\( \beta \)3.1 (\( p < 0.002 \)).

**Fig. 2.** Functionality of Kv\( \beta \)3 subunits depends on the expression system. A, Kv1.5/Kv\( \beta \)chi currents recorded with the patch clamp technique in CHO cells. Kv\( \beta \)chi cDNA was microinjected at a 20-fold higher concentration than Kv1.5 cDNA. Note that all tested Kv\( \beta \) chimera conferred rapid inactivation to Kv1.5 channels. B, oocyte currents mediated by Kv1.5 and Kv1.5/Kv\( \beta \)3.1 channels. Oocytes were injected with 1 ng of Kv1.5 and 20 ng of Kv\( \beta \)3.1 cRNA (1:20 ratio). Despite the high expression level, Kv\( \beta \)3.1 subunits failed to rapidly inactivate Kv1.5 channels in the *Xenopus* oocyte expression system. Note, however, that the relative current amplitude (in %) remaining at the end of a 1-s pulse was smaller in the presence of Kv\( \beta \)3.1 as shown in C.

Factors (\( k = 19–27 \) mV) of the conductance-voltage relationships were similar for Kv1.5/Kv\( \beta \)1.1, Kv1.5/Kv\( \beta \)3.1, and Kv1.5/Kv\( \beta \)chi currents except for Kv1.5/Kv\( \beta \)chiB (\( V_{0.5} = +36 \) mV), Kv1.5/Kv\( \beta \)chiK (\( V_{0.5} = +38 \) mV), and Kv1.5/Kv\( \beta \)chiF currents (\( V_{0.5} = +27 \) mV) (Table I). Obviously, the distinct inactivation time courses of the various Kv1.5/Kv\( \beta \)chi currents at +80 mV, especially the rapid decay of Kv1.5/Kv\( \beta \)chi1 currents, are not due to an altered voltage-dependent steady-state activation.

**CHO Cells—**We considered the possibility that the structural differences at specific sites between Kv\( \beta \)1.1 and Kv\( \beta \)3.1 lead to improper protein folding and thereby to the observed functional inactivity of the Kv\( \beta \) chimeras. Because the wild-type Kv\( \beta \)3.1 is functionally active in CHO cells (12), we tested the activity of certain Kv\( \beta \) chimeras in the same expression system. We chose Kv\( \beta \)chiE, Kv\( \beta \)chiF, and Kv\( \beta \)chiG for these experiments because this series exhibited both gain of functional activity (from Kv\( \beta \)chiE to Kv\( \beta \)chiF) and loss of functional activity (from Kv\( \beta \)chiF to Kv\( \beta \)chiG) in *Xenopus* oocytes. When we coexpressed Kv1.5 channels with these chimeras in CHO cells, all
versus Kv were obtained as described under "Experimental Procedures." The values in brackets are from oocytes, which were injected with a 1:5 ratio of Kv1.5 Shaker domain (3) and thus may confer rapid N-type inactivation to decay accounted for by insufficient protein expression represented the most unlikely Kv mutation affected rapid inactivation behavior to a different 

importance of Kv activities in their oxidoreductase active sites, we mutated the putative catalytic residues in Kv1.1 (Asp-119, Tyr-124, and Lys-152), which are highly conserved in the superfamilies of oxidoreductase enzymes (1). We investigated the effect of the mutations on Kv1.1-mediated N-type inactivation. Coexpression of the mutants Kv1.1D119A, Kv1.1Y124F, and Kv1.1K152A with Kv1.5 at cRNA ratios of 1:1 (Fig. 3A) and 1:5 (Fig. 3C) showed that the respective outward currents did not decay as rapidly as Kv1.5/Kv1.1 currents (Table II). Each mutation affected rapid inactivation behavior to a different degree. The most marked effect was observed with Kv1.1D119A, which did not confer rapid inactivation to Kv1.5 channels. However, 15-s test pulses revealed that Kv1.1D119A did accelerate the slow inactivation of Kv1.5 currents (Fig. 3, B and D), which most likely represents a C-type inactivation (23, 24). As N-type and C-type inactivation are coupled (25), the observed acceleration of Kv1.5 inactivation was probably due to some residual inactivating activity of the Kv1.1D119A subunit.

**DISCUSSION**

It has been shown that rapid N-type inactivation of Shaker Kv channels requires the presence of an N-terminal inactivating domain and of a receptor close to the inner entrance of the Shaker channel pore (4). Upon depolarization the inactivating domain rapidly binds to the receptor and thereby occludes the pore. Kvβ3.1, like Kvβ1.1, contains a functional inactivating domain (3) and thus may confer rapid N-type inactivation to Shaker type channels. However, the inactivating activity of Kvβ3.1 depends on the in vitro expression system; in CHO cells Kvβ3.1 confers rapid inactivation to Kv1.5 channels (12) but fails to do so in Xenopus oocytes (3, 13). Our results demonstrate that the lack of function of Kvβ3.1 in Xenopus oocytes was correlated with two C-terminal Kvβ3.1 domains encompassing the NADPH and substrate binding sites, respectively, of the Kvβ oxidoreductase active site. Domain I in Fig. 1C and Fig. 4 contributes to the Kvβ NADPH cofactor binding site (Ser-325, Gln-329, Glu-332, and Asn-333 in Kvβ2.1; Ref. 1). Seven of the eight domain I residues that differ between Kvβ1.1 and Kvβ3.1 (Fig. 4A) are near or at the Kvβ adenosine-binding pocket (Fig. 4, B and C). Residues in domain II (Kvβ1.1 amino acids 303–323; Fig. 4A) have been proposed to participate in substrate binding, in particular Kvβ1.1 residue Trp-306 that corresponds to Trp-272 in Kvβ2 (1). When domains I and II in Kvβ3.1 were replaced by those in Kvβ1.1, the resulting Kvβ3.1/Kvβ1.1 chimeras were able to confer rapid inactivation to Kv1.5 channels in the Xenopus oocyte expression system. The results indicated that the functional activity of Kvβ3.1 in Xenopus oocyte could be rescued by replacing Kvβ3.1 amino acid residues in the oxidoreductase site by the ones of Kvβ1.1. In agreement with the assumption that C-terminal Kvβ domain(s) are responsible for the observed lack of function, Kvβ1.1 was rendered non-functional when the C-terminal half of the protein was replaced by Kvβ3.1 sequences. Three main alternatives may be considered to understand the results: i) Kvβ3.1 is not active because the oxidoreductase site is not properly folded; ii) Kvβ3.1 activity is inhibited in Xenopus oocytes by an as yet unknown factor; and iii) Kvβ3.1 is not active because the Xenopus oocytes do not provide a substrate for the Kvβ3.1 oxidoreductase. The results showed that Kvβ3.1 and Kvβ chimeras are functionally active in CHO cells. This demonstrates that active and properly folded Kvβ3.1 protein can be expressed in in vitro expression systems. Most likely, translation and folding of Kvβ3.1 protein in Xenopus oocytes is not different from that in CHO cells. Therefore, it is unlikely that Kvβ3.1 (and the Kvβ chimeras) is not properly folded when expressed in Xenopus oocytes. The existence of a putative inhibitory factor in Xenopus oocytes, which is specific for Kvβ3.1, cannot be rigorously excluded but seems to be also unlikely. Thus, we assume that Kvβ3.1 fails to confer rapid inactivation to Kv1.5 channels because its oxidoreductase activity is not functioning in Xenopus oocytes. In agreement with this assumption we find that a replacement of the NADPH and the putative substrate binding domains by those of Kvβ1.1...
reconstitutes the inactivating activity of Kvβ3.1 in Xenopus oocytes.

Because Kvβ3.1 oxidoreductase activity is apparently important for conferring rapid inactivation to Kv1.5 channels, we explored the possibility that mutations of catalytic residues in Kvβ1.1 (Asp-119, Tyr-124, Lys-152) may attenuate the Kvβ1.1 inactivating activity. The catalytic residues are highly conserved among the active sites of oxidoreductases (1), and comparable mutations in established oxidoreductase enzymes have been shown to impair catalytic activity (26, 27). The results showed that the mutations severely affected the ability of Kvβ1.1 to confer rapid inactivation to Kv1.5 channels. Although the putative Kvβ enzymatic activity could not be tested directly, it is likely that the mutations of catalytic residues in Kvβ1.1 affected its putative oxidoreductase activity. We propose that Kvβ oxidoreductase catalytic activity is required for the inactivating activity of Kvβ1.1. Thus, manipulations of the putative Kvβ1.1 and Kvβ3.1 oxidoreductase active sites were correlated with a loss and, respectively, gain of inactivating activity in the Xenopus oocyte expression system.

Although we have not carried out biochemical experiments to test directly the binding of Kvβ3.1 to Kv1.5, the effects of Kvβ3.1 and the different Kvβ1.1/Kvβ3.1 chimeras on the voltage-gating properties of Kv1.5 are a clear indication that Kvβ3.1 binds to Kv1.5. Changes in the voltage-gating properties of Kv1 channels are typically observed upon assembly of Kvo with Kvβ subunits (3, 14, 19–21). In conclusion, Kvβ3.1 assembles with Kv1.5 channels, but the activity of the N-terminal Kvβ3.1 inactivating domain is impaired. Apparently, the effects of Kvβ3.1 on the voltage-dependent activation of Kv1.5 channels are distinct from those leading to rapid inactivation. This is in agreement with the previous observation that removal of 10 amino acids from the Kvβ1.3 N terminus eliminated the inactivation activity but not the voltage shift of activation of Kv1.5 channels (28).

Kvβ2 subunits do not have an N-terminal inactivating domain. When coexpressed with Kvo subunits, Kvβ2 may also alter the voltage-gating properties of Kv channels and, in addition, enhance trafficking of Kv channels to the plasma membrane. In agreement with our results, it has been shown in a recent report (29) that mutating active site residues in Kvβ2 did not interfere with its binding to Kv1.4 channels. These Kvβ2 mutants still affected the voltage-gating properties of Kv1.4 channels like wild-type Kvβ2, but the enhancing effects on Kv1.4 channel surface expression were attenuated. Apparently, the putative Kvβ oxidoreductase activity is important for distinct aspects of Kvβ function.

Previously, we have shown that N-type inactivation can be prevented by a NIP-domain in Kv1.6 subunits (15). Now, we show that N-type inactivation of Kv channels may be coupled to the putative Kvβ1.1 and Kvβ3.1 oxidoreductase activity. This observation indicates that the gating mode of Kv channels linked to N-type inactivation may be regulated by a variety of cellular mechanisms. We propose that the presence of an oxidoreductase activity in Kv channels may couple cellular redox regulation to the gating mode of Kv channels allowing the channels to switch between a rapidly inactivating and a non-inactivating mode. Identifying the Kvβ oxidoreductase substrate(s) will bring us closer to understanding the cellular function of such potential energetic coupling.

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Coupling of Voltage-dependent Potassium Channel Inactivation and Oxidoreductase Active Site of Kv β Subunits
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