Comparison of Binding and Block Produced by Alternatively Spliced Kvβ1 Subunits*

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Voltage-gated K⁺ (Kv) channels consist of α subunits complexed with cytoplasmic Kvβ subunits. Kvβ1 subunits enhance the inactivation of currents expressed by the Kv1 α subunit subfamily. Binding has been demonstrated between the C terminus of Kvβ1.1 and a conserved segment of the N terminus of Kv1.4, Kv1.5, and Shaker α subunits. Here we have examined the interaction and functional properties of two alternatively spliced human Kvβ subunits, 1.2 and 1.3, with Kvα subunits 1.1, 1.2, 1.4, and 1.5. In the yeast two-hybrid assay, we found that both Kvβ subunits interact specifically through their conserved C-terminal domains with the N termini of each Kvα subunit. In functional experiments, we found differences in modulation of Kv1α subunit currents that we attribute to the unique N-terminal domains of the two Kvβ subunits. Both Kvβ subunits act as open channel blockers at physiological membrane potentials, but hKvβ1.2 is a more potent blocker than hKvβ1.3 of Kv1.1, Kv1.2, Kv1.4, and Kv1.5. Moreover, hKvβ1.2 is sensitive to redox conditions, whereas hKvβ1.3 is not. We suggest that different Kvβ subunits extend the range over which distinct Kvα subunits are modulated and may provide a variable mechanism for adjusting K⁺ currents in response to alterations in cellular conditions.

The electrical properties of excitable cells such as neurons and cardiomyocytes are strongly influenced by the K⁺ currents they express. A variety of voltage-gated K⁺ (Kv) channels control the falling phase of the action potentials of excitable cells. Kv channels are also important in many nonexcitable cells, where they contribute to diverse processes such as volume regulation, hormone secretion, and activation of mitogenes (1). Functional Kv channels assemble as tetramers of pore-forming α subunits (Kvα). Many mammalian Kvα genes have been cloned and assigned to four subclasses based on sequence similarities: Kv1, Kv2, Kv3, and Kv4 (2). In heterologous expression systems, individual Kvα subunits confer characteristic properties of gating, selectivity, and ion conduction, but several lines of evidence suggest that native Kv channels are more complex. First, within subfamilies, Kvα subunits are able to form functionally distinct heterotetramers, which contribute to increased K⁺ channel diversity (3–5). Second, accessory or Kvβ subunits that modify the gating properties of coexpressed Kvα subunits have been cloned (6–12) and found to be associated with Kvα subunits in native membranes (6, 13).

To date, five distinct mammalian Kvβ subunits have been cloned: Kvβ1.1, Kvβ1.2, Kvβ1.3, Kvβ2, and Kvβ3, according to recently proposed terminology (11, 12). Kvβ subunits have been shown to alter the phenotype of a subset of Kvα subunit currents within the Kv1 subfamily (7–10, 12, 14) primarily by introducing inactivation into noninactivating delayed rectifier channels (i.e. Kv1.1 and Kv1.5) and accelerating the intrinsic inactivation of rapidly inactivating channels (i.e. Kv1.4). The Kvβ N terminus is thought to act as a “ball peptide” to mimic the N-type inactivation characteristic of Shaker and Kv1.4 (7). There are differences between Kvβ-induced and N-type inactivation, however. Whereas N-type inactivation is complete, Kvβ-induced inactivation is generally partial with significant sustained currents remaining at the end of the pulse.

Recent biochemical studies have shown that Kvβ1.1 interacts with the N-terminal domains of the Kv1 subfamily α subunits, Kv1.4, Kv1.5, and Shaker, but not Kv2, Kv3, or Kv4 (15, 16). A recently proposed model of Kvα-Kvβ interactions suggests that the inactivation conferred on Kv1 currents by Kvβ subunits is the result of two sequential interactions: 1) physical association of the two subunits through the interaction of the conserved Kvβ1 C terminus with conserved regions in the Kv1α N terminus, and 2) plugging of the Kv1α pore by functional interaction of the Kvβ1 N terminus with its corresponding receptor site on the Kv1α subunit (15, 16). One resulting hypothesis is that all Kv1α subunits would interact with all Kvβ1 subunits but that the functional effects on a particular Kvα might differ depending upon which Kvβ1 N terminus is present.

To test this hypothesis, we have studied the interaction of two human Kvβ1 subunits with a variety of Kv channels. hKvβ1.2 and hKvβ1.3 share identical C-terminal domains but have unique, nonhomologous N termini. Our results indicate that both Kvβ1 subunits interact selectively with Kv1α subunits and produce open channel block, but hKvβ1.2 is much more potent than hKvβ1.3. In addition, hKvβ1.2 is sensitive to redox potentials, while hKvβ1.3 is not. The results are consistent with our hypothesis that functional differences originate with distinct Kvβ1 N-terminal domains.

EXPERIMENTAL PROCEDURES

Cloning of hKvβ1.3 by 5’-RACE and Reverse Transcription-PCR—5’-RACE Ready human heart cDNA (Clontech) was used as a template in a nested PCR with antisense oligonucleotides encoding portions of the Kvβ1 subunit C terminus near the N-terminal junction point in combination with the sense oligonucleotide anchor primer supplied with the 5’-RACE Ready cDNA. The first PCR consisted of the β subunit-specific
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Protein-protein interactions were tested in two host yeast strains, SFY526 and SFY527, transformed with cotransformants. The product was used as template in the second reaction with an internal β subunit-specific antisense oligonucleotide (R6): 5'-TCCAAATGTCAC-CCATGTTCC-3' and the anchor primer oligonucleotide. Without purification, the products from the second PCR were cloned into the pCRII vector (Invitrogen). Resulting clones were sequenced with the Sequenase kit (U.S. Biochemical Corp.), and compared to hKvβ1.2, Kvβ1.1, Kvβ1.3 and the Drosophila Kvβ, hK. This protocol generated several overlapping clones, the longest of which resulted in an open reading frame of 90 amino acids (hKvβ1.3). This clone was identical to hKvβ1.2 in the region between oligonucleotide R6 and the arginine (R) residue marking the junction point between the N and C termini. Since this partial clone did not possess a putative initiating methionine residue, another 5' antisense transcript (90 bases for hKv1.4) was performed to obtain the full N-terminal coding region. The second 5'-RACE reaction was done with the antisense β3.5'-RACE oligonucleotide 5'-TGAGGGACTAAGGCTGCTGTC-3' in combination with the anchor primer, and generated a clone that contained a putative initiating methionine by virtue of two upstream in-frame stop codons.

To obtain the full-length hKvβ1.3 sequence from human heart, we performed in vitro transcription-PCR with human atrial total RNA using oligonucleotides spanning the proposed initiating methionine of hKvβ1.3 and the C-terminal residues plus stop codon of hKvβ1.2. A single band of approximately 1.3 kilobase pairs was obtained. Sequencing of full-length hKvβ1.3 revealed a unique N terminus identical to what had been obtained from the 5'-RACE protocol and a C terminus identical to hKvβ1.2.

RNAase Protection—Fragments encoding the unique N-terminal 91 amino acids of hKvβ1.3 and 79 amino acids of hKvβ1.2 were prepared by PCR and cloned into pCRII (Invitrogen) to be used as the template to prepare β subunit-specific probes for RNAase protection assays. The fragments were sequenced to confirm that no mutations were introduced by PCR, and to determine the orientation in the pCRII vector. Plasmid DNA was purified on QiaGen Midi-prep plasmid purification columns and linearized with HindIII. 32P-Labeled antisense transcripts were prepared from linearized template with T7 RNA polymerase using the MAXiScript transcription kit (Ambion) according to the manufacturer's protocol. Full-length antisense transcripts (385 bases for hKvβ1.3; 420 bases for hKvβ1.2) were gel-purified on a denaturing 5% polyacrylamide gel containing 8% urea and eluted from the gel slices by overnight incubation at 37 °C in the following buffer: 0.5 mM ammonium acetate, 1 mM EDTA, 0.2% SDS.

Total RNA from human atrial appendage tissue, right atrium, right ventricle, and left ventricle was isolated using RNA-STAT-60 (Tel-Test). The ventricular and right atrial samples were obtained in accordance with Tulane University School of Medicine Institutional guidelines and the anchor primer oligonucleotide. Without purification, the products from the second PCR were cloned into the pCRII vector (Invitrogen) to be used as the template to prepare β subunit-specific probes for RNAase protection assays. The fragments were sequenced to confirm that no mutations were introduced by PCR, and to determine the orientation in the pCRII vector. Plasmid DNA was purified on QiaGen Midi-prep plasmid purification columns and linearized with HindIII. 32P-Labeled antisense transcripts were prepared from linearized template with T7 RNA polymerase using the MAXiScript transcription kit (Ambion) according to the manufacturer's protocol. Full-length antisense transcripts (385 bases for hKvβ1.3; 420 bases for hKvβ1.2) were gel-purified on a denaturing 5% polyacrylamide gel containing 8% urea and eluted from the gel slices by overnight incubation at 37 °C in the following buffer: 0.5 mM ammonium acetate, 1 mM EDTA, 0.2% SDS.

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Macropatch Recording—Macropatch currents were measured in cell-attached and inside-out configuration by using pipettes made from borosilicate glass with tip openings of ~10–15 μm. The electrodes were connected to a patch-clamp amplifier (Axopatch 1-C, Axon Instrument, Foster City, CA). The pClamp suite of programs was employed for data acquisition and analysis. The bath solution contained (in mM): NaCl, 100; KCl, 5; CaCl2, 0.3; MgCl2, 2; and HEPES, 10. pH was adjusted to 7.4 with Tris base. Records were digitized at 10 KHz and filtered at 3 KHz. Experiments were conducted at room temperature (20–22 °C).

Data Analysis—For whole-cell and macropatch recordings, the peak and steady-state currents were measured as the maximal current amplitude relative to the zero base-line level during a 100-ms pulse or as the current amplitude at the end of the pulse by Clampfit in pClamp. Single-channel data were analyzed with Ftest in pClamp, and open and closed transitions were detected using a half-amplitude threshold criterion. Group data are presented as mean ± S.E. Student's t tests (paired or unpaired) were used to evaluate the statistical significance of differences between means. A two-tailed probability of 0.5% was taken to indicate statistical significance. For analysis of current kinetics, data points were fitted by Clampfit in pClamp. For other curve-fitting procedures, a nonlinear curve-fitting technique
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**RESULTS**

Expression of hKv1.2 and hKv1.3 in Brain and Heart—The Kv1 subfamily of Kvβ subunits consists of three members, which result from alternative splicing of a single gene: Kv1.1 (7), Kv1.2 (8), and Kv1.3 (11). We originally cloned hKv1.2 from human atrium and found that it shared an identical C terminus to rat brain Kv1 (subsequently renamed Kv1.1). When this study was initiated, only two Kvβ subunits had been cloned: Kv1.1 and hKv1.2. Anticipating that there might be other members of this family, we searched for distinct Kvβ subunit family members with a common structural plan, i.e. a conserved C-terminal domain with a variable N terminus, by using 5’-RACE on human heart RNA. With this strategy we cloned hKv1.3 from human atrium, which proved to be identical to a recently published hKv1.3 clone from human ventricle (11). Each Kvβ subunit consists of an identical C-terminal domain of 329 amino acids spliced to a unique N-terminal domain. The nonhomologous N termini of the three Kvβ subunits are shown in Fig. 1.

We examined the expression of hKv1.3 in human atrium, ventricle, and brain with RNase protection assays. To distinguish the expression of hKv1.3 from other β subunits, a radio-labeled probe covering the unique N terminus of hKv1.3 was hybridized to total RNA from human atrium, right ventricle, left ventricle, and brain (Fig. 2, panel A). Compared to heart RNA, 5 times less brain total RNA was used in the assay. Specific protection of a 273-nucleotide (nt) band was observed in all tissues with the strongest signals coming from left ventricle and brain. In addition, a smaller band of approximately 250 nt was observed in all tissues. The source of this second band is unknown, but could represent a second isoform of hKv1.3 with sequence divergence at one or the other end of the N-terminal probe. Interestingly, the relative abundance of the 250-nt band increased in atrium such that the 273- and 250-nt bands were present in at least relatively equal proportions, whereas the 250-nt band appeared to be only a minor component in ventricle and brain. For comparison, the expression of hKv1.2 was also examined with an N-terminal hKv1.2 probe (Fig. 2, panel B). hKv1.2 was expressed in all tissues by virtue of the protected 237-nt band seen in all lanes. Expression was highest in ventricle and brain relative to atrium, however. These data are consistent with higher levels of hKv1.2 expression in human ventricle compared to atrium using Northern blotting (10).

hKv1.3 and hKv1.2 Bind to the N Terminus of Kv1 α Subunits—To determine whether there is any specificity in the interaction of hKv1.2 and hKv1.3 with members of the Kv1 subfamily, we used the yeast two-hybrid system to identify the Kv1 α subunits to which hKv1.2 and hKv1.3 bind as well as to map the domains that mediate this interaction. As seen in Fig. 3, hKv1.3 interacts with the N terminus of hKv1.4 (aa 1–305) but not the C terminus (aa 562–654), as evidenced by the growth pattern on media lacking histidine. hKv1.3 also interacts with the truncated Kv1.4 N terminus, Kv1.4-N2–146, indicating the binding site is within the 159 amino acids immediately preceding the putative S1 transmembrane domain. β-Galactosidase filter assays were also performed on these cotransformants and were positive for the expression of the lacZ reporter gene in those that grew on media lacking histidine (Table I). These experiments, yeast fusion vectors were cotransformed into the SY526 yeast strain, and the activation of the lacZ reporter gene was monitored. Table I shows that hKv1.3 interacts with the N-terminal sequences of not only Kv1.4, but also Kv1.5 (aa 1–248), Kv1.1 (aa 1–168), and Kv1.2 (aa 1–124). Thus, hKv1.3 is able to bind to all four Kv1 α subunits. hKv1.2 exhibits the same binding properties. As controls, we observed no interaction with either Kvβ subunit and the N terminus of the unrelated K+ channels, HERG (aa 1–396) and hIRK (aa 1–86).

Table I also shows that it is the conserved C-terminal core of the Kvβ subunits which mediates their binding to the Kv1α subunit N terminus. Yeast fusion proteins were constructed with the hKv1.2 N terminus (aa 1–79), hKv1.3 N terminus (aa 1–91), and the conserved Kvβ C-terminal core region of 329 residues and tested for interaction with the N termini of Kv1.4 and Kv1.5. Only the Kvβ C-terminal core region interacts with the Kv1α N terminus.

Relative Selectivity and Sensitivity of K+ Channels to Kvβ
**Subunit Modulation**—Since both Kvα subunits are able to interact with each of the Kv1α N-terminal domains tested, we performed a detailed characterization of the functional effects of hKv1.3 and hKv1.2 by coexpression in *Xenopus* oocytes with a variety of Kvα subunits: Kv1.1, Kv1.2, Kv1.4, Kv1.5, Kv2.1, and Kv3.1. In order to compare different combinations of Kvα and β subunits, the amounts of α subunit cRNAs injected were controlled to yield steady-state currents of 4–15 μA at a potential of +60 mV when Kvβ subunits were absent. Kvβ subunits were coinjected with Kvα subunits at concentrations resulting in maximal Kvβ effects.

When coinjected with hKv1.2, both β subunits introduced rapid but partial inactivation in the currents (Fig. 4A). In the presence of hKv1.2, hKv1.2 peak whole-cell currents are reduced and inactivation is introduced at potentials above 0 mV. By contrast, at saturating concentrations of hKv1.3, there is a smaller reduction in peak currents and the amount of inactivation, which is only apparent at very positive potentials (+60 mV), is less pronounced. hKv1.2 is a more potent modulator of Kv1.2 currents. This is further emphasized in Fig. 4B, in which I-V curves for steady-state Kv1.2 currents, plus and minus Kvβ subunits, are shown. Clearly, hKv1.2 reduces Kv1.2 currents over a much larger potential range than hKv1.3.

The effects of hKv1.2 and hKv1.3 on other Kv1 channels are presented in Fig. 4C. For each Kvα-Kvβ subunit combination, the percent (%) block, defined as the difference between the peak current and the steady-state current remaining at the end of a pulse to +60 mV, is indicated. For each Kv1 channel examined (hKv1.1, hKv1.2, hKv1.4ΔN2–146, and hKv1.5), hKv1.2 produced significantly more block compared to hKv1.3. In the most dramatic example, hKv1.2 elicited an approximately 6-fold greater block of hKv1.2 than hKv1.3 did (60.7 ± 1.2% (n = 9) versus 9.8 ± 1.9% (n = 14), p < 0.01). hKv1.2 introduced less inactivation into Kv1.5 (52.0 ± 3.7%; n = 7), Kv1.4ΔN (48.2 ± 1.3%; n = 7), and Kv1.1 currents (31.3 ± 4.5%; n = 6). Interestingly, hKv1.3 produced the most marked effects on Kv1.4ΔN (% block = 32.0 ± 2.9; n = 10) and Kv1.5 (30.0 ± 4.5%; n = 7). More modest inactivation was introduced into hKv1.1 (10.6 ± 2.2%; n = 4) and hKv1.2 (see above).

For Kv1.4, which exhibits intrinsic rapid inactivation, both Kvβ subunits accelerated the inactivation time course of the currents (data not shown). When intrinsic fast inactivation was removed by deletion of 146 amino acids from the N terminus (hKv1.4ΔN2–146), the channel expressed noninactivating currents that are also sensitive to Kvβ subunits.

![Fig. 4. Functional effects of hKvβ1.2 and hKvβ1.3 on coexpressed Kv1α currents in Xenopus oocytes.](image)

**Mechanism of hKvβ1.2 and hKvβ1.3 Modulation of hKv1.2 Currents: Single-channel Properties**—To probe the mechanism by which hKv1.2 and hKv1.3 introduce inactivation and reduce peak whole-cell currents, we examined the single-channel properties of hKv1.2 with and without coexpressed Kvβ subunits. Kv1.2 currents were chosen for analysis, since the difference in the magnitude of the effects of hKv1.2 and hKv1.3 was greatest for this channel.

Kv1.2 single channels recorded under different conditions from the same patches at a test potential of +70 mV, where both Kvβ subunits introduce inactivation, are presented in Fig. 5. Unitary currents from hKv1.2 in the cell-attached configuration (panel A, left) activated rapidly (see rising phase of ensemble average) and remained in the open state with tran-
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FIG. 5. Single-channel recordings from hKv1.2 (A) and hKv1.2 coexpressed with hKvβ1.2 (B) or hKvβ1.3. Representative records of unitary currents produced by 100-ms voltage steps to +70 mV from a holding potential of ~80 mV. Channel openings are displayed as upward deflections, and dashed lines indicate zero current levels. Ensemble averages are displayed below each set of traces and the numbers refer to the number of records averaged. The panels on the left show traces recorded in the cell-attached configuration and on the right, single channels from the same patch excised in the inside-out configuration.

sient, brief closures. In the presence of either hKvβ1.2 (panel B, left) or hKvβ1.3 (panel C, left), Kv1.2 channels open transiently at the onset of the voltage step and reopen infrequently during the test pulse. Just as with whole-cell currents, the effects of hKvβ1.2 are more pronounced than hKvβ1.3 as seen in the respective ensemble average currents.

After recording in the cell-attached mode, the same patch was excised in the inside-out configuration. For Kv1.2 alone, there was no apparent change in the appearance of either the single channels or the ensemble currents. Single Kv1.2 channels coexpressed with hKvβ1.2, however, consistently showed longer, flickery openings, more characteristic of Kv1.2 alone, upon excision (Fig. 5B). By contrast, excision of membrane patches containing Kv1.2 coexpressed with hKvβ1.3 (Fig. 5C, left panel) did not change either the single-channel phenotype or the ensemble current properties. This observation may reflect different responses of the Kvβ subunits to the redox state of the membrane and was reexamined with macropatch recordings as shown later.

Single-channel analysis is presented in Fig. 6. Panel A shows that the unitary conductance of Kv1.2 (8.9 ± 0.9 pS, n = 4) was not significantly reduced upon coexpression with either hKvβ1.2 (7.1 ± 0.7 pS; n = 4) or hKvβ1.3 (8.4 ± 0.8 pS; n = 5).

We evaluated the block conferred by hKvβ1.2 and hKvβ1.3 at both +70 mV and at ~30 mV, a more physiological potential. At +70 mV, both Kvβ subunits significantly reduced the mean open probability (Po) of Kv1.2 channels (Fig. 6B). hKvβ1.2 decreased the Po about 6-fold from 65.3 ± 10.3% (n = 3) to 11.2 ± 1.8% (n = 3; p < 0.05), while hKvβ1.3 reduced the Po to 26.5 ± 11.2% (n = 4; p < 0.05). This was the result of a dramatic shortening of the burst duration (Fig. 6C) and open time (Fig. 6D) along with an increase in the long-lived closed state (Fig. 6E, right). The open time of Kv1.2 channels (4.10 ± 0.30 ms; n = 3) was significantly reduced by coexpression with both hKvβ1.2 (1.01 ± 0.06 ms; n = 3; p < 0.05) and hKvβ1.3 (1.80 ± 0.09 ms; n = 4; p < 0.05). The closed time distribution was best fitted with two exponentials: for Kv1.2, a brief one of 0.44 ± 0.09 ms (n = 3) (closed time 1; Fig. 6E, left panel) and a longer one of 3.87 ± 1.27 ms (n = 3) (closed time 2; Fig. 6E, right panel). Closed time 2 was lengthened significantly in the presence of hKvβ1.2 (11.83 ± 1.83 ms; p < 0.05) and hKvβ1.3 (7.95 ± 1.01 ms; p < 0.05) contributing to the observed decrease in Po with the Kvβ subunits, while closed time 1 was unaffected.

At ~30 mV, the mean Po burst duration, open time, and closed time 2 of Kv1.2 were all significantly altered in the presence of hKvβ1.2 even though inactivation was not apparent at the macroscopic level. The mean Po was reduced about 4-fold from 38.8 ± 11.7% (n = 3) with Kv1.2 alone to 12.6 ± 1.1% (n = 3; p < 0.05) when coexpressed with hKvβ1.2. The burst duration of Kv1.2 was also reduced approximately four-fold in the presence of hKvβ1.2. hKvβ1.2 decreased the mean open time of Kv1.2 from 4.78 ± 0.75 ms to 0.87 ± 0.07. Just as at +70 mV, closed time 1 was not altered by hKvβ1.2 at ~30 mV. hKvβ1.3 did not change either the single-channel phenotype or the ensemble current properties.
mV while the longer closed time 2 was significantly increased (2.90 ± 0.15 ms for Kv1.2 (n = 3) versus 8.27 ± 0.07 ms for Kv1.2 plus hKvβ1.2 (n = 3)). Thus, even at a potential at which no inactivation is apparent, hKvβ1.2 still blocked Kv1.2. hKvβ1.3 had similar effects; the mean open time was significantly decreased (1.86 ± 0.29 ms; n = 4; p < 0.05), and closed time 2 significantly increased (6.30 ± 0.64 ms; n = 4; p < 0.05). hKvβ1.3 effects were less dramatic than hKvβ1.2, the current amplification introduced was less with hKvβ1.3 than with hKvβ1.2. Note that the inactivation induced by hKvβ1.2 was eliminated in inside-out patches or after perfusion with H2O2. Similar results were obtained from another six cells tested. Perfusion with GSH (5 mM) restored inactivation within 5–15 min. The vertical calibration is 200 pA. B, hKv1.2 + hKvβ1.2. No changes in macropatch currents were found either in the inside-out patches or with application of H2O2. Similar results were obtained from another five cells tested. The vertical calibration is 2500 pA.

An important finding is that with removal of inactivation in Kv1.2 currents coexpressed with hKvβ1.2, the current amplitude markedly increased under oxidizing conditions. The difference in current amplitudes between oxidizing and reducing conditions in the same patch approximates the amount of Kv1.2 current blocked by hKvβ1.2. The ratio of currents in oxidizing versus reducing conditions measured at the end of the pulse (5.2 ± 1.3 at −20 mV and 4.0 ± 0.5 at +60 mV (n = 4)) shows that Kv1.2 currents are reduced by 75–80% in the presence of hKvβ1.2 at a wide range of potentials. These values are consistent with the reduction in whole-cell current amplitudes observed upon coexpression of Kv1.2 with hKvβ1.2.

These experiments also indicate that the binding of the Kvβ to the α subunit is very stable. In some cases, excised patches in which Kvβ inactivation was stable (hKvβ1.3) or restored upon application of GSH (hKvβ1.2) were maintained for over 1 h without loss of Kvβ functional effects.

DISCUSSION

hKvβ1.2 and hKvβ1.3, both cloned from human heart, derive from alternative splicing of a single gene and contain identical C-terminal (329-amino acid) domains spliced to unique N-terminal (79 and 91 amino acids, respectively) regions (11, 14). By examining the interaction of hKvβ1.2 and hKvβ1.3 with Kv1α subunits both biochemically and electrophysiologically, we have been able to assess the roles of both the Kvβ C terminus and the variable N termini.

With the yeast two-hybrid system, we have shown that Kvβ subunits are able to interact with the N terminus of each Kv1 α subunit tested: Kv1.1, Kv1.2, Kv1.4, Kv1.5. This interaction is mediated by the C termini of the Kvβ subunits, as the individual N termini alone do not interact with the Kvα subunits. These results are consistent with recent reports, which also assign Kvβ binding to the N termini of Kv1 α subunits (15, 16), as well as with a recent report indicating association of Kvβ1.1 with all Kv1α subunits upon transient transfection in mammalian cells (19).

Both hKvβ1.2 and hKvβ1.3 accelerate or introduce partial inactivation into all Kv1 channels tested: Kv1.1, Kv1.2, Kv1.4, and Kv1.5. We had previously reported that hKvβ1.2 had no effect on Kv1.1 and Kv1.2 currents upon heterologous expression in Xenopus oocytes (8). The difference between these two studies is likely to be due to the levels of Kv1α currents that were expressed. In the first study whole-cell Kv1.1 and Kv1.2 peak currents at +80 mV reached upwards of 60 μA, whereas Kv1.4 and Kv1.5 currents were approximately 15–20 μA. Thus, it is unlikely that sufficient Kvβ subunits were coexpressed with Kv1.1 and Kv1.2 to produce an effect in the first study. In the experiments reported here, equivalent levels of whole-cell Kv1α currents (no greater than 15 μA at +80 mV) were recorded in the presence of saturating amounts of Kvβ subunits. When recordings were made under such conditions, hKvβ1.2 had the most dramatic effect on Kv1.2 currents.

hKvβ1.3 and hKvβ1.2 differ in the potency with which they block Kvα subunit currents. In all cases the amount of inactivation introduced was less with hKvβ1.3 than for hKvβ1.2. Since both Kvβ subunits have identical C-terminal domains,
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we attribute these differences to the actions of the unique N-terminal domains. We cannot exclude the possibility, however, that the translational efficiency of hKvβ1.3 cRNA is lower than hKvβ1.2 or that hKvβ1.3 protein is less stable than hKvβ1.2. All factors that result in lower local concentrations of hKvβ1.3 available for interaction with Kv1α subunits may contribute to weaker effects of hKvβ1.3 relative to hKvβ1.2.

In this report, we provide the first single-channel recordings of Kv1α currents in the presence of Kvβ subunits. These data clearly show how hKvβ1.2 and hKvβ1.3 produce only partial inactivation of nonactivating Kv1α subunit currents as seen with whole-cell and macropatch recordings. The sustained currents remaining at the end of the pulse are reflected in the reopening of single channels observed during the pulse period. Thus, complete inactivation is not achieved since channels are blocked by Kv1α depolarizations, which do not produce inactivating currents, the decrease in macroscopic currents. Thus, even with weak geting of channel proteins to the membrane or 2) Kv1α subunits that are able to modulate Kv1α currents to differ-

The major effects of Kvβ subunits as assessed electrophysiologically in heterologous expression systems are to control S1-Kv1 current levels. It is intriguing that a number of different Kvβ subunits that are able to modulate Kv1 currents to different extents have evolved. Different cell types may modulate Kv1 currents in unique ways by assembling various combinations of Kv1α and Kvβ subunits. Clearly, a characterization of Kv1α-Kvβ interactions in native cells will be critical for determining the molecular composition of individual Kv currents and the physiological relevance of these interactions.

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