Auxiliary Kvβ subunits form complexes with Kv1 family voltage-gated K⁺ channels by binding to a part of the N terminus of channel polypeptide. This association influences expression and gating of these channels. Here we show that Kv4.3 proteins are associated with Kvβ2 subunits in the brain. Expression of Kvβ1 or Kvβ2 subunits does not affect Kv4.3 channel gating but increases current density and protein expression. The increase in Kv4.3 protein is larger at longer times after transfection, suggesting that Kvβ-associated channel proteins are more stable than those without the auxiliary subunits. This association between Kv4.3 and Kvβ subunits requires the C terminus but not the N terminus of the channel polypeptide. Thus, Kvβ subunits utilize diverse molecular interactions to stimulate the expression of Kv channels from different families.

Voltage-gated K⁺ channels are multimeric proteins that consist of four pore-forming α subunits in association with auxiliary subunits. Unlike α subunits that exhibit overall structural similarity to various voltage-gated ion channels, auxiliary subunits are diverse and show specificity for association with particular classes of K⁺ channels. Kvβ subunits belong to the NADPH-dependent oxidoreductase superfamily (1) and contain a conserved catalytic domain with a NADPH-binding site (2). These proteins by themselves form a tetramer (3), making an αβ₄ channel complex (4–6). There are at least four mammalian genes that encode Kvβ subunits. Previous studies have established that Kvβ1, -2, and -3 gene products are components of Kv1 family channels. Indeed, all products from the four genes contain a conserved core region with variable N-terminal peptides. The core region of β subunits was found to be sufficient for association with a part of the N terminus of Kv1 family α subunits that is highly conserved within this family (5, 6). However, more recent studies indicate that Kvβ subunits can interact with heterologously expressed Kv4.2 (7, 8) and EAG1 family (9) channels. Furthermore, it appeared that Kvβ4 subunits are present as complexes with Kv2.2 proteins in rat brain (10). Likewise, plant Kvβ subunits (KAB) are associated with KAT1 channels (11). Hence, auxiliary Kvβ subunits are structurally well characterized, yet the specificity and mechanism of interaction between Kvα and β subunits remain obscure.

Kvβ subunits influence expression and function of K⁺ channels. Specifically, distinct Kvβ subunits differentially affect heterologously expressed Kv1 family channels. A long-stretched N-terminal peptide in Kvβ1 and Kvβ3 gene products produces rapid inactivation on most of Kv1 family channels by a mechanism similar to the action of a ball peptide present at the N terminus of some channel α subunits (12). Furthermore, Kvβ2 subunits have been shown to increase stability and cell surface expression of Kv1 family channels (13) without producing rapid inactivation. Thus, expression of distinct Kvβ subunits controls excitability by differentially affecting the expression and gating of Kv1 family channels.

Although many studies have shown that Kvβ1 and Kvβ2 subunits can associate with heterologously expressed channels from diverse families, it remains unclear whether these auxiliary subunits are present as complexes with non-Kv1 family channels in native cells. Furthermore, structural features of the interaction between Kvβ subunits and non-Kv1 family channel polypeptides remain unknown. To address these questions, we examined complexes consisting of Kvβ and Kv4.3 channel subunits. We show here that Kv4.3 proteins are associated with Kvβ2 subunits in the brain and that this interaction requires the C terminus of the channel polypeptide.

**EXPERIMENTAL PROCEDURES**

**Constructions**—Rat Kv4.3 short (14) and long (15) isoform cDNAs were subcloned into pcDNA3 (Invitrogen, Carlsbad CA). Rat Kv2.1, β1.1, and β2.1 expression constructs were previously obtained (7). Chimeric constructs between Kv4.3 short splicing form and Kv2.1 were made using a two-step overlapped polymerase chain reaction with primers that corresponded to the border region of Kv4.3 and Kv2.1 sequences. Kv4.3-Kv2.1N contains amino acids 1–184 of rat Kv2.1 polypeptide (16) linked to amino acids 183–636 of rat Kv4.3 polypeptide (14). Kv4.3-Kv2.1C consists of amino acids 1–406 of the Kv4.3 polypeptide connected to amino acids 413–853 of the Kv2.1 polypeptide. For cosolubilization experiments, wild type and chimeric channel cDNAs were subcloned in-frame into pcDNA3.1/HisC vector (Invitrogen) using a polymerase chain reaction-based method. GFP-tagged Kvβ subunit constructs were prepared by subcloning the whole coding region of Kvβ1.1 and Kvβ2.1 cDNAs at the end of enhanced green fluorescent protein-coding sequence of EGFP-C1 (CLONTECH, Palo Alto CA). All of the obtained constructs were verified by DNA sequencing.

**Cell Culture and Transfection**—HEK 293 cells (American Type Culture Collection, Manassas, VA) were maintained at 37 °C under 5% CO₂ atmosphere in high glucose Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. Transient transfection was carried out by the calcium phosphate-DNA coprecipitation method (Transfection, Life Technologies, Inc.).

For patch clamp recording, pCMV-Kv4.3 alone (0.3 µg for 60-mm dish) or in combination with 5× excess pCMV-Kvβ1.1 or pCMV-Kvβ2.1 were used. In addition, EGFP-C1 plasmid (50 µg/60-mm dish) was cotransfected to aid in the identification of transfected cells. Transfected cells in 60-mm plates were split into 35-mm dishes 5 h after transfection and used for whole-cell recordings 48–72 h after...
transfection.

For immunoblot analysis, cells on 100-mm plates were transfected with expression constructs at the same ratio as for patch clamp recording (0.9 μg of pCMV-Kv4.3/dish). Transfected cells were divided into five 60-mm plates at various densities 5 h after transfection and used for immunoblot analysis at various days after transfection. Cell extracts were prepared by suspending the collected cell pellet in 100 μL of lysis buffer (20 mM Tris-HCl (pH 7.4), 0.15 M NaCl, 1 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride, and 1 mM EGTA). The suspension was kept on ice for 10 min and centrifuged at 10,000 g for 5 min to remove nuclear debris.

Electrophysiological Recording—Whole-cell voltage-clamp recording (17) was performed with an EPC-9 patch-clamp amplifier using the Pulse program (HEKA Electronik, Lambrecht, Germany) on a Power Macintosh computer. Patch pipettes were filled with a solution containing 140 mM KCl, 1 mM MgCl2, 1 mM EGTA, and 10 mM HEPES (pH 7.4). Bath solution contained 155 mM NaCl, 5 mM KCl, 2 mM MgCl2, 20 mM glucose, 10 mM HEPES (pH 7.4). Series resistance compensation was set at 70%. Peak currents were converted into conductance (G) by the formula G = I/(V_m - V_rev) assuming a reversal potential V_rev of −84 mV, where V_m is the membrane voltage of depolarization pulses. Using the first-order Boltzmann equation G_{G_{1/2}} = 1/[1 + \exp(V_m - V_{1/2})/\text{slope factor}], the half-maximal voltages (V_{1/2}) and the slope factors were acquired. Statistical analysis was carried out using the Mann Whitney two-tailed test. All the data in the text are presented as means ± S.E.

Biochemical Association Assays—Two days after transfection, transfected HEK 293 cells on 100-mm dishes were harvested with ice-cold phosphate-buffered saline. Triton extract was prepared by suspending the pelleted cells in 0.4 mL of solution containing 1% Triton X-100, 20 mM Tris-HCl (pH 7.9), 50 mM NaCl, and 5 mM imidazole. The extract was mixed with 100 μL (50% slurry) of preactivated His-bind resin (Novagen, Milwaukee WI) for 2 h with gentle shaking. The resin was washed 5× with the same solution, except that the imidazole concentration was 40 mM. The bound materials were then eluted with 0.1 M EDTA.

Immunoprecipitation—Immunoprecipitation was performed with polyclonal anti-panKvβ antibody (18) or monoclonal anti-Kv4.3 antibody. The latter antibody was generated against a synthetic peptide corresponding to a part of the N terminus of rat Kv4.3 polypeptide (amino acids 25–40) CPMAPLADKKNKRQDE. Whole rat brain tissue was homogenized in 0.32 M sucrose solution supplemented with 1 mM iodoacetamide, 0.2 mM phenylmethylsulfonyl fluoride, and 1 mM EGTA at a protein concentration of ~10 mg/mL.

Triton extract was then obtained by centrifugation of the suspension at 100,000 × g for 30 min. After preclearing with fixed protein A containing Staphylococcus aureus (Pansorbin, Calbiochem), Triton extract was incubated overnight with monoclonal anti-KV4.3 or polyclonal anti-Kvβ (20) antibody and Pansorbin. The bound materials were collected by centrifugation and washed 4× with the same Triton-containing solution. The bound materials were eluted by heating in 2× SDS sample buffer and subjected to immunoblot analysis.

Immunoblot Analysis—Proteins were separated on a 7.5% polyacrylamide gel and transferred to a nitrocellulose membrane. The membrane was coated with 5% nonfat dry milk in phosphate-buffered saline containing 0.1% Tween 20 and probed with primary antibody followed by incubation with horseradish peroxidase-conjugated secondary antibody. Primary antibodies against Kv4.3, GFP, and Express tag were purchased from Alomone labs (Jerusalem, Israel), MBL International Corp. (Watertown MA), and Invitrogen, respectively. Anti-Kv1.4 (18), anti-Kv2.1 (19), and polyclonal anti-panKvβ (20) antibodies were previously generated. Bound antibody was detected by chemiluminescence method (PerkinElmer Life Sciences). Immunoreactivity was quantified using densitometry of the developed films.

Confocal Microscopy—Confocal Images of GFP fluorescence were taken on a Molecular Dynamics 2001 scanning laser confocal microscope with a 60× oil immersion objective lens (1.4 NA) using 488-nm excitation and 510-nm emission filters with 3% maximal laser intensity. Cell surface localization was evaluated by comparing the location of fluorescence with bright field images of cells.

RESULTS

Kv4.3 Proteins Are Present in Association with Kvβ Subunits in the Brain—To test for the presence of Kv4.3-Kvβ subunit complexes, we first used anti-Kv4.3 monoclonal antibody for immunoprecipitation from rat brain extract. Anti-Kv4.3 antibody effectively and specifically precipitated its targeted channel proteins but not Kv1.4 proteins (Fig. 1A). Importantly, the immunoprecipitated material was found to contain significant immunoreactive Kvβ subunit proteins detected with polyclonal anti-panKvβ antibody (20). This antibody detects two bands on the blot with distinct sizes. The larger and smaller bands are known to correspond to Kvβ1 and Kvβ2, respectively (13, 19, 20). In addition, the larger band may also contain Kvβ3 subunits. We found that only smaller molecular weight Kvβ2 subunits were significant in the precipitated material. To further obtain evidence for the presence of Kv4.3-Kvβ complexes in the brain, anti-panKvβ antibody was used for immunoprecipitation from the brain extract (Fig. 1B). The antibody precipitated significant Kv4.3 proteins in addition to its targeted proteins. In contrast, no detectable Kvβ1 proteins were found in the precipitated material. Hence, brain Kv4.3 channel proteins are present in association with Kvβ2 subunits.

Fig. 1. Kv4.3 proteins are associated with Kvβ2 subunits in rat brain. Triton extract of whole rat brain was subjected to immunoprecipitation (IP) with monoclonal anti-KV4.3 (A) or polyclonal anti-panKvβ (B) antibody. Brain extract, unbound fraction (sup), and precipitated material (ppt) were examined by immunoblot (IB) analysis with anti-Kv1.4, anti-Kv2.1, anti-panKvβ, and polyclonal anti-Kv4.3 antibodies. The larger and smaller bands detected with anti-panKvβ antibody have been shown to correspond to Kvβ1/3 and Kvβ2 subunits, respectively (13, 19, 20). Note that a fraction of immunoreactive Kvβ subunits (Kvβ2) is recovered in the anti-Kv4.3 antibody-precipitated material. Anti-panKvβ antibody also precipitated a portion of Kv4.3 proteins.
**Kvβ Subunits Increase Kv4.3 Current Density and Proteins—** Functional consequences of Kvβ subunit association on Kv4.3 channels was examined in transfected HEK293 cells. We first examined the effect of Kvβ subunits on Kv4.3 current density. Coexpression of either Kvβ1.1 or Kvβ2.1 subunits led to a 2–3-fold increase in the peak current density (Fig. 2; at +50 mV, peak current density was 0.5 ± 0.05 nA/pF (n = 6) for Kv4.3 alone, 1.7 ± 0.4 nA/pF (n = 5) for Kv4.3 + Kvβ1.1, and 1.8 ± 0.5 nA/pF (n = 5) for Kv4.3 + Kvβ2.1). Increases in current density produced by Kvβ1.1 or Kvβ2.1 subunits were significant at test pulses higher than −20 mV (p < 0.05). No significant change in HEK293 cell endogenous current was detected; peak current density at +50 mV was 39.2 ± 8.7 pA/pF (n = 4) for mock transfection, 29.3 ± 9.9 pA/pF (n = 4) for Kvβ1.1, and 39.7 ± 7.1 pA/pF (n = 4) for Kvβ2.1. Thus, Kvβ subunits increase functional cell surface Kv4.3 channels.

To test if this elevation in current density was correlated with an increase in Kv4.3 protein level, we measured channel proteins by immunoblot analysis (Fig. 3A). The Kv4.3 protein level significantly increased when coexpressed with Kvβ2.1 subunits 3 or 4 days after transfection (Fig. 3B, n = 6, p < 0.05). In contrast, coexpression of these auxiliary subunits did not produce changes in Kv2.1 protein levels. Similar increases in Kv4.3 proteins were also produced by Kvβ1.1 at 3 days after transfection (Fig. 3C). Hence, Kvβ subunits increase total cellular Kv4.3 protein level.

**Effects of Kvβ Subunits on Kv4.3 Channel Gating—** Next we examined the gating properties of Kv4.3 channels with or without coexpression of Kvβ subunits. Excess Kvβ subunits (αβ = 1:5) were used to enhance formation of Kv4.3-Kvβ complexes. Kvβ subunits have been shown to shift voltage dependence of activation of Kv1 family channels to the left. However, we found that Kvβ1.1 or Kvβ2.1 subunits produce no significant change in voltage dependence of activation of Kv4.3 channels (Fig. 4A). The voltage for half-maximal activation and the slope factor were −7.00 ± 1.08 mV and 14.0 ± 0.99 mV (n = 5) for Kv4.3 alone, −7.83 ± 1.01 mV and 15.8 ± 0.96 mV (n = 4) for Kv4.3 + Kvβ1.1, and −8.43 ± 1.13 mV and 14.4 ± 1.05 mV (n = 4) for Kv4.3 + Kvβ2.1, respectively. Thus, Kvβ subunits do not influence activation of Kv4.3 channels.

The most profound effect produced by Kvβ subunits on Kv1 family channels is acceleration of inactivation, due to the ball and chain mechanism. Thus, the effect of Kvβ1.1, which contains a ball peptide, as well as Kvβ2.1 on inactivation properties of Kv4.3 channels was examined. Coexpression of these auxiliary subunits did not significantly affect time constant of inactivation (Fig. 4B); time constant for inactivation at +50 mV was 50.5 ± 5.1 ms for Kv4.3 alone (n = 5), 52.6 ± 4.3 ms (n = 4) for Kv4.3 + Kvβ1.1, and 57.2 ± 2.2 ms (n = 4) for Kv4.3 + Kvβ2.1. We also measured the steady-state inactivation using a test pulse to +40 mV after a conditioning prepulse at various voltages (Fig. 4C and D). The voltage for half-maximal inactivation and the slope factor were −48.4 ± 0.33 mV and −5.2 ± 0.3 mV (n = 5) for Kv4.3 alone, −41.5 ± 0.23 mV and −6.8 ± 0.2 mV (n = 4) for Kv4.3 + Kvβ1.1, and −47.2 ± 0.45 mV and −6.85 ± 0.4 mV (n = 4) for Kv4.3 + Kvβ2.1. Therefore, Kvβ subunits, regardless of the presence of a ball peptide, do not influence inactivation of Kv4.3 channels.

We also determined whether association with Kvβ1.1 or Kvβ2.1 might influence the recovery from inactivation (Fig. 4E). A protocol of two consecutive depolarizing test pulses interrupted by variable interpulse intervals at −70 mV was used to determine the time course of recovery from inactivation (Fig. 4E). The recovery from inactivation was fitted by a single exponential function. Time constant for recovery from inactivation was 181 ± 24 ms for Kv4.3 alone (n = 6), 169 ± 11 ms for Kv4.3 + Kvβ1.1 (n = 5), and 176 ± 38 ms for Kv4.3 + Kvβ2.1 (Fig. 4F, n = 5). Thus, Kvβ subunits do not affect recovery from inactivation. Taken together, Kvβ1.1 and Kvβ2.1 subunits produce no marked effects on Kv4.3 channel gating.

**The C Terminus of Kv4.3 Proteins Is Required for Association with Kvβ Subunits—** To assess association of Kv channel α subunits with Kvβ subunits, we first examined localization of GFP-tagged Kvβ subunits upon coexpression of various channel proteins. Confocal microscopy revealed that GFP-Kvβ1 (Fig. 5A) or GFP-Kvβ1.1 (data not shown) were predominantly present in the cytosol in the absence of channel α subunits. Coexpression of Kv4.2 or Kv4.3 proteins as well as Kv1.4 proteins, but not Kv2.1 proteins, localized the fluorescence to plasma membrane (Fig. 5A). Similarly, a splicing variant of Kv4.3, which contains a 19-amino acid insertion at the C terminus (15), targeted GFP-Kvβ fusion proteins to plasma membrane. Thus, Kv4 family channel proteins regardless of the presence or absence of the insertion can associate with Kvβ subunits.

Kv1 family channels interact with Kvβ subunits via a highly conserved region of the N terminus. Although the corresponding region of Kv4 family polypeptides exhibits significant sequence homology, this peptide itself was insufficient for association with Kvβ subunits (5, 6). To identify the region important for association, we generated chimeric channel proteins consisting of Kv4.3 and Kv2.1 polypeptides. If chimeric proteins are capable of interacting with Kvβ subunits, the fluorescence would be expected in plasma membrane or other membrane-associated compartments. Replacing the N terminus of Kv4.3 protein with that of Kv2.1 polypeptide (Kv4.3-Kv2.1N) did not affect the ability to localize Kvβ2.1 (Fig. 5B) and Kvβ1.1 (data not shown) subunits to plasma membrane and other membrane-associated regions. In contrast, substituting the C terminus of Kv4.3 protein with that of Kv2.1 protein (Kv4.3-Kv2.1C) eliminated plasma membrane localization of the fluorescence. This chimeric channel (Kv4.3-Kv2.1C) was functional as confirmed by patch clamp recording (data not shown). Thus, the C terminus, but not the N terminus, of Kv4.3 polypeptide is required for localizing Kvβ subunits at plasma membrane.

We also used protein biochemical assays to test association.
Histidine (His)-tagged wild type and chimeric channel proteins were expressed with Kvβ1 or Kvβ2 subunits. After purification with His-binding beads, copurified Kvβ subunit proteins were examined by immunoblot analysis (Fig. 6). Significantly higher levels of immunoreactive Kvβ1.1 or Kvβ2.1 proteins were recovered from cells coexpressed with Kv4.3 or Kv4.3-Kv2.1N than those with Kv2.1 or Kv4.3-Kv2.1C. These results demonstrate that the C terminus, but not the N terminus, of Kv4.3 channels is necessary for association with Kvβ subunits.

**DISCUSSION**

Kvβ subunits have been suggested to interact with various K⁺ channels including Kv2.2 (10), Kv4.2 (7, 8), and several EAG family (9) and plant KAT1 (11) channels in addition to Kv1 family channels. In particular, Kv4.2 channels were found to interact with Kvβ1 and -2 subunits in heterologous expression systems (7, 8), suggesting that the same Kvβ subunits might form complexes with K⁺ channels from the two different families. However, previous studies had not addressed whether such association actually occurs with native channels and did not identify a physiological effect of the association. In this study, we have shown that Kv4.3 proteins are associated with Kvβ2 subunits in the brain. We also found that coexpression of Kvβ subunits leads to increases in Kv4.3 current density and protein level without altering gating properties. Finally, this association requires the C terminus, but not the N terminus, of the channel polypeptide. Thus, the same Kvβ2 subunits influence expression and function of channels from the two different families by distinct interaction mechanisms.

Association of Kvβ subunits appeared to produce different effects on interacting channels. Our results indicate that the ball peptide of Kvβ1.1 does not alter inactivation of Kv4.3 channels. Similarly, it has been shown that Kvβ1 does not markedly influence inactivation kinetics of Kv4.1 (21), Kv4.2 (8) and Drosophila Shal (5) channels. Likewise, Drosophila Kvβ subunit HK was unable to produce rapid inactivation on
recent study revealed that sensitivity to O\textsubscript{2} tension differs between Kv\textsubscript{4.2} and Shaker channels (8). In analogy to the difference in rapid inactivation, this difference in hypoxia response may arise from distinct ability of channels to respond O\textsubscript{2} tension signals. Alternatively, specific interaction of Kv\textsubscript{4.2} channels with Kv\textbeta subunits may be essential for the regulation. Further structural and functional information of channel complexes may resolve these issues.

In contrast to specific alterations in channel gating, association of Kv\textbeta subunits commonly increases current amplitude or density of various channels. This has been observed with Kv\text{1 family (13), Kv\textsubscript{2.2} (10), and EAG family (9) channels. Our results also revealed that Kv\textbeta subunits increase Kv\textsubscript{4.3} current density and proteins. In addition to Kv\textbeta subunits, other channel auxiliary subunits for K\textsuperscript{+} channels as well as Na\textsuperscript{+} and Ca\textsuperscript{2+} channels have been shown to increase associating channel current density. It is assumed that the exit from endoplasmic reticulum is the rate-limiting step for plasma membrane protein targeting. Therefore, the generally observed increase in current density by various auxiliary subunits may be due to masking of endoplasmic reticulum retention signals present in channel proteins. This mechanism has been implicated for controlling selective cell surface expression of heteromeric ATP-sensitive K\textsuperscript{+} channel complexes (23, 24) and voltage-gated Ca\textsuperscript{2+} channel (25). Thus, it is possible that some of the Kv\textbeta subunit effect on Kv\textsubscript{4.3} channel current density and proteins may be due to masking potential endoplasmic reticulum retention signals in the channel polypeptide. However, our previous study found that GFP-tagged Kv\textsubscript{1.4} and Kv\textsubscript{1.5} channels are efficiently transported to plasma membrane in the absence of Kv\textbeta subunits (26, 27). Similarly, we found efficient plasma membrane localization of GFP-tagged Kv\textsubscript{4.3} (data not shown). Furthermore, coexpression of Kv\textbeta subunits produced no apparent changes in localization of these GFP-tagged channel proteins. Thus, it is likely that Kv\textbeta subunits increase Kv\textsubscript{4.3} as well as Kv\text{1 family proteins in endoplasmic reticulum and at the plasma membrane. This stabilization effect is further supported by our finding that the Kv\textbeta effect on Kv\textsubscript{4.3} protein level is larger at longer times after transfection. Hence, Kv\textsubscript{4.9}-Kv\textbeta complexes are likely more stable than those without these auxiliary subunits.

Despite the similarity between Kv\textsubscript{4.4} and Kv\text{1 family polypeptides, our data indicate that the two family proteins exhibit distinct requirements for interaction with Kv\textbeta subunits. A part of the N terminus of Kv\text{1 family polypeptide is sufficient for association (5, 6). In contrast, our results demonstrated that the corresponding region of Kv\textsubscript{4 family peptide is not necesa-
Association of Kv4.3 Channels with Kvβ Subunits

sary. Instead, the association requires the C terminus of Kv4.3 polypeptide. The importance of the C terminus for interaction with Kvβ subunits was also suggested in Kv2.2-Kvβ4 complex formation; a part of the C terminus of Kv2.2 protein is required for the increase in current density produced by Kvβ4 coexpression in Xenopus oocytes (10). Thus, the C terminus of Kv2.2 and Kv4 family polypeptides is likely to be involved in association with Kvβ subunits. The apparent lack of sequence similarity between the N terminus of Kv1 family and the C termini of Kv2.2 or Kv4.3 polypeptides suggest that the interaction between these subunits may be more complex than previously assumed. To further elucidate interaction mechanisms, we generated a chimeric Kv2.1 channel containing the C terminus of Kv4.3 polypeptide. We found that this chimera does not efficiently associate with Kvβ subunits (data not shown), suggesting that the Kv4.3 C terminus may not be sufficient for association. However, this chimera was found to be nonfunctional. Therefore, misfolding of this chimeric channel protein might be responsible for the observed lack of interaction. Thus, a simple explanation for the requirement of the C terminus is that this peptide interacts with a site of Kvβ polypeptide that is distinct from one for the Kv1 family N terminus. Alternatively, the C terminus may indirectly participate in interaction. For example, the C-terminal peptide interacts with other part of the channel protein to place an association site in a position for efficient interaction with these auxiliary subunits. More detailed analyses are required to differentiate these possibilities.

Recently identified Ca\(^{2+}\)-binding subunits (KChIP) are likely to play important roles in controlling the expression and function of Kv4 family channels (28). In addition, our results likely to play important roles in controlling the expression and function of Kv4 family channels. Consequently, the association of Kvβ subunits with Kv4 family polypeptides is likely to be involved in association with Kvβ subunits. The apparent lack of sequence similarity between the N terminus of Kv1 family and the C termini of Kv2.2 or Kv4.3 polypeptides suggest that the interaction between these subunits may be more complex than previously assumed. To further elucidate interaction mechanisms, we generated a chimeric Kv2.1 channel containing the C terminus of Kv4.3 polypeptide. We found that this chimera does not efficiently associate with Kvβ subunits (data not shown), suggesting that the Kv4.3 C terminus may not be sufficient for association. However, this chimera was found to be nonfunctional. Therefore, misfolding of this chimeric channel protein might be responsible for the observed lack of interaction. Thus, a simple explanation for the requirement of the C terminus is that this peptide interacts with a site of Kvβ polypeptide that is distinct from one for the Kv1 family N terminus. Alternatively, the C terminus may indirectly participate in interaction. For example, the C-terminal peptide interacts with other part of the channel protein to place an association site in a position for efficient interaction with these auxiliary subunits. More detailed analyses are required to differentiate these possibilities.

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