Selective Interaction of Voltage-gated K⁺ Channel β-Subunits with α-Subunits*

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To begin to study the molecular bases that determine the selective interaction of the β-subunits of voltage-gated K⁺ channels with α-subunits observed in situ, we have expressed these polypeptides in transfected mammalian cells. Analysis of the specificity of α/β-subunit interaction indicates that both the Kvβ1 and Kvβ2 β-subunits display robust and selective interaction with the five members of the Shaker-related (Kv1) α-subunit subfamily tested. The interaction of these β-subunits with Kv1 α-subunits does not require the β-subunit N-terminal domains. Thus, the previously observed failure of N-terminal mutants of Kvβ1 to modulate inactivation kinetics of Kv1 family members is not simply due to a lack of subunit interaction. Interaction of these β-subunits with members of two other subfamilies (Shab- and Shaw-related) could not be detected. Somewhat surprisingly, a member of the Shal-related subfamily was found to interact with β-subunits; however, this interaction had biochemical characteristics distinct from the β-subunit interaction with Kv1 family members. In all cases, Kvβ1 and Kvβ2 exhibited indistinguishable α-subunit selectivity. These studies point to a selective interaction between K⁺ channel α- and β-subunits mediated through conserved domains in the respective subunits.

Voltage-dependent K⁺ channels are fundamental and diverse components of neuronal activity. Molecular cloning studies have identified over a dozen distinct K⁺ channel genes and shown that the encoded pore-forming α-subunits are members of a large, multigene superfamily that includes Na⁺ and Ca²⁺ channel α-subunits (1). Although expression of these individual α-subunits alone is sufficient to generate voltage-gated channels exhibiting many features of the corresponding channels in situ, studies on native Na⁺ and Ca²⁺ channels in neurons and other excitable cells have confirmed the existence of auxiliary polypeptides in tight association with α-subunits (2). Cloning of these auxiliary subunits and their subsequent co-expression with α-subunits has shown that the expression level, gating, and conductance properties of expressed channels are profoundly influenced by the presence of auxiliary subunits (2).

Recently, it has been discovered that K⁺ channels also have auxiliary (β) subunits. A cDNA encoding a β-subunit copurifying with the bovine brain DTX acceptor complex was recently isolated (3). Subsequently, cDNAs encoding three highly related yet distinct β-subunit isoforms were isolated from rat brain (Kvβ1 and Kvβ2, Ref. 4) and from ferret (Kvβ3, Ref. 5) and human (hKvβ3, Refs. 6 and 7) heart. Although dissimilar in their primary structures, β-subunits of K⁺ and Ca²⁺ channels exhibit general structural similarity in that they are basic (pI ~ 9.5), hydrophilic, and presumably peripheral membrane proteins present at the cytoplasmic face of the plasma membrane (2).

Co-expression of Kvβ1 was found to greatly accelerate the rate of inactivation of K⁺ currents expressed from the Kv1.1 or Kv1.4 α-subunit cDNAs in Xenopus oocytes (4). Kvβ3, which is an alternatively spliced product of the Kvβ1 gene, accelerates the rate of inactivation of K⁺ currents expressed from Kv1.4 or Kv1.5 but not from Kv1.1, Kv1.2, or Kv1.3 cDNAs (5–7). These results suggest that β-subunit modulation of α-subunit gating can contribute additional functional diversity to K⁺ channels in excitable cells. Surprisingly, co-expression of the highly related Kvβ2 had no effect on inactivation, apparently due to the lack of the N-terminal “ball” domain present in Kvβ1 that is both necessary and sufficient for the observed modulation of inactivation (4). However, from the published electrophysiological analysis of α/β-subunit interaction presented, it was also possible that the lack of observed Kvβ2 effects was simply due to a lack of Kvβ2 interaction with the co-expressed α-subunits.

We previously used an antibody raised against the C terminus of the bovine β-subunit, predicted to recognize both Kvβ1 and Kvβ2 in rat brain, to investigate the expression of these β-subunits in situ (8). A major 38-kDa polypeptide and a minor 41-kDa polypeptide were detected in rat brain membrane fractions by immunoblot analysis. These two bands correspond closely to the predicted sizes of Kvβ2 and Kvβ1, respectively. Immunoprecipitation experiments showed that the major 38-kDa polypeptide is associated and colocalizes with Kv1.2 and Kv1.4, but not Kv2.1, in rat brain (8), suggesting the selective interaction of K⁺ channel α- and β-subunits.

As a first step toward understanding the molecular mechanisms that determine subunit composition of K⁺ channels, rat Kvβ1 and Kvβ2 were transfected either alone or together with K⁺ channel α-subunits into mammalian cells lacking these proteins. Kvβ1 and Kvβ2 exhibited selective interactions with Shaker- and Shal-related α-subunits but did not interact with Shab- and Shaw-related α-subunits. The interaction with α-subunits did not require the N-terminal domain necessary for the previously observed effects of some β-subunits on α-subunit inactivation.

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The abbreviations used are: DMEM, Dulbecco's modified Eagle's medium (DMEM); [35S]Methionine (Expre35S35S); [35S]Methionine was from Hyclone Laboratories. [35S]Methionine (Expre35S35S) was from DuPont NEN. Restriction and other enzymes were from Boehringer Mannheim. Horseradish peroxidase-conjugated secondary antibody was from Cappel (West Chester, PA). The enhanced chemiluminescence (ECL) reagents were from Amersham Corp. Pansorbin was from Calbiochem. Pre stained molecular weight standards were from Sigma. All other reagents were from Sigma or Boehringer Mannheim.

Subunit-specific Antibodies—The rabbit polyclonal antibodies anti-β and anti-Kv1.2 were produced and affinity purified essentially as described previously (8). Anti-Kv1.1, Kv1.5, Kv1.6, and Kv4.2 antibodies were made against synthetic peptides listed below: Kv1.1, residues 483–496 CEEDMNSSIAHYQANIRGTG (9); Kv1.5, 586–602 ECRSLV-ACLDTSRETL (10); Kv1.6, 506–524 CRERRSLYPHTHAYAEKR (10); and Kv4.2, 484–502 CLEKTNNHFVQIEES (11). Antibodies were produced and purified essentially as described previously (8, 12). Anti-Kv2.1 Kv2.2 antibodies were produced as described previously (12). Anti-Kv1.3 antibody T4 (13), generated by Dr. J. Douglass (Vollum Institute), was kindly provided by Dr. L. Levitan (Brandeis University). Anti-Kv3.1 antibody 602 (14) was kindly provided by Dr. T. Perney (Rutgers University).

Cloning of Kv1.2 and Kv1.2 cDNAs—We initially cloned a 475 bp fragment of Kv1.2 cDNA by reverse transcriptase-PCR. Total adult rat brain RNA (1 μg) was reverse transcribed, and the resultant cDNA was subcloned into Bluescript SK+ (Stratagene) at PstI-Sall sites. Eight cDNAs corresponding to the predicted size were identified by restriction mapping. One of these clones was analyzed by I sites. Eight cDNAs corresponding to the predicted size were obtained and subcloned into pRBG4 at the NsiI site. The resultant product predicted size (642 bp) was isolated and subjected to 15 additional rounds of amplification using the same primers. The resultant product was subjected to PCR. Oligonucleotide primers B1–5-1 (21-mer, 5′-CTATCGATGACTTAGGATCTATAGTCC-3′) and B1–3-1 (20-mer, 5′-GGAAT-3′) were used for 20 rounds of PCR. Amplified product was fractionated by agarose gel, and a specific product of predicted size (642 bp) was isolated and subjected to 15 additional rounds of amplification using the same primers. The resultant product was isolated from an agarose gel, digested with EcoRI and HindIII, and cloned into Bluescript SK+. Its identity as a fragment of Kv1.2 cDNA was verified by sequencing. The clone was then used as a probe to screen a rat brain cDNA library (in λZAPII, kindly provided by Dr. T. Snutch (University of British Columbia)). From a total of 3.5 × 106 plaques, 9 Kv1.2 clones were obtained. The identity of these clones was confirmed by restriction mapping and partial sequencing. One of the cDNA clones, pKB16, which contains the full-length Kv1.2 coding region, 0.3 kbp of 5′-untranslated region, and 1.7 kbp of 3′-untranslated region, was used for further experiments.

As a result of this cDNA screening, we also obtained several Kv1.2 clones. However, none of these clones contained full coding sequences. We again employed reverse transcriptase-PCR using the following primers: B2–3 (5′-CTATCGATGACTTAGGATCTATAGTCC-3′), a primer that is complementary to the coding region of Kv1.2, and B2–5 (5′-CTGATCTAGATAAGTGAGGC-3′), a primer that is complementary to the untranscribed region of Kv1.2. PCR primers were chosen to avoid amplification of a fragment of approximately 475 bp, which could result in a false positive. The amplified fragment was isolated, digested with EcoRI and HindIII, and cloned into Bluescript SK+.

Construction of Mammalian Expression Vectors—cDNAs encoding α-subunits of voltage-gated K⁺ channel were kindly provided by investigators listed below: Kv1.1 (RBK1, Ref. 9), Dr. J. Adelman (Vollum Institute); Kv1.2 (rat RAK, Ref. 15), Dr. K. Rhodes (Wyeth Ayerst Research); Kv1.3 (Kv3, Ref. 10), and Kv1.6 (Kv2, Ref. 10), Dr. R. Swanson (Merck Research Labs); Kv1.5, Kv1.5 (Kv2, Ref. 10), Dr. L. Kaczmarek (Yale Medical School); Kv1.1 (dK1, Ref. 16), Dr. R. J. Oho (University of Texas Southwestern Medical Center); and Kv3.1 (Kv4, Ref. 17), Dr. T. Perney (Rutgers University). The Kv1.2 DNA RAK was cloned from rat heart and differs at three nucleotides from rat brain cDNA (BK2, Ref. 18) kindly provided by Dr. D. McKinnon (SUNY, Stony Brook). We obtained similar results using either the RAK heart (RAK) or brain (BK2) cDNA; the data presented here were obtained with the RAK clone.

A 1.7-kbp EcoRI fragment of pKB16, which contains the entire coding region, was isolated and ligated into pRBG4 to generate Kv1.2/ RBG4. Expression vectors containing α-subunit cDNAs were constructed by cloning the respective coding regions into pRBG4 as follows: Kv1.1/RBG4 was generated by digesting Kv1.1/ps-9 with PstI and HindIII, followed by ligation with Pst/HindIII-digested pRBG4. To generate

Kv1.2 (rat RAK)/RBG4, a BglII fragment containing a coding region was isolated from rat RAK/pSP64T (15) and cloned into Bluescript SK+.

Kv1.2, a vector in the EcoRI site was changed to BglII, which was then digested with XbaI and HindIII, and the fragment was cloned into pRBG4. Kv1.2/RBG4 was constructed by digesting pBSII/GEM2 (16) with EcoRI, followed by ligation into EcoRI-digested pRBG4. Kv1.3/RBG4 was generated by digesting clone D469 (10) with BstEII, followed by blunting with Klenow and ligation into EcoRV-digested pRBG4. Kv1.6/RBG4 was generated by digesting pBluescript GEM2 (10) with NotI, followed by blunting with Klenow, digestion with EcoRI, and ligation with EcoRV/EcoRI-digested pRBG4. The construction of Kv1.2/RBG4 was described previously (9). Kv1.2/RBG4 was generated by digesting ratShal1/5K− with HindIII and ligating the resultant fragment into EcoRV-digested pRBG4. The mammalian expression plasmid for Kv3.1 (in pRcCMV) was obtained from Dr. T. Perney (Rutgers University).

Expression and Analysis of K⁺ Channel α- and β-Subunits—Procedures for COS-1 cell culture, DNA transfection, immunoblot analysis, and immunoprecipitation reactions were performed essentially as described in Shi et al. (19) with the following exceptions. For immunoblots, cells were extracted as described previously in 500 μl of lysis buffer, and the soluble lysate was insoluble pellet was separated by centrifugation in the microcentrifuge at 15,800 × g for 2 min. The supernatant (lysate) was diluted with an equal volume of 2 × reducing SDS sample buffer. For metabolic labeling in [35S]methionine, cells grown on 60-mm tissue culture dishes were pre-incubated in methionine-free DMEM for 10 min at 37 °C followed by incubation in methionine-free DMEM containing 33 μCi/ml of [35S]methionine at 37 °C for 2–4 h. Cells were then washed and extracted with 1 ml of lysis buffer. For immunoprecipitation reactions, 100 μl of lysate was used, and the resultant products were analyzed on 9% SDS-polyacrylamide gel electrophoresis and visualized by fluorography on Kodak Biomax film or by phosphorimaging (Molecular Dynamics).

RESULTS

Expression of β-Subunits by Transient Transfection in COS-1 Cells

We have previously characterized the expression of β-subunits in rat brain using an anti-β-subunit antibody (8). This antibody, raised against the conserved C-terminal region of Kv1.1 and Kv1.2 (and Kv3), recognizes several polypeptides in rat brain, among these a predominant polypeptide species of 38 kDa, a polypeptide of 41 kDa, and minor species at 44 kDa (Fig. 1, lane 1). As a first step toward correlating these brain polypeptides with the recombinant β-subunits, we expressed Kv1.1 and Kv1.2 cDNAs by transient transfection into COS-1 cells (19) and investigated the expressed polypeptides by immunoblots. Surprisingly, a minor immunoreactive polypeptide species of 44 kDa in rat brain membranes comigrates with the

1 The abbreviations used are: DMEM, Dulbecco’s modified Eagle’s medium (DMEM); PCR, polymerase chain reaction; bp, base pair(s); kb, kilobase pair(s).
recombinant Kvβ1 polypeptide (Fig. 1, lane 2). Comigration of the major β-subunit immunoreactive polypeptide of 38 kDa with recombinant Kvβ2 (Fig. 1, lane 3) is consistent with our previous proposal (8) that this abundant brain polypeptide is in fact Kvβ2. Similar electrophoretic mobilities for these recombinant β-subunits are obtained in two other mammalian cell lines (HEK293, PC12; not shown), suggesting that cell typespecific post-translational modifications do not contribute significantly to the mobility of β-subunit polypeptides. This suggests, but does not prove, that the prominent 41-kDa immunoreactive band in rat brain is not Kvβ1 and that Kvβ1 apparently corresponds to the 44-kDa polypeptide.

**Specific Association of Kvβ1 and Kvβ2 with Kv1.2**

To study the selectivity of αβ-subunit interaction, we undertook a biochemical approach utilizing co-immunoprecipitation from cotransfected COS-1 cells. Except where explicitly stated otherwise, all immunoprecipitation reactions were performed under conditions designed to maintain subunit association, resulting in some nonspecific background, even in reactions performed in the absence of antibody. Initially, we focused on β-subunit interaction with Kv1.2, based on previous studies in brain (3,8). Fig. 2 shows a fluorographic image of immunoprecipitation products fractionated on an SDS gel. Kv1.2-, Kvβ1-, and Kvβ2-transfected cells express 65-, 44-, and 38-kDa proteins, respectively (Fig. 2), and in each case subunit-specific antibodies show no detectable cross-reactivity to heterologous samples. As expected, Kvβ1 and Kvβ2 are immunoprecipitated with the pan-β antibody from Kv1.2/Kvβ1- or Kv1.2/Kvβ2-cotransfected cells (Fig. 2). Both β-subunits could also be co-immunoprecipitated with the anti-Kv1.2 antibody. The presence of Kv1.2Kvβ interaction was confirmed by reciprocal co-immunoprecipitation reactions by the presence of Kv1.2 in the β-subunit immunoprecipitation products.

Addition of a denaturing agent, such as the detergents SDS and deoxycholate, should affect polypeptide folding and disrupt the noncovalent protein-protein interactions typical of most multisubunit membrane protein complexes (20). To test if K+ channel subunit association was through similar noncovalent interactions, immunoprecipitation reactions were performed in the presence of such denaturing agents. The coprecipitation of Kv1.2 with Kvβ2 could be disrupted by the addition of 0.2% SDS and 0.5% sodium deoxycholate during the immunoprecipitation reactions; this treatment has no effect on the direct immunoprecipitation of the subunits themselves (Fig. 3A). Similar results were obtained for Kv1.2-Kvβ1 interaction (not shown). Thus, K+ channel αβ-subunit interaction has similar sensitivity to denaturing detergents as exhibited for other multisubunit membrane protein complexes (20).

To test whether co-expression within the same cell is necessary for subunit interaction, individually transfected dishes of COS-1 cells expressing either Kv1.2 or Kvβ2 were harvested. The cells were then pooled, and the pooled mixture of cells was extracted under standard conditions. The resultant lysates...
were then subjected to immunoprecipitation with subunit-specific antibodies. These experiments yielded no co-immunoprecipitation of α- and β-subunits above background (no antibody lanes), showing that co-expression within the same cell is necessary for subunit interaction (Fig. 3B). Previous studies had shown that deletion of the N terminus of Kv1.2 destroyed its ability to modulate inactivation (4). To test whether this was simply due to a lack of interaction, an N-terminal truncation mutant, Kv1.2ΔN70, which lacks amino acids 1–70, was co-expressed with Kv1.2. As shown in Fig. 4A, Kv1.2ΔN70 can be efficiently co-immunoprecipitated with anti-KV1.2 antibody and vice-versa. Thus, removal of the domain necessary for Kv1.2-mediated modulation of inactivation does not disrupt αβ-subunit interaction, showing that the loss of the ability of such mutants to modulate inactivation is not due to an inability to interact with α-subunits. A similar N-terminal deletion of Kvβ2 (Kv2ΔN22) also exhibited interaction with Kv1.2 that was indistinguishable from wild-type Kvβ2 (Fig. 4B). These data indicate that the N-terminal domains of β-subunits are not necessary for the interaction with α-subunits and that the interaction domain lies somewhere else in the β-subunit sequence.

Selective Association of Kvβ1 and Kvβ2 with α-Subunits

To investigate the selectivity of αβ-subunit interaction, co-immunoprecipitation from cells co-expressing pairwise combinations of recombinant mammalian α-subunits and Kvβ1 and Kvβ2 was performed. Control experiments, as detailed above, were performed for each set of αβ-subunit combinations. However, due to space limitations, only the relevant co-immunoprecipitation reactions are presented here.

Kv1 Subfamily—Five members of the mammalian Shaker-related (Kv1) subfamily were tested for interaction with Kvβ1 and Kvβ2. All of the Kv1 family members tested (Kv1.1, Kv1.2, Kv1.3, Kv1.5, and Kv1.6) exhibit direct and specific interaction with both Kvβ1 and Kvβ2, as evidenced by reciprocal co-immunoprecipitation (Fig. 5). However, distinctions are apparent in the extent of co-immunoprecipitation among the specific pairwise combinations. Kv1.3 and Kv1.6 are similar to Kv1.2, in that high levels of co-immunoprecipitation of both the α- and β-subunits are observed in reactions using either anti-α-subunit or anti-β-subunit antibody. Kv1.1 and Kv1.5, however, show lower than expected levels of coprecipitated α-subunits in the anti-β-subunit immunoprecipitation reactions, perhaps due to an overabundance of β-subunits such that only a small fraction of the large total β-subunit pool is associated with the small α-subunit pool. However, it is not possible to determine from these types of experiments whether these differences reflect quantitative differences in αβ-subunit association. Control experiments on singly transfected cells expressing α-subunits alone show no detectable immunoprecipitation with anti-β-subunit antibodies. This verifies that the low levels of Kv1.1 and Kv1.5 seen in anti-β-subunit immunoprecipitation reactions performed on cells co-expressing α- and β-subunits are specific and significant.

Kv2 and Kv3 Subfamily—Analysis of cells cotransfected with Shab-related Kv2.1 and either Kvβ1 or Kvβ2 show no coprecipitation by anti-β subunit antibody (Fig. 6A). Low levels of Kvβ1 and Kvβ2 are seen in immunoprecipitation reactions performed with the anti-α-subunit antibody; however, comparable levels are observed in similar immunoprecipitation reactions performed on cells expressing β-subunits alone (not shown), indicating that these products are due to minor cross-reactivity of the anti-KV2.1 antibody to these β-subunits and

![Figure 4. Association of N-terminal truncation mutants of Kvβ1 and Kvβ2 with Kv1.2.](image)

![Figure 5. Association of Kvβ1 and Kvβ2 with Shaker-related α-subunits.](image)
not to α/β-subunit interaction. Similar nonspecific immunoprecipitation of low levels of β-subunits was also seen in immunoprecipitation reactions performed on cotransfected cells in the absence of antibody (not shown). The addition of denaturing agents, SDS and deoxycholate, during the immunoprecipitation reactions shows that this treatment has no or very weak effect on the relatively low but detectable level of co-immunoprecipitation (Fig. 6B). Taken together, these data indicate that the observed co-immunoprecipitation is not due to specific noncovalent interactions between Kv2.1 and Kv2.2, as these sorts of intermolecular associations are typically disrupted by denaturing agents (see Fig. 3A) but is due to low levels of antibody cross-reactivity or other nonspecific precipitation. When these β-subunits were co-expressed with the mammalian Shaw homolog Kv3.1, no detectable co-immunoprecipitation was observed (Fig. 6C), although strong subunit-specific immunoprecipitation was observed.

Kv4 Subfamily—Strong reciprocal co-immunoprecipitation was observed between both Kv4.2 and Kv4.2 and the mammalian Shal homolog Kv4.2 (Fig. 7A). The interaction of Kv4.2 and Kv4.2 is relatively resistant to treatment with the denaturing detergent SDS (SDS treatment) in that the co-immunoprecipitation is not disrupted by the addition of SDS at concentrations less than 0.6% (Fig. 7B). This is distinct from the characteristics of the interaction of Kv1.2 with Kv1.1 and Kv1.2, where interaction is partially disrupted by the addition of SDS to only 0.2%, with complete disruption observed at 0.4% SDS (Fig. 7B).

DISCUSSION

Our previous study using an antibody against a sequence conserved in both Kv1.1 and Kv1.2 revealed the existence of multiple immunoreactive β-subunits in rat brain (8). Here, analysis of transfected cells expressing recombinant Kv1.1 and Kv1.2 reveals that a minor 44-kDa rat brain β-subunit comigrates with Kv1.1, while the major 38-kDa β-subunit comigrates with Kv1.2. The other immunoreactive β-subunit at 41 kDa, which is recognized by the β-subunit antibody, is apparently neither Kv1.1 nor Kv1.2 and suggests the existence of an additional, as yet uncharacterized member of the β-subunit gene family in rat brain. Recent cloning of a partial cDNA for a rat Kv1.3 β-subunit, which shares the same nucleotide sequence with Kv1.1 except for its unique N-terminal region and is predicted to encode a polypeptide of 45 kDa, strongly suggests the presence of at least one alternatively spliced product of the Kv1.1 gene (5). Studies with subtype-specific antibodies will allow for the eventual unequivocal identification and localization of each of the individual components of the β-subunit.
K⁺ Channel αβ-Subunit Interactions

In brain, the ability to interact functionally with Kvα subunits is a major factor in the identification of other α-subunits. Thus, the ability to interact with Kvα subunits is a major factor in the identification of other α-subunits. In COS-1 cells, these two α-subunits are unable to interact with Kvα and Kvβ. Subcellular localization of Kvα2 and β-subunits in transfected cells is consistent with this model in that immunofluorescence staining of cells co-expressing Kvα2 and Kvβ shows no overlap of α- and β-subunit staining, while cells co-expressing Kvα2 and Kvβ2 show extensive overlap throughout the cells.3

Our results provide direct biochemical evidence for selective interaction of K⁺ channel αβ-subunits with only a subset of the αβ-subunit family, have greatly expanded the initial observations of Rettig et al. (4) who showed that Kvα1.1 and Kvα1.4 interact functionally with Kvβ1 in oocytes (4), and provide the evidence for a direct, noncovalent interaction between αβ-subunits. These results also confirm and extend our previous studies of rat brain αβ-subunit association in situ, where we found that neuronal αβ-subunits could be coprecipitated with rat brain Kvα1.2 and Kvα1.4 but not with Kvα2.1 (8). A detailed characterization of purified bovine brain dendrotoxin acceptors, which were later found to contain Kvα1β and Kvβ3 (3), showed that these K⁺ channel complexes contain Kvα1.1, Kvα1.2, Kvα1.4, and Kvα1.6 (22). Our findings provide a first step toward understanding the molecular determinants of αβ-subunit interaction by showing that the subunit selectivity observed in rat brain can be recapitulated in transfected cell lines, indicating that selectivity is mainly determined by the primary structure of the interacting subunits.

The voltage-gated K⁺ channel αβ-subunit genes segregate into four subfamilies based on the similarity of primary structure of each member (23). As discussed above, our results show that Kvα1 and Kvα2 interaction seemed to be restricted to Shaker- and Shal-related subfamilies. Interestingly, proposed phylogenetic trees place the Shaker (Kvα1) and Shal (Kvα4) subfamilies on one major branch, while Shab (Kvα2) and Shaw (Kvα3) members are placed on a separate branch (1, 24). Thus, the ability to interact with Kvα1 and Kvα2 appears to reside in the relatedness of their primary sequences as evidenced by their phylogenetic groupings and allows for the design of structure-function analyses aimed at defining the domains of αβ-subunits mediating αβ-subunit interaction. In the case of voltage-sensitive Ca²⁺ channel αβ-subunit interaction, the β-subunit binds to a conserved cytoplasmic motif in the α-subunit (25). Taken together with the fact that K⁺ channel β-subunits are also cytoplasmic proteins, it is likely that the interaction domain on K⁺ channel α-subunits is present on a cytoplasmic domain.

No distinct domain of α-subunits stands out as a clear candidate for mediating interaction with β-subunits based simply on the positive interaction of both Kvα and Kvβ family members. However, our experiments using SDS treatment to disrupt αβ-subunit interaction imply that the interaction of αβ-subunits with Kvα1.2 and Kvα2 are somewhat distinct. In addition, only Kvα1 and not Kvα2, Kvβ3, or Kvβ4 subfamily α-subunits have been found associated with Kvα1 and Kvα2 in rat brain in situ (8). Together, these data may imply that the only physiologically relevant subunit interactions are between Kvα (Shaker-related) α-subunits and Kvα1 and Kvα2. Using this assumption, a conserved N-terminal, presumably cytoplasmic domain of about 130 amino acids is striking in that it is highly conserved among Kvα- subunits but not among members of the other (Kvβ, Kvα3, and Kvβ4) subfamily members. This highly conserved region, known as the “T1” (26) or “NAB” (27) domain, is thought to be important in mediating efficient αβ-subunit interaction (26–28). This may raise the interesting scenario whereby both αα- and αβ-subunit interactions are mediated through similar domains. Extensive mutational analysis of αβ-subunit proteins will lead to the elucidation of the specific αβ-subunit binding region.

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