Association and Colocalization of K+ Channel α- and β-Subunit Polypeptides in Rat Brain

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Recent cloning of auxiliary subunits associated with voltage-gated ion channels and their subsequent coexpression with the channel forming α-subunits has revealed that the expression level, gating and conductance properties of the expressed channels can be profoundly affected by the presence of an auxiliary subunit polypeptide. In the present study, we raised antibodies against the β-subunit associated with the bovine dendrotoxin sensitive K+-channel complex and used these antibodies to characterize the related β-subunit polypeptides in rat brain. The anti-β-subunit antibodies displayed a specific reaction on immunoblots of rat brain membranes with a major 38 kDa polypeptide, and a minor 41 kDa polypeptide, which correspond closely to the predicted sizes of the Kvβ2 and Kvβ1 β-subunit polypeptides, respectively, recently cloned from rat brain. Reciprocal coimmunoprecipitation experiments revealed that the β-subunit polypeptides are associated with Kv1.2 and Kv1.4, but not Kv2.1, α-subunits. Immunohistochemical staining revealed that the β-subunit polypeptides were widely distributed in adult rat brain. Moreover, the cellular distribution of β-subunit immunoreactivity corresponded closely with immunoreactivity for Kv1.2, and to a lesser extent Kv1.4, but not with Kv2.1. These results suggest that neuronal mechanisms may exist to direct the selective interaction of K+ channel α- and β-subunit polypeptides, and that the properties of K+ channels in specific subcellular domains may be regulated by the formation of heteromultimeric K+ channel complexes containing specific combinations of α- and β-subunits.

[Key words: ion channel, CNS, auxiliary subunits, tissue-specific gene expression, hippocampus, cerebellum]

Voltage-gated cation channels selective for K+, Na+, or Ca2+ are fundamental components in the generation and control of excitability in neurons (Hille, 1992). Molecular cloning studies have

determined that the α-subunits of these channels are members of a large, multigene superfamily (Catterall, 1988). Although expression of these α-subunits alone is sufficient to generate voltage-gated channels possessing 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 the α-subunit complex. Cloning of these auxiliary subunits and their subsequent coexpression with α-subunits has revealed that the expression level, gating and conductance properties of the expressed channels can be profoundly affected by the presence of these auxiliary subunit polypeptides (reviewed in Isom et al., 1994).

Recent biochemical and immunological studies have demonstrated that there are also auxiliary "β-subunit" polypeptides in association with voltage-gated K+ channels. Immunoprecipitation of K+ channel complexes from radioiodinated rat brain membranes using antibodies specific for the Kv2.1 K+ channel α-subunit (nomenclature according to Chandy, 1991) revealed the presence of a 38 kDa β-subunit polypeptide in tight association with this delayed rectifier K+ channel complex (Trimmer, 1991). Similarly, affinity purification and immunoprecipitation of the α-dendrotoxin (DTX) sensitive K+ channel complex from bovine brain revealed, in addition to α-subunit polypeptides, the presence of 38 kDa and 41 kDa β-subunit polypeptides in association with the DTX acceptor complex (Parcej and Dolly, 1989; Scott et al., 1990; Muniz et al., 1992). A cDNA encoding a DTX acceptor β-subunit was recently isolated from bovine brain by Scott et al. (1994b). Using this bovine cDNA as a probe, cDNAs encoding two homologous polypeptides, termed Kvβ1 and Kvβ2, were isolated from rat brain cDNA libraries (Rettig et al., 1994). Coexpression of Kvβ1 with either the Kv1.1 or Kv1.4 α-subunit showed that the presence of Kvβ1 greatly accelerated the rate of inactivation of the expressed K+ currents, compared to the rate of inactivation of currents arising from these α-subunit polypeptides expressed alone. However, coexpression of Kvβ2 with either Kv1.1 or Kv1.4 had no measurable effect on the macroscopic properties of the expressed currents (Rettig et al., 1994).

Individual α-subunits of voltage-gated K+ channels have been shown to associate to form hetero-oligomeric complexes in brain. Immunoprecipitation of the DTX acceptor complex using antisera specific for the Kv1.2 polypeptide indicated that Kv1.1, Kv1.4, and Kv1.6 are also components of this acceptor (Scott et al., 1994a). Interestingly, while virtually all DTX acceptors appeared to contain the Kv1.2 α-subunit, the relative amounts

of the other α-subunits in the DTX acceptor complex varied across brain regions. Thus, there was a larger proportion of Kv1.1 in DTX acceptor complexes isolated from the cerebral cortex and brainstem than in hippocampus, where there was a greater proportion of Kv1.4 (Scott et al., 1994a). These observations extended findings of prior biochemical and immunohistochemical analyses which demonstrated that Kv1.2 coimmunoprecipitates with Kv1.1 from rat brain membranes, and is colocalized with Kv1.1 at nodes of Ranvier and in cerebellar basket cell terminals (Wang et al., 1993). Furthermore, these observations indicated that Kv1.2 coprecipitates with Kv1.4 from rat brain membranes and is colocalized with Kv1.4 in the dentate gyrus (Sheng et al., 1993).

The formation of hetero-oligomeric channels containing multiple α-subunit polypeptides has been proposed as a substrate for generating functional diversity among voltage-gated K^+ channels *in situ* (Sheng et al., 1993; Wang et al., 1993; Scott et al., 1994a). To better understand the heteromeric nature of K^+ channel complexes in general, and the contribution of β-subunits to channel diversity in particular, we have investigated the association and colocalization of K^+ channel α- and β-subunit polypeptides in rat brain. We produced antibodies against a peptide sequence derived from the cloned bovine K^+ channel β-subunit, and used these antibodies to characterize the related β-subunit polypeptides in rat brain. We also used these antibodies to investigate, biochemically and neuroanatomically, the coassociation and colocalization of these K^+ channel β-subunits with specific α-subunit polypeptides. These studies provide the first direct biochemical and neuroanatomical data to indicate that heteromultimeric complexes containing specific combinations of K^+ channel α- and β-subunits exist *in situ*.

Materials and Methods

Materials. All reagents were molecular biology grade from Sigma (St. Louis, MO) or Boehringer-Mannheim (Indianapolis, IN), except where noted otherwise.

Production of synthetic peptides and antibodies. A synthetic peptide (CEIDSILGNKPYSKKDYRS) corresponding to the C-terminal 18 amino acids (350–367) of the bovine β-subunit polypeptide (Scott et al., 1994b) was produced (Quality Controlled Biochemicals, Hopkinton, MA). This synthetic peptide was conjugated to keyhole limpet hemocyanin (1 mg of peptide per milligram of carrier protein) using sulfo-*m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester (Pierce, Rockford, IL), and injected into rabbits for the production of antisera (Pocono Rabbit Farm, Canadensis, PA). For affinity purification, the β-subunit peptide was conjugated to SulfoLink coupling gel (Pierce) via the terminal cysteine residue, and anti-β-subunit antibodies were affinity-purified by standard procedures (Harlow and Lane, 1988). Antibodies to the Kv2.1 (Trimmer, 1991) and Kv1.4 (Sheng et al., 1992) K^+ channel α-subunit polypeptides were prepared as previously described. A rabbit polyclonal antibody specific for the Kv1.2 K^+ channel α-subunit polypeptide was generated using a synthetic peptide (CGVNNSNED-FREENLKTAN) corresponding to amino acids 463–480 of the Kv1.2 polypeptide (Stühmer et al., 1989). Immunization, affinity purification, and characterization were performed essentially as for the anti-β-subunit antibody, and will be described in more detail elsewhere (Rhodes and Trimmer, unpublished observations).

Brain membrane preparations. A crude synaptosomal membrane fraction was prepared from freshly dissected adult rat brains, essentially as described (Trimmer, 1991, 1993). Briefly, brains were homogenized in 0.3 M sucrose, 10 mM sodium phosphate, pH 7.4, 10 mM sodium fluoride, containing a protease inhibitor cocktail (1 mM phenylmethyl sulfonyl fluoride, 1 μg/ml leupeptin, 2 μg/ml aprotinin, and 1 μg/ml pepstatin). The resultant homogenate was subjected to centrifugation at 3000 $\times$ g for 10 min to remove nuclei and debris. The supernatant was centrifuged at 45,000 $\times$ g for 60 min to pellet the membranes. Membranes were suspended in the homogenization buffer and protein was determined using the BCA method (Pierce).

SDS-polyacrylamide gels and Western blotting. For immunoblots, 100 μg of membrane protein was added to reducing SDS sample buffer, boiled, and fractionated on 7.5 or 12% polyacrylamide-SDS gels (Maizel, 1969). Disulfide bonds were reduced by the addition of 20 mM 2-mercaptoethanol in the sample buffer. Nonreducing sample buffer contained 60 mM iodoacetamide. Lauryl sulfate (Sigma) was the SDS source used for all SDS-PAGE (Shi et al., 1994). After electrophoretic transfer to nitrocellulose paper, the resulting blots were blocked in 0.15 M NaCl, 10 mM Tris-HCl, pH 8.0 (TBS) containing 4% low fat milk (Blotto; Johnson et al., 1984), incubated in affinity-purified antibody diluted 1:20–1:2000 in Blotto for 1 hr, and washed three times in Blotto for 30 min total. Blots were then incubated in HRP-conjugated anti-rabbit secondary antibody (Organon Teknika, West Chester, PA; 1:2000 dilution in Blotto) for 1 hr and then washed in TBS 3$\times$ for 30 min total. The blots were then incubated in substrate for enhanced chemiluminescence (ECL) for 1 min and autoradiographed on preflashed (to $OD_{545} = 0.15$) Fuji RX or Kodak XAR-5 film.

For blocking experiments, antisera or affinity-purified antibodies were first incubated overnight at 4°C in 1 ml of Blotto alone, or Blotto containing either 50 μg peptide-BSA conjugate, or 50 μg BSA alone. Before use, the samples were brought to a final volume of 2 ml with Blotto. For strip blots, 3 mg of adult brain membranes were size fractionated on a curtain gel, electrophoretically transferred to nitrocellulose, and the resultant blot (11 $\times$ 14 cm) cut into 22–0.5 cm wide strips. Strips were stained with antisera, affinity-purified antibodies or samples prepared in blocking experiments.

Immunoprecipitations. Immunoprecipitates were performed using detergent lysates of brain membranes. All procedures were performed at 4°C. Rat brain membranes (1 mg of membrane protein/tube) were solubilized to 1 ml final volume/tube in lysis buffer (1% Triton X-100, 0.15 M NaCl, 1 mM EDTA, 10 mM sodium azide, 10 mM Tris-HCl, pH 8.0) containing the protease inhibitor cocktail (see above). Samples were precleared by incubation with fixed *S. aureus* (Pansorbin, CalBiochem, La Jolla, CA) for 30 min, followed by centrifugation at 10,000 $\times$ g for 5 min. Various volumes of antisera, preimmune sera or affinity-purified antibodies were added, and the volume adjusted to 1 ml with lysis buffer. Samples were incubated for 18 hr on a rotator, followed by addition of fixed *S. aureus* and further incubation for 45 min. Samples were centrifuged at 10,000 $\times$ g for 1 min, and pellets washed by resuspension and centrifugation 3$\times$ with lysis buffer. Final pellets were resuspended in reducing sample buffer, electrophoresed on 7.5% or 12% SDS-PAGE, and subjected to immunoblotting as above.

Immunohistochemistry. Fifteen adult male Sprague-Dawley rats were deeply anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and perfused through the ascending aorta with 150 ml of 0.9% saline followed by 500 ml of fixative containing 4% paraformaldehyde in 0.1 M NaPO$_4$ buffer. The brains were removed, cryoprotected for 18–48 hr in 20% sucrose, frozen in a bed of pulverized dry ice, and then cut into 35 μm sections on a sliding microtome. Consecutive 1 in 10 series of sections were collected in 0.1 M NaPO$_4$ buffer and processed immediately for immunohistochemistry as described below.

For immunohistochemistry, sections were incubated for 1 hr in 10 mM NaPO$_4$ (pH 7.4) buffer containing 0.9% NaCl (PBS) and 0.5% Triton X-100. The sections were then washed in PBS and incubated for 1 hr in antibody vehicle (4% goat serum, 0.1% Triton X-100 in PBS). After additional washes in PBS, sections were incubated overnight at 4°C in vehicle containing affinity-purified rabbit anti-β-subunit antibody at dilutions ranging from 1:25 to 1:400. For comparison of K^+ channel α- and β-subunit polypeptides, series of adjacent sections were processed using affinity-purified polyclonal antibodies against Kv1.2 (Kv1.2C), Kv1.4 (Kv1.4N; Sheng et al, 1992), and Kv2.1 (KC; Trimmer, 1991). Following overnight incubation in the primary antibody, sections were washed in PBS, incubated for 1 hr in vehicle containing affinity-purified goat anti-rabbit IgG (1:250; Jackson ImmunoResearch, West Grove, PA), washed again in PBS, and then incubated for 1 hr in vehicle containing rabbit peroxidase–antiperoxidase complex (1:500; Chemicon International, Temecula, CA). To increase the sensitivity of the immunocytochemical reaction, some sections were processed using a double-bridge procedure whereby the secondary antibody and PAP steps were repeated using the original antibody solutions diluted 1:1. After additional washes in PBS, the PAP complex was visualized by developing sections for 5–15 min in 50 mM Tris buffer (pH 7.6) containing 0.04% diaminobenzidine (DAB) and 0.003% H$_2$O$_2$. In some cases, 0.3% nickel ammonium sulfate was included in the DAB solution to intensify the reaction product. Sections were subsequently washed in

Tris buffer, mounted onto chrome-alum and gelatin subbed slides, and then dehydrated and coverslipped using DPX (Fluka, Ronkonkoma, NY). Some of the immunohistochemically stained sections were lightly counterstained with thionin to facilitate identification of cytoarchitectonic landmarks.

To verify the specificity of the anti-α- and anti-β-subunit immunohistochemistry, some sections were processed either without addition of the primary antibody, using a nonspecific affinity-purified rabbit IgG (Jackson ImmunoResearch) as the primary antibody, or using antibodies previously incubated (24 hr) in vehicle containing an excess of the synthetic peptide-BSA conjugate (50–100 μg/ml). No specific staining was observed in these control sections (not shown).

Results

Characterization of anti-β-subunit polypeptide antibodies

A polyclonal antiserum was produced by the immunization of rabbits with a synthetic β-subunit peptide immunogen, corresponding to the last 18 amino acids (450–467) of the deduced bovine β-subunit sequence (Scott et al., 1994b). This region of the C-terminal cytoplasmic tail was chosen to increase the probability that the synthetic β-subunit sequence would project from the carrier protein as it does from the authentic β-subunit polypeptide, and because this sequence was noted by Scott et al. (1994b) to be conserved across species. Subsequent isolation of cDNAs encoding the Kvβ1 and Kvβ2 β-subunit polypeptides from rat brain revealed that this peptide sequence was conserved in both isoforms (Kvβ1, 16/18 amino acids identical; Kvβ2, 18/18 identical; Rettig et al., 1994). Thus, it is likely that both of these β-subunit polypeptide isoforms, and other as yet unidentified members of the β-subunit family that contain this sequence, would be detected by this antibody. That this antibody recognizes both Kvβ1 and Kvβ2 has been confirmed in studies on transfected mammalian cells expressing these β-subunit polypeptide isoforms (Nakahira, Shi, Hammond and Trimmer, unpublished results).

The anti-β-subunit polyclonal rabbit antiserum displayed a reaction on immunoblots of crude adult rat brain membranes with two major bands at 50 kDa and 38 kDa, and a less prominent band at 41 kDa (Fig. 1A). Preimmune serum exhibited a pronounced reaction to the 50 kDa polypeptide, indicating that this component of the immunoreactivity was nonspecific (not shown). Preincubating the antiserum with a β-subunit peptide-BSA conjugate, but not with BSA alone, blocked immunoreactivity to the major 38 kDa band and the minor band at 41 kDa, but not to the major nonspecific band at 50 kDa (Fig. 1A).

Affinity purification of anti-β-subunit antibodies resulted in a loss of the immunoreactivity to the 50 kDa band, and yielded an antibody preparation specific for the 38 and 41 kDa bands (Fig. 1A). The immunoreactivity of the affinity-purified antibody to the 38 and 41 kDa bands was eliminated by preincubation with the synthetic β-subunit peptide-BSA conjugate, but not with BSA alone (Fig. 1A). All subsequent experiments were performed using the affinity purified rabbit polyclonal antibody.

The mobilities on SDS gels of the major 38 kDa and minor 41 kDa immunoreactive bands in adult rat brain were comparable to, but slightly lower than those predicted for the rat brain Kvβ2 (41.0 kDa) and Kvβ1 (44.7 kDa) core polypeptides deduced from the cDNA sequences (Rettig et al., 1994). The mobility and relative intensity of both the major immunoreactive band of 38 kDa and minor band at 41 kDa were virtually identical to the pattern observed on Coomassie blue stained gels of the DTX acceptor β-subunit polypeptide purified from bovine brain (Scott et al., 1994b). The 38 kDa rat brain β-subunit polypeptide also has a similar mobility to the polypeptide that has

been coimmunoprecipitated with the rat brain Kv2.1 α-subunit polypeptide (Trimmer, 1991). The mobility of these 38 kDa and 41 kDa adult rat brain β-subunit polypeptides was similar whether the SDS gels were run under reducing or non-reducing conditions, indicating that these polypeptides did not contain extensive intra- or inter-chain disulfide bridges (not shown).

Identification of K⁺ channel α-subunits interacting with the β-subunits

To determine which K⁺ channel α-subunit polypeptides are associated with Kvβ1, Kvβ2, and other related β-subunits recognized by this antibody, we performed reciprocal coimmunoprecipitation experiments. Rat brain membrane lysates were subjected to immunoprecipitation using antibodies specific for α- or β-subunit polypeptides. The resultant immunoprecipitation products were then analyzed by immunoblotting, using the either α- or β-subunit specific antibodies. As both the immunoprecipitation reactions and immunoblots were performed using rabbit antibodies, background immunoreactivity is visible in the immunoblots, and is due to immunoglobulin heavy and light chains and other nonspecific proteins in the rabbit antibodies. Consequently, the electrophoretic mobility of each of the K⁺ channel polypeptides is denoted with an arrow.

Immunoprecipitation reactions performed with the anti-β-subunit antibody were first analyzed for β-subunit immunoreactivity on immunoblots (Fig. 1B). High levels of β-subunit immunoreactivity were present in immunoprecipitation reactions containing the β-subunit antibody, used alone or in the presence of BSA, but not in reactions performed in the presence of antibody plus the β-subunit peptide-BSA conjugate (Fig. 1B). No β-subunit immunoreactivity was precipitated in the absence of primary antibody. Products of immunoprecipitation reactions performed with anti-α-subunit antibodies were then analyzed for coprecipitation of β-subunits by immunoblotting (Fig. 2). β-subunit immunoreactivity is present in the Kv1.4 and Kv1.2 α-subunit immunoprecipitates, showing that Kv1.4 and Kv1.2 associate with these β-subunits in rat brain. However, no β-subunits could be detected in the Kv2.1 immunoprecipitate (Fig. 2). Aliquots of these same α- and β-subunit immunoprecipitation reactions were then analyzed for the presence of α-subunit immunoreactivity by immunoblotting (Fig. 3). The Kv2.1, Kv1.4, and Kv1.2 immunoprecipitations contain high levels of the expected α-subunit polypeptides.

Figure 3 shows that Kv1.4 and Kv1.2 could be coprecipitated with the β-subunit antibody, while Kv2.1 could not. These results are consistent with the reciprocal experiment shown in Figure 2. Comparison of the signal from the identical aliquots of rat brain membranes on each immunoblot to that from the anti-β-subunit immunoprecipitations allows a determination of the relative amount of α-subunit polypeptide associated with the rat brain β-subunit polypeptide pool. Densitometric analysis of such immunoblot data shows that the β-subunit antibody was able to coprecipitate 36.7% of the Kv1.2 α-subunits present in the detergent lysate of adult rat brain membranes, while only 8.4% of the Kv1.4 α-subunits were coprecipitated by the β-subunit antibody. The results of these reciprocal immunoprecipitation experiments show that Kv1.2 and Kv1.4 are present in brain membranes in association with β-subunit polypeptides, while Kv2.1 exists in complexes that do not contain these particular β-subunit polypeptides.

Immunohistochemical localization of β-subunit polypeptides in adult rat brain. Analysis of immunohistochemically stained
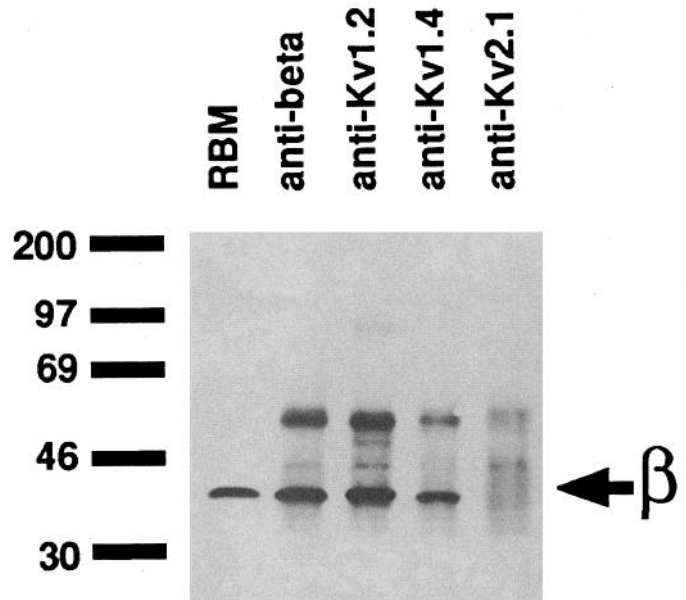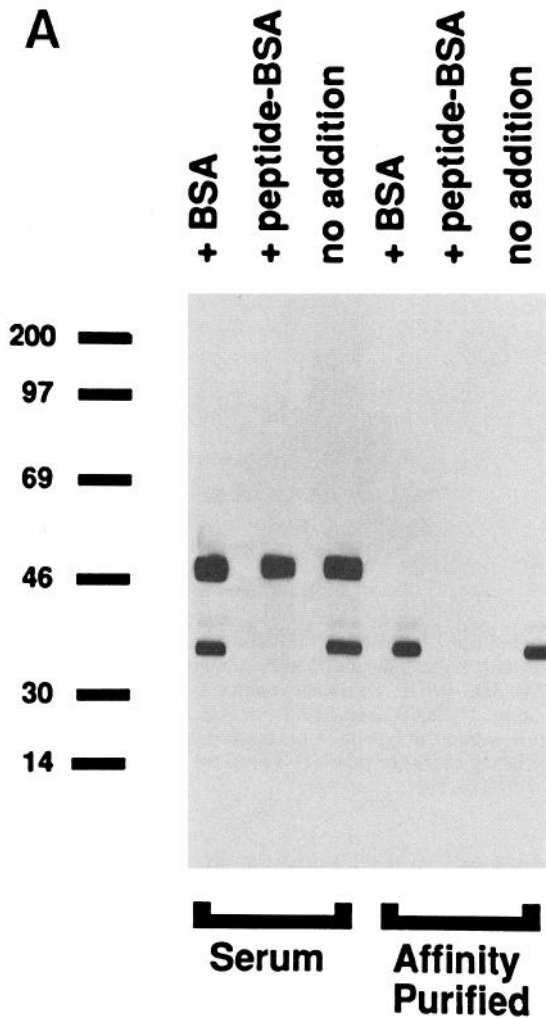
A

Serum   **Affinity Purified**

Figure 2. Detection of β-subunit polypeptides in anti-α- and anti-β-subunit immunoprecipitates. Samples of adult rat brain membranes (*RBM*, 60 μg), and products of immunoprecipitation from detergent extract of 500 μg of RBM with either the β-subunit antibody at 1:400 (*anti-beta*), Kv1.2C antibody at 1:200 (*anti-Kv1.2*), Kv1.4N antibody at 1:2000 (*anti-Kv1.4*), or KC antibody at 1:200 (*anti-Kv2.1*) were size fractionated by 12.5% SDS-PAGE. Samples were transferred to nitrocellulose and probed with anti-β-subunit antibody at 1:20, and bound antibody detected by ECL/autoradiography for 30 sec. *Arrow* points to mobility of β-subunit polypeptide.
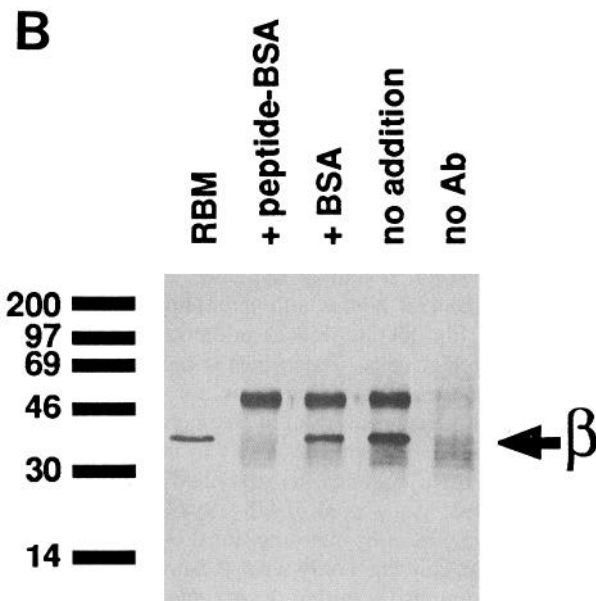
sections indicated that the β-subunit polypeptides are present in neurons and fiber tracts within many areas of adult rat brain (Fig. 4). Regions containing moderate to intense β-subunit immunoreactivity were the neocortex, hippocampus, piriform cortex, striatum, cerebellum, cranial nerve nuclei, and most major white matter pathways. The cellular and subcellular distribution of β-subunit immunoreactivity and the relative intensity of somatodendritic, axonal, and terminal staining varied considerably across brain regions and appeared to be limited to neurons as opposed to glial cells. It is beyond the scope of this paper to provide an exhaustive account of β-subunit immunohistochemistry in every brain region. Therefore, only the major features of the immunohistochemical staining are described below.

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B

Figure 1. Characteristics of the adult rat brain β-subunit polypeptides. *A*, Immunoblot analysis of antibody binding to rat brain membranes. Membranes were electrophoresed on 12.5% SDS-PAGE. Lanes denoted "antiserum" were probed with anti-β-subunit crude antiserum at 1:1000, preincubated with 50 μg/ml BSA alone (*+BSA*), 50 μg/ml β-subunit peptide-BSA conjugate (*+peptide-BSA*), or no addition. Lanes denoted *Affinity Purified* were probed with affinity purified anti-β-subunit polypeptide antibody at 1:20 preincubated as above. Numbers at left of panel denote M_r of prestained molecular weight standards. *B*, Immunoblot analysis of specificity of anti-β-subunit immunoprecipitations. Samples of adult rat brain membranes (*RBM*, 60 μg), and products of immunoprecipitation from a detergent extract of 500 μg RBM with either the β-subunit antibody at 1:200 in the presence of 50 μg/ml peptide-BSA conjugate (*+peptide-BSA*), 50 μg/ml BSA alone (*+BSA*) or with no addition (*no addition*), or without antibody (*no Ab*) were size fractionated on 12.5% SDS-PAGE. After transfer, blots were probed with affinity purified anti-β-subunit antibody at 1:25, and bound antibody detected with ECL/autoradiography for 30 sec. *Arrow* points to mobility of β-subunit polypeptide.
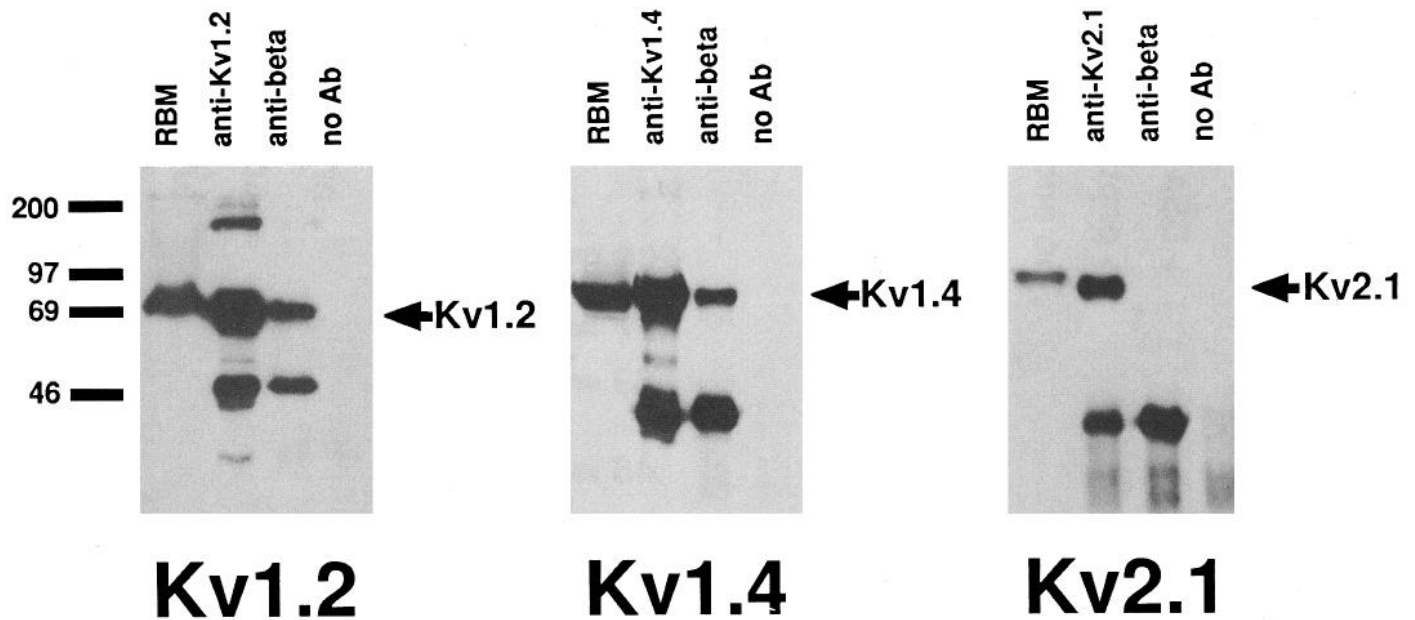
Figure 3. Detection of α-subunit polypeptides in anti-α- and β-subunit immunoprecipitates. Samples of adult rat brain membranes (*RBM*, 60 μg), and aliquots of products of immunoprecipitations from Figure 2 above performed with either the indicated anti-α-subunit antibody, the anti-β-subunit antibody (*anti-beta*), or with no antibody (*no Ab*) were size fractionated by 7.5% SDS-PAGE. Samples were transferred and probed with specific anti-α-subunit antibodies: Kv1.2C antibody at 1:500 (*anti-Kv1.2*), Kv1.4N antibody at 1:2000 (*anti-Kv1.4*), or KC antibody at 1:50 (*anti-Kv2.1*). Arrows at right of each panel denotes the mobility of the specific K⁺ channel α-subunit polypeptide on these gels. *Numbers to the left* denote M_r of prestained molecular weight standards. Bands at ≈50 kDa in each panel are heavy chains of rabbit IgG used for immunoprecipitations, which react with anti-rabbit secondary antibody.

Neocortex and hippocampus. Although immunoreactivity for the β-subunit polypeptides was present within neurons of all neocortical layers, pyramidal cells in layers III and V were the most intensely labeled (Fig. 4A). Within these cells, immunoreactivity was concentrated within the cell body, proximal portions of the basal dendrites, and throughout the entire apical dendritic tree, including fine branches and apical tufts. Staining within apical dendrites was uniformly distributed along the dendritic membrane, and at the level of resolution of the light microscope this staining appeared to be associated with the dendritic shaft (Fig. 5a). Axons in the cortical neuropil, subcortical white matter and corpus callosum contained a moderate density of β-subunit immunoreactivity. The staining in many of these axons was discontinuous, with a higher density of reaction product adjacent to nodes of Ranvier. This pattern of β-subunit immunoreactivity in subcortical white matter corresponded closely to the pattern reported for the Kv1.1, Kv1.2, and Kv1.4 α-subunits (Sheng et al., 1993; Wang et al., 1993, 1994). However, close inspection of staining within individual axons revealed that immunoreactivity for the β-subunit polypeptides was not as clearly confined to juxtaparanodal regions. Rather, immunoreactivity for the β-subunit polypeptides was less restricted and was often observed in the axonal cytoplasm leading up to and extending across the nodes.

In the hippocampal formation, there was a heterogeneous distribution of β-subunit immunoreactivity with moderate to intense staining of granule and pyramidal cells as well as in terminal zones of the mossy fiber projection and perforant path (Fig. 6A). In the dentate gyrus, granule cells and hilar polymorph cells were labeled with moderate intensity, as were scattered interneurons in the molecular layer. There was also a prominent band of immunoreactivity in the middle third of the molecular layer of the dentate gyrus (Fig. 7A). The location of this band

corresponds closely to the termination zone of the medial perforant path (see Rosene and Van Hoesen, 1987). In the CA subfields and subiculum, β-subunit immunoreactivity was concentrated in cell bodies of pyramidal cells and was diffusely distributed throughout the neuropil, with a somewhat greater density in stratum radiatum than in stratum moleculare. In CA3, there was moderate to intense staining in the mossy fiber zone. This staining was concentrated in the apical dendrites of CA3 pyramidal cells and in what appeared to be the mossy fiber terminals. Surprisingly, there was little or no β-subunit immunoreactivity in stratum moleculare of CA1-CA4 or the subiculum.

Basal ganglia and cerebellum. In the dorsal striatum (Fig. 4B), nucleus accumbens, and globus pallidus, there was a moderate density of β-subunit immunoreactivity within neurons of all sizes and morphologies and throughout the neuropil. There was also a moderate density of immunoreactivity in the subcommissural extension of the globus pallidus, and within axons of the internal capsule. Within the substantia nigra, axons, and apparent terminal fields in the pars reticulata were more intensely labeled than in the pars compacta.

In the cerebellum, β-subunit immunoreactivity was concentrated within the cell bodies and throughout the dendrites of Purkinje cells (Fig. 8A) as well as in the cell bodies and axon terminals of basket cells. These basket cell terminals form a characteristic plexus surrounding the initial segment of Purkinje cell axons, and the concentration of β-subunit immunoreactivity in these terminals corresponded closely to immunoreactivity for Kv1.1 and Kv1.2 α-subunits, as described previously (McNamara et al., 1993; Wang et al., 1993, 1994). The cell bodies of basket cells, granule cells, and other local circuit neurons in the cerebellar cortex contained only weak β-subunit immunoreactivity. Numerous fine axons within the granule cell layer also contained β-subunit immunoreactivity as did large neurons within
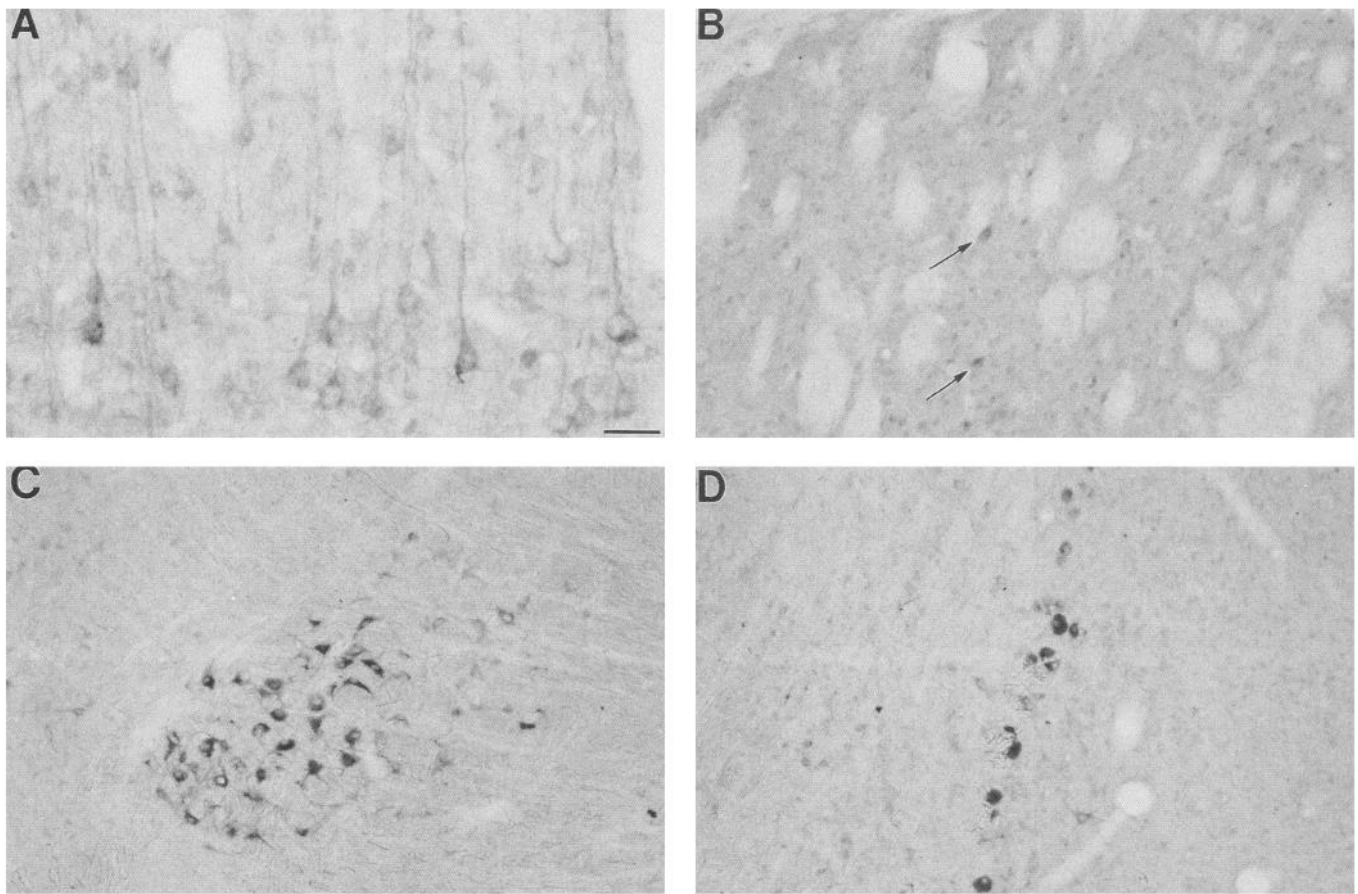
Figure 4. Immunohistochemical localization of β-subunit polypeptides in adult rat brain. Photomicrographs taken to show β-subunit immunoreactivity in neocortical pyramidal cells in layers III–V (*A*), the caudate nucleus (*B*), the red nucleus (*C*, and the mesencephalic nucleus of cranial nerve V (*D*). *Arrows* in *B* point to large striatal interneurons containing intense β-subunit immunoreactivity. Scale bar in *A*, 50 μm.

the deep cerebellar nuclei and axons within the cerebellar peduncles.

Midbrain and brainstem. Large motor and sensory neurons within structures such as the red nucleus (Fig. 4*C*), brainstem cranial nerve nuclei (Fig. 4*D*) as well as midbrain and pontine reticular formation were moderately to intensely labeled by anti-β-subunit antibodies, as were axons within midbrain and brainstem white matter pathways and cranial nerve efferents. As described above, immunoreactivity within axons was not continuous, as there appeared to be a greater density of reaction product adjacent to nodes of Ranvier.

Comparative distribution of β-subunit, Kv1.2, Kv1.4, and Kv2.1 immunoreactivity. To examine the colocalization of β-subunit polypeptides with specific α-subunits, we compared the distribution of β-subunit immunoreactivity with staining for Kv1.2, Kv1.4, and Kv2.1 α-subunits in adjacent sections of adult rat brain. Immunohistochemical staining using our Kv1.2C antibody revealed intense staining in many regions of adult rat brain in a pattern consistent with that reported for Kv1.2 in the mouse (McNamara et al., 1993; Wang et al., 1993, 1994), but somewhat different from that previously reported for Kv1.2 in the rat (Sheng et al., 1994; see below). Immunohistochemical staining with antibodies specific for the Kv1.4, and Kv2.1 α-subunit was indistinguishable from that reported by others (Kv1.4, Sheng et al., 1992, 1993; Kv2.1, Trimmer, 1991; Hwang et al., 1993). As a result, the description that follows does not reca-

pitulate results reported previously by others, but focuses on the areas of apparent co-localization of these α- and β-subunit polypeptides.

Cerebral cortex. Examination of adjacent sections stained for the α- and β-subunit polypeptides indicated that pyramidal cells in layers II, III, and V were strongly immunoreactive for β-subunit polypeptides and Kv2.1, and were less strongly immunoreactive for Kv1.2 and Kv1.4 (Fig. 5A–C). Within layer III and V pyramidal cells, immunoreactivity for β-subunit polypeptides and Kv1.4 was evenly distributed along the membrane of apical dendrites, whereas immunoreactivity for Kv1.2 appeared to be concentrated in small, discrete patches scattered along the apical dendrite (Fig. 5a–c). However, at this level of resolution it was not possible to determine whether the patches of Kv1.2 labeling were located presynaptically on afferent terminals or postsynaptically in the dendritic membrane. Immunoreactivity for the β-subunit polypeptides and Kv1.2 was also concentrated in the neuropil in what appeared to be numerous fine axonal processes (arrows in Fig. 5a,b). This pattern of immunoreactivity was most dense in layers II/III and V.

Although immunoreactivity for Kv2.1 was also concentrated in discrete patches along the membrane of cortical pyramidal cells, these patches were substantially larger than those observed for Kv1.2 and were confined to the cell soma and the proximal portion of apical and basal dendrites (Fig. 5d).

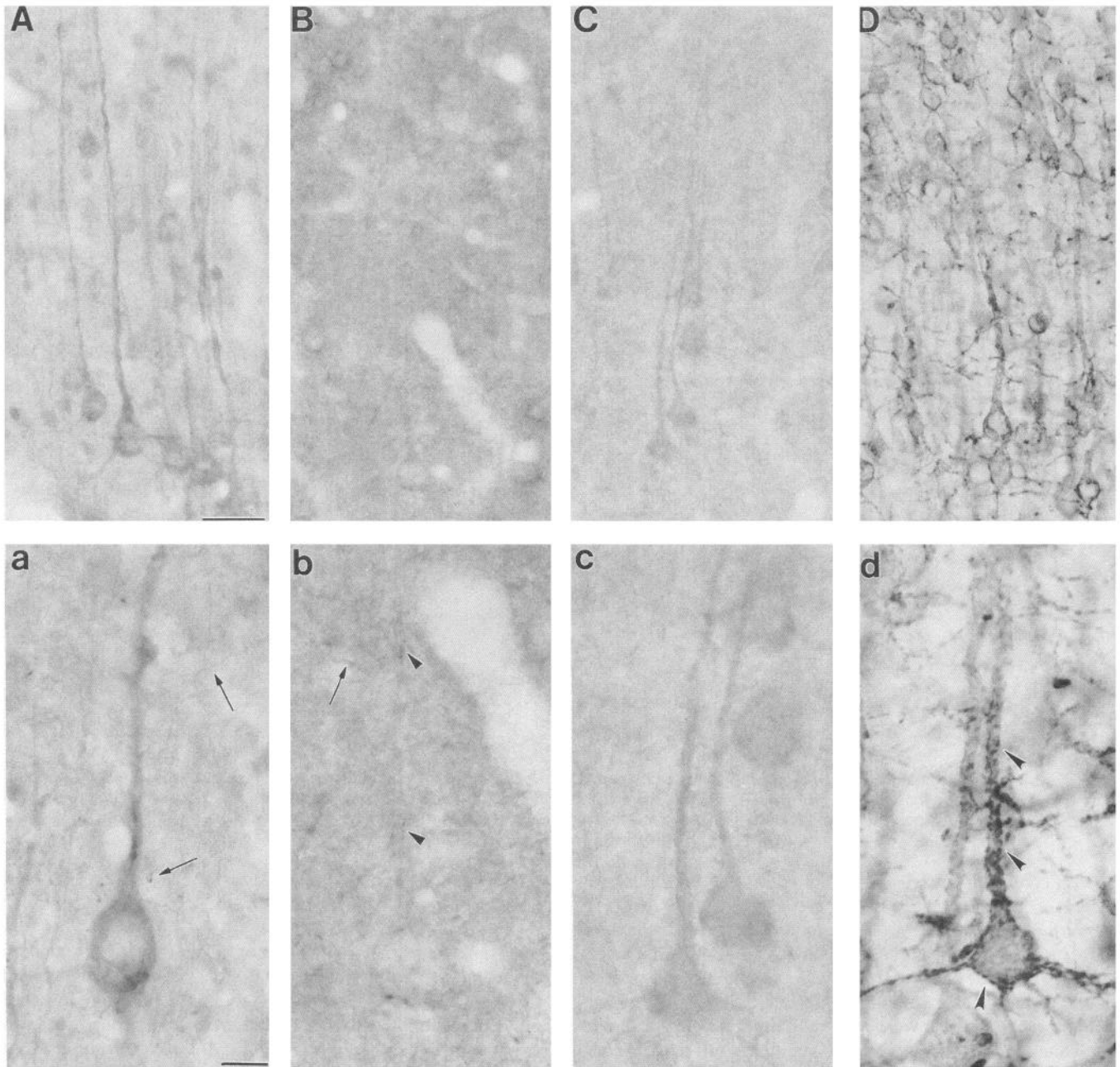*Hippocampal formation.* As in the neocortex, analysis of sec-

Figure 5. Immunohistochemical localization of β-subunit, Kv1.2, Kv1.4 and Kv2.1 immunoreactivity in rat neocortex. Photomicrographs taken to show the distribution of β-subunit, Kv1.2, Kv1.4, and Kv2.1 immunoreactivity (*A–D*, respectively) in layer V pyramidal cells. At higher magnification, immunoreactivity for Kv1.2 (*b*) appears to be concentrated in small, discrete patches in the apical dendrite (*arrowheads*). In addition, immunoreactivity for the β-subunits and Kv1.2 appear to be concentrated in fine axonal processes (*arrows* in *a* and *b*). Note that immunoreactivity for Kv2.1 is also present in discrete patches along the membrane of apical and basal dendrites (*arrowheads* in *d*). However, these patches are considerably larger than those observed for Kv1.2. Scale bars: *A*, 30 μm (for *A–D*); *a*, 10 μm (for *a–d*).

tions taken through the hippocampal formation indicated that in the dentate gyrus, the cell bodies of granule cells were weakly immunoreactive for the β-subunit polypeptides, Kv1.2, and Kv1.4, and were strongly immunoreactive for Kv2.1 (Figs. 6, 7*A–D*). Interestingly, the β-subunit polypeptides and Kv1.4 appeared to be concentrated and colocalized in the mossy fiber pathway, where there was a moderate density of immunoreactivity for β-subunit polypeptides and a high density of immunoreactivity for Kv1.4. In the molecular layer of the dentate gyrus there was a prominent band of immunoreactivity for the

β-subunit polypeptides, Kv1.2 and Kv1.4, but not Kv2.1 (Fig. 7). As mentioned above, the position of this band in the middle one-third of the molecular layer suggests that this staining is associated with terminals of the medial perforant path.

In the CA subfields, the cell bodies of CA1–CA4 pyramidal cells and scattered interneurons contained a moderate intensity of immunoreactivity for the β-subunits, Kv1.2 and Kv1.4, and a high density of immunoreactivity for Kv2.1 (Figs. 6, 7*a–d*). As in neocortical pyramidal cells, immunoreactivity for Kv2.1 was distinct from the other subunits and was concentrated in
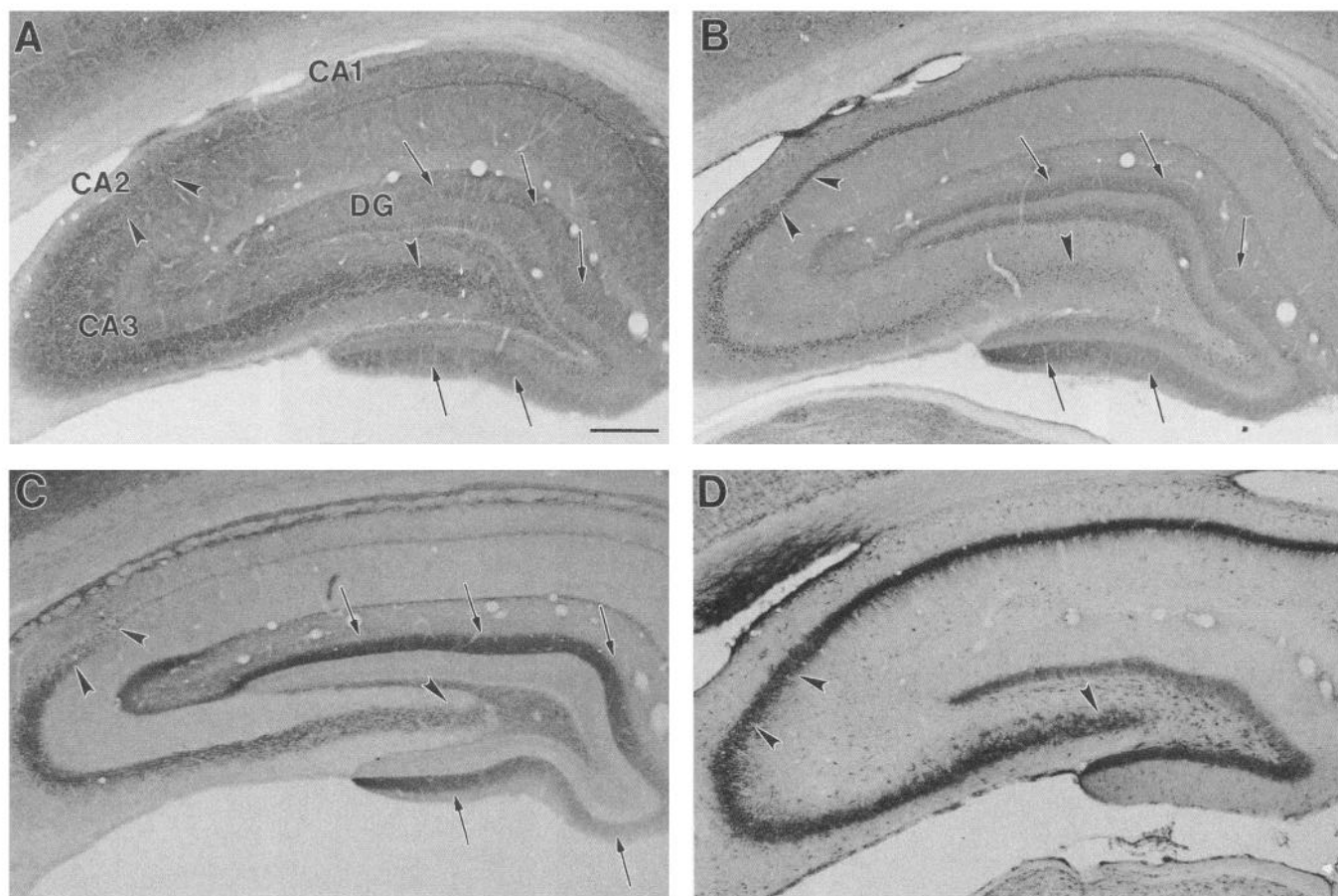
Figure 6. Immunohistochemical localization of β-subunit polypeptides Kv1.2, Kv1.4, and Kv2.1, in the hippocampal formation. *A–D*, Photomicrographs taken to show the distribution of β-subunit, Kv1.2, Kv1.4, and Kv2.1, respectively, in the hippocampal formation. *Arrowheads* mark approximate cytoarchitectonic boundaries. The *arrows* in *A–C* mark the corresponding bands of β-subunit, Kv1.2 and Kv1.4 immunoreactivity in the middle third of the molecular layer of the dentate gyrus (*DG*). Scale bar in *A*, 300 μm.

large, discrete patches along the neuronal membrane. In stratum moleculare of CA1–CA3 there was a prominent band of immunoreactivity for Kv1.2 and Kv1.4 which overlapped with the termination zone of the perforant path. Surprisingly, there was not a similar band of immunoreactivity for the β-subunit polypeptides. The comparatively lower density of β-subunit immunoreactivity in stratum moleculare of these CA subfields raises the intriguing possibility that the composition of K$^+$ channels is distinct within different segments of the perforant path; these β-subunit polypeptides are present in terminals located in the molecular layer of the dentate gyrus, but not in terminals located in CA1–CA3.

Cerebellum and brainstem. In the cerebellum, many neurons contained immunoreactivity for the β-subunit polypeptides, Kv1.2, Kv1.4, and Kv2.1. The most striking feature of the immunohistochemical staining in the cerebellum was the presence of immunoreactivity for the β-subunit polypeptides and Kv1.2 in the cerebellar basket cell terminals which surround the initial segment of Purkinje cell axons (Fig. 8*B*). We did not observe immunoreactivity for Kv1.4 or Kv2.1 in these basket cell terminals.

Although there was clear overlap and apparent colocalization of Kv1.4 and β-subunit polypeptides in some regions as described above, there were also several regions that contained intense immunoreactivity for Kv1.4, but no corresponding den-

sity of immunoreactivity for the β-subunits (see Discussion). These regions may either lack β-subunit polypeptides or contain β-subunits that are immunologically distinct from Kvβ1 and Kvβ2. Moreover, these regions may either express K$^+$ channel complexes composed of Kv1.4 homomultimers, or Kv1.4 in association with another, as yet undetermined, α-subunit.

In several brain regions, including the hippocampus, cerebral cortex, and cerebellum, the pattern of immunoreactivity observed using our Kv1.2C antibody differed somewhat from that reported previously by Sheng et al. (1994). For example, we did not observe staining throughout the entire apical dendritic tree of layer V pyramidal cells as reported by Sheng et al. (see Fig. 5 of Sheng et al., 1994) or in hippocampal mossy fiber terminals (see Fig. 3 of Sheng et al., 1994). In the cerebellum, we observed staining in the somata and proximal dendrites of cerebellar basket cells (Fig. 8*B*) in addition to staining of basket cell terminals, but Sheng et al. did not report staining of basket cell somata by their antibody. In general, the results obtained using our Kv1.2 antibody in the rat are more similar to those of Wang et al. (1994), who used polyclonal antibodies against the C-terminus of the mouse Kv1.2 protein for immunohistochemical studies of mouse brain. Moreover, our data more closely match the pattern of ^{125}I-α-DTX binding sites visualized by quantitative autoradiography (Awan and Dolly, 1991). One possible explanation for the discrepancies between our findings and those of
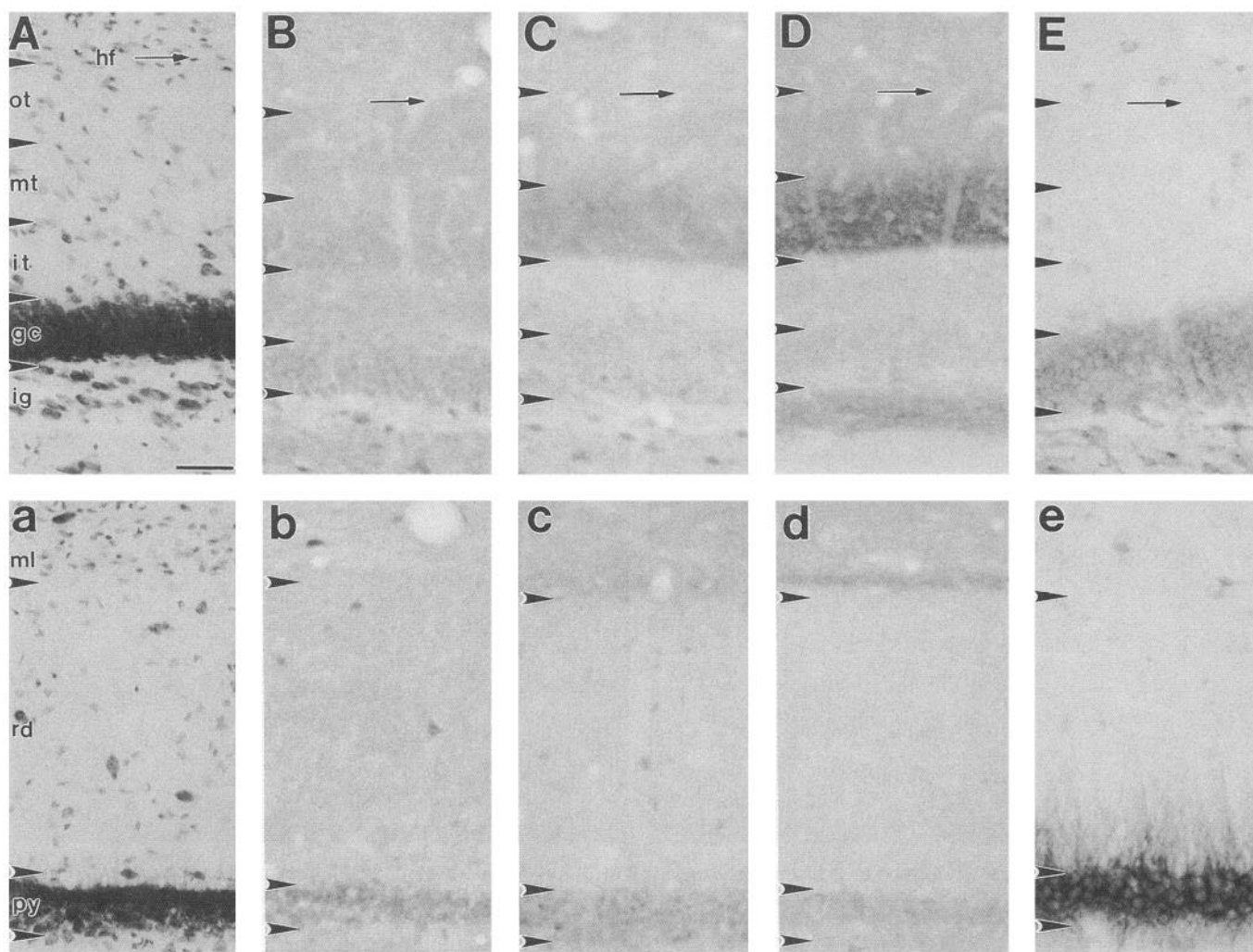
Figure 7. Immunohistochemical localization of β-subunits, Kv1.2, Kv1.4, and Kv2.1 in the dentate gyrus and CA1 subfield. Photomicrographs taken to show the cytoarchitecture (Nissl stain, *A*) as well as the laminar distribution of β-subunit, Kv1.2, Kv1.4, and Kv2.1 immunoreactivity in the dentate gyrus (*B–E*, respectively). Note that there is a greater density of β-subunit, Kv1.2 and Kv1.4, but not Kv2.1, immunoreactivity in the middle third (*mt*) of the molecular layer as compared to the inner third (*it*) or outer third (*ot*). Photomicrographs taken to show the cytoarchitecture (Nissl stain; *a*) and laminar distribution of β-subunit, Kv1.2, Kv1.4, and Kv2.1 immunoreactivity in the CA1 subfield (*b–e*, respectively). Note that there is a band of intense immunoreactivity for Kv1.2 and Kv1.4 in stratum moleculare (*ml*) that is not matched by a similarly intense band of immunoreactivity for the β-subunit polypeptides. Abbreviations: *ig*, infragranular zone; *hf*, hippocampal fissure; *gc*, granule cells; *py*, stratum pyramidale; *rd*, stratum radiatum. Scale bars in *A* and *a*, 50 μm.

Sheng et al. (1994) is a slight difference in the Kv1.2 C-terminal peptide sequence used in our immunizations, which excluded a cysteine residue contained within the peptide use by Sheng et al. (1994). Exclusion of this cysteine may have led to more uniform coupling of the synthetic peptide to the KLH carrier protein.

Discussion

Antibodies raised against the cloned bovine voltage-gated K$^+$ channel β-subunit polypeptide were used to determine the biochemical characteristics, spatial expression patterns, and the extent of α-subunit polypeptide interaction of the corresponding β-subunit polypeptides in rat brain. We observed immunoreactive 38 kDa and 41 kDa β-subunit polypeptide species; of these the 38 kDa β-subunit polypeptide is far more abundant. Results from reciprocal immunoprecipitation reactions indicate that the Kv1.2 and Kv1.4, but not Kv2.1, α-subunit polypeptides asso-

ciate with these β-subunits in rat brain membranes. Immunohistochemical localization of these K$^+$ channel α- and β-subunits reveals consistent results, in that β-subunit immunoreactivity corresponds closely with immunoreactivity for Kv1.2, and to a lesser extent Kv1.4. Although many neurons contained immunoreactivity for the β-subunit polypeptides and Kv2.1, the subcellular distribution of β-subunit polypeptides and Kv2.1 was distinct. More specifically, Kv2.1 was concentrated in discrete patches associated with neuronal somata and proximal dendrites, and the β-subunit polypeptides were diffusely distributed in the neuronal cytoplasm and evenly distributed along the neuronal membrane. Taken together, these biochemical and immunohistochemical analyses point to interactions between specific rat brain K$^+$ channel α- and β-subunits.

On immunoblots, the immunoreactive adult rat brain β-subunit polypeptide pool is virtually identical in both electrophoretic mobility and stoichiometry to that obtained from SDS-PAGE
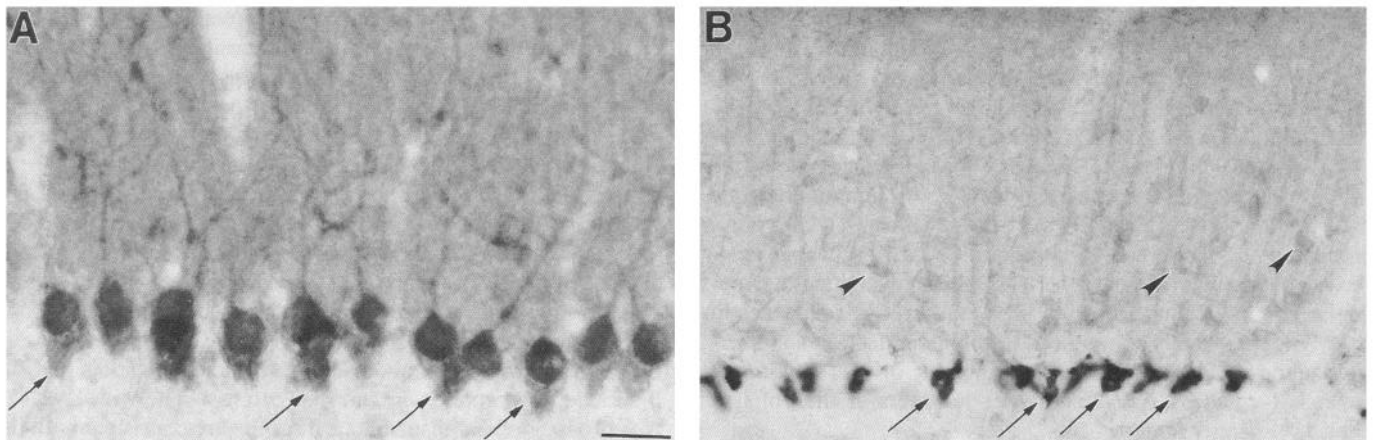
Figure 8. Immunohistochemical localization of β-subunits polypeptides and Kv1.2 in the cerebellar cortex. Photomicrographs taken to show the correspondence between β-subunit and Kv1.2 immunoreactivity in the cerebellar cortex. Note that the β-subunit polypeptides and Kv1.2 are concentrated in the cell bodies (*arrowheads* in *B*) and terminals (*arrows* in *A* and *B*) of cerebellar basket cells. Scale bar in *A*, 30 μm.

analysis of the β-subunit pool associated with purified bovine brain DTX acceptor K⁺ channel complexes (Scott et al., 1994b). This suggests that the relative stoichiometry of the 41 kDa and 38 kDa subunits in these purified bovine channel preparations is not an artifact of the extensive purification procedures, but represents the authentic stoichiometry of these polypeptides in adult brain. Scott et al. (1994b) reported that sequencing of proteolytic fragments generated from these purified bovine β-subunit preparations yielded nine peptide sequences: eight of these nine sequences are present in the deduced amino acid sequence of the cloned bovine β-subunit cDNA. Subsequent cloning of β-subunit cDNAs from rat brain (Rettig et al., 1994) showed the existence of a rat homolog of the bovine β-subunit (designated rat Kvβ2), and what is predicted from the cDNA sequence to be a slightly larger, distinct β-subunit termed Kvβ1. Surprisingly, the ninth peptide sequence obtained from the purified bovine DTX acceptor was present in the deduced Kvβ1 sequence, showing that both Kvβ1 and Kvβ2 are components of the purified DTX acceptor complex. Assuming that the relative yield of sequence data is proportional to the relative abundance of the Kvβ1 and Kvβ2 polypeptides in the DTX acceptor complex, it would be plausible to assign the minor component of 41 kDa as Kvβ1, and the major 38 kDa component as Kvβ2. Consistent with this designation is the larger size (by about 3 kDa) of the deduced Kvβ1 polypeptide relative to Kvβ2 (Rettig et al., 1994). It is interesting to note that coexpressed Kvβ1, but not Kvβ2, is a potent modulator of the inactivation gating properties of expressed Kv1.1 and Kv1.4 α-subunits (Rettig et al., 1994). Thus, it is possible that a function has been assigned for the relatively minor 41 kDa component of the DTX acceptor complex (Kvβ1), while the function of the major 38 kDa component (Kvβ2) remains unresolved. Recent expression in our laboratory of the cloned Kvβ1 and Kvβ2 polypeptides by transient transfection in COS-1 cells supports this model, in that the expressed Kvβ2 polypeptide comigrates with the rat brain 38 kDa β-subunit, while the expressed Kvβ1 polypeptide has mobility similar to, but slightly larger than, the rat brain 41 kDa β-subunit (K. Nakahira, G. Shi, and J. S. Trimmer, unpublished observations). However, the specific correlation of the 38 kDa β-subunit polypeptide to Kvβ2, and the 41 kDa β-subunit polypeptide to Kvβ1, will require future studies utilizing antibodies that can

differentiate between these two gene products (e.g., generated against the novel amino termini of Kvβ1 and Kvβ2), or direct microsequencing of the N-termini of individually purified 38 and 41 kDa immunoreactive proteins from rat brain.

Coimmunoprecipitation and immunohistochemistry indicate that these β-subunits associate in complexes with Kv1.2 and Kv1.4, but not Kv2.1, α-subunit polypeptides. Purified DTX acceptors from bovine brain have been shown to contain (in order of relative abundance) Kv1.2, Kv1.1, Kv1.6, and Kv1.4 (Scott et al., 1994a). As our β-subunit-specific antibody was raised to a sequence derived from a component of purified DTX acceptors, we predicted that the Kv1.2 and Kv1.4 α-subunit polypeptide components of this complex would be coimmunoprecipitated by this antibody. Our results also show that the β-subunit antibody does coprecipitate both Kv1.2 and Kv1.4. However, this antibody more efficiently coprecipitates Kv1.2 than Kv1.4, indicating that relative to Kv1.4, a greater proportion of the total rat brain Kv1.2 pool is associated with these β-subunit polypeptides. Scott et al. (1994a) determined that most of the bovine brain DTX acceptors contained Kv1.2 (83%), whereas only a small fraction (8%) contained Kv1.4. These data corroborate our immunohistochemical findings, which indicate that the overlap of Kv1.2 and β-subunit immunoreactivity is more extensive than the overlap of Kv1.4 and β-subunit immunoreactivity. Moreover, comparison of autoradiograms of ¹²⁵I-α-DTX binding in the hippocampus and cerebellum (Awan and Dolly, 1991) with immunohistochemical visualization of β-subunit polypeptides, Kv1.2 and Kv1.4 in these brain regions indicates that there is a closer correspondence between the distribution of ¹²⁵I-α-DTX binding, β-subunits and Kv1.2 than these same markers and Kv1.4. Taken together, these results suggest that in these β-subunit polypeptides may be preferentially associated with the majority of the Kv1.2-containing channel complexes, and with the relatively small fraction of the total Kv1.4 pool found in heteromultimeric DTX acceptor complexes. The remaining Kv1.4 pool would not be predicted to be associated with either DTX acceptor complexes or these β-subunit polypeptides.

The lack of Kv2.1 α-subunit polypeptides in these immunoprecipitates implies that this α-subunit polypeptide is present in complexes lacking β-subunits, or associates with auxiliary subunits immunologically distinct from the DTX acceptor β-subunit

polypeptide. We have previously identified a 38 kDa polypeptide associated with Kv2.1 by coimmunoprecipitation (Trimmer, 1991). Immunoprecipitation reactions performed under identical conditions do not yield any polypeptide species recognized by our β-subunit antibody, nor does our β-subunit antibody coprecipitate Kv2.1 complexes. These data strongly suggest that the 38 kDa polypeptide associated with Kv2.1 is immunologically distinct from the Kvβ1 and Kvβ2-related 38 and 41 kDa polypeptides. Purification and characterization of K⁺ channel complexes containing these and other β-subunit polypeptides, or molecular cloning studies using the Kvβ1 and Kvβ2 sequences to identify related cDNAs, may lead to the identification and characterization of auxiliary subunits important in the function of channels formed by Kv2.1 and other members of the voltage-gated K⁺ channel family.

Immunohistochemical analysis of β-subunit polypeptides indicates that these polypeptides are present in diverse neuronal types, and are concentrated in multiple subcellular domains, including somata, dendrites, axons, and axon terminals. Comparison of the immunohistochemical staining patterns for the β-subunit polypeptides as well as the Kv1.2, Kv1.4, and Kv2.1 α-subunits confirmed and extended the results of the immunoprecipitation analyses, and indicated that in adult rat brain the β-subunit polypeptides are likely to associate with the Kv1.2 and Kv1.4, but not the Kv2.1, α-subunits. Moreover, the immunohistochemical findings suggest that there is an extensive overlap in the distribution of these β-subunit polypeptides and Kv1.2, and less of an overlap with Kv1.4. As described above, the close correspondence between the immunohistochemical distribution of the β-subunit polypeptides and Kv1.2 is not surprising in view of the association of these polypeptides in the DTX acceptor complex purified from several regions of bovine brain (Scott et al., 1994a). Nonetheless, our biochemical and immunohistochemical data strongly suggest that the majority of K⁺ channel complexes containing the Kv1.2 α-subunit also contain one or more of these β-subunit polypeptides. However, some regions contained immunoreactivity for the β-subunits but no matching pattern of immunoreactivity for Kv1.2. One example is in the CA3 subfield of the hippocampus, where immunoreactivity for the β-subunit polypeptides was present in the mossy fiber zone, but we and others (Wang et al., 1994) have not observed a similar pattern of Kv1.2 immunoreactivity in this region. Thus, in the mossy fiber zone these β-subunit polypeptides may be associated with other α-subunits, for example, Kv1.4 (Sheng et al., 1992, 1993). A second example is in neocortical pyramidal cells, where immunoreactivity for the β-subunit polypeptides was more heavily concentrated than Kv1.2 in the cell soma and distal regions of the dendritic tree. In these cells, the pattern of β-subunit immunoreactivity more closely resembled Kv1.1 (Wang et al., 1994) than Kv1.2, suggesting that in the dendritic compartment of cortical pyramidal cells these β-subunit polypeptides are found in K⁺ channel complexes containing a comparatively greater amount of Kv1.1 than Kv1.2.

In agreement with data obtained from the reciprocal immunoprecipitations, the immunohistochemical staining also indicates that the β-subunit and Kv1.4 immunoreactivity corresponded closely in only a limited number of brain regions. For example, in the middle third of the molecular layer of the dentate gyrus there was good agreement between the patterns of β-subunit and Kv1.4 immunoreactivity. However, several brain regions that contain particularly intense immunoreactivity for Kv1.4 lacked a correspondingly high density of immunoreactivity for the β-subunit polypeptides. For example, in the substantia nigra pars reticulata there was a high density of immunoreactivity for Kv1.4, but there was not a correspondingly high density of immunoreactivity for the β-subunit polypeptides. The existence of areas where Kv1.4 and the β-subunit polypeptides do not overlap supports the biochemical observations reported here, and the findings implicit in the data of Scott et al. (1994a), that K⁺ channel complexes containing Kv1.4 exist in brain that either lack β-subunits, or contain β-subunits that are immunologically distinct from Kvβ1 and Kvβ2.

Although there is extensive overlap between the β-subunit polypeptides and Kv2.1, as described above, the subcellular distribution of these proteins is quite distinct, indicating that the β-subunit polypeptides are not likely to associate with Kv2.1 in vivo. These immunohistochemical results are consistent with the results of immunoprecipitation reactions using β-subunit and Kv2.1-specific antibodies, which indicate that these β-subunit polypeptides are not present in K⁺ channel complexes containing Kv2.1. Taken together, the biochemical and anatomical data suggest that neuronal mechanisms may exist to direct the selective interaction of β-subunits with members of a specific K⁺ channel gene family. Hydropathy analyses of the deduced sequences of Kvβ1 and Kvβ2 have led to the proposal that these polypeptides exist as peripheral membrane proteins (Rettig et al., 1994), and would thus be predicted to interact with cytoplasmic domains of K⁺ channel α-subunit polypeptides. Future structure–function analyses of K⁺ channel α- and β-subunit polypeptides will allow for the identification of peptide segments involved in mediating K⁺ channel α/β subunit interactions.

Wang et al. (1993, 1994) recently demonstrated that the Kv1.1 α-subunit is colocalized with Kv1.2 in several areas of mouse brain, including the apical dendrites of cortical pyramidal cells, the termination zone of the medial perforant path, and in cerebellar basket cell terminals. Assuming that the distribution of Kv1.1 is similar in rat and mouse, it appears likely that K⁺ channel complexes in neocortical pyramidal cells and cerebellar basket cell terminals are composed of a combination of Kv1.1, Kv1.2 as well as the β-subunit polypeptides, and that K⁺ channel complexes in the terminals of the medial perforant path contain the Kv1.1, Kv1.2, and Kv1.4 α-subunits as well as the β-subunit polypeptides. It remains to be determined whether the Kv1.6 α-subunit, which is also a component of the bovine DTX acceptor complex, is present in the same neuronal compartments that contain the β-subunits, Kv1.1, Kv1.2, or Kv1.4. Nonetheless, the codistribution of Kv1.1 and Kv1.2 in cerebellar basket cell terminals, and Kv1.1, Kv1.2, and Kv1.4 in perforant path terminals, suggests that the electrophysiological properties of the expressed K⁺ channels are distinct in these two systems.

The localization of individual K⁺ channel subunits to multiple neuronal types and subcellular domains raises the intriguing question of how these proteins are targeted within the neuron and whether K⁺ channel complexes of specific α- and β-subunit composition are preferentially located in certain subcellular domains or associated with neurons of a specific neurotransmitter phenotype. The presence of multiple, functionally distinct α- and β-subunit polypeptides within the same neuron, axon, and nerve terminal is likely to contribute to the enormous functional diversity observed electrophysiologically for voltage-gated K⁺ channels in situ. The observed subunit selectivity and cell-specific heterogeneity of α/β subunit interaction also suggests a novel mechanism whereby neuronal excitability can be regulated at the molecular level.

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