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Immunological identification and characterization of a delayed rectifier K^+ channel polypeptide in rat brain

(excitability/mammalian central nervous system/ion-channel molecular biology/tissue-specific gene expression)

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ABSTRACT **Antibodies specific for the drk1 polypeptide were used to characterize the corresponding protein in rat brain. Recombinant and synthetic immunogens containing fragments of the drk1 polypeptide were produced. Antibodies raised to these immunogens display monospecific reactions with the same 130-kDa polypeptide on immunoblots of adult rat brain membranes. Immunoprecipitation of ^{125}I-labeled brain membranes identifies a 38-kDa peptide in tight association with the drk1 polypeptide. Immunohistochemical staining of sections of adult rat cortex shows that drk1 protein is restricted to neurons, where staining is present on dendrites and cell bodies but not on axons. These studies point to the value of such immunological reagents to the further characterization of the components of this delayed rectifier K^+ channel in the mammalian central nervous system.**

Voltage-sensitive K^+ channels are fundamental components in the control of excitability in neurons. Studies in a number of mammalian central neurons have identified delayed rectifier K^+ currents (I_K; ref. 1). I_K has been seen in >12 mammalian central nervous system preparations (2), and some neurons contain more than one type of delayed outward current. Whether this diversity represents different states of a single polypeptide or different gene products is unknown. The role of I_K in the mammalian central nervous system has been difficult to define; no compound is selective in blocking this conductance, and, thus, the ability to determine the behavior of neurons after blocking I_K has not been feasible (3).

Recently, a number of cDNAs have been isolated from rat brain that, when expressed in *Xenopus* oocytes from *in vitro*-transcribed mRNAs, produce I_K-like currents (4–9). Among these *in vitro* transcription products, those from the drk1 cDNA template produce currents in oocytes that most closely resemble neuronal I_K in voltage-dependence and kinetics of activation, pharmacological characteristics, and lack of inactivation over tens of milliseconds (6). Thus, probes derived from the drk1 cDNA could be of great use in characterizing components of neuronal I_K.

Biochemical characterization of K^+ channel proteins has lagged behind similar studies of voltage-sensitive Na^+ and Ca^{2+} channels. This fact is primarily due to the lack of naturally occurring high-affinity toxins for most K^+ channel classes; this situation is especially true for neuronal I_K. The generation of antibodies to immunogens derived from cloned K^+ channel cDNAs provides an approach to characterize biochemical properties of the corresponding proteins in the absence of purified material. This report describes the use of such immunochemical reagents to begin to characterize the drk1 polypeptide in adult rat brain.

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MATERIALS AND METHODS

Materials. ^{125}I-labeled protein A was from ICN, and Na^{125}I was from NEN. All other reagents were molecular biology grade from Sigma or Schwarz/Mann.

Production of Fusion Proteins and Antibodies. An 85-base-pair (bp) insert of a drk1 cDNA (pMK4; J.S.T., unpublished work) was ligated into the vector pGEX-2 (25) to create pGEXdrk1, containing amino acids 516–533 of the drk1 polypeptide, which was used to immunize rabbits for production of antisera.

A synthetic peptide, KC, corresponding to the C terminus (amino acids 837–853) of the drk1 polypeptide, was conjugated to keyhole limpet hemocyanin and injected into rabbits for production of antisera. A recombinant pGEX fusion protein corresponding to the C terminus of drk1 polypeptide was also constructed as follows. The PCR was used to amplify a genomic fragment of drk1-coding region corresponding to nucleotides 1537–2590. A 126-bp *Hin*cII fragment (nucleotides 2465–2590, polylinker *Hin*cII site) was ligated into pGEX-2 to create pGEXKC.

Anti-pGEXdrk1, anti-glutathione-*S*-transferase protein from *Schistosoma japonicum* (*sj*26), and anti-pGEXKC antibodies were affinity-purified from crude antisera on nitrocellulose strips containing 1 mg of purified pGEX protein as described (10).

Brain Membrane Preparations. A crude synaptosomal membrane fraction was prepared from freshly dissected adult rat brains by homogenization in 0.3 M sucrose/10 mM sodium phosphate, pH 7.4/1 mM EDTA containing 1 mM phenylmethylsulfonyl fluoride, leupeptin at 1 μg/ml, aprotinin at 2 μg/ml, and pepstatin at 1 μg/ml. This homogenate was centrifuged at 3000 × *g* for 10 min to remove nuclei and debris. The supernatant was centrifuged at 45,000 × *g* for 90 min to pellet the crude membranes. Membranes were suspended in the homogenization buffer, and protein was determined using the BCA method (Pierce).

SDS/Polyacrylamide Gels and Immunoblotting. Membranes were size fractionated on 9% polyacrylamide gels (11) and transferred to nitrocellulose membranes as described (12). After transfer, nonspecific protein-binding sites were blocked by incubation in 5% (wt/vol) Carnation nonfat dry milk/0.15 M NaCl/20 mM Tris, pH 7.5 (Blotto; ref. 13), for 1 hr at room temperature. Antisera or affinity-purified antibodies diluted 1:100 in Blotto were incubated with the blocked membranes for 2 hr at room temperature. After washes in Blotto, membranes were incubated in ^{125}I-labeled protein A at 0.5 μCi/ml (1 Ci = 37 GBq) for 1 hr at room temperature, followed by three washes in 0.15 M NaCl/10 mM Tris, pH 7.5, and autoradiography.

Abbreviations: I_K, delayed rectifier K^+ current; *sj*26, glutathione-*S*-transferase protein from *Schistosoma japonicum*.; KC, synthetic peptide corresponding to the C terminus (amino acids 837–853) of the drk1 polypeptide.

For blocking experiments, antisera or affinity-purified antibodies were preincubated overnight at 4°C in 1.5 ml of Blotto with fusion protein or *sj*26 parent protein at 35 μg/ml. Before use, the samples were brought to a final dilution of 1:100 with Blotto.

***In Vitro* Transcription and Translation.** Transcription of RNA from the pDRK1/SK- (a full-length drk1 cDNA provided by R. Joho, Baylor School of Medicine; ref. 5) template using T7 RNA polymerase was as described (14). *In vitro* translation in rabbit reticulocyte lysates was done exactly as instructed by supplier (Promega), except that 0.1% Triton X-100 was present in the reaction mixture.

Iodination and Immunoprecipitation. Three milligrams of rat brain membranes was solubilized in lysis buffer (0.5% Nonidet P-40/0.15 M NaCl/1 mM EDTA/10 mM Tris·HCl, pH 8.0) containing protease inhibitors for 1 hr at 4°C, followed by centrifugation at 40,000 × *g* for 1 hr. The resultant supernatant was either used directly for immunoprecipitation (unlabeled lysate, see Fig. 4) or radioiodinated with 1.25 mCi of $Na^{125}I$ for 5 min at 4°C using one Iodo-Bead (Pierce). After removing the Iodo-Bead to stop the reaction, the sample was applied to a Sephadex G-10 spin column to remove unincorporated $Na^{125}I$. A volume of the resultant iodinated lysate corresponding to 2×10^6 cpm (≈30 μg of protein) was used for each immunoprecipitation reaction.

"Cold" immunoprecipitates were done by using equivalent amounts of unlabeled lysate (≈30 μg of protein). Samples were diluted to 0.9 ml with lysis buffer and precleared by incubation with fixed *Staphylococcus aureus* (Pansorbin, Calbiochem) for 30 min, followed by centrifugation at 10,000 × *g* for 30 min. Various volumes of antiserum or preimmune serum were added, and the volume was adjusted to 1 ml with lysis buffer. Samples were incubated for 1 hr at 4°C, followed by addition of fixed *S. aureus*, further incubation for 1 hr, and centrifugation at 10,000 × *g* for 1 min; the pellets were then washed five times with lysis buffer. Final pellets were resuspended in reducing sample buffer, electrophoresed on SDS/9% PAGE, and autoradiographed (for iodinated samples) or immunoblotted (for unlabeled samples).

Immunohistochemical Procedures. Deeply anesthetized animals were perfused transcardially with 0.1 M phosphate-buffered saline, pH 7.4, followed by 4% (wt/vol) paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. Brains were dissected free, postfixed in paraformaldehyde for 24 hr, and cryoprotected in 30% sucrose/0.1 M phosphate buffer before cryostat sectioning at 40 μm.

Free-floating sections were treated with affinity-purified primary antibodies at established dilutions with 2% Triton X-100 overnight and with horseradish peroxidase-conjugated goat anti-rabbit IgG at established dilutions for 2–4 hr. Secondary antibodies were visualized with 3,3′-diaminobenzidine hydrochloride (0.03%) with H_2O_2 (0.003%). Sections were mounted on gelatinized slides, dehydrated, cleared, and coverslipped.

RESULTS

Identification of the drk1 Polypeptide. Polyclonal antisera were produced by immunizing rabbits with two drk1 immunogens, pGEXdrk1 (amino acids 507–533) and KC (amino acids 837–853). Regions of the C-terminal cytoplasmic tail were chosen to increase the probability that drk1 sequences would project from the fusion or carrier proteins as they do from drk1 polypeptide. Both antisera display monospecific reactions on immunoblots of crude adult rat brain membranes with a somewhat diffuse band of 130 kDa (Fig. 1*a*, lanes A and C). Neither preimmune serum exhibited any reaction to adult rat brain membranes (Fig. 1*a*, lanes B and D).

In vitro translation of drk1 cRNA yields a 95-kDa polypeptide, showing that the increased size of rat brain drk1

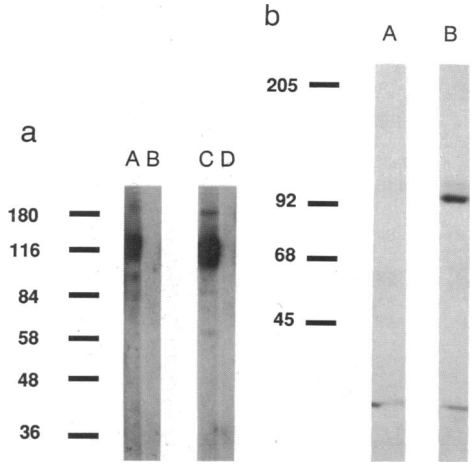

FIG. 1. Characteristics of adult rat brain drk1 polypeptide. (*a*) Immunoblot analysis of adult rat brain membranes with anti-drk1 antisera. Autoradiograms of rat brain membrane samples (75 μg) size-fractionated by SDS/9% PAGE, transferred to nitrocellulose, and treated with 1:100 dilutions of immune (lanes A and C) and preimmune (lanes B and D) sera from rabbits injected with the pGEXdrk1 (lanes A and B) and KC (lanes C and D) immunogens. Strips were then incubated with ^{125}I-labeled protein A and autoradiographed for 18 hr. (*b*) Autoradiogram of rabbit reticulocyte lysate translation products fractionated on SDS/9% PAGE. The drk1 polypeptide translated *in vitro* has a molecular mass of ≈95 kDa. Lanes contain translation reactions with no RNA addition (lane A) and with addition of 10 ng of *in vitro*-transcribed drk1 mRNA (lane B). Numbers at left denote molecular weight of ^{14}C-methylated molecular mass standards (in kDa).

polypeptide (130 kDa) is not from anomalous migration of the core polypeptide (predicted size, 95.3 kDa; ref. 6) on SDS gels (Fig. 1*b*). The molecular mass of the brain drk1 polypeptide was ≈130 kDa on SDS gels of different acrylamide concentrations whether SDS gels were run under reducing or nonreducing conditions (data not shown).

Preincubation of KC antiserum with the pGEXKC fusion protein resulted in a loss of reactivity to the 130-kDa brain polypeptide (Fig. 2, lane B). Reactions to higher-molecular-mass bands (≈160 and 180 kDa) were not affected, indicating that these polypeptides are not related to drk1. The *sj*26 parent protein (pGEX-2) had no effect on KC reactivity to the 130-kDa drk1 polypeptide (Fig. 2, lane C). Anti-drk1 antibodies in KC serum were affinity-purified against pGEXKC fusion protein (10). These antibodies retain their reactivity to the 130-kDa drk1 polypeptide but lose their reaction to the 160- and 180-kDa bands (Fig. 2, lane D). That the anti-130-kDa immunoreactivity of this anti-peptide serum is retained after affinity purification against a fusion protein containing fragments of drk1 cDNA supports the conclusion that the 130-kDa band represents endogenous rat brain drk1 polypeptide. The reaction of the affinity-purified KC antibodies to the 130-kDa drk1 polypeptide is blocked by pGEXKC fusion protein (Fig. 1, lane E) but not by the pGEX parent protein (Fig. 2, lane F).

Similarly, reaction of pGEXdrk1 serum to the 130-kDa brain polypeptide was blocked by pGEXdrk1 fusion protein, whereas *sj*26 had no effect (data not shown). Reciprocal adsorption of pGEXdrk1 to pGEXKC protein, and *vice versa*, had no effect on immunoreactivity, showing that the competition for binding seen above was specific to the region of drk1 polypeptide used as the immunogen (data not shown).

Fig. 3 shows that antibodies affinity-purified by binding to immobilized pGEXdrk1 fusion protein recognize both the 130-kDa drk1 polypeptide in adult rat brain membranes and *sj*26 on immunoblots. However, the antibodies affinity-purified by binding to immobilized *sj*26 parent protein rec-

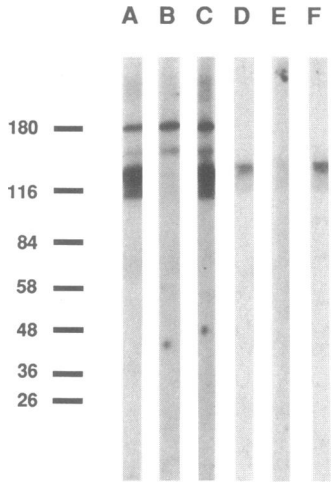

FIG. 2. Preincubation with soluble fusion protein blocks antibody binding to the 130-kDa drk1 polypeptide. Immunoblot analysis of antibody binding to rat brain membranes electrophoresed on SDS/9% PAGE. Lanes A–C: KC serum at 1:100 preincubated with no addition (lane A), 50 μg of pGEXKC fusion protein (lane B), and 50 μg of pGEX-2 parent protein (lane C); D–F, Affinity-purified KC IgG at 1:100 preincubated with no addition (lane D), 50 μg of pGEXKC fusion protein (lane E), and 50 μg of pGEX-2 parent protein (lane F). Numbers at left denote relative molecular mass of prestained standard proteins.

ognize only *sj*26 and do not display reactivity to any component of the rat brain membrane preparation on these immunoblots (Fig. 3, lane B). This result shows conclusively that the specific reaction to the 130-kDa protein in brain membranes is from antibodies in the serum that recognize the part of the fusion protein derived from drk1 polypeptide and not to antibodies in the serum that recognize *sj*26 parent protein.

To determine whether pGEXdrk1 and KC antibodies were recognizing the same 130-kDa drk1 antigen in rat brain membranes, immunoprecipitation with the KC antibodies

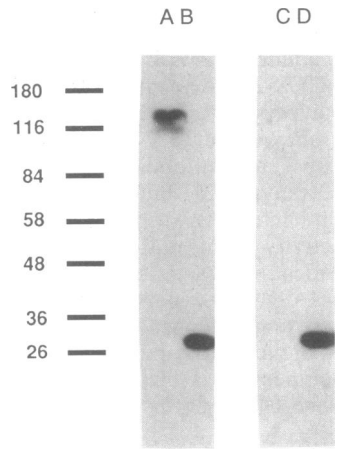

FIG. 3. Antibodies affinity-purified on expressed drk1 fragments recognize brain 130-kDa drk1 polypeptide. Duplicate samples of adult rat brain membranes (75 μg, lanes A and C) or purified *sj*26 protein (5 μg; lanes B and D) were size-fractionated on SDS/12.5% PAGE and transferred to nitrocellulose. Nitrocellulose strips were incubated in a 1:100 dilution of antibodies affinity-purified from pGEXdrk1 serum by binding to pGEXdrk1 (lanes A and B) or in a 1:100 dilution of antibodies affinity-purified from pGEXdrk1 serum by binding to the *sj*26 parent protein (lanes C and D). Strips were then incubated with ^{125}I-labeled protein A and autoradiographed for 18 hr. Numbers at left denote relative molecular mass of prestained standard proteins.

was done on detergent lysates of brain membranes. The immunoprecipitation product was analyzed on immunoblots using either pGEXdrk1 or KC antiserum. Fig. 4 shows that the brain protein immunoprecipitated by KC antiserum displays immunoreactivity with both KC and pGEXdrk1 sera.

Identification of Neuronal Proteins Interacting with drk1 K^+ Channel in Brain. To determine whether other proteins associate with the 130-kDa drk1 polypeptide, immunoprecipitations were done on radiolabeled detergent lysates of adult rat brain membranes under the same conditions used for the experiment of Fig. 4. Immunoprecipitation with increased amounts of KC serum yields increased amounts of the 130-kDa drk1 polypeptide (Fig. 5), corresponding to the KC immunoprecipitation product detected by immunoblotting with KC or pGEXdrk1 sera (Fig. 4). No 130-kDa drk1 polypeptide was precipitated by the preimmune serum at any dilution, although an 80-kDa protein was precipitated nonspecifically by both immune and preimmune sera (Fig. 5).

Surprisingly, a second peptide of 38 kDa is specifically precipitated by KC immune serum (Fig. 5). The amount of 38-kDa peptide in the immunoprecipitate parallels the levels of 130-kDa drk1 polypeptide. As shown above, neither KC nor pGEXdrk1 antiserum recognize the 38-kDa polypeptide on immunoblots of these same membranes (Figs. 1*a*, 2, and 3) or on immunoblots of immunoprecipitated drk1 complexes (Fig. 4). These results show that the 38-kDa protein is immunologically distinct from the drk1 polypeptide, suggesting that specific precipitation of the 38-kDa polypeptide is from interaction of this protein with the immunoreactive 130-kDa polypeptide.

Localization of drk1 Polypeptide in Rat Cortex. Cellular localization of the drk1 protein in brain was determined to better correlate distribution of this delayed rectifier isoform to K^+ conductance pathways previously identified in neurons and glia. The drk1 cDNA was isolated from a whole-brain library (6); thus, it was unknown whether this channel isoform was present in neurons or glia. Fig. 6*A* shows that anti-drk1 labeling in adult cortex is detected only in the membranes of cell bodies and dendrites of a subpopulation of cortical neurons. No detectable staining is seen to glial cells. Fig. 6*B* shows a higher magnification of the same field that demonstrates that the immunoreactivity is prominent on both apical and distal dendrites of a large pyramidal cell. This figure also demonstrates the punctate, membrane-associated nature of the staining. Similar neuronal-specific staining has been seen in other brain regions (data not shown). Staining was eliminated by adsorbing the antiserum with pGEXdrk1

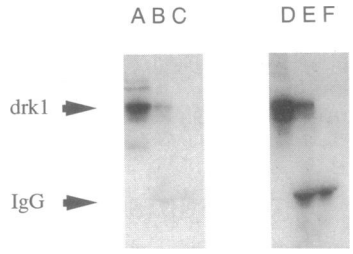

FIG. 4. KC and pGEXdrk1 sera recognize the same 130-kDa drk1 antigen. The 130-kDa drk1 polypeptide was isolated from detergent lysates of adult rat brain membranes by immunoprecipitation with KC immune serum; control immunoprecipitations were also done with preimmune serum. Rat brain membranes (75 μg; lanes A and D) and immunoprecipitation products of immune (lanes B and E) and preimmune (lanes C and F) serum were size-fractionated by SDS/9% PAGE, transferred to nitrocellulose, and treated with 1:100 dilutions of KC serum (lanes A–C) or pGEXdrk1 serum (lanes D–F), followed by incubation in ^{125}I-labeled protein A and autoradiography for 20 hr. Arrow labeled drk1 denotes migration of drk1 polypeptide; arrow labeled IgG denotes IgG heavy chains (≈50 kDa) in the immunoprecipitation products.

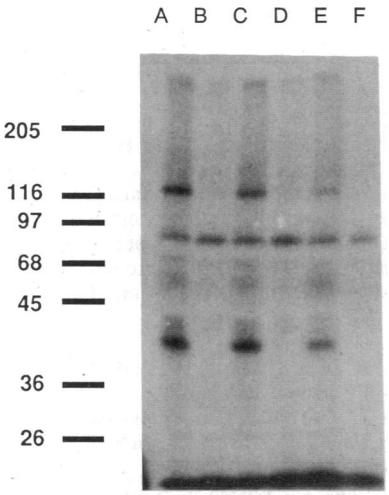

FIG. 5. Immunoprecipitation of radiolabeled neuronal drk1 K^+ channels. ^{125}I-labeled detergent lysates of adult rat brain membranes were subjected to immunoprecipitation with KC immune (lanes A, C, and E) and preimmune (lanes B, D, and F) sera. Immunoprecipitation products were analyzed on SDS/9% PAGE and autoradiographed for 72 hr. Lanes: A and B, 50 μl of serum; C and D, 20 μl of serum; E and F, 10 μl of serum. Numbers denote relative molecular mass of standard proteins.

fusion protein but not with the *sf*26 parent protein, showing the specificity of the antiserum.

DISCUSSION

To begin to study the biochemical characteristics, subunit structure, and localization of components of neuronal I_K, it was necessary to generate high-affinity probes specific for the polypeptide components of the channels that underlay these currents. Recently, a cDNA encoding a delayed rectifier K^+ channel polypeptide, drk1, was identified and isolated by expression cloning in *Xenopus* oocytes (6). Of the currents expressed from mammalian K^+ channel cDNAs to date, those from drk1 cDNA have properties most similar to the classically defined I_K. Thus, it seemed reasonable that antibodies raised to drk1 protein could be applied to the study of polypeptide components underlying neuronal I_K in a manner analogous to the use of neurotoxins in the analysis of other ion channels.

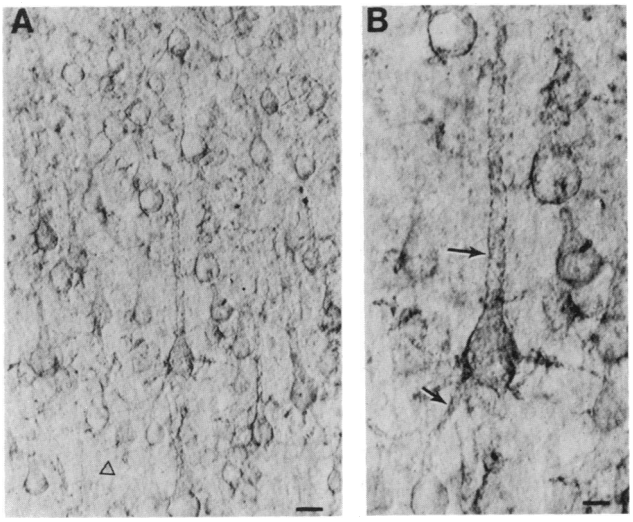

FIG. 6. Immunocytochemical localization of drk1 polypeptide in adult rat cortical neurons. Photomicrographs were taken with Normarski optics. (*A*) Low-magnification photomicrograph of a 40-μm-thick section of adult rat brain containing cortical layers 5/6, stained with affinity-purified pGEXdrk1 antibodies at a 1:100 dilution. △, region where neuronal cell bodies are not labeled. Note variety of cells exhibiting some cell-body staining. (Bar = 19 μm.) (*B*) Higher-magnification photomicrograph of the same stained area in *A*. Arrows point to apical and distal dendrites of a large layer-5/6 pyramidal cell. Note punctate texture of staining that appears associated with plasma membrane. (Bar = 9.5 μm.)

Biochemical Characteristics of drk1 K^+ Channel in Adult Rat Brain. The pGEXdrk1 and KC sera identify the adult rat brain drk1 polypeptide as a 130-kDa protein. This molecular mass is 35% larger than the calculated molecular mass of the core peptide (95.3 kDa) derived from drk1 cDNA. The increased size is not due to anomalous mobility of the core peptide on SDS gels, as the peptide translated *in vitro* has a molecular mass of ≈95 kDa. Furthermore, Ferguson analysis (15) of mobility of the 130-kDa adult rat brain drk1 polypeptide on SDS gels of different acrylamide composition shows that drk1 protein does not exhibit any anomalous behavior on SDS gels. As yet, the molecular basis for the increased molecular mass of the mature brain drk1 polypeptide is not known.

The immunoreactive drk1 band on immunoblots is quite broad. Whether this microheterogeneity is due to expression of alternative core polypeptides or differential posttranslational modification of a single core polypeptide and whether this heterogeneity reflects differences between cell types, subcellular locations, or physiological conditions are unanswered questions. Studies in less heterogeneous preparations may resolve the nature and role of microheterogeneity in the adult rat brain drk1 protein population.

Rat Brain drk1 K^+ Channel May Exist as a Heteromultimeric Complex. KC serum specifically immunoprecipitates both the 130-kDa drk1 polypeptide and an immunologically distinct 38-kDa protein. Neither the KC or pGEXdrk1 antiserum reacts directly with the 38-kDa protein, as shown by immunoblot analysis of both crude brain membranes and the immunoprecipitated drk1 complex. The amount of coprecipitated 38-kDa protein is proportional to the amount of drk1 polypeptide present, indicating that a stoichiometric relationship, the relative proportions of which are unknown, exists between these two brain membrane proteins in the precipitated complex.

This complex remains intact under relatively harsh detergent conditions; these conditions are known to disrupt other multisubunit membrane protein complexes, such as the T-lymphocyte antigen receptor (16) and the acetylcholine (17) and glycine (18) receptors. The interaction between the drk1 and 38-kDa polypeptides is noncovalent, as the drk1 polypeptide does not change mobility upon reduction of disulfide bonds. All procedures involved in these analyses were done at 4°C with protease inhibitor mixtures; however, the 38-kDa peptide could be a proteolytic fragment of the drk1 polypeptide that remains associated during the immunoprecipitation procedure. Further molecular characterization of the coprecipitating 38-kDa peptide will be needed to determine its relationship to the drk1 polypeptide and its role in the quaternary structure of the drk1 channel oligomeric complex.

Interestingly, the one class of K^+ channels amenable to biochemical characterization—those sensitive to the epileptogenic neurotoxins dendrotoxin and β-bungarotoxin—are also formed as a oligomeric complex containing an α-peptide and a 38-kDa protein (19, 20). The similar sizes of the β subunit of inactivating K^+ channels and the coprecipitating 38-kDa peptide seen here make it interesting to speculate that different functional classes of mammalian neuronal K^+ channels may share β subunits.

It is interesting that no other α-peptide-sized proteins are present in the immunoprecipitated drk1 channel complex. Studies on Shaker (21) and mammalian (22) K^+ channel

α-peptides expressed in *Xenopus* oocytes have shown that heterooligomers can be formed with different α-peptides. It is not clear whether such structures exist *in situ*, although electrophysiological evidence suggests that in heterologous expression systems some K^+ channels are formed as homotetramers of α-peptides (23). drk1 is unusual among K^+ channel polypeptides in its large size; thus, it should be possible to detect other K^+ channel polypeptides (50–70 kDa) in the drk1 immunoprecipitation products. The absence of other K^+ channel-sized polypeptides in the immunoprecipitates could be from disruption of heteromultimeric complexes during immunoprecipitation procedures or by poor labeling of other K^+ channel α-peptides present in drk1 complexes by radioiodination. However, this result could also mean that the predominant drk1 channel complexes exist as homotetramers of the drk1 polypeptide, perhaps in association with the 38-kDa peptide.

drk1 Is Expressed in Neurons. Delayed rectifier-like K^+ currents have been found in both mammalian central neurons and glial cells. All mammalian neuronal K^+ channel cDNAs isolated to date have come from libraries derived from both neurons and glia. Cellular localization of protein components of any of these K^+ channels has yet to be determined. The cDNA encoding drk1 polypeptide was originally isolated from a library prepared from whole adult rat brain mRNA (6) and, thus, contained cDNAs encoding both neuronal and glial K^+ channels.

Immunocytochemical localization of the drk1 polypeptide in the adult rat cortex shows that at the detection level by immunocytochemistry, drk1 polypeptide is specific to neurons; no detectable staining was seen in glial cells. The finding that the drk1 polypeptide is preferentially expressed in neurons is essential to any eventual correlation of the role of the corresponding channel in a specific neuronal K^+ conductance pathway. Localization of Shaker channels in *Drosophila* brain (24) revealed that these K^+ channel α-peptides are not uniformly distributed within the fly nervous system and that they are found in nerves composed of axons as well as in regions rich in synapses.

The drk1-specific staining was cell-surface-associated in these cortical neurons, supporting the premise that the drk1 polypeptide plays a role in surface K^+ conductance pathways in the plasma membrane. drk1 immunoreactivity appears localized to the cell bodies and proximal dendrites of these neurons. It is not clear whether this immunoreactivity represents postsynaptic staining on these neurons or presynaptic staining on the nerve terminals that cover these cells. A more detailed understanding of the subcellular distribution of the drk1 polypeptide will require EM.

The results presented here provide information on the biochemical characteristics of the endogenous drk1-delayed rectifier K^+ channel in adult rat brain. The antibodies employed in this study should be useful tools to characterize further the biochemical properties, biosynthesis, and distribution of this neuronal delayed rectifier K^+ channel. These antibodies will be especially helpful in isolating drk1 for chemical analysis after treatment of neurons with modulators of neuronal delayed rectifier currents, such as protein kinase C. In addition, a description of the biosynthesis, processing, assembly, surface appearance, and turnover of drk1 channel components will lead to a better understanding of the processes that control surface channel abundance and distribution. These antibodies should also prove useful in purifying neuronal drk1 channel complexes to isolate and determine the structural and functional contributions of channel components other than the α-peptide.

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