Localization of A-type K^+ Channel Subunit Kv4.2 in Rat Brain

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Abstract

Kv4.2, a voltage-gated K^+ (Kv) channel subunit, has been suggested to be the key component of the subthreshold A-type K^+ currents (I_{SA}s) recorded from the specific subcellular compartments of certain CNS neurons. To correlate Kv4.2 localization with the I_{SA}s detected, immunohistochemistry will be useful. Although the Kv4.2 immunostaining pattern in the hippocampus and cerebellum has been reported, the Kv4.2 antibody used was not specific. Furthermore, Kv4.2 localization in other brain regions remains unclear. In this report, we first demonstrated the specificity of a new Kv4.2 antibody, and then used it to examine Kv4.2 localization throughout adult rat brain by immunohistochemistry. At the cellular level, Kv4.2 was found in neurons but not glias. At the subcellular level, Kv4.2 was localized in the somatodendritic compartment of most neurons examined. Nevertheless, our preliminary data indicated that Kv4.2 might be also present in the axon/terminal compartment. At the functional level, our data indicates that Kv4.2 localization and I_{SA} correlate quite well in some CNS neurons, supporting that Kv4.2 is the key component of some I_{SA}s recorded in vivo.

Key Words: voltage-gated potassium channel, Kv4.2, subthreshold A-type K^+ currents, immunohistochemistry, subcellular localization, immunoprecipitation

Introduction

A-type K^+ currents (I_As) are activated transiently and inactivated rapidly even during membrane still in depolarizing state. I_As can be activated over a very wide range of membrane potentials (-80 to −10 mV). Subthreshold A-type K^+ current (I_{SA}), a type of I_As, has been specified because it can be activated below the threshold of action potential (-55 mV). Since I_{SA} delays the time required for membrane depolarization to reach the threshold for action potential firing, it can regulate the frequency of action potentials (1). Native I_{SA}s have been recorded from the somatodendritic compartment of many CNS neurons, such as the cerebellar granule cells (2), hippocampal CA1 and CA3 pyramidal neurons (3,4), thalamic relay neurons (5), and a type of pyramidal neurons in the layer V of cerebral cortex (6). In addition, I_{SA}s have been also recorded from the axon/terminal compartment, such as the nerve terminals of posterior pituitary (7,8), and the axons of hippocampal CA3 pyramidal cells (9).

So far only the voltage-gated K^+ (Kv) channel Kv4 members (Kv4.1, Kv4.2, Kv4.3) have the ability to evoke I_{SA}s in heterologous expression systems (10). In *Xenopus* oocytes, Kv4.2 subunit alone is significantly activated between -40 mV to -50 mV (1,11). When Kv4.2 is co-expressed with the unidentified factor(s) encoded in low molecular weight brain mRNAs or the Kv channel-interacting protein (KChIP), its activation $V_{1/2}$

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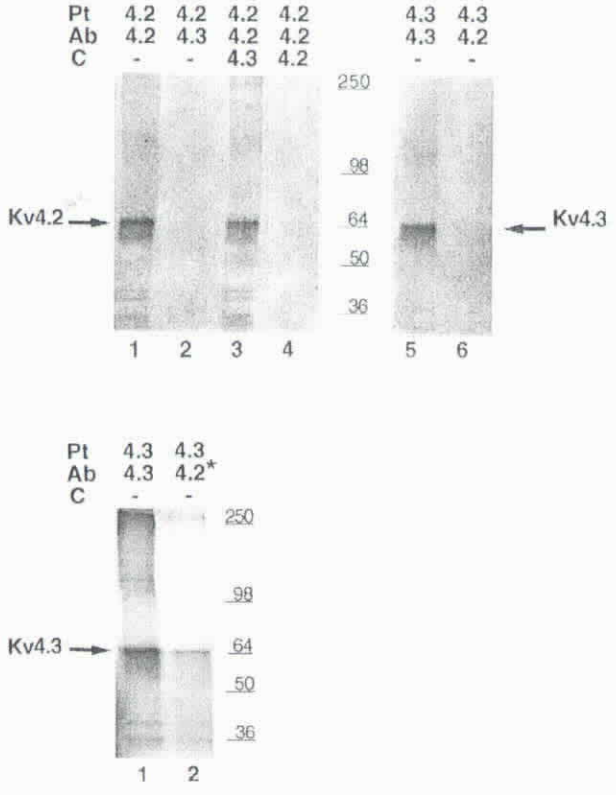

Fig. 1. Specificity of two Kv4.2 antibodies. Upper panel, the Kv4.2 antibody used in this study (Alomone Labs) can recognize the Kv4.2 polypeptide, but not the Kv4.3 polypeptide. Lower panel, the Kv4.2 antibody (Kv4.2*) used in previous reports (12-15) can also recognize the Kv4.3 polypeptide. Identical amounts of [^{35}S-methionine]-labeled in vitro synthesized proteins (Pt): Kv4.2 or Kv4.3 polypeptides, were immunoprecipitated by the indicated antibody (Ab): Kv4.2, Kv4.2*, or Kv4.3 antibody. Excess competitor peptides (C): Kv4.2 or Kv4.3 antigenic peptide, were added to the immunoprecipitation reactions as indicated. The positions of molecular mass standards (in kilodaltons) are indicated. The minor bands at 30-40 kDa are degradation products.

shifts to more negative membrane potentials by 10-30 mV(1,10), similar to the I_{SA}s recorded *in vivo* (2-9).

To correlate Kv4.2 localization with I_{SA}s detected in the specific subcellular compartments of those CNS neurons, immunohistochemistry will be a useful apporaoch. Although the immunohistochemical localization of Kv4.2 has been reported (12-15), the Kv4.2 antibody seems non-specific. Comparing the immunogenic peptide sequence of that Kv4.2 antibody (Kv4.2C:residues484-502CLEKTTNHEFVDEQVFEES) (12-15) and the corresponding Kv4.3 peptide sequence (residues 482-500, CLEKTTNHEFIDEQMFEQN) (16), only two amino acids at the C-terminal are not similar. It is possible that the Kv4.2 antibody used previously could also recognize Kv4.3. Furthermore, only immunostaining in the hippocampus and cerebellum has been addressed (12-15, 17), while other brain regions remain unclear. In this report, we first demonstrated the Kv4.2 antibody used here is specific to Kv4.2, but not the Kv4.2C antibody. Then, by immunohistochemistry, we examined the localization of Kv4.2 throughout the whole adult rat brain.

Materials and Methods

Antibodies and AntigenicPeptides

Anti-Kv4.2 and anti-Kv4.3 antibodies, as well as their antigenic peptides, were purchased from Alomone Labs (Jerusalem, Israel). The sequence of the Kv4.2 antigenic peptide is (C)SNQLQSSEDEPAFVSKS, corresponding to residues 454-469 of rat Kv4.2 polypeptide (11), with an additional N-terminal cysteine. The sequence of the Kv4.3 antigenic peptide is (Y)NEALELTGTPEEEH[Norleucine]GK, corresponding to residues 451-467 of rat Kv4.3 polypeptide (16), with an additional N-terminal tyrosine and methionine replaced with norleucine. According to the manufacturer, the Kv4.2 or Kv4.3 polyclonal antibody was raised in rabbits against the antigenic peptide described above, and purified by affinity column chromatography immobilized with the same antigenic peptide. Another Kv4.2 antibody, Kv4.2C, has been described (12).

In Vitro Transcription/Translation

Rat Kv4.2 cDNA was a gift from Dr. Lily Jan (University of California at San Francisco). Plasmids containing rat Kv4.2 cDNA (11) or rat Kv4.3 cDNA (16) were linearized with NotI and ApaI, and transcribed *in vitro* with GpppG capping using T7 and T3 RNA polymerases, respectively (Ambion, Austin, TX). In the presence of [^{35}S]-methionine (Pharmacia, Uppsala, Sweden), 1 μg of transcript was translated *in vitro* using a rabbit reticulocyte lysate system (Promega, Madison, WI).

Immunoprecipitation

Aliquots of the protein mixture synthesized by *in vitro* transcription/translation were diluted with cold protein buffer (100 mM NaCl, 1 mM EDTA, 20 mM Tris [pH 8.0], 0.5% NP-40) and preabsorbed with normal rabbit sera. For immunoprecipitation, primary antibody (1:100 dilution) was added for 1 hour, followed by

Table 1. Relative Intensity of Kv4.2 Immunoreactivity in Selected Regions of Adult Rat Brain

Brain region		Brain region	
Cortex		Basal ganglia	
Piriform cortex	-	Caudate putamen	+++
Parietal cortex	++	Globus pallidus	-
Perirhinal cortex	+++	Lateral septum	-
Temporal cortex	+	Epithalamus	
Hippocampal formation		Medial habenula	++++
CA1		Lateral habenula	-
S. oriens	++++	Thalamus	
Pyramidal cell layer	-	Anterodorsal n.	+
S. radiatum	++++	Anteroventral n.	+
S. lacunosum mol.	++	Anteromedial n.	+
CA3		Laterodorsal n.	+/-
S. oriens	+++	Lateroposterior n.	+
Pyramidal cell layer	-	Paraventricular n.	++
S. lucidum	-	Ventromedial n.	+/-
S. radiatum	+++	Ventroposterior n.	+/-
S. lacunosum mol.	++	Posterior n.	+/-
Dentate gyrus		Reticular n.	+++
Granule cell layer	-	Geniculate	
Molecular layer	+++	Dorsolateral	+++
Hilus	-	Ventrolateral	+/-
Entorhinal cortex	++	Medial	++
Parasubiculum	++	Brain stem	
Presubiculum	+	Substantia nigra	
Olfactory bulb		Pars compacta	+/-
Glomerular layer	+	Pars reticulata	+/-
External plexiform layer	++++	Superior colliculus	
Mitral cell layer	++	Superficial gray layer	++
Internal plexiform layer	+++	Optic layer	-
Granule cell layer	++	Intermediate gray layer	+/-
Anterior olfactory n.	+	External cuneate n.	++
Cerebellum		Inferior colliculus	++
Molecular layer	-	Central gray	-
Purkinje cell layer	-	Dorsal cochlear n.	+
Granule cell layer	++++	Inferior olive	++
Deep n.	+/-	Pontine	+++
Hypothalamus		Reticulotegmental n.	++
Supramammillary n.	+/-	Sp5 spinal trigeminal n.	++
Lateral mammillary n.	++	Amygdala	+/-

Intensity was rated in an arbitratory scale according to the darkness of immunostaining. Intensity: ++++ the strongest; +++ strong; ++ moderate; + low; +/- very low but clearly above background; - background level. Abbreviations: n., nucleus; S., stratum.

protein A-sepharose (Pharmacia, Uppsala, Sweden) for 45 minutes, with gentle mixing at 4 °C. Competition analysis was performed by pre-incubating antigenic peptides with primary antibody for 1 hour before addition to the immunoprecipitation reactions, at a final concentration of 10 μg/ml for each peptide. Immunoprecipitates were washed with cold protein buffer for 5 times, boiled in SDS sample buffer in the presence of β-mercaptoethanol, and separated by SDS/polyacrylamide gel electrophoresis. Labeled polypeptide bands were visualized by exposing dried gels to x-ray films.

Animals

The experiments were carried out on Sprague-Dawley rats provided by the Animal Center, National Yang-Ming University. Adult male rats (275-300 g) were used. Rats were housed and handled according the guidelines of Animal Center (National Yang-Ming University).

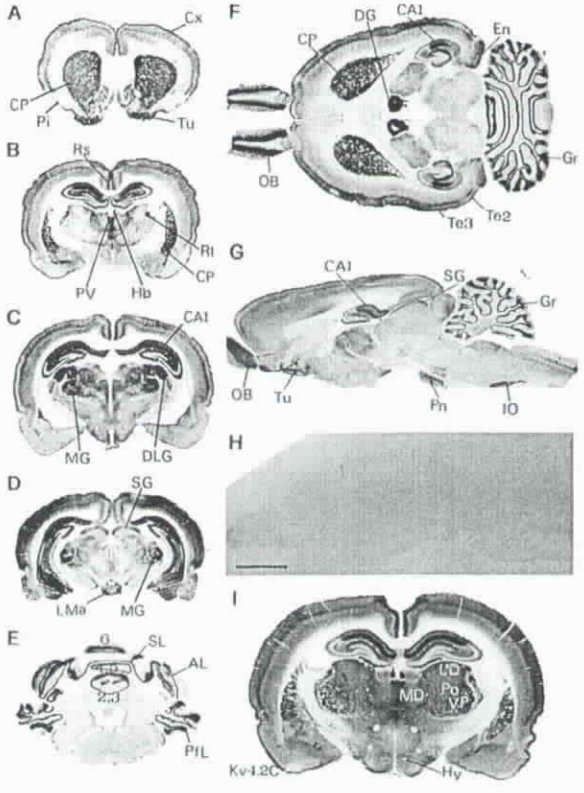

Fig. 2. General distribution of Kv4.2 in adult rat brain. (A-G) Kv4.2 antibody (Alomone Labs) was used for immunohistochemistry in a series of coronal sections from rostral to caudal (A-F), a horizontal section (F), and a sagittal section (G). The strongest immunoreactivity was detected in the olfactory bulb (OB), hippocampus CA1 region (CA1), habenular nucleus (Hb), and cerebellar granular layer (Gr). Lobules of cerebellar vermis are indicated by Arabic numerals in (E). (H) When the primary antibody was preincubated with excess antigenic peptides, staining pattern in (G) could be completely abolished. (I) When applying the Kv4.2C antibody to a coronal section similar as (B), non-specific staining was found in the posterior (Po), ventroposterior (VP), laterodorsal (LD), and mediodorsal (MD) thalamus, as well as hypothalamus (Hy). Other abbreviations: AL, ansiform lobule; CP, caudate putamen; Cx, cerebral cortex; DLG, dorsal lateral geniculate; En, entorhinal cortex; Hy, hypothalamus; IO, inferior olive; LD, laterodorsal thalamus; LMa, lateral mammillary nucleus; MD, mediodorsal thalamus; MG, medial geniculate; Po, posterior thalamus; Pi, piriform cortex; PfL, paraflocculus; Pn, pontine; PV, paraventricular thalamus; Rs, retrosplenial cortex; Rt, reticular thalamus; RTg, reticulotegmental nucleus; SG, superficial gray layer of superior colliculus; SL, simplex lobule; Te2, the area 2 of temporal cortex; Tu, olfactory tubercle; VP, ventroposterior thalamus. Scale bar: A-H, 3.5 mm; I, 2.1 mm.

Immunohistochemistry

Animals were injected intraperitoneally (i.p.) with 2000 units/kg heparin to prevent blood clotting. After

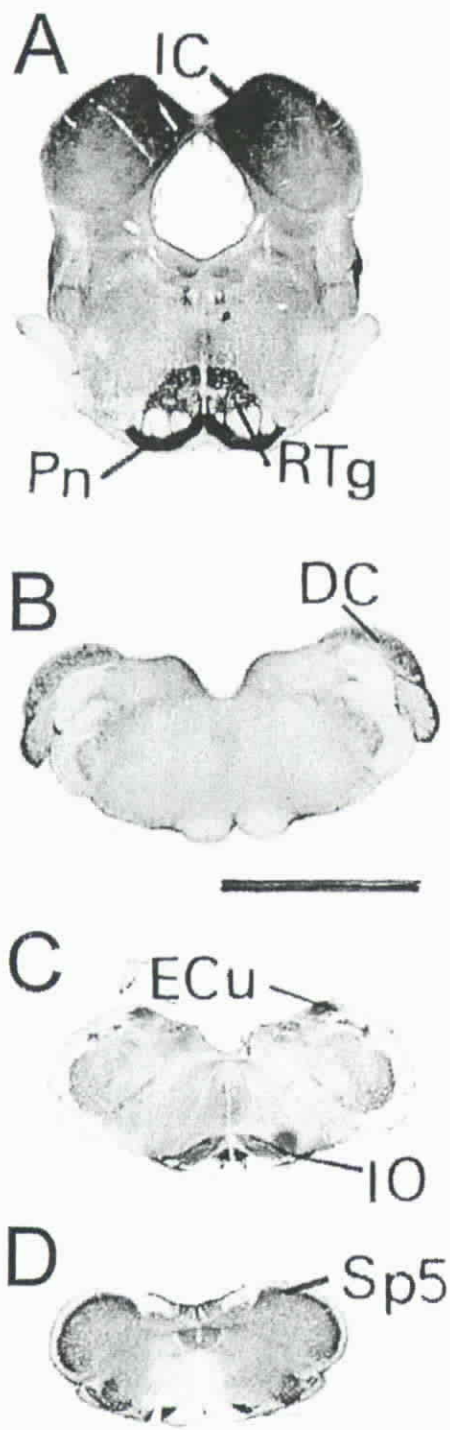

Fig. 3. Distribution of Kv4.2 in the brain stem. (A-D) Immunohistochemistry was performed in coronal sections from rostal to caudal. Abbreviations: DC, dorsal cochlear nucleus; ECu, external cuneate nucleus; IC, inferior colliculus; IO, inferior olive; Pn, pontine; RTg, reticulotegmental nucleus; Sp5, spinal trigeminal nucleus. Scale bar: 3.5 mm.

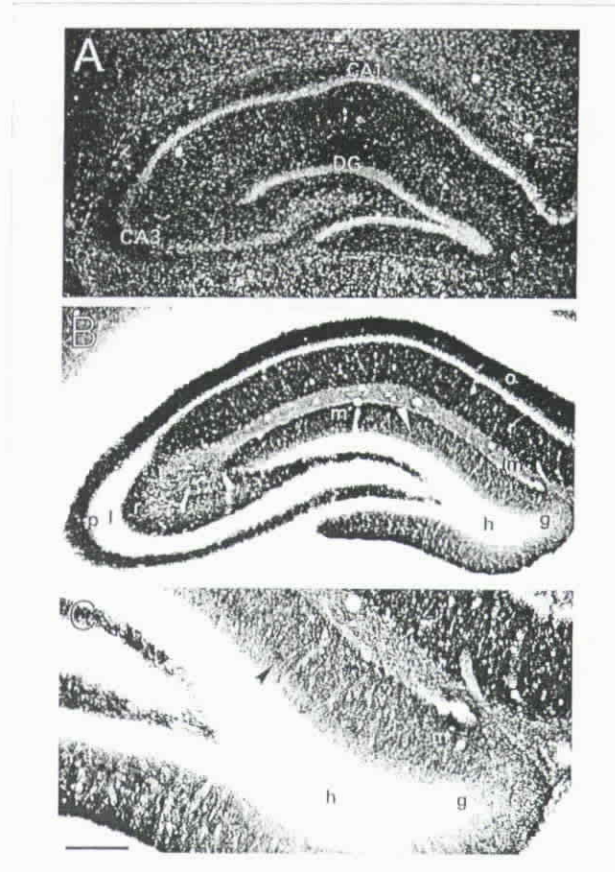

Fig. 4. Subcellular localization of Kv4.2 in the hippocampal neurons. (A) Coronal sections were processed for in situ hybridization with Kv4.2 antisense cRNA probe. (B) Immunostaining with Kv4.2 antibody. The white triangle indicates a narrow zone in the lower part of dentate molecular layer. (C) A higher magnification of the narrow zone (arrowhead). Abbreviations: CA1, CA3, CA1 or CA3 sub-region of hippocampus; DG, dentate gyrus of hippocampus; g, dentate granule cell layer; h, dentate hilus; l, stratum lucidum; lm, stratum lacumosum-moleculare; m, dentate moleular layer; o, stratum oriens; p, pyramidal cell layer; r, stratum radiatum. Scale bar: A, B, 375 μm; C, 150 μm.

10 minutes, animals were anesthetized with sodium pentobarbital (120 mg/kg, i.p.), and perfused transcardially with normal saline first and then 4% paraformaldehyde in PBS. The brains were removed, dehydrated in 30% sucrose, and cut with a cryostat into 50 μm for free floating sections, or 16 μm for sections attached onto slides. After washing in Tris-buffer saline (TBS, 250 mM Tris [pH 7.5], 8.5% NaCl), sections were treated with 0.2% hydrogen peroxide for 10 minutes for the floating sections, or 20 minutes for the sections on slides. Non-specific binding was blocked by 3% normal goat serum plus 2% BSA in TBS containing 0.3% Triton X-100 for 1.5 hours. Primary antibody was applied at a 1:200 dilution for the floating sections, or a 1:100 dilution for the sections on slides, with gentle shaking at room temperature overnight. Sections were washed with TBS, and incubated with biotinylated goat anti-rabbit secondary antibody (1:1000 dilution) and avidin-biotin-horseradish peroxidase

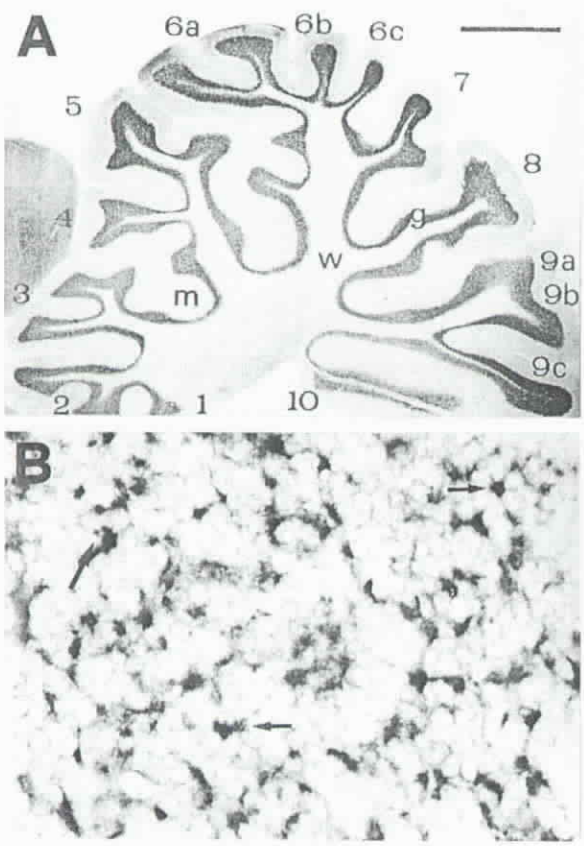

Fig. 5. Localization of Kv4.2 in the cerebellum. (A) Lobules of vermis are numbered 1 to 10 from the rostral to caudal in a sagittal section. Staining was evenly distributed in the granule cell layer (g). (B) High magnification of granule cell layer shows that immunoreactivity is concentrated in the glomeruli (arrows). Other abbreviations: m, molecular layer; w, white matter. Scale bar: A, 1.1 mm; B, 75 μm.

complex (Pierce, Rockford, IL) for 1.5 hours each, with intervening washes in TBS containing 0.3% Triton X-100. Antigen was visualized by combining equal volumes of ammonium nickel sulfate solution (30 mg/ml in 0.1 M sodium acetate, pH 6.0) and diaminobenzidine solution (4 mg/ml in TBS) in the presence of 0.01% hydrogen peroxide. Brain regions were identified according to Paxinos and Watson (18).

In Situ Hybridization

Synthesis of [α-^{35}S]UTP labeled Kv4.2 anti-sense cRNA probe and in situ hybridization on 10 mm cryostat sections of rat brain have been described (19).

Kainic Acid Lesion

Adult rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and fixed in a stereotacxic apparatus. By the insertion of a glass micropipette through a hole on rat skull, 100 nl of 10 mg/ml kainic acid was injected very slowly into the ventromedial/ventroposterior thalamic areas (AP +6.7 mm, ML +1.8 mm, DV +6.8 mm) (18, 20, 21). Animals recovered gradually after the skin was sutured. Eight days after lesion, animals were processed for immunohistochemistry as described above.

Results

Specificity of Two Kv4.2 Antibodies

Using rat brain plasma membrane proteins for immunoblots, the Kv4.2 antibody (Alomone Labs) could recognize a protein band ~70 kDa, which could be competed away by preincubation with excess Kv4.2 antigenic peptide (see Catalog of Alomone Labs, 1999 Spring). Since Kv4.2 and Kv4.3 polypeptides are similar in molecular weight (72 and 69 kDa, respectively), it is not enough to confirm the specificity of Kv4.2 antibody simply by immunoblot. Therefore, to test whether the Kv4.2 antibody can also recognize Kv4.3 polypeptide, we synthesized both Kv4.2 and Kv4.3 polypeptides by *in vitro* transcription/translation. [^{35}S]-methionine labeled Kv4.2 gene product could be immunoprecipitated by the Kv4.2 antibody, which was blocked by preincubation with excess Kv4.2 antigenic peptide (Fig. 1, upper panel, lanes 1-4). Most significantly, Kv4.3 gene product was not immunoprecipitated by the Kv4.2 antibody (Fig. 1, upper panel, lanes 5-6). In contrast, when applying the Kv4.2C antibody (12-15), 40%-60% of the Kv4.3 gene product (bands on x-ray films estimated by densitometry) was immunoprecipitated (Fig. 1, lower panel). These data demonstrates that the Kv4.2 antibody (Alomone Labs) is specific to Kv4.2 polypeptide, while the Kv4.2C antibody used in previous reports can also recognize Kv4.3 polypeptide and is therefore non-specific.

General Distribution of K4.2 in Rat Brain

We used the Kv4.2 antibody (Alomone Labs) for immunohistochemistry. Kv4.2 immunostaining was widely distributed in adult rat brain (Figs. 2A-G, 3A-D), and the characteristic pattern could be completely abolished by preincubation with excess Kv4.2 immunogenic peptide (Fig. 2H). The strongest immunoreactivity was detected in the hippocampus,

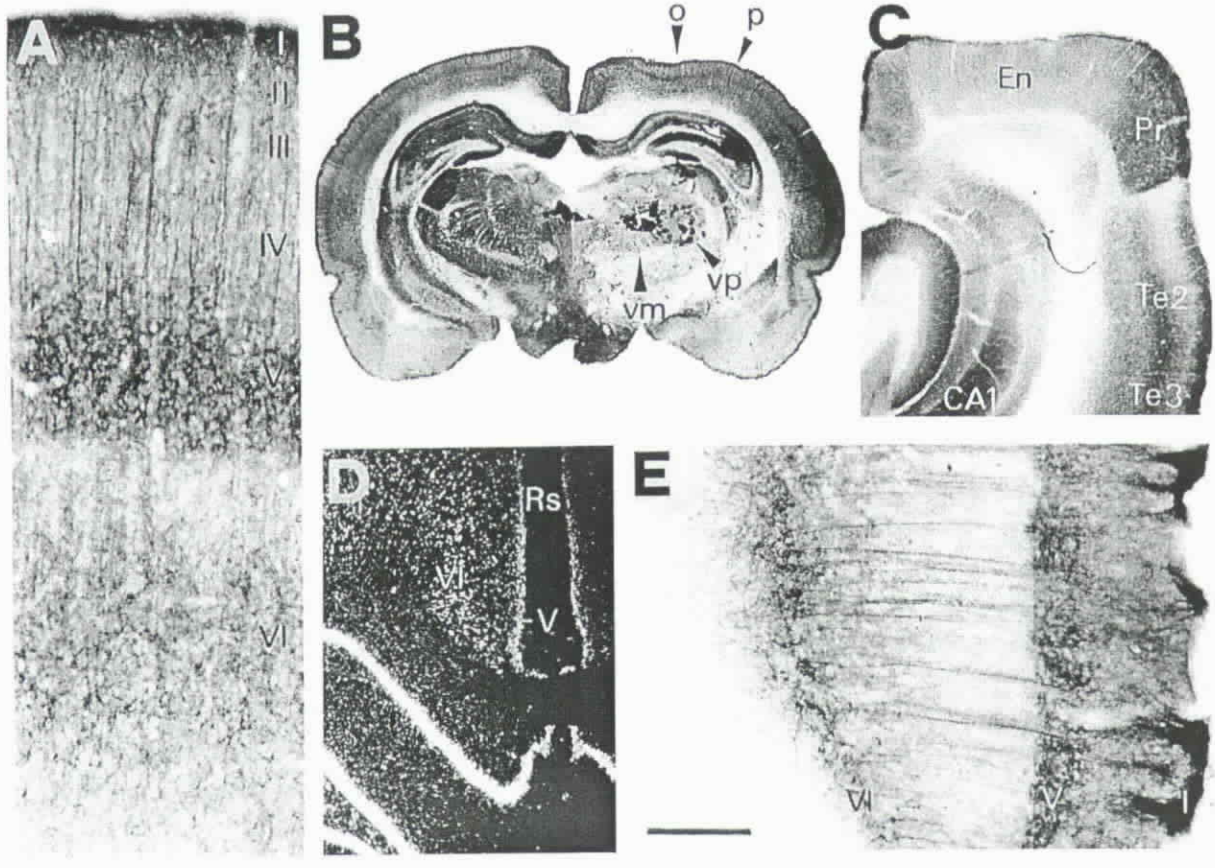

Fig. 6. Localization of Kv4.2 in the cerebral cortex. Cortical layers are labeled with Roman numerals. (A) In the parietal cortex (a coronal section), Kv4.2 immunoreactivity was strong in the apical dendrites of layer V neurons terminating in layer I, and moderate in their somata and basal dendrites located in layer V. (B) The ventroposterior thalamus (vp) and ventromedial thalamus (vm) were unilaterally lesioned by kainic acid. However, the staining pattern in the occipital cortex (o) and parietal cortex (p) was not affected. (C) Staining pattern in the entorhinal cortex (En), perirhinal cortex (Pr), and the area 2 of temporal cortex (Te2) (a horizontal section). (D) In the retrosplenial cortex (Rs, a coronal section), Kv4.2 mRNA was expressed strongly in the layer V neurons, and weakly in the layer VI neurons. (E) Higher magnification of Kv4.2 immunostaning in the retrosplenial cortex. In addition to layer V neurons, staining was localized on the somata and basal dendrites of layer VI neurons, as well as their apical dendrites extending to layer I. Scale bar: A, 150 mm; B, 3.5 mm; C, D, 750 μm; E, 150 μm.

cerebellar granular layer, olfactory bulb, and habenular nucleus (Figs. 2A-G, 3A-D). The relative intensity in various brain regions is summarized in Table 1. When compared with the Kv4.2C immunostaining pattern reported previously (12), a major difference appeared in the thalamus. Immunostaining in the thalamus was generally low, except the reticular thalamic nuclei, dorsal lateral geniculate, and paraventricular thalamic nuclei (Figs. 2B, C; Table 1). For comparision, we also did immunohistochemistry with the Kv4.2C antibody in adjacent sections. Indeed, strong immunostaining was detected in almost all thalamus (Fig. 2I). We also found that Kv4.2C staining was present in the hypothalamus (Fig. 2I). Coincidently, heavy Kv4.3 immunoreacitivty was detected in the thalamus and hypothalamus where strong Kv4.2C staining was located (Tsaur et al., unpublished observation), indicating that the Kv4.2C antibody could also recognize Kv4.3. In addition to immunoprecipitation described above, immunostaining presented here also demonstrates that the Kv4.2C antibody is not specific.

Localization of Kv4.2 protein in other selected brain regions is analyzed below.

Hippocampus

The most characteristic pattern of Kv4.2 staining was in the hippocampal CA1 region (Figs. 2B-D, F, G; Fig. 4B). Although the Kv4.2 mRNA signal appeared equally abundant in the CA1 pyramidal cells and dentate granule cells (Fig. 4A), it could be higher in the CA1 pyramidal cells, because signal is more easily trapped in the highly compacted granule cells than the loosely

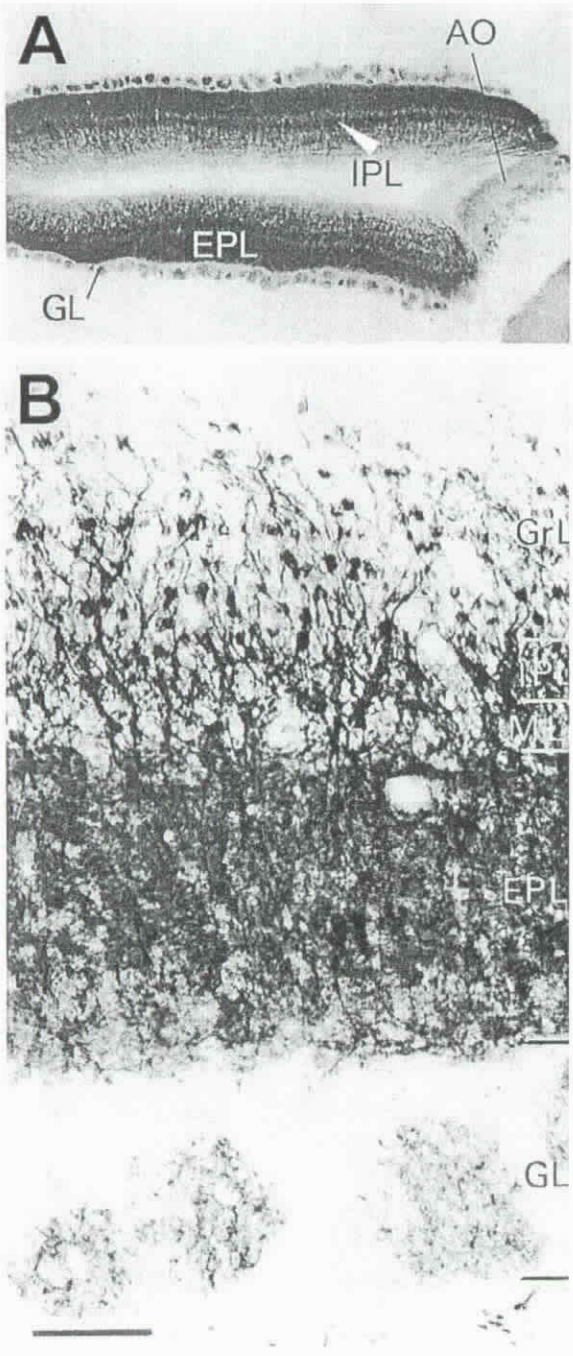

Fig. 7. Localization of Kv4.2 in the olfactory bulb. (A) Immunostaining pattern of olfactory bulb in horizontal sections. Strong staining was distributed in the external plexiform layer (EPL) and the internal plexiform layer (IPL, indicated by a white triangle). (B) A higher magnification shows staining in different layers of olfactory bulb. Short lines on the right side indicate boundaries between layers. Abbreviations: AO, anterior olfactory nucleus; GL, glomerular layer; GrL, granule cell layer; ML, mitral cell layer. Scale bar: A, 750 μm; B, 75 μm.

packed pyramidal cells. This could be observed clearly at the protein level (Fig. 4B). Extremely strong immunoreactivity was detected in the stratum oriens, and stratum radiatum, where the basal and apical dendrites of CA1 pyramidal cells arborize, respectively. Moderate staining was detected in the stratum lacunosome moleculare. Nevertheless, their somata or proximal dendrites located within the pyramidal cell layer showed only background level of staining. Similar but weaker expression pattern was found in the CA3 region, except that staining was also absent from the stratum lucidum (Fig. 4B). In the dentate gyrus, immunostaining was concentrated in the molecular layer where the dendrites of granule cells arborize, but absent from their somata and proximal dendtrites located in the granule cell layer (Figs. 4B, C). Surprisingly, we found a stronger immunoreactivity in a narrow zone in the molecular layer near the hilus, which was composed of discontinuous varicosities (Figs. 4B, C). These might be the nerve terminals of axons projecting from the supramammillary area of hypothalamus (22). However, since the immunoreactivity in the molecular layer was quite strong, sometimes this zone could not be observed clearly. This may be the reason why it has not been addressed in previous reports.

Cerebellum

Strong Kv4.2 immunoreactivity was detected only in the granule cell layer, but not in the Purkinje cell layer or molecular layer (Fig. 5A). Within the granule cell layer, staining was concentrated in the glomeruli and lightly at the boundaries of granule cell somata (Fig. 5B), indicating that more Kv4.2 proteins are localized on the dendrites than somata of granule cells. A recent report indicated that Kv4.2 mRNA displayed a decreasing rostral to caudal gradient in the cerebellar granule cell layer (23). Nevertheless, we did not observe this difference in the distribution of Kv4.2 mRNA in an earlier study (19). At the protein level, since previous reports did not analyze Kv4.2 expression in various lobules of vermis and hemispheres (12,15), it was unknown whether Kv4.2 protein also displayed a rostral to caudal gradient. To verify this controversy, we further examined the intensity of Kv4.2 immunostaining in different cerebellar lobules. Our results indicated that Kv4 2 staining was distributed in the granule cell layer evenly throughout the cerebellum, including the ten lobules of vermis, and the lobules in both hemispheres, such as the simplex lobule, ansiform lobules and paraflocullus (Figs. 2E, 5A). This finding demonstrates that Kv4.2 protein is expressed in the somatodendritic compartment of granule cells evenly throughout the whole cerebellum.

Cerebral Cortex

An intense zone-like staining was detected in the middle of most cerebral cortex (Fig. 2), except the piriform cortex (Fig. 2A). Taking the parietal cortex as an example, under higher magnification (Fig. 6A), immunoreactivity was concentrated on the basal dendrites and somata of pyramidal neurons in layer V, as well as their apical dendrites extending to layer I. Since layer V is next to layer IV, to confirm that Kv4.2 is not present on the thalamocortical projections located in layer IV, unilateral lesion in the ventroposterior and ventromedial thalamus by kainic acid was made. Axons from these two thalamic regions terminate in the layer IV of parietal cortex and occipital cortex. We found that staining in the layer IV of parietal cortex and occipital cortex was not affected (Fig. 6B), demonstrating that Kv4.2 was not localized on the thalamocortical projections. In the retrosplenial cortex, due to compression of layers I-V (Figs. 2B; 6D), staining on these layers became more intense than other cerebral cortex (Fig. 6E), especially the patch-like immunoreactivity in layer I (Fig. 6E). In addition to layer V neurons, a weaker staining was detected in layer VI neurons, in their somata, basal dendrites, and apical dendrites extending to layer I (Figs. 6A, E).

Two different patters were observed in the perirhinal cortex and the temporal cortex area 2 (Te2), although they are two parts of cerebral cortex link together anatomically. Te2 showed strong staining in layer V (except at the junction right next to perirhinal cortex), a pattern similar to parietal cortex (Fig. 6C). Oppositely, expression of Kv4.2 in the perirhinal cortex was not the same as the rest of cerebral cortex. Staining was strong in layers I-IV but absent from layer V in the perirhinal cortex, similar to that in the entorhinal cortex, a part of hippocampal formation (Fig. 6C).

Olfactory Bulb

Heavy Kv4.2 staining was detected on fibers throughout the entire external plexiform layer (EPL), and many cell bodies scattered in the granule cell layer (Figs. 7A,B). Since the olfactory granule cells lack axons, we reasoned that Kv4.2 protein was localized on the somata of superficial granule cells (G_{III}), deep granule cells (G_{II}) and intermediate granule cells (G_{I}), as well as their dendrites ramifying in the superficial EPL, deep EPL and all levels of the EPL, respectively. Consistently, Kv4.2 mRNA was abundantly expressed in the granule cell layer of olfactory bulb (23). Another strong staining was found in a thin zone at the junction of the mitral cell layer and the internal plexiform layer (Fig. 7A). There are many interneurons located at this junction, such as Cajal cells and horizontal cells (24). However, due to the heavy staining on the granule cell dendrites passing through this region, it has been hard to tell whether Kv4.2 is localized on these interneurons. Although staining is weak in the glomerular layer, many dot-like cells scattered outside this layer showed significant staining (Fig. 7B). These cells are likely to be olfactory sensory neurons.

Summary

At the cellular level, Kv4.2 was localized in neurons but not glial cells in adult rat brain. At the subcellular level, Kv4.2 was mainly detected in the somatodendritic compartment of most CNS neurons examined. Nevertheless, Kv4.2 staining was also found in the axon/terminal compartment, namely, in the terminal field of hippocampal molecular layer and the internal plexiform layer of olfactory bulb. At the regional level, expression of Kv4.2 protein in the cerebellar granule cells is evenly throughout the granule cell layer in the whole cerebellum.

Discussion

In this report, by immunoprecipitation and immunohistochmistry, we first demonstrated that the Kv4.2C antibody used in previous studies was not specific, because it could also recognize Kv4.3. Then, combining in situ hybridization and immunohistochemistry, as well as a lesion approach, a comprehensive picture of Kv4.2 localization in adult rat brain has been provided. New insights have been raised from the re-examination of hippocampus and cerebellum. The localization of Kv4.2 in the cerebral cortex and olfactory bulb has also been analyzed carefully. These data is useful in the identification whether Kv4.2 is responsible for the native I_{SA}s recorded from the specific subcellular compartment of some CNS neurons. Most importantly, information provided here should be helpful in elucidating the role of Kv4.2 in the CNS. The significance of Kv4.2 expression in the specific subcellular compartment of some CNS neurons is discussed below.

In the hippocampus, in the CA1/CA3 pyramidal neurons and dentate granule cells, we found that tremendous amount of Kv4.2 protein was expressed in their distal dendrites, but only in background levels on the somata and proximal dendrites. However, Kv4.2 staining was found on the somata of these hippocampal

neurons previously (12). This discrepancy may be due to different immunostaining methods. Floating sections were used in this report, while sections attached to slides were used in previous study (12). The latter might cause more background. Consistently, a prominent I_{SA} was detected from the distal dendrites but not from the proximal dendrites or somata of CA1 pyramidal neurons (3). I_{SA} has been also detected from the somatodendritic compartment of CA3 pyramidal neurons (4). Kv4.2 should be the major channel subunit responsible for these I_{SA}s. Since I_{SA} plays a key role in dendritic excitability of hippocampal pyramidal neurons, Kv4.2 may have an influence in the long-term potentiation (LTP), a fundamental phenomenon of learning and memory (3, 25). Recent reports indicated that Kv4.2 was regulated by arachidonic acid (26, 27), protein kinase A, and protein kinase C (28). These molecules are highly involved in the modulation of LTP, further supporting that Kv4.2 may play an important role in the learning and memory.

In the cerebellum, Kv4.2 was abundantly expressed in the dendrites of granule cells. Consistently, prominent I_{SA} has been detected from the somatodendritic compartment of cerebellar granule cells (2). Kv4.2 should be the key channel molecule evoking this K^+ current. Dendrites of cerebellar granule cells form excitatory synapses with mossy fibers, which originate from neuclei in the spinal cords and brain stem that give rise to the spinocerebellar tracts. Since these tracts are important in adjusting ongoing movement (29), it is likely that Kv4.2 plays a significant role in this function.

In most cerebral cortex, Kv4.2 was prominently expressed in layer V. Consistently, a type of I_A activated between −40 to −50 mV has been recorded from the somatodendritic compartment of layer V neurons (6). As described in the Introduction, without auxiliary subunit (1,10), Kv4.2 alone is significantly activated at membrane potential positive than the threshold of action potential. Kv4.2 is probably the main component responsible for the I_A detected from the layer V neurons, although we are not as sure as the cases with I_{SA}s. Perirhinal cortex is part of cerebral cortex anatomically, however, expression of Kv4.2 in this region was different from the rest of cerebral cortex. Instead, it is more similar to the entorhinal cortex, a part of hippocampal formation. Perirhinal cortex has been found to be involved in cognition as the hippocampal formation (30). Perirhinal cortex may have a similar function as the entorhinal cortex. Abundant expression of Kv4.2 in the perirhinal cortex, as well as the hippocampus discussed above, again suggesting that Kv4.2 may play an important role in cognition, such as learning and memory.

Deep in the olfactory bulb, Kv4.2 was abundantly expressed in the dendrites of all types of GABAergic granule cells (types I, II, and III). In the EPL, the dendrites of granule cells receive excitatory input from the secondary dendrites of mitral and tufted cells. Excitation of these granule cell interneurons then make negative feedback to the secondary dendrites of mitral and tufted cells, and results in proloned inhibition of mitral and tufted cells. Consistently, a type of I_A (not I_{SA}) recorded from the somatodendritic compartment of olfactory granule cells, has been found to be important in regulating the inputs from mitral and tufted cells (31). Based on the intense immunoreactivity in the granule cells presented here, Kv4.2 is probably the channel molecule responsible for this I_A.

Finally, it has been demonstrated that Kv4.2 is localized only in the somatodendritic compartment at the subcellular level (12-15). Here, our preliminary evidence suggests that Kv4.2 is also present in the axon/terminal compartment. Since only Kv4 subunits can evoke I_{SA}s, our finding could be supported by the electrophysiological experiments reported previously, that is, I_{SA}s have been recorded from the axon/terminal compartment (7-9). However, to provide a direct evidence for presynaptic localization, further analysis using electronic microscopy will be required.

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