Inward rectifier K$^+$ channel Kir2.3 is localized at the postsynaptic membrane of excitatory synapses

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Inanobe, Atsushi, Akikazu Fujita, Minoru Ito, Hitonobu Tomoike, Kiyoshi Inagada, and Yoshihisa Kurachi. Inward rectifier K$^+$ channel Kir2.3 is localized at the postsynaptic membrane of excitatory synapses. Am J Physiol Cell Physiol 282: C1396–C1403, 2002; 10.1152/ajpcell.00615.2001.—Classical inwardly rectifying K$^+$ channels (Kir2.0) are responsible for maintaining the resting membrane potential near the K$^+$ equilibrium potential in various cells, including neurons. Although Kir2.3 is known to be expressed abundantly in the forebrain, its precise localization has not been identified. Using an antibody specific to Kir2.3, we examined the subcellular localization of Kir2.3 in mouse brain. Kir2.3 immunoreactivity was detected in a granular pattern in restricted areas of the brain, including the olfactory bulb (OB). Immunoelectron microscopy of the OB revealed that Kir2.3 immunoreactivity was specifically clustered on the postsynaptic membrane of asymmetric synapses between granule cells and mitral/tufted cells. The immunoprecipitants for Kir2.3 obtained from brain contained PSD-95 and chapsyn-110, PDZ domain-containing anchoring proteins. In vitro binding assay further revealed that the COOH-terminal end of Kir2.3 is responsible for the association with these anchoring proteins. Therefore, the Kir channel may be involved in formation of the resting membrane potential of the spines and, thus, would affect the response of N-methyl-D-aspartic acid receptor channels at the excitatory postsynaptic membrane.

dendritic spine; ion channel localization; excitatory synaptic transmission

INWARDLY RECTIFYING K$^+$ (Kir) channels play pivotal roles in controlling the excitability of various cells, including neurons. The Kir channel subunit family contains >16 members, which can be classified into 4 major groups: background Kir channels (Kir2.0), G protein-gated Kir channels (Kir3.0), ATP-sensitive Kir channels (Kir6.0), and K$^+$ transporters (Kir1.0, Kir4.0, and Kir7.0) (14, 15). The classical Kir2.0 channels are responsible for formation of the deep resting membrane potential near the K$^+$ equilibrium potential in a variety of cells, such as cardiac and skeletal myocytes, glial cells, and some neurons. It was shown at the level of mRNA that Kir2.0 subunits are expressed differentially in various regions of the brain (11, 16, 36): Kir2.1 is expressed diffusely but weakly in the whole brain, Kir2.2 mainly in the cerebellum, Kir2.3 mainly in the forebrain, and Kir2.4 in motor neurons of the spinal cord. These observations indicate that members of the Kir2.0 subfamily may play different physiological roles in control of neuronal function. The subcellular localization of Kir2.0 channels in the brain, however, has not been extensively examined.

The mRNA of Kir2.3 is expressed predominantly in the forebrain (6, 21, 26, 35). In situ hybridization studies revealed that most Kir2.3 mRNA is found in neurons, rather than in glial cells (5, 11, 16). Furthermore, Kir2.3 binds to PSD-95, which scaffolds various functional proteins at the postsynaptic membrane of asymmetric synaptic junctions (5, 7, 23, 30). Although these results suggest that Kir2.3 is localized at the postsynaptic membrane of excitatory synapses, light microscopy indicated that neuronal Kir2.3 distributes diffusely in the nuclei and on the plasma membrane of pyramidal cells in the CA3 region of rat hippocampus (33). To resolve this paradox, we produced a polyclonal antibody specific to Kir2.3 and examined the localization of the channel protein in mouse brain at light- and electron-microscopic levels. The Kir2.3 immunoreactivity was detected in a granular pattern in limited regions of the forebrain, including the olfactory bulb (OB), neocortex, hippocampus, and caudate putamen. Immunoelectron-microscopic analysis in the OB revealed that Kir2.3 is specifically localized at the postsynaptic membrane of the dendritic spines of granule cells facing the secondary dendrites of mitral/tufted cells. The distribution of Kir2.3 at sites of excitatory postsynaptic specialization suggests that this channel participates in the formation of the membrane potential of the spines.

MATERIALS AND METHODS

Antibodies. Animals were treated in accordance with the guidelines for the use of laboratory animals of Osaka Uni-
A rabbit polyclonal antibody (mI3C3) was generated against a synthetic polypeptide, LQEGAS-KEEAHRMLPEGSHL, corresponding to amino acids 400–422 of mouse Kir2.3 (21). Cysteine was added at the NH\(_2\) terminus of the peptide to couple to ovalbumin for the preparation of immunogen and to SulfoLink coupling gel (Pierce, Rockford, IL) for purification of antigenic peptide-specific IgG. Affinity purification of mI3C3 antibody was carried out with sequential column chromatography through protein A-Sepharose (Amersham Pharmacia Biotech, Uppsala, Sweden) and antigenic peptide-coupled resins from antisera. To detect PDZ domain-containing proteins, we used monoclonal anti-PSD-95 and anti-dlg/SAP97 antibodies (clones 16 and 12, respectively; Transduction Laboratories, Lexington, KY), rabbit anti-SAP102 and anti-S-SCAM (kindly provided by Dr. Y. Takai) and anti-chapsyn-110 antibodies (APZ-002, Alomone Laboratory, Jerusalem, Israel), and goat anti-ZO-1 antibody (N-19; Santa Cruz Biotechnology, Santa Cruz, CA).

**Immunoblotting, immunocytochemistry, and immunohistochemistry.** Mouse Kir2.3 (21) and rat PSD-95 (kindly provided by Dr. D. Richmond) were transiently expressed in human embryonic kidney (HEK) 293T cells with the LipofectAMINE PLUS reagent (Invitrogen, San Diego, CA). The constructs and rat chapsyn-110 in GW1 (generous gift from Dr. M. Sheng) were transiently expressed in human embryonic kidney (HEK) 293T cells with the LipofectAMINE PLUS reagent (Invitrogen). The cell lysate and membrane fraction of various tissues from Balb/c mice were prepared as described previously (13). The cell lysate and membrane fraction of various tissues from Balb/c mice were prepared as described previously (13). The proteins were subjected to SDS-PAGE and transferred to polyvinylidine difluoride membranes. Kir2.3 immunoreactivity was developed with the SuperSignal chemiluminescence kit (Pierce).

Cells expressing Kir2.3 were also fixed with 4% (wt/vol) paraformaldehyde (PFA) in 0.1 M sodium phosphate buffer (PB), pH 7.4, at 4°C overnight. After the cells were washed with phosphate-buffered saline (PBS), they were incubated with mI3C3 antibody and then with FITC-conjugated anti-rabbit antibody and propidium iodide for staining nuclei, as described previously (13). Fluorescence was measured using laser scanning confocal microscopy (system LSM510, Carl Zeiss, Oberkochen, Germany).

Immunohistochemistry was carried out as described previously (13). Briefly, Balb/c mice were deeply anesthetized with pentobarbital sodium (60 mg/kg ip) and perfused transcardially with 4% PFA in 0.1 M PB (pH 7.4). The brains were dissected, postfixed in the same solution at 4°C for 12 h, and then dehydrated in 30% (wt/vol) sucrose and 0.05% (wt/vol) Na\(_2\)SO\(_4\) in PBS at 4°C. The parasagittal sections (20 μm thick) were prepared with a cryostat and stored in PBS supplemented with 0.1% (wt/vol) Triton X-100 and 0.05% Na\(_2\)SO\(_4\) at 4°C. Cryosections were immunostained by the free-floating method with incubation of mI3C3 (0.5 μg/ml) at 4°C for 48 h and then biotinylated goat anti-rabbit IgG (Vectorstain ABC kit, Vector Laboratories, Burlingame, CA). The immunoreactivity was detected with H\(_2\)O\(_2\), dianobenzidine (DAB), and nickel ammonium. The same antibody preincubated with 100-fold antigenic peptide was also used for control staining.

**Immunoelectron microscopy.** Postembedding immunogold electron-microscopic examination was performed with mouse brain fixed by the “pH-shift” protocol (10, 19). Ultrathin sections were cut from small blocks of OB with a Reichert ultramicrotome and mounted on nickel grids. Immunoreactivity of Kir2.3 was developed with mI3C3 (1 μg/ml) and then with 10-nm colloidal gold particle-conjugated F(ab\(_2\)) fragment (GFAR10, British BioCell International, Cardiff, UK). Electron-microscopic examination for the DAB-developed Kir2.3 immunoreactivity was carried out with mouse brain fixed by periodate-lysine-PFA (13). Sections were incubated with mI3C3 antibody and then with biotinylated goat anti-rabbit IgG (Vectorstain ABC kit). After color development with DAB, nickel ammonium, and platinized potassium chloride, the sections were postfixed in reduced osmium, filtered with epoxy resin, and then sectioned with an ultramicrotome. Finally, the sections obtained with both procedures were counterstained with uranyl acetate and Reynolds’ lead citrate and examined in an electron microscope (model 7100, Hitachi, Tokyo, Japan).

**Immunoprecipitation.** Three forebrains dissected from Wistar rats were homogenized with Physcoton (NITI-ON Medical Physical Instrument, Tokyo, Japan) and then with a tight-fitting glass-Teflon homogenizer in 30 ml of 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, and protease inhibitor cocktail (1 mM phenylmethylsulfonyl fluoride, 0.2 mg/ml benzamidine-HCl, and 5 μg/ml each of pepstatin, leupeptin, and chymostatin). After centrifugation at 100,000 g for 30 min, the sedimented membrane fraction was suspended with 30 ml of 50 mM Tris-HCl (pH 8.6), 1 mM Na-EDTA, 1% (wt/vol) deoxycholate (DOC), and protease inhibitor cocktail, homogenized with a glass-Teflon homogenizer, and then centrifuged at 100,000 g for 30 min. The soluble fraction was supplemented with Triton X-100 to give a final concentration of 0.3% and dialyzed against 3 liters of 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 0.1% Triton X-100 at 4°C overnight. After the addition of protease inhibitor cocktail, the DOC extract was clarified by centrifugation and stored at −80°C.

Immunoprecipitation was carried out as described previously (13). Briefly, Kir2.3 in 1 ml of the DOC extract (30 mg of protein) was recovered with 2 μg of mI3C3 and protein G-Sepharose beads (Amersham Pharmacia Biotech). Rabbit nonimmune IgG or mI3C3 antibody preincubated with a 100-fold amount of its antigenic peptide was used for negative control. Protein retained on beads was eluted with 20 μl of loading buffer, resolved by SDS-PAGE, and detected by immunoblotting with a series of antibodies.

**Affinity chromatography.** Amino acids 298–445 and 298–441 of mouse Kir2.3, amino acids 1434–1482 of rat N-methyl-D-aspartic acid (NMDA) receptor 2B (NR2B), and amino acids 605–655 of rat voltage-dependent K+ channel Kv1.4 were amplified by polymerase chain reaction to tag BamHI and XhoI sites at 5’ or 3’ ends, respectively, and subcloned into pGEX-4T-1 (Amersham Pharmacia Biotech). Each GST-fusion protein (10 μg of protein) expressing PSD-95 chapsyn-110 and amino acids 605–655 of rat voltage-dependent K+ channel Kv1.4 were amplified by polymerase chain reaction to tag BamHI and XhoI sites at 5’ or 3’ ends, respectively, and subcloned into pGEX-4T-1 (Amersham Pharmacia Biotech). Each GST-fusion protein (10 μg of protein) expressing PSD-95 was mixed with the DOC extract (10 mg of protein) from rat brain or the cell extract (10 μg of protein) expressing PSD-95 or chapsyn-110 and rotated at 4°C for 2 h. The resin was washed once with 1 ml of 20 mM HEPES-NaOH (pH 7.4), 1 mM Na-EDTA, 1 mM dithiothreitol, and protease inhibitor cocktail (base solution) supplemented with 1% Triton X-100 and 0.15 M NaCl, twice with 1 ml of base solution with 1% Triton X-100 and 0.5 M NaCl, and twice with 1 ml of base solution with 0.15 M NaCl. Bound proteins were analyzed with the immunoblotting technique described above.

**Nomenclature.** Anatomic examinations were carried out with the main OB. Therefore, the term OB represents the main OB. Nevertheless, we could not detect any significant difference in Kir2.3 localization between the main OB and accessory OB at the light-microscopic level. The term mitral
cell represents mitral and tufted cells, because we could not distinguish between their dendrites under our conditions.

RESULTS

Characterization of anti-Kir2.3 antibody. To examine the localization of Kir2.3 channel protein expressed in neural tissues, we developed a polyclonal antibody against a synthetic peptide corresponding to the COOH terminus of mouse Kir2.3 (21). After purification of the IgG fraction through protein A- and antigenic peptide-coupled resins from antiserum, we characterized the IgG (mI3C3) by immunoblotting and immunocytochemical analyses (Fig. 1). As shown in Fig. 1A, the antibody developed several bands at $\sim$55 kDa in the lysate from HEK 293T cells expressing Kir2.3 (lane 1). These bands were not detected in the control cell lysate (lane 2). The antibody preincubated with excess antigenic peptide failed to detect any bands (lane 3). The duplicated major protein bands at $\sim$55 kDa were also detected in the membrane fraction obtained from mouse forebrain (lane 4), but not from cerebellum (lane 5). Kir2.3 immunoreactivity was examined in other tissues by overexposing the blot until the duplicated bands of Kir2.3 in the forebrain were indistinguishable (Fig. 1B). There were no significant signals in the tissues examined other than the forebrain. The distribution of Kir2.3 immunoreactivity agreed with that of Kir2.3 mRNA shown by Northern blotting (6, 21, 26, 35). The specificity of the mI3C3 antibody was tested with immunocytochemistry (Fig. 2). Kir2.3, transiently expressed in HEK 293T cells, was detected with this antibody (Fig. 2A), but not with the antibody preincubated with antigenic peptide (Fig. 2B). The signal was not detected in the cells that were not transfected with Kir2.3 cDNA (Fig. 2C). The mI3C3 antibody used in this study, therefore, specifically recognizes mouse Kir2.3.

Immunolocalization of Kir2.3 in mouse brain. Using the mI3C3 antibody, we first examined the distribution of Kir2.3 in sagittal slices of mouse brain at the level of light microscopy (Fig. 2). Kir2.3 immunoreactivity was preferentially detected in several areas of the forebrain (Fig. 2A), which is consistent with the result of immunoblotting analysis (Fig. 1, A and B). No significant signal was developed with the antibody preincubated with the antigenic peptide (Fig. 2B). The Kir2.3 signal was found strongly in the OB, olfactory tubercle, neocortex, caudate putamen, and hippocampus and weakly in the inferior colliculus. In these areas, the immunoreactivity showed a granular pattern (Fig. 2D–F). The distribution of Kir2.3 immunoreactivity was consistent with that of its mRNA shown by in situ hybridization studies (5, 11, 16, 17).

Figure 2, C–F, depicts the immunolocalization of Kir2.3 in mouse OB at higher magnification. Kir2.3 immunoreactivity was distributed in the external plexiform (EPL), mitral cell (MCL), and granule cell layers (GRL). The density of dots was high in the EPL, while it was relatively weak in the MCL and GRL. Little signal was detected in the glomeruli (Fig. 2D) or the...
centrifugal axonal tracts of mitral cells in the GRL (arrowheads, Fig. 2F). Whereas Kir2.3 mRNA was expressed predominantly in the granule cells (5, 11, 16), no clear immunoreactivity was detected in somata of granule cells (Fig. 2F). Granule cells in the OB spread their dendrites into the EPL and GRL (Fig. 2G) (32). These observations suggest that Kir2.3 is located in neuronal processes, rather than in the somata, of granule cells in the OB.

Electron-microscopic study of Kir2.3 in mouse OB. The dendritic spines of granule cells contact with the secondary dendrites of mitral cells in the EPL, and most such synapses exist as pairs of reciprocal contacts (Fig. 2G) (32). In the electron-microscopic images of the EPL (Fig. 3), the reciprocal synapses could be easily identified as the contacts between the relatively smooth dendrites of mitral cells, which contain round vesicles, and the dendritic spines of granule cells, which contain flattened vesicles. The preembedding method was applied first to detect immunoreactivity of Kir2.3 (Fig. 3, A and B). Dark-colored DAB-immunoreactive Kir2.3 was detected in granule cell spines (Fig. 3A). Substantial immunoreactivity was detected in the vicinity of the plasma membrane of granule cells facing mitral cell dendrites (Fig. 3B). Although we searched for Kir2.3 immunoreactivity in the superficial area of the OB, no signal could be found at the synaptic contacts between periglomerular cells and mitral cells or at synapses between nerve termini of olfactory epithelium and primary dendrites of mitral cells (data not shown). Kir2.3 immunoreactivity was specifically localized on the plasma membrane of the dendritic spines of granule cells in contact with the dendrites of mitral cells in the EPL of the OB.

Fig. 2. Immunohistochemistry of Kir2.3 in mouse brain. A and B: parasagittal sections of mouse brain immunostained with mI3C3 antibody. Sections were stained with antibody in the absence (A) and presence (B) of an excess of antigenic peptide. Strong diamobenzidine (DAB) immunoreactivity was found in the olfactory bulb (OB), olfactory tubercle (Tu), neocortex (Cx), caudate putamen (CP), and hippocampus (Hip), and weak immunoreactivity was found in the inferior colliculus (IC); no immunoreactivity was found in the cerebellum (Cb). C: distribution of Kir2.3 immunoreactivity in mouse OB. Kir2.3 immunoreactivity was strongly developed in the external plexiform layer (EPL) and weakly developed in the mitral cell layer (MCL) and granule cell layer (GRL). No immunoreactivity was found in the olfactory nerve layer (ONL) or the glomerular layer (GL). D–F: higher magnification of areas within boxes in C. Dense immunoreactive signals were distributed in a granular pattern, and immunoreactivity could not be found in the glomerulus (D) and the nerve tracts in the GRL (arrowheads in F). G: major nerve connections in the OB. Axon termini of olfactory sensory neurons (OSN) connect with the primary dendrites of mitral cells (M) in the glomerulus. In the EPL, secondary dendrites of mitral cells contact superficial dendrites of the granule cells (G). Dendrites of granule cells also contact with the axon branches of mitral cells and the centrifugal afferents in the GRL. Output signals pass through the axons of mitral cells to the higher cortical structure. Scale bars, 2 mm (A and B), 1 mm (C), and 50 μm (D–F).
The reciprocal synapse is a pair of mitral-to-granule asymmetric and granule-to-mitral symmetric junctions (32). Although the former synapse is considered to be excitatory, the latter is inhibitory. In Fig. 3, C and D, we examined the localization of Kir2.3 with immunogold electron microscopy. Kir2.3 was localized on the postsynaptic membranes of asymmetric synapses in granule cell spines (Fig. 3C). More highly magnified electron microscopy (Fig. 3D) was used to carry out quantitative analysis of the localization of the gold particles corresponding to Kir2.3 immunoreactivity (Fig. 3, E and F). In the perpendicular axis to the synaptic membrane, the distribution of gold particles peaked at ~4 nm into the granule cell spine from the postsynaptic membrane (Fig. 3E). In the horizontal axis, the gold particles distributed within the excitatory synaptic contact (Fig. 3F). Therefore, Kir2.3 was concentrated at the excitatory postsynaptic membrane of granule cell spines in the OB.

**Molecular basis for specific localization of Kir2.3 on excitatory postsynaptic membrane.** At the postsynaptic membrane of excitatory synapses, PDZ (PSD-95/dlg/ZO-1) domain-containing proteins scaffold many functional proteins, including NMDA receptors and voltage-dependent K⁺ channels (7, 30). Kir2.3 possesses a COOH-terminal sequence corresponding to a class I PDZ domain-binding motif, i.e., Glu-Ser-Ala-Ile, and has been reported to interact with the anchoring protein PSD-95 (5, 23). Because Kir2.3 was specifically distributed at the excitatory postsynaptic membrane of dendritic spines of granule cells in the OB, we looked for possible PDZ domain-containing anchoring proteins that could associate with it (Fig. 4A). With the use of a series of antibodies, PSD-95 and chapsyn-110 were identified in the immunoprecipitants with m3C3 obtained from the DOC extract of rat brain (lane 3). Neither nonimmune IgG nor m3C3 preincubated with excess antigenic peptide immunoprecipitated the protein complex containing these PDZ domain-containing proteins (lanes 2 and 4, respectively). Neither SAP102, SAP97, S-SCAM, nor ZO-1 was detected in the immunoprecipitants. These results suggest that PSD-95 and/or chapsyn-110 forms protein complexes with Kir2.3 in the brain.

Next we tested the specificity of interaction between Kir2.3 and various PDZ domain-containing proteins. The COOH termini of Kir2.3 (Kir2.3/298–445), a deletion mutant of Kir2.3 (Kir2.3/298–441), NR2B (NR2B/1434–1482), and Kv1.4 (Kv1.4/605–655) were constructed to concatenate GST. Using these GST-fusion constructs, the pull-down assay was carried out with the DOC extract from rat brain (Fig. 4B). PSD-95, chapsyn-110, and SAP97 bound to the COOH termini of NR2B and Kv1.4; SAP102 was isolated only with the COOH terminus of NR2B and not with Kv1.4 (Fig. 4B, lanes 5 and 6). Under the same conditions, only PSD-95 and chapsyn-110 and neither SAP97 nor SAP102 bound to the COOH terminus of Kir2.3 (Kir2.3/298–445; Fig. 4B, lane 3). The deletion mutant of Kir2.3 (Kir2.3/298–441) did not interact with PSD-95 or chapsyn-110 (Fig. 4B, lane 4). These results indicate that the four amino acids (-Glu-Ser-Ala-Ile) at the COOH-terminal end of Kir2.3 are responsible for the association between Kir2.3 and the anchoring proteins PSD-95 and chapsyn-110.

PSD-95 and chapsyn-110 are reported to form homomeric and heteromeric assemblies (18). To clarify the interaction between Kir2.3 and both PDZ domain-containing proteins, we prepared cell lysates expressing PSD-95 or chapsyn-110 alone and then mixed with the COOH terminus of Kir2.3 fused with GST (Fig. 4C). Both PDZ domain-containing proteins clearly bound to the COOH terminus of Kir2.3 (Kir2.3 amino acids...
298–445), but not to the COOH terminus without the last four amino acids (Kir2.3 amino acids 298–441). Therefore, PSD-95 and chapsyn-110 interact with the COOH terminus of Kir2.3.

**DISCUSSION**

This study shows that Kir2.3 protein is specifically localized on the excitatory postsynaptic membrane of dendritic spines of granule cells in the OB. At the reciprocal synapses in the EPL of the OB, the NMDA receptor NR1 and α-amino-3-hydroxyl-5-methylisoxazole-4-propionic acid receptors GluR2 and GluR3 are present at the asymmetric postsynaptic membrane on granule cell spines (28). The distribution of these channel proteins within the postsynaptic membrane is essentially the same as in other excitatory synapses (2, 8, 19); therefore, the molecular organization at the postsynaptic density of reciprocal asymmetric synapses in the OB may be similar to that of other excitatory synapses. At the light-microscopic level, Kir2.3 immunoreactivity exhibited a granular pattern not only in the OB but also in other regions in the forebrain (Fig. 2). Thus Kir2.3 may play an important functional role in excitatory synaptic transmission in the forebrain.

The COOH-terminal end of Kir2.3 possesses a PDZ domain-binding motif, which is considered to account for the interaction with PSD-95 (5, 23). Cohen et al. (5) biochemically isolated the protein complex of PSD-95 and Kir2.3, not only from rat forebrain but also from the OB. In addition to PSD-95, we showed that chapsyn-110 forms a protein complex with Kir2.3 through the interaction with its COOH-terminal end (Fig. 4). In rat forebrain, the expression pattern of chapsyn-110 mRNA largely overlaps with that of PSD-95 mRNA (3). Because chapsyn-110 is closely related to PSD-95 (85% similarity) and has a potency comparable to that of PSD-95 in NMDA receptor-clustering activity (3, 18), PSD-95 and chapsyn-110 may be responsible for the specific localization of Kir2.3 at the postsynaptic membrane of excitatory synapses.

Dendritic spines are neuronal compartments that protrude from the dendrite, and it is postulated that they behave separately from the dendrite biochemically and electrically (for review see Ref. 31). The spine also functions as the site of excitatory synaptic input (31). Therefore, the situation of the Kir2.3 channel on the excitatory postsynaptic membrane may contribute to form a resting membrane potential for the spines that is more negative than that of the dendrites. Most glutamatergic synaptic input terminates on the spines, and ionotropic and metabotropic glutamate receptors are found at the excitatory postsynaptic membrane of the spines (31). Because the behavior of NMDA receptor channels is voltage dependent due to block by Mg2+ (20, 25), the Kir2.3 channel might be a factor contributing to the response of NMDA receptor channels at the postsynaptic membrane.

Although specific modulation of Kir2.3 channels at excitatory postsynaptic membranes has not been reported, Nehring et al. (23) showed that association of PSD-95 causes reduction of the single-channel conductance of Kir2.3, although the physiological role is not clear. Various background Kir channel currents have been shown to be suppressed by the activation of Gq-coupled neurotransmitter receptors. They in-
clude group I metabotropic glutamate receptors in various neurons, including granule cells in the OB (1, 34), substance P receptors in nucleus basalis neurons (22), 5-hydroxytryptamine receptors in nucleus accumbens neurons (24), and muscarinic receptors in locus ceruleus neurons (29). Inhibition may be due to reduction of phosphorylidyinositol 4,5-bisphosphate (PIP₂) content in the cell membrane through the activation of phospholipase C. PIP₂ is an essential lipid for the maintenance of the activity of Kir channels (12). Therefore, Kir2.3 channel activity modulated by G₅-coupled receptors may affect synaptic transmission at some excitatory synapses. In addition to PIP₂, cytoplasmic ATP (6), inter- and extracellular pH (27, 37), phosphorylation by protein kinase C (9, 38), and intracellular Mg²⁺ (4) have been found to modulate Kir2.3 activity. Thus Kir2.3 is potentially an important target of various signaling systems in the spines. Further studies are needed to clarify the physiological role of Kir2.3 channels at excitatory postsynaptic membranes.

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