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Surface Expression of the AMPA Receptor Subunits GluR1, GluR2, and GluR4 in Stably Transfected Baby Hamster Kidney Cells

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Abstract: The surface expression of the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)-type glutamate receptor (GluR) subunits GluR1, GluR2, and GluR4 was studied in cultures of stably transfected baby hamster kidney (BHK)-570 cells. Two methods were used to quantify surface expression: cross-linking with the membrane-impermeant reagent bis(sulfosuccinimidyl)suberate (BS^3) and labeling of surface receptors with the membrane-impermeant biotinylating reagent sulfosuccinimidyl 2-(biotinamido)ethyl-1,3-dithiopropionate (NHS-ss-biotin) followed by precipitation with neutravidin beads. Western blot analyses of control versus treated cultures revealed that, for all three GluR subunits examined, 25–40% of the total GluR population is located in the plasma membrane of the BHK-570 cells. This finding was corroborated by analyses of the surface expression of [^{3}H]AMPA binding sites in the GluR-expressing BHK-570 cells performed via the biotinylation/precipitation method; these studies revealed that 30–40% of the total binding site population is found in the plasma membrane. Analyses of combinations of the subunits, both GluR1 + GluR2 and GluR2 + GluR4, revealed that heteromeric combinations of the subunits are not trafficked to the surface more efficiently than homomeric receptors. For each of the three subunits, western blots revealed two distinct bands; removal of surface receptors reduced immunoreactivity for the upper band of each subunit by >90%, whereas immunoreactivity for the lower band was reduced by only 10–20%. Treatment of extracts from the various cell lines with glycopeptidase F resulted in the collapse of the two bands into a single band of lower molecular weight, suggesting that the two original bands represent differentially glycosylated forms of the same polypeptides. These data indicate that the majority of the stably expressed GluR subunits in these cell lines are incompletely glycosylated and that complete glycosylation is associated with trafficking of the GluR subunits to the cell surface. **Key Words:** Glutamate receptors—Glycosylation — Phosphorylation — GluR1 — GluR2 — GluR4—Binding—Biotinylation—Cross-linking.
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In the mammalian CNS, there are three main classes of channel-linked receptors that mediate responses to the neurotransmitter glutamate: the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors, which comprise various combinations of the glutamate receptor (GluR) subunits GluR1–4, the kainate receptors, which consist of the GluR5–7 and kainate KA1 and 2 subunits, and the *N*-methyl-D-aspartate (NMDA) receptors, which comprise the subunits NR1 and NR2A–D (reviewed by Seeburg, 1993). An understanding of the development and plasticity of glutamatergic synapses depends crucially on knowledge of how GluRs are assembled and eventually inserted into the plasma membrane. The subcellular distribution of AMPA receptors has been studied via three different methods: immunohistochemistry with subunit-specific antibodies (Petralia and Wenthold, 1992; Craig et al., 1993; Eshhar et al., 1993; Martin et al., 1993; Molnar et al., 1993; Baude et al., 1994), fractionation studies (Henley, 1995), and modification of surface receptors with membrane-impermeant reagents (Stern-Bach et al., 1994; Hall and Soderling, 1996). All three methods have revealed that the GluR subunits are abundantly found in the plasma membrane but that there is also a substantial pool of subunits located intracellularly. These studies raise the question of whether factors such as subunit composition or posttranslational modification play a role in determining which AMPA receptors are found at the cell surface and which are not.

AMPA receptors are believed to be pentameric subunit assemblies (Wenthold et al., 1992), with the vari-

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Abbreviations used: AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; BHK, baby hamster kidney; BS^3, bis(sulfosuccinimidyl)suberate; CaMKII, calcium/calmodulin-dependent protein kinase II; GluR, glutamate receptor; NHS-ss-biotin, sulfosuccinimidyl 2-(biotinamido)ethyl-1,3-dithiopropionate; NMDA, *N*-methyl-D-aspartate; PAGE, polyacrylamide gel electrophoresis; PNGase F, glycopeptidase F; SDS, sodium dodecyl sulfate; SS, saline solution; TBS, Tris-buffered saline.

ous GluR subunits conferring different properties to the receptor complexes that they constitute (Seeburg, 1993). The contribution of individual GluR subunits to the trafficking of AMPA receptors is difficult to study in neurons, as most native receptors are believed to be heteromeric combinations of subunits (Seeburg, 1993; Wenthold et al., 1996). Moreover, it is not known whether the subcellular distribution of the GluR subunits in neurons is determined primarily by signals intrinsic to the subunits themselves or by other factors in the neuronal environment. Problems such as these are best addressed by studying receptor subunits expressed in nonneuronal cells. In the present study, we examined the surface expression of GluR1, GluR2, and GluR4 subunits that were stably expressed, individually and in combinations, in baby hamster kidney (BHK)-570 cells.

MATERIALS AND METHODS

Materials

Culture plates were from Falcon. Dulbecco's modified Eagle's medium and heat-inactivated fetal calf serum were from GIBCO. Mito+ serum extender was from Collaborative Research. Sulfosuccinimidyl 2-(biotinamido)ethyl-1,3-dithiopropionate (NHS-ss-biotin), bis(sulfosuccinimidyl)-suberate (BS^3), and neutravidin beads were from Pierce. Anti-GluR1 and anti-GluR2/3 antibodies were a gift from Robert J. Wenthold (National Institutes of Health); anti-GluR4 was obtained from Chemicon. Anti-actin monoclonal antibodies, alkaline phosphatase, and glycopeptidase F (PNGase F) were from Boehringer Mannheim. Anti-β-tubulin monoclonal antibodies were from Sigma. Goat anti-rat brain calcium/calmodulin-dependent protein kinase II (CaMKII; α subunit) antibodies were developed by Bethyl Laboratories. Donkey anti-rabbit and donkey anti-mouse horseradish peroxidase-linked secondary antibodies were from Amersham; swine anti-goat antibody was from Boehringer Mannheim. ReadySafe liquid scintillation fluid was obtained from Beckman. GF/C filters were from Whatman. [^{3}H]AMPA and the Renaissance chemiluminescence kit were obtained from Du Pont/NEN. All other reagents were from Sigma.

Cell cultures

GluR subunit cDNA, in a mammalian expression vector (Zem219b) using a constitutively active mouse metallothionein promoter, was transfected into BHK-570 cells; stable cell lines were created and maintained as described (Tygesen et al., 1994; Andersen et al., 1996). Unsuccessful attempts were made to create stable cell lines for GluR3; Southern blots of these cells indicated incorporation of the GluR3 DNA, but northern and western blots revealed no GluR3 RNA or protein. Thus, the present study examined only the GluR1, GluR2, and GluR4 subunits ("flip" versions).

Cross-linking and biotinylation

Cultures were washed twice with 1.0 ml of an osmotically balance saline solution (SS; 137 m*M* NaCl, 5.3 m*M* KCl, 0.17 m*M* Na_2HPO_4, 0.22 m*M* KH_2PO_4, 10 m*M* HEPES, 33 m*M* glucose, 44 m*M* sucrose, and 0.024 g/L phenol red, pH 7.3; 325 mOsm) and then incubated with either 1 mg/ml BS^3 or 1 mg/ml NHS-ss-biotin in SS for 10 min with agitation at 37°C. The SS was then aspirated, and the plates were washed three times in ice-cold harvest buffer (SS with 1 m*M* EDTA, 1 m*M* phenylmethylsulfonyl fluoride, 1 m*M* benzamidine, 100 μ*M* leupeptin, 10 m*M* NaF, 1 μ*M* microcystin-LR, and 50 m*M* ethanolamine; the ethanolamine is present to quench any unreacted BS^3 or NHS-ss-biotin). After the third wash, 1.0 ml of fresh, ice-cold harvest buffer was added, and the cells were scraped off, transferred into a 1.5-ml snap-cap vial, and frozen at −80°C. To prepare the cross-linked samples for western blots, the samples were simply thawed on ice, sonicated for 8–10 s with a Branson Sonifier 250 set at 20% of peak power, and then mixed with sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE) sample buffer to final concentrations of 2% SDS, 5% β-mercaptoethanol, and 5% glycerol. To prepare biotinylated samples for western blots and binding assays, an extra step was taken: After thawing on ice, the samples were centrifuged for 20 min at 10,000 *g* at 4°C. The supernatant was removed, and the pellet was resuspended via an 8–10-s sonication in fresh ice-cold solubilization buffer (see below). This step of preparing a lysed membrane pellet was taken to remove excess glutamate that might interfere with the binding assay.

Precipitation of biotinylated samples

Pellets of lysed membranes prepared from control intact cultures and intact cultures that had been biotinylated with NHS-ss-biotin were resuspended via sonication for 8–10 s in 0.5 ml of solubilization buffer (50 m*M* HEPES, 0.5 *M* NaCl, 1% Triton X-100, 1 m*M* EDTA, 1 m*M* phenylmethylsulfonyl fluoride, 1 m*M* benzamidine, 100 μ*M* leupeptin, 10 m*M* NaF, and 1 μ*M* microcystin-LR) and incubated with agitation for 30 min at 4°C. The samples were then centrifuged for 30 min at 10,000 *g* (4°C); the resulting supernatants were defined as the soluble fraction. Virtually all (>90%) of the AMPA receptors in the BHK-570 cells were solubilized under these conditions. The solubilized samples were incubated for 2 h at 4°C with 50 μl of neutravidin-linked beads. The bead/soluble fraction mixtures were then spun for 1 min at 10,000 *g*, and the supernatants were removed. The amounts of GluR immunostaining and [^{3}H]-AMPA binding remaining in these samples were then quantified and expressed as a percentage of matched control samples that had been processed identically but were derived from cultures that had not been biotinylated.

SDS-PAGE and western blotting

Lysate samples were incubated with sample buffer for 5 min in boiling water, loaded into 9% acrylamide gels, and electrophoresed at 150 V for 1 h. The dye front was not allowed to exit the bottom of the gel. Proteins were then blotted onto nitrocellulose for 4 h at 60 V. Blots were blocked for 30 min with 2% milk in Tris-buffered saline (TBS; 50 m*M* NaCl and 10 m*M* Tris, pH 7.4) with 0.1% Tween-20 and then incubated with primary antibodies in 2% milk in TBS with 0.1% Tween-20 ("wash buffer") for 1 h at room temperature. Blots were then washed three times (10 min each) in wash buffer and incubated for 1 h at room temperature with an appropriate horseradish peroxidase-linked secondary antibody at a dilution of 1:2,000 in wash buffer. Following three more 10-min washes with wash buffer and one 10-min wash with TBS, blots were developed via a 1-min incubation with the Renaissance chemiluminescence reagent (luminol in an oxidizing solution) followed by exposure to sheets of Kodak X-Omat film for varied

intervals. Films were developed such that all bands resulting from a given blot exposure were in the most linear range of intensity, as determined from preliminary experiments in which standard curves were constructed by plotting the relative OD of the immunoreactivities for increasing concentrations of lysed membranes versus the amount of membranes loaded per lane. Reductions in immunoreactivity in experimental samples were determined from these standard curves.

[^{3}H]AMPA binding assay

[^{3}H]AMPA binding was assayed via the filtration method as previously described (Murphy et al., 1987; Hall et al., 1992). Samples were incubated in borosilicate test tubes in a final volume of 100 μl with 20 n*M* [^{3}H]AMPA in the presence of 50 m*M* KSCN; incubations were for 40–60 min in an ice bath. The assay was terminated via addition of 4 ml of ice-cold assay buffer with 50 m*M* KSCN followed rapidly by filtration through GF/C filters that had been soaked in 0.03% polyethylenimine for at least 30 min. The filters were rapidly washed twice with the assay buffer (with 50 m*M* KSCN) and then placed into scintillation vials to which 4 ml of scintillation fluid was added. Results were expressed as the percentage of specific binding (in cpm) in treated samples relative to control samples. Nonspecific binding was defined as binding that occurred in the presence of 2.5 m*M* L-glutamate; nonspecific binding was ~15% of the total and was subtracted to yield the specific binding values.

Immunoprecipitation

To study the assembly of coexpressed receptor subunits, cells from the BHK-570 cell line expressing both GluR1 and GluR2 were harvested and solubilized as described above. Each soluble sample was then incubated for 2 h at 4°C with 10 mg of protein A-Sepharose beads and 2 μl of anti-GluR1 antibody according to the method of Wenthold et al. (1992). Following this incubation, the supernatants were removed, and the beads were washed five times with solubilization buffer. After the final wash, the beads were resuspended in 100 μl of SDS sample buffer, boiled for 5 min, and briefly centrifuged; the supernatants were taken and loaded on 9% SDS-PAGE gels as described above.

Dephosphorylation and deglycosylation

To examine posttranslational modifications of the GluR proteins, samples were denatured via boiling for 5 min in the presence of 0.5% SDS and 250 m*M* β-mercaptoethanol. The samples were then diluted five times in a buffer containing either 1× dephosphorylation buffer (Boehringer Mannheim) for the alkaline phosphatase-treated samples or 50 m*M* HEPES (pH 7.4) for the PNGase F-treated samples, along with 1 m*M* phenylmethylsulfonyl fluoride, 1 m*M* benzamidine, 100 μ*M* leupeptin, and 1 m*M* EDTA. The diluted samples were incubated for 12 h at 35°C in the absence and presence of either 10 units/ml alkaline phosphatase or 10 units/ml PNGase F. The samples were then prepared for SDS-PAGE as described above.

RESULTS

Western blots of extracts from BHK-570 cells expressing the flip forms of GluR1, GluR2, or GluR4 were probed with subunit-specific antibodies. The total level of expression of all three subunits per unit protein was slightly lower than that observed in whole brain

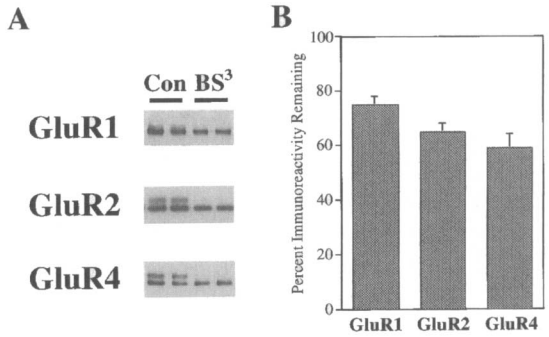

FIG. 1. A: Western blots demonstrate immunoreactivity for the AMPA receptor subunits GluR1, GluR2, and GluR4 stably expressed in BHK-570 cells. The two lanes to the left represent duplicate control (Con) samples, and the two lanes to the right represent duplicates of lysate prepared from intact cells that had surface receptors removed via treatment with the membrane-impermeant cross-linking reagent BS^3. **B:** Quantification of total immunoreactivity for the various GluR subunits after treatment with BS^3, expressed as a percentage of control immunoreactivity. Data are mean ± SEM (bars) values for four to six independent determinations.

tissue. The western blots of the BHK-570 cell extract revealed two distinct bands for each subunit that were more distinctly separated for GluR2 and GluR4 than for GluR1: The two bands migrated with approximate molecular masses of 104/102 kDa for GluR1 and 106/102 kDa for GluR2 and GluR4 (Fig. 1A, left two lanes). In all cases, the bottom band was the major band, but the top band more closely comigrated with brain tissue anti-GluR immunoreactivity (data not shown).

To examine whether or not the GluR subunits expressed in the BHK-570 cells were in the plasma membrane, the cells were treated with the membrane-impermeant cross-linking reagent BS^3. Preliminary experiments revealed that intracellular proteins were not affected by this treatment: Western blot immunoreactivities for the cytoskeletal proteins actin and tubulin and the abundant intracellular enzyme CaMKII were not altered by exposure of intact cells to the cross-linking reagent, as previously reported (Hall and Soderling, 1996). All of these proteins were, however, readily cross-linked into higher-molecular-weight species when the cross-linking was performed in a lysate preparation, suggesting that the reason they were not affected in the whole-cell experiments was because the BS^3 did not have access to intracellular compartments. The preliminary experiments with lysate preparations also demonstrated that a 10-min incubation with 1 mg/ml BS^3 removed virtually all of the 102–106-kDa immunoreactivity from anti-GluR western blots (data not shown), presumably because cross-linked GluR subunits form aggregates that are so large that they are barely able to penetrate SDS-PAGE gels. When such cross-linking treatments were performed on intact BHK-570 cells expressing GluR1, GluR2, or GluR4, total immunoreactivity for each of the subunits was

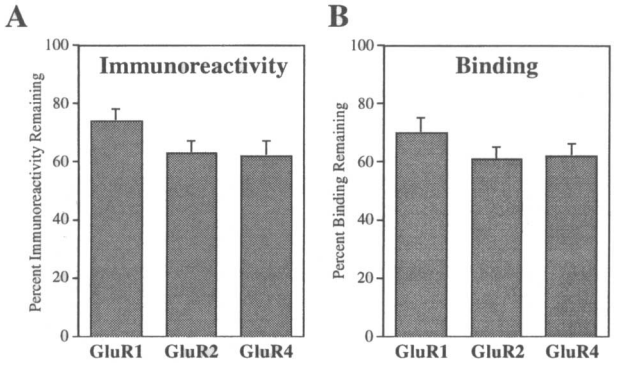

FIG. 2. A: Quantification of the amount of immunoreactivity removed by treating intact GluR-expressing BHK-570 cells with the membrane-impermeant reagent NHS-ss-biotin and then solubilizing and precipitating with neutravidin beads. Data are mean ± SEM (bars) values for four to six independent determinations. **B:** Removal of [^{3}H]AMPA binding from GluR-expressing BHK-570 cells via biotinylation/precipitation. Data are mean ± SEM (bars) values for four to six independent determinations.

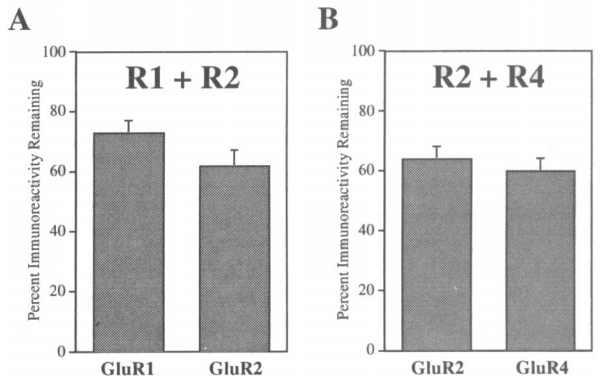

FIG. 3. Reduction in total GluR immunostaining induced by cross-linking of surface receptors with BS^3 in BHK-570 cells expressing a combination of two GluR subunits, either (**A**) GluR1 and GluR2 or (**B**) GluR2 and GluR4. Data are mean ± SEM (bars) values for four independent determinations.

reduced by 25–40% (Fig. 1). The two bands observed for each subunit, however, were not affected equally by the surface cross-linking; the top band was reduced in all cases by >90%, whereas the bottom band of all three subunits was reduced by only 10–20%.

The surface expression of the GluR subunits was also examined by treating intact cultures of the BHK-570 cells with the membrane-impermeant biotinylating reagent NHS-ss-biotin and then solubilizing the cultures and removing biotinylated species via neutravidin precipitation. Preliminary experiments demonstrated that actin, tubulin, and CaMKII were not biotinylated by treatment of whole cells with NHS-ss-biotin, suggesting that this treatment resulted in modification of only transmembrane proteins. These experiments yielded results very similar to those from the cross-linking studies in that ~25–40% of the total immunoreactivity for each subunit was removed (Fig. 2A), with immunoreactivity for the top band being reduced >90% in all cases. The biotinylation/precipitation method was also used to study the surface expression of [^{3}H]AMPA binding sites. For each subunit, ~30–40% of the total binding site population was removed by treatment with NHS-ss-biotin followed by neutravidin precipitation (Fig. 2B).

BHK-570 cell lines stably expressing combinations of GluR subunits (GluR1 + GluR2 and GluR2 + GluR4) were constructed to examine the surface expression of heteromeric GluR combinations. The surface expression of the subunits in these cell lines was examined via the cross-linking method; in both of the combination cell lines, 25–40% of the total subunit population was removed by the cross-linking (Fig. 3). As in the cases of the individually expressed subunits, surface cross-linking reduced immunoreactivity for the top band of each of the subunits expressed in the combination cell lines by >90%, whereas immunoreactivity for the lower band of each subunit was reduced by <20%. To confirm that the subunits were assembling into heteromeric combinations, samples of the GluR1/GluR2-expressing cell line were solubilized and immunoprecipitated with an anti-GluR1 antibody. The precipitates were then examined via western blot and found to contain immunoreactivity for both GluR1 and GluR2, with a concomitant loss of immunoreactivity for each subunit in the supernatant fractions; both the upper and lower bands of GluR1 and GluR2 were present in the GluR1-precipitated fraction in roughly the same proportions found in the starting extract (data not shown).

To examine if the two bands observed for each of the GluR subunits represented species with different levels of posttranslational modification, samples of extract from the GluR1, GluR2, and GluR4 homomeric cell lines were treated with either alkaline phosphatase, to remove phosphate groups, or PNGase F, to remove N-linked glycosyl residues. The alkaline phosphatase treatment had no effect on the mobility of any of the GluR-immunoreactive bands (data not shown). Treatment with PNGase F, however, resulted in the collapse of the GluR1, 2, or 4 doublets into single bands of markedly lower molecular weight (Fig. 4). The molecular mass estimates for the deglycosylated species of

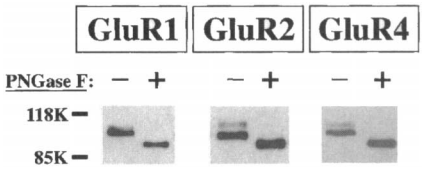

FIG. 4. Western blots demonstrate the enzymatic deglycosylation of GluR1, GluR2, and GluR4 by a 12-h incubation at 35°C in the absence (−) or presence (+) of 10 units/ml PNGase F. The lines along the left side of these blots indicate the positions of two molecular mass markers, 118 and 85 kDa.

each subunit were ~98 kDa, which is close to the predicted size value for unmodified GluR subunits based on their primary sequences.

DISCUSSION

The present results demonstrate that the AMPA receptor subunits GluR1, GluR2, and GluR4 are trafficked to the plasma membrane of BHK-570 cells with roughly similar efficiency; our finding that ~25–40% of the total GluR protein is surface-expressed is very similar to an estimate derived from biotinylation studies of GluR3 transiently expressed in oocytes (Stern-Bach et al., 1994). Furthermore, we show in the present study that expression of heteromeric combinations of GluR subunits does not increase the percentage of total receptors found on the cell surface. Thus, it seems that all known AMPA receptor subunits exhibit similar trafficking properties when expressed in nonneuronal cells and that assembly of heteromeric combinations does not affect trafficking. In these respects, AMPA receptor subunits differ profoundly from NMDA receptor subunits, which exhibit striking differences in surface expression if expressed individually. For example, it has recently been shown in immunofluorescence studies that the NR1 subunit expressed by itself cannot be detected in the plasma membrane, but coexpression of NR1 with the NR2A subunit leads to robust surface expression of heteromeric NR1 + NR2A receptors (McIlhinney et al., 1996).

It has previously been shown that GluR subunits are *N*-glycosylated (Blackstone et al., 1992; Kawamoto et al., 1994). The two bands observed for all of the expressed GluR subunits in the present study represent differentially glycosylated forms of the same polypeptides. However, it is not simply the case that the upper band represents the glycosylated form and the lower band the nonglycosylated form; rather, both bands represent glycosylated GluR protein, with the upper band having either one or more extra glycosylation sites or a larger glycosyl attachment. This extra glycosylation is associated with trafficking of the subunits to the cell surface, because >90% of the fully glycosylated GluR species but only ~10–20% of the incompletely glycosylated GluR species are found in the plasma membrane. Nevertheless, the incompletely glycosylated subunits can still assemble into homomeric or heteromeric complexes with functional agonist binding sites. The evidence for this is (a) that the proportion of total [^{3}H]AMPA binding sites on the surface of the GluR-expressing kidney cells is very similar to the proportion of total GluR protein on the surface and (b) that immunoprecipitation of GluR1/GluR2 heteromeric receptors with an anti-GluR1 antibody brings down both the upper (fully glycosylated) and lower (partially glycosylated) bands of GluR2 immunostaining.

Each of the GluR subunits contains multiple consensus glycosylation sites in the N-terminal region (Keinänen et al., 1990). Our observation that at least one of these sites is associated with insertion into the plasma membrane but is not necessary for receptor assembly is reminiscent of the case for a glycosylation site conserved in all nicotinic acetylcholine receptor subunits (Gehle and Sumikawa, 1991), which was reported to be necessary for surface expression but not for assembly. It is possible, however, that the multiple glycosylation sites in the GluR subunits have different regulatory roles, as it has been shown that total blockade of GluR glycosylation with tunicamycin results in nonfunctional receptors as determined by both ligand binding (Kawamoto et al., 1994) and physiological response to applied agonist (Mußhoff et al., 1992).

Western blots of brain GluR1–4 immunoreactivity reveal single bands for each subunit (Blackstone et al., 1992; Wenthold et al., 1992). We found in the present work that brain and hippocampal culture GluR subunits comigrate on SDS-PAGE with the fully glycosylated GluR subunits from the stable BHK cell lines. This suggests that all GluR subunits in neurons are fully glycosylated. Given this, it might be expected that the vast majority of neuronal GluR subunits should be found at the cell surface, because virtually all fully glycosylated GluR subunits in the BHK cells are surface-expressed. We have previously shown, however, that only about half of total GluR subunits in cultured hippocampal neurons are found at the cell surface (Hall and Soderling, 1996). It is not clear at present why neurons possess such large intracellular pools of fully glycosylated GluR subunits.

In summary, we have examined in the present study the surface expression of the AMPA receptor subunits GluR1, GluR2, and GluR4 stably expressed in BHK-570 cells. We have found that 25–40% of total GluR protein and 30–40% of total binding sites are localized to the cell surface. A majority of the expressed GluR subunits are incompletely glycosylated, and these subunits are preferentially found intracellularly. These data suggest a key role for glycosylation in the trafficking of GluR subunits. A further important finding in the present work is that fully glycosylated GluR subunits are all trafficked with roughly equal efficiency to the cell surface and that heteromeric combinations are not trafficked more efficiently than homomeric combinations. The subunits of certain other ionotropic receptors, such as NMDA-type glutamate receptors (McIlhinney et al., 1996) and nicotinic acetylcholine receptors (Claudio et al., 1991), are trafficked much more efficiently to the cell surface as heteromeric assemblies than as homomeric assemblies. In cases such as these, where efficient receptor trafficking is dependent on proper subunit stoichiometry, the number of subunit combinations that might potentially yield functional receptors is somewhat restricted. In contrast, fully glycosylated GluR subunits are all trafficked to the cell surface efficiently, as both homomeric and heteromeric assemblies, and thus there would seem to be an enormous number of potential GluR subunit

arrangements that can result in the formation of functional, surface-expressed AMPA receptors.

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