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Evidence that High- and Low-Affinity DL-α-Amino-3-Hydroxy-5-Methylisoxazole-4-Propionic Acid (AMPA) Binding Sites Reflect Membrane-Dependent States of a Single Receptor

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Abstract: Binding of DL-α-[^{3}H]amino-3-hydroxy-5-methylisoxazole-4-propionic acid ([^{3}H]AMPA) to lysed rat brain membranes in the presence of potassium thiocyanate resulted in curvilinear Scatchard plots that could be resolved by regression analysis into a large low-affinity component and a small high-affinity component. Solubilization with Triton X-100 resulted in solubilized and nonsolubilized fractions that were considerably enriched in the high-affinity component and correspondingly reduced in the low-affinity component. It thus appears that solubilization converts low-affinity AMPA receptors into high-affinity receptors. Also, synaptic plasma membranes were found to be greatly enriched in the low-affinity form and deficient in the high-affinity form of the AMPA receptor. These experiments provide evidence for the hypothesis that the high- and low-affinity components of AMPA binding are interconvertible states of the same receptor rather than separate binding sites and that the conversion of these receptors from their native high-affinity state to the low-affinity state occurs on insertion of the receptors into synapses. **Key Words:** Glutamate receptors—DL-α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid—Solubilization—Affinity. **Hall R. A. et al.** Evidence that high- and low-affinity DL-α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) binding sites reflect membrane-dependent states of a single receptor. *J. Neurochem.* **59,** 1997–2004 (1992).

Glutamate receptors, the primary mediators of excitatory synaptic transmission in mammalian forebrain, are usually classified into three groups based on agonist preference: *N*-methyl-D-aspartate (NMDA), kainate, and DL-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors (Watkins and Evans, 1981). In addition to their role in transmission, glutamate receptors are also central to the plasticity of telencephalic synapses. It is well established that NMDA receptors participate in the induction of long-term potentiation (LTP) (Collingridge et al., 1983), and recent studies indicate that modifications of AMPA receptors likely contribute to LTP expression (Muller et al., 1988; Staubli et al., 1990, 1992). Thus, an understanding of the factors that control AMPA receptors should provide insights into how the characteristics of excitatory postsynaptic currents are achieved as well as how they are modified by physiological activity.

There is considerable evidence that AMPA receptors in mammalian brain homogenate are not homogeneous (Murphy et al., 1987; Olsen et al., 1987; Honore and Drejer, 1988; Terramani et al., 1988; Hunter et al., 1990; Nielsen et al., 1990; Morgan et al., 1991; Massicotte et al., 1991). Scatchard analyses of [^{3}H]AMPA binding are curvilinear and two-site models with affinities of 5–40 n*M* ("high") and 200–1000 n*M* ("low") consistently provide a better fit than do one-site models. Two classes of AMPA receptors—low affinity/high conductance and high affinity/low conductance—are also evident in physiological studies of excised patches from cultured hippocampal neurons (Tang et al., 1989). The low-affinity/high-conductance group exhibits rapid desensitization that subsequent work has shown to be partially blocked by the drug aniracetam (Tang et al., 1991); the finding that aniracetam prolongs fast excitatory postsynaptic currents in cultured cells (Tang et al., 1991) and slices of hippocampus (Staubli et al., 1992) implies that the low-affinity/high-conductance receptor is the synaptic receptor.

The existence of two apparent AMPA receptors in

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Abbreviations used: AMPA, DL-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; KSCN, potassium thiocyanate; LTP, long-term potentiation; NMDA, *N*-methyl-D-aspartate; PEG, polyethylene glycol; SPM, synaptic plasma membrane.

the biochemical and physiological experiments raises the question whether these are different receptors or different states of the same receptor. In support of the latter idea is the observation that a number of manipulations are known to affect markedly the affinity of the AMPA binding site. These include chaotropic ions (Honore and Drejer, 1988) and sulfhydryl reagents (Terramani et al., 1988), which reversibly increase the affinity of membrane-associated AMPA receptors, and phospholipase A_2 treatment (Massicotte et al., 1991) and high-energy irradiation (Honore and Nielsen, 1985), which irreversibly increase their affinity. These treatments produce graded effects and thus are not direct evidence of a two-state receptor; they do, however, indicate that the receptor is malleable and hence support the idea that it can change binding configurations in situ. One approach to testing the single receptor hypothesis is to attempt explicitly to convert one group into the other. Membrane environments are known to influence receptor properties (Stephenson et al., 1982; Wikberg et al., 1983; Florio and Sternweis, 1985; Luedtke and Molinoff, 1987; Sidhu, 1988; Oriowo et al., 1991) and hence, assuming a single-receptor hypothesis, might be expected to affect whether the AMPA receptor is in one state or the other. If so, solubilization should result in marked decreases in the numbers of one of the two varieties of AMPA receptors, with an equivalent increase in the population of the other type. Solubilization is known to modify AMPA binding, but there is disagreement in the literature as to whether the affinity of the solubilized fraction increases (Hunter et al., 1990) or decreases (Henley and Barnard, 1991), and explicit tests for inverse changes in the numbers of the two subtypes have not been conducted.

The present experiments (a) reexamined the effects of solubilization on membrane-bound AMPA receptors and asked whether they accord with a two-state model and (b) tested whether, as expected from physiological experiments, low-affinity binding sites are concentrated in synaptic fractions. The results indicate that solubilization causes an increase in high-affinity sites and a large decrease in low-affinity sites. Purified synaptic plasma membranes (SPMs) were found to be enriched in low-affinity sites and to be deficient in high-affinity binding, suggesting that the former may correspond to the high-conductance, rapidly desensitizing receptors identified in physiological experiments. Together these findings support the single-receptor hypothesis and raise the possibility that high- and low-affinity sites represent extrasynaptic vs synaptic versions of that receptor.

MATERIALS AND METHODS

Preparation of membranes

Whole brains were collected from adult male Sprague–Dawley rats that had been anesthetized with ether before decapitation. The brains were kept frozen at −80°C for up to 3 months until use. After thawing, the brains were homogenized in 0.32 *M* sucrose, 1 m*M* EGTA ("sucrose solution"; 20 ml/brain) using a glass/Teflon homogenizer. The homogenate was centrifuged at 800 *g* for 10 min and the supernatant was recentrifuged at 48,000 *g* for 30 min. The resultant membrane pellet (P_2) was lysed by resuspending it in 20 ml of 1 m*M* EGTA, followed by a 20-min incubation on ice and centrifugation at 48,000 *g* for 30 min. This lysis and centrifugation cycle was repeated one more time. The pellet was then resuspended in the assay buffer (in general, 500 m*M* Tris acetate, 50 μ*M* EGTA, pH 7.2) to approximately 1 mg/ml; this sample is referred to as "lysed membranes" or "starting material." All procedures for membrane preparation were carried out at 0–4°C.

Solubilization

Triton X-100 was added to lysed membranes at 0.4% (wt/vol) to achieve solubilization. The membrane/detergent mix was incubated for 20 min at 37°C with constant agitation. The incubation was followed by centrifugation at 48,000 *g* for 2 h. The resultant supernatant was removed and dialyzed for 24 h against a 100-fold excess of assay buffer containing 0.4% Triton X-100, with one buffer change. This dialysate is termed the "soluble" fraction. The pellet obtained in the 2-h centrifugation was resuspended in 30 ml of the assay buffer and centrifuged again for 2 h at 48,000 *g*. The resultant pellet was resuspended in assay buffer to approximately 1 mg protein/ml; this fraction is termed the "nonsolubilized" fraction. All fractions were stored at −80°C until use.

In some experiments, the membranes were treated with detergent under milder solubilization conditions. In this case, the lysed membranes were suspended in a buffer of reduced ionic strength containing 100 m*M* Tris acetate, 50 μ*M* EGTA, pH 7.2, and the incubation with Triton X-100 was done at 0 instead of 37°C. All other steps were the same as described above.

Preparation of SPMs

To form a Percoll gradient, 2 ml each of 3, 10, 15, and 25% Percoll in 100 m*M* Tris acetate, 50 μ*M* EGTA, pH 7.2, was layered into a 15-ml centrifuge tube. One milliliter of material from the P_2 fraction, resuspended in sucrose solution (see above), was layered on top of the gradient, and the tube was centrifuged at 48,000 g_{max} for exactly 5 min. The interface between the 15% Percoll and the 25% Percoll layers was extracted, diluted in sucrose solution, and centrifuged at 48,000 *g* for 30 min (Dunkley et al., 1986). The resultant pellet was then lysed as described above for the regular membrane preparation. The final pellet was resuspended in 100 m*M* Tris acetate, 50 μ*M* EGTA, pH 7.2, to approximately 1 mg of protein/ml. All procedures for SPM preparation were carried out at 0–4°C.

Receptor binding assays

[^{3}H]AMPA binding was studied using the centrifugation method. Typically, 50–100 μg of protein in a final volume of 100 μl was equilibrated with AMPA for 90–120 min at 0°C in the presence of 50 m*M* potassium thiocyanate (KSCN). To determine binding constants, AMPA concentrations between 2 n*M* and 1.5 μ*M* were chosen. Concentrations between 2 and 50 n*M* were measured by increasing the concentration of radiolabeled AMPA, whereas those above 50 n*M* were reached by adding unlabeled AMPA; preliminary studies had shown that both methods produce identical saturation curves in the case of [^{3}H]AMPA binding. Non-

specific binding was defined as that occurring in the presence of 2.5 mM unlabeled L-glutamate. Samples were centrifuged at 48,000 g (4°C) for 20 min, then placed back in the ice water bath for 10–20 min. After this, the supernatants were aspirated and the pellets superficially rinsed with 0.5 ml of ice-cold assay buffer and dissolved in 10 μl of Beckman tissue solubilizer. Radiolabel content was assayed by liquid scintillation spectroscopy with a counting efficiency of 0.40 in 1 ml of aqueous counting scintillant. For assaying soluble fractions, 10 μl of 25 mg/ml γ-globulin in assay buffer and 90 μl of a 30% polyethylene glycol (PEG) solution in assay buffer were added to each sample at the end of the incubation period to produce protein aggregation; thus, the final volume for the soluble samples was 200 μl. The twofold dilution of the ligand was taken into account in the calculations; in preliminary studies γ-globulin and PEG were shown to have no effect on ligand binding. The pellets were dissolved in 100 μl of dH_2O and counted with an efficiency of 0.35. Results are expressed as specifically bound [^{3}H]AMPA, which equals the difference between the total bound and the nonspecifically bound ligand. Two-site analysis of binding data was performed by nonlinear regression using the Inplot program by GraphPAD Software, Inc.

^{3}H-Labeled 6-cyano-7-nitroquinoxaline-2,3-dione ([^{3}H]-CNQX) binding was determined at ligand concentrations ranging from 10 to 300 nM; KSCN was omitted from the assay buffer. Nonspecific binding was defined as the amount of [^{3}H]CNQX bound in the presence of 25 μM unlabeled CNQX.

The affinity for L-glutamate was determined in centrifugation assays by measuring displacement of 5 nM [^{3}H]AMPA by increasing concentrations of L-glutamate (30 nM–30 μM).

Protein concentrations were determined according to Bradford (1976) with the protein assay reagent obtained from Bio-Rad and with bovine serum albumin as standard. Separate standard curves were constructed for samples with and without Triton X-100.

[^{3}H]AMPA and [^{3}H]CNQX were purchased from NEN/Dupont. AMPA was obtained from Tocris; CNQX, from Cambridge Research Biochemicals. γ-Globulin, PEG, and other reagents were from Sigma.

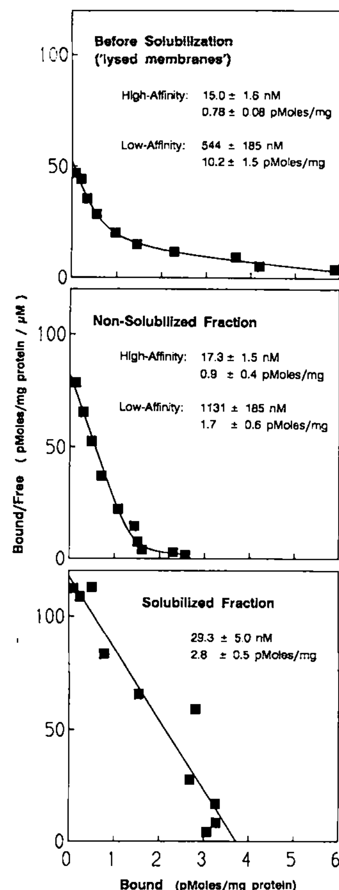

FIG. 1. Scatchard plots of [^{3}H]AMPA binding before and after solubilization. Representative Scatchard plots are shown from an experiment in which a lysed whole-brain membrane preparation was treated with 0.4% Triton X-100 at 37°C in a high-ionic strength buffer as described in Materials and Methods. Material that did not sediment in a 2-h 48,000 g centrifugation was designated the "solubilized fraction"; the pelleted material that represents the "nonsolubilized fraction" was resuspended in buffer and recentrifuged before use to remove residual detergent. [^{3}H]-AMPA binding was measured in triplicate at 2, 5, 10, 20, 50, 100, 200, 400, 800, and 1,500 nM except for the solubilized fraction, where the [^{3}H]AMPA concentrations were half of those listed. The assay buffer contained 50 mM KSCN. Lines were fitted by nonlinear regression analysis of the primary binding data (top and middle) or by linear regression of the Scatchard transformed data (bottom). K_D and B_{max} values (mean and SEM) averaged from three such solubilization experiments are shown as insets.

RESULTS

Scatchard analysis of lysed whole rat brain membranes resulted in a curvilinear plot (Fig. 1, top). Two-site fitting of these graphs consistently revealed binding sites with affinities of 5–40 and 200–1,000 nM and B_{max} values on the order of 0.8 and 10 pmol/mg of protein, respectively; thus, the low-affinity component was typically 10–12 times more abundant. After detergent treatment, the Scatchard plots for the resultant fractions containing solubilized and nonsolubilized proteins were distinctly different (Fig. 1, middle and bottom). In the solubilized fraction, binding appeared to be uniformly of the high-affinity type, with an average K_D of 29 ± 5 nM (three experiments); the B_{max} was 2.8 ± 0.5 pmol/mg of protein and thus was increased three- to fourfold over the B_{max} of the high-affinity component seen in the starting material. The binding sites remaining in the nonsolubilized material still contained some low-affinity sites but their number was greatly reduced compared to the starting material, and the proportion of high-affinity sites in the nonsolubilized material was considerably higher than in the starting material.

Table 1 lists the amounts of high- and low-affinity sites in the fractions obtained by multiplying the B_{max} values by the protein content. Of 100 pmol of binding sites present in the starting material, a total of 33.3 pmol was recovered after solubilization, 24.6 pmol in the solubilized fraction and 8.6 pmol in the nonsolubilized fraction. Combining all the high-affinity sites,

TABLE 1. *[^{3}H]AMPA binding before and after solubilization*

	Percentage of total number of sites in starting material		
	Total number of sites	High-affinity sites	Low-affinity sites
Before solubilization	100	7.1 ± 1.9	92.2 ± 1.9
Solubilized fraction	24.6 ± 5.3	24.6 ± 5.3	~0
Nonsolubilized fraction	8.6 ± 2.9	3.5 ± 1.5	5.2 ± 1.5
Total after solubilization	33.3 ± 6.6	28.1 ± 6.1	5.2 ± 1.5
Ratio, after/before		3.9	0.06

The combined number of high- and low-affinity [^{3}H]AMPA binding sites in the starting material was set as 100% and the number of each type of site in the other fractions is expressed as a percentage of this. In each instance, the number of binding sites was obtained by multiplying the B_{max} by the amount of protein in that particular fraction. The data shown are averages (with SEM) from the same three preparations for which averaged K_D and B_{max} values are given in Fig. 1. For the "total after solubilization," the numbers of sites in solubilized and nonsolubilized fractions were combined for each preparation and then averaged. The solubilized fraction contained 70–85% of the protein present in the starting material.

TABLE 2. *[^{3}H]CNQX binding before and after solubilization*

	K_D (n*M*)	Percentage of sites relative to starting material
Before solubilization	132 ± 9	100
Solubilized fraction	70 ± 5	25.1 ± 5.2
Nonsolubilized fraction	133 ± 16	12.6 ± 4.6
Total after solubilization		37.8 ± 7.2

The total number of binding sites was calculated for each fraction by multiplying the B_{max} by the amount of protein. The data shown are the mean and SEM of three preparations.

one obtains a total of 28 pmol that accounts for 84% of all sites after solubilization. Thus, solubilization increases the amount of binding sites of the high-affinity type from 7 to 28 pmol. Expressed as the percentage of low- plus high-affinity sites, the increase is from 7 to 84% (Table 1).

As the total number of sites after solubilization was only one-third of the original number, it appeared that a number of binding sites were lost during solubilization. To test this, Scatchard analyses were performed using [^{3}H]CNQX. This AMPA receptor antagonist labels the same population of sites as AMPA but does not discriminate between low- and high-affinity variants (Honore et al., 1989; Nielsen et al., 1990). Its binding properties are not as readily manipulated as AMPA binding (unpublished observations) and appear to change minimally after solubilization treatments (Table 2). The recovery of receptors estimated from [^{3}H]CNQX binding was 38% (Table 2) and thus was similar to the value obtained using AMPA as a ligand.

To test if the above results were due to detergent effects on the receptors or to some facet of the solubilization process, membranes were exposed to the same concentration of Triton X-100 as in the previous experiment but under milder incubation conditions. These experiments resulted in Scatchard plots for both soluble and membrane-bound fractions that were different from those seen following regular solubilization (Fig. 2). Scatchard analysis revealed a curvilinear plot for the nonsolubilized fraction similar to that of the starting material, with only a slight reduction in the number of low-affinity sites and a comparable increase in high-affinity sites (data not shown). The solubilized fraction, which contained 50–60% of the protein, contained only a small number (5–10%) of binding sites. These solubilized receptors, like those in the regular solubilized fraction, were exclusively of the high-affinity type. In two experiments, lysed membranes were exposed to 5% octylglucoside for 60 min at 0°C. The resultant solubilized fractions were processed identically to the post-Triton soluble fractions as described in Materials and Methods (including dialysis); the K_D for AMPA of these two samples was between 15 and 30 n*M*.

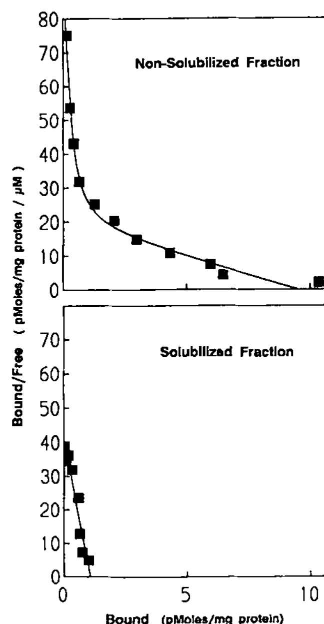

FIG. 2. Scatchard plots of [^{3}H]AMPA binding after mild detergent treatment. A lysed whole-brain membrane preparation was subjected to Triton X-100 treatment in which the solubilization temperature was 0 instead of 37°C and the ion concentration was reduced to 100 m*M* (see Materials and Methods). Conditions for [^{3}H]AMPA binding were the same as in the legend to Fig. 1. The solubilized fraction contained 50–60% of the total protein and 5–10% of the binding sites present in the starting material.

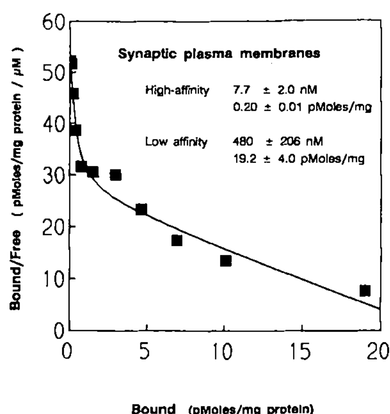

FIG. 3. Scatchard plot of [^{3}H]AMPA binding to SPMs. SPMs were prepared on Percoll gradients as described in Materials and Methods. Assay conditions were the same as in the legend to Fig. 1. K_D and B_{max} values (mean and SEM) averaged from three SPM preparations are shown as insets and in Table 3.

In another set of control experiments, lysed membranes were incubated at 37°C using the same protocol as in solubilization experiments but without the addition of Triton X-100. This treatment did not produce a noticeable change in the [^{3}H]AMPA binding characteristics (data not shown). Additional experiments were conducted using filtration through Whatman GF/B glass-fiber filters instead of the centrifugation assay. The binding profiles of most fractions were similar to those seen with the centrifugation assay; however, the B_{max} values in general were about 50% lower than those found using the centrifugation method and binding in the nonsolubilized fraction revealed only the high-affinity form. This loss of binding is presumably due to dissociation from low-affinity sites, which occurred in spite of the precautions taken to minimize it, e.g., the inclusion of high concentrations of KSCN in the ice-cold filtration buffer. Thus, it appears that the filtration assay is not an appropriate choice for quantitative studies of [^{3}H]-AMPA binding and data obtained with that method were excluded from the present analysis.

The above results indicate that membrane-bound AMPA receptors are predominantly of the low-affinity variety, whereas solubilized receptors are exclusively of high affinity. From this it might be expected that synapses would contain high concentrations of low-affinity sites. To test this, binding was compared between SPMs and lysed membrane starting material. Scatchard analysis of the SPMs resulted in a curvilinear plot (Fig. 3). A two-site fit of this plot revealed affinities similar to those seen in normal lysed membranes (Table 3). However, the B_{max} of the low-affinity component in the SPMs was 88% higher than in lysed membranes, whereas the B_{max} of the high-affinity component was 74% lower; this meant that the ratio of low- to high-affinity sites was nearly sevenfold higher in SPMs. Thus, the prevalence of low-affinity sites increased, whereas that of high-affinity sites decreased. It is also of interest that the total B_{max} of AMPA-binding sites (high- and low-affinity combined) was nearly twice as high in SPMs as in lysed membranes.

L-Glutamate is thought to be the endogenous ligand for the AMPA receptor, and it was of interest to determine whether the change in affinity seen in AMPA binding with solubilization also held for the putative transmitter. This proved to be the case: glutamate displacement of 5 nM AMPA in membranes resulted in an apparent K_i of 1.8 ± 0.8 μM, whereas in the dialyzed soluble fractions the apparent K_i of glutamate was 0.45 ± 0.03 μM (average of three experiments with SEM). Thus, the affinity for L-glutamate

TABLE 3. *Comparison of [^{3}H]AMPA binding in lysed membranes and SPMs*

	B_{max} (pmol/mg of protein)			
	Total	High-affinity sites	Low-affinity sites	Ratio, low/high
Lysed membranes	11.0 ± 1.7	0.71 ± 0.06	10.3 ± 1.7	14
SPMs	19.4 ± 4.0	0.20 ± 0.01	19.2 ± 4.0	96
Ratio, SPMs/lysed membranes	1.8	0.28	1.9	6.8

	K_D (nM)	
	High-affinity sites	Low-affinity sites
Lysed membranes	15.0 ± 1.6	544 ± 185
SPMs	7.7 ± 2.0	480 ± 206

Averaged data (mean and SEM) from three preparations of synaptic plasma membranes (SPMs) and six preparations of lysed whole-brain membranes. Three of the lysed membranes preparations were prepared in matched sets, with SPM preparations starting from the same P_2 material. A representative Scatchard plot of an SPM preparation is shown in Fig. 3.

was nearly fourfold higher in solubilized fractions than in lysed membranes.

DISCUSSION

The results described above indicate that solubilization of brain membranes results in an increase in high-affinity AMPA sites and an almost-complete loss of low-affinity AMPA sites. The most straightforward interpretation of this is that solubilization converts low-affinity into high-affinity sites. A more complex explanation is that the solubilization procedure inactivates low-affinity receptors while, at the same time, uncovering high-affinity sites normally undetected by binding assays. The relative magnitudes of the observed effects suggest that the first, and simpler, of the two hypotheses is correct. Our results indicate that about two-thirds of all AMPA sites are lost or rendered nonfunctional during the solubilization procedures. This was confirmed with [^{3}H]CNQX, which binds to the same receptor population as AMPA but does not discriminate between low- and high-affinity sites (Honore et al., 1989; Nielsen et al., 1990) and which is less easily disturbed than AMPA binding. Similar losses of NMDA receptors following solubilization with Triton X-100 have also been observed (unpublished data). It therefore appears that the post-solubilization fractions contain the same population of receptors as before solubilization, only minus the 67% of the receptors that were lost during solubilization. One-third of the low-affinity sites expected from the CNQX binding data to be present after solubilization corresponds reasonably well with the number of high-affinity AMPA binding sites found after solubilization. Thus, our data offer strong evidence that low-affinity AMPA receptors in the starting material were converted into high-affinity receptors on solubilization. This conversion was contingent on solubilization, as no change in binding affinity was observed when membranes were incubated at 37°C in the absence of Triton X-100, nor were low-affinity sites eliminated from the postsolubilization membrane-bound fractions after exposure to Triton that was insufficient to solubilize the receptors (the existence of low-affinity sites despite exposure to Triton is more clearly seen in the fractions subjected to less stringent solubilization conditions).

In previous work, Honore and Drejer (1988) used kinetic data to argue that the two apparently different AMPA binding sites are states of the same receptor. However, the kinetic model used by these authors resulted in calculated equilibrium binding constants of 0.37 and 2.4 nM, values that have not been confirmed in any equilibrium study. With regard to the effects of solubilization, Hunter et al. (1990) obtained a marked increase in high-affinity AMPA binding sites in solubilized rat brain tissue as reported here; however, the AMPA binding Scatchard plots of the soluble fractions in these experiments still appeared curvilinear, indicating the existence of low-affinity binding sites. Henley and Barnard (1991), conversely, found a single population of AMPA binding sites in solubilized chick cerebellum, with a fivefold decrease in affinity compared to membranes. In solubilized frog brain membranes, the same group (Henley et al., 1989) also found a decrease in affinity, which was, however, followed by a large increase in affinity on partial purification of the receptors (Ambrosini et al., 1990). These differences from our data may arise from species differences or from variations in experimental techniques. One aspect that appears to be of particular importance is dialysis of the solubilized fraction. Despite lysis and repeated washings during membrane preparation, it was found in our experiments that solubilized fractions before dialysis still contained glutamate at a concentration sufficient to shift apparent K_D values for [^{3}H]AMPA binding from 20–30 nM into the range of 250–500 nM. Dialysis periods longer than that described in Materials and Methods did not result in a change in the binding characteristics of the solubilized samples.

Studies with recombinantly expressed glutamate receptors have revealed a single class of high-affinity AMPA binding sites (K_D = 12 nM), which were shown by patch-clamp experiments to be of a low conductance (1 pS) (Keinanen et al., 1990). Thus, it appears that the AMPA receptor, in its native state and unincorporated into a synapse, is of a high affinity and a low conductance. This observation, combined with our finding that SPMs are considerably enriched in low-affinity receptors and deficient in high-affinity receptors, suggests that newly synthesized, high-affinity receptors are converted into lower-affinity receptors on insertion into synapses. There is a precedent for this: it has been hypothesized from studies on peripheral cholinergic receptors that extrajunctional nicotinic acetylcholine receptors may have properties different from those of receptors incorporated into the postsynaptic specialization (Neher and Sakmann, 1976; Reiser et al., 1989). Pertinent to the above argument, it should be noted that the concentration of low-affinity sites in synaptic membranes agrees with predictions derived from physiological/pharmacological experiments (see the introductory section). This agreement points to the conclusion that the two classes of glutamate receptors identified in such studies (high affinity/low conductance vs. low affinity/high conductance) correspond to the two types of binding sites (high vs. low affinity) described here and elsewhere. Thus, from the two-state, single-receptor hypothesis, it would be predicted that solubilized AMPA receptors reconstituted into artificial membranes should exhibit a high affinity and a low conductance. More generally, the likely correspondence between physiologically and biochemically defined receptor classes, viewed from the perspective of the interconvertible receptor hypothesis, raises the possibility that interactions in the synaptic region af-

fect both binding and conductance properties of the AMPA receptor.

As discussed earlier, AMPA receptors are thought to be central to synaptic plasticity in the mammalian brain (Staubli et al., 1992), and recent studies have also implicated them in epilepsy (Hosford et al., 1991) and excitotoxicity (Koh et al., 1990; Buchan et al., 1991). Thus, the means by which modulation of AMPA receptors occurs is a topic of considerable interest. As all sites in solubilized fractions were of a high affinity, and given that the membrane-bound fraction of the solubilized samples had a much higher percentage of high-affinity sites than did normal lysed membranes, it appears that the affinity-modulating factor(s) is disrupted by the solubilization process. This is in agreement with the report by Honore et al. (1989), who found that high-energy irradiation treatment irreversibly raised the affinity of AMPA binding sites; this was interpreted to mean that the AMPA receptor was held in a lower-affinity state by a large modulatory protein. Another possibility is that the membrane fluidity and/or lipid environment is the key factor, as added phospholipids are known to affect AMPA receptor affinity (Baudry et al., 1991). In addition, AMPA receptors may be conformationally constrained by the cytoskeleton of synaptic membranes. Finally, interactions between receptors could maintain them in a low-affinity/high-conductance state; thus, in the synapse, where receptor density is highest according to our data, a greater proportion of receptors would be modulated to a low affinity. Experiments to discriminate among these possibilities are in progress.

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