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Effects of Thiol-Reagents on [^{3}H]α-Amino-3-hydroxy-5-methylisoxazole-4-propionic Acid Binding to Rat Telencephalic Membranes

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SUMMARY

The binding of [^{3}H]α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid ([^{3}H]AMPA), a ligand for the quisqualate subtype of excitatory amino acid receptors, was measured after chemical modifications of rat brain synaptic membranes. Treatment with oxidizing or thiol-alkylating agents did not modify [^{3}H]AMPA binding, whereas treatment with several sulfhydryl reagents produced marked increases in binding. The involvement of free sulfhydryl groups in the regulation of the properties of [^{3}H]AMPA binding sites was suggested by the specificity of *p*-chloromercuribenzoic acid (PCMB), its sulfonate analog *p*-chloromercuriphenyl-sulfonic acid (PCMBS), and $HgCl_2$, plus the reversal of their effects after reduction with dithiothreitol. Pretreatment of synaptic membranes with the oxidizing agent 5,5′-dithiobis(2-nitrobenzoic acid) or the alkylating agent *N*-ethylmaleimide did not significantly affect [^{3}H]AMPA binding but markedly reduced the enhancing effect of PCMBS. On the other hand, the increase in [^{3}H]AMPA binding produced by PCMBS was not prevented by treatment with agonists such as quisqualate or L-glutamate and was produced equally well in resealed postsynaptic membranes with both lipophilic or nonlipophilic SH-reagents. Using filtration assays, two types of binding sites could be detected with high and low affinity for [^{3}H]AMPA. Treatment with SH-reagents produced an increase in the B_{max} for the high affinity component and a decrease in the B_{max} for the low affinity component, accompanied by an increase in its affinity for the ligand. Using centrifugation assays, the same two types of sites could be detected under control conditions but treatment with SH-reagents produced an increase in affinity of the large component that prevented the analytical differentiation of the two sites. Treatment with SH-reagents also increased the binding of [^{3}H]glutamate to the *N*-methyl-D-aspartate receptors but did not modify the binding of [^{3}H]kainate to the kainate receptors or the strychnine-insensitive [^{3}H]glycine binding. These results suggest that free sulfhydryl groups allosterically modulate the affinity of the quisqualate subtype of excitatory amino acid receptors and also indicate that different types of glutamate receptors might be differentially affected by chemical modification.

Several types of excitatory amino acid receptors have been defined on the basis of biochemical, pharmacological, and electrophysiological studies (1–3). They are generally designated the NMDA, kainate, and quisqualate receptors, although it has proven difficult to distinguish between the kainate and the quisqualate receptors. Binding techniques have confirmed this classification and it is generally agreed that each subtype of receptor can be studied with appropriate ligands as follows: [^{3}H]glutamate or [^{3}H]2-amino-5-phosphonopentanoate for NMDA receptors (1, 4–6), [^{3}H]kainate for kainate receptors (7), and [^{3}H]AMPA for quisqualate receptors. A number of groups have now shown that in the presence of the chaotropic ion, potassium thiocyanate (KSCN), the latter ligand appears to label sites in brain tissues with properties expected of quisqualate receptors (8–10). In addition, it has recently been shown that the NMDA receptor is linked to a glycine site that is labeled with [^{3}H]glycine in a strychnine-insensitive manner (11, 12).

Following the initial discovery that disulfide and sulfhydryl groups play a critical role in the regulation of the nicotinic and muscarinic receptors, respectively (13–17), it has been shown that these groups are involved in the functioning of numerous receptor complexes (18–21). In some cases it has been reported that these groups are very close to or at the recognition site for agonists or antagonists (20, 21) and their chemical modification directly modifies the properties of ligand binding. In other cases, although they might be distant from the binding sites, their chemical modification results in some alterations in the conformation of receptors producing allosteric changes in affin-

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ABBREVIATIONS: NMDA, *N*-methyl-D-aspartate; AMPA, DL-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; PCMB, *p*-chloromercuribenzoic acid; PCMBS, *p*-chloromercuriphenyl-sulfonic acid; DTT, dithiothreitol; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); NEM, *N*-ethylmaleimide; EGTA, ethylene glycol-bis-(β-aminoethyl ether)tetraacetic acid.

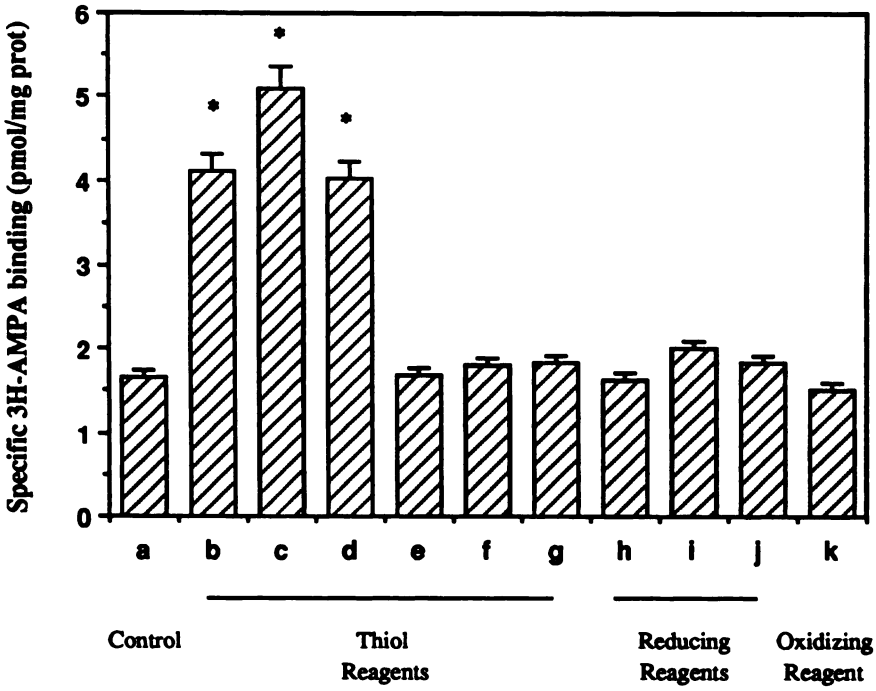

Fig. 1. Effects of different chemical reagents on [^{3}H]AMPA binding to rat brain synaptic membranes. Synaptic membranes were prepared as described under Materials and Methods and incubated with different chemical reagents for 30 min at 35°. After two cycles of centrifugation and resuspension in Tris/acetate buffer, the membranes were resuspended in the same buffer and the binding of [^{3}H]AMPA (50 nM) was determined. Results are expressed as pmol/mg of protein and are mean ± standard error of four to six experiments. a, Control; b, PCMB (1 mM); c, PCMBS (2 mM); d, $HgCl_2$ (2 mM); e, NEM (2 mM), f, iodoacetic acid (2 mM); g, $CdCl_2$ (5 mM); h, cysteine (2 mM); i, DTT (2 mM); j, glutathione (2 mM); and k, DTNB (2 mM). (*, significantly different from control ($p < 0.001$, Student's t test).

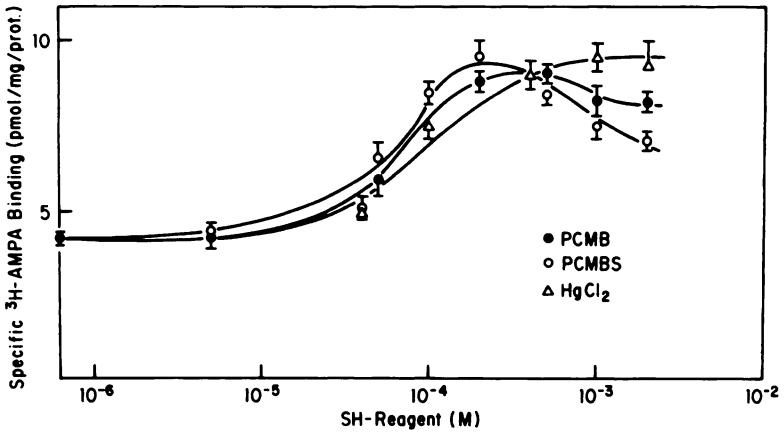

Fig. 2. Effects of various concentrations of PCMB, PCMBS, and $HgCl_2$ on [^{3}H]AMPA binding. Synaptic membranes were prepared and treated with the indicated concentrations of PCMB, PCMBS, and $HgCl_2$ at 35° for 30 min. The binding of [^{3}H]AMPA (150 nM) was then assayed as described under Materials and Methods. Results are expressed as pmol/mg of protein and are mean ± standard error of four to six experiments.

TABLE 1

Reversal of thiol-reagent effects by DTT

Synaptic membranes were first incubated with 2 mM PCMBS or 5 mM $HgCl_2$ for 30 min at 35°. After two cycles of centrifugation and resuspension in Tris/acetate buffer, membranes were incubated in the absence or presence of (2 mM) DTT at 35° for 20 min. [^{3}H]AMPA binding was then measured as described under Materials and Methods at a concentration of [^{3}H]AMPA of 100 nM. Results are mean ± standard error of four experiments.

Addition	[^{3}H]AMPA Bound	
	Control	DTT
	pmol/mg of protein	
Control	3.45 ± 0.09	3.47 ± 0.29
PCMBS (2 mM)	7.43 ± 0.24	3.28 ± 0.06
$HgCl_2$ (5 mM)	6.06 ± 0.24	4.44 ± 0.22

TABLE 2

Effects of glutamate and quisqualate on the stimulation of [^{3}H]AMPA binding elicited by PCMBS

Synaptic membranes were first preincubated with 50 μM quisqualate or 50 μM L-glutamate for 15 min at 35°. The incubation was then continued in the absence or presence of 2 mM PCMBS for 30 min. Synaptic membranes were then washed by several cycles of centrifugation and resuspension in Tris/acetate buffer and the binding of 80 nM [^{3}H]AMPA was determined as described under Materials and Methods. Results are mean ± standard error of four experiments.

Addition	[^{3}H]AMPA Bound	
	Control	PCMBS
	pmol/mg of protein	
Control	2.10 ± 0.06	7.50 ± 0.30
Quisqualate (50 μM)	2.46 ± 0.08	8.37 ± 0.47
Glutamate (50 μM)	2.76 ± 0.05	8.75 ± 0.82

ity for agonists or antagonists or in some other characteristics of the receptor binding properties (22, 23).

A recent publication examined the effects of chemical modifications of sulfhydryl residues on glutamate receptor/channel complexes in hippocampal pyramidal neurons (24). The results of this study showed that the responses to glutamate and kainate were markedly depressed after treatment with a variety of compounds acting on SH-groups, thus suggesting the involvement of SH-groups in the regulation of the properties of some glutamate receptor/channel complexes. The present study investigated the effect of chemical modification of SH- or disulfide-groups on the binding characteristics of [^{3}H]AMPA, a ligand for the quisqualate receptors. The results indicate that only the former type of chemical group participates in the

TABLE 3

Effect of DTNB and NEM on the stimulation of [³H]AMPA binding elicited by PCMBS

Synaptic membranes were first incubated in the absence or presence of DTNB (5 mM) or NEM (2 mM) at 35° for 30 min. They were then washed by two cycles of centrifugation and resuspension in Tris/acetate buffer and were further incubated for 30 min at 35° with or without PCMBS (2 mM). [³H]AMPA (100 nM) binding was then measured as described under Materials and Methods. Results are mean ± standard error of 6–10 experiments. *Numbers in parentheses*, the percentage stimulation of [³H]AMPA binding elicited by PCMBS. *, $p < 0.001$ (Student's *t* test) compared with PCMBS treatment in control.

Addition	[³H]AMPA Bound		
	Control	DTNB	NEM
	pmol/mg of protein		
Control	3.10 ± 0.08	2.85 ± 0.22	3.48 ± 0.22
PCMBS (2 mM)	7.54 ± 0.26 (+143%)	4.35 ± 0.24* (+53%)	5.42 ± 0.04* (+56%)

regulation of the receptor binding properties, with reagents directed at free SH-groups producing an increase in affinity whereas reagents directed at disulfide bonds produce no effect on binding properties.

Materials and Methods

Preparation of membranes. Male Sprague-Dawley rats were sacrificed by decapitation and the telencephalon was rapidly dissected and homogenized in 10 volumes of 0.32 M sucrose containing 1 mM EGTA. Crude synaptic membranes were prepared as previously described (25, 26). The final pellet was resuspended in Tris/acetate buffer (100 mM Tris/acetate, pH 7.4, containing 50 μM EGTA) (approximately 5 mg of protein/ml) and the membrane preparation was incubated with 0.1% saponin at 35° for 30 min. Membranes were washed free of the detergent by two more steps of centrifugation and resuspension in Tris/acetate buffer. After resuspension of the membranes, aliquots were frozen at −70°.

Preparation of synaptoneurosomes. Synaptoneurosomes were prepared according to the method of Hollingsworth *et al.* (27). Briefly, rat cerebral cortex was cut manually with a cooled razor blade into small cubes. The tissue was homogenized by hand using a glass-glass homogenizer in 7 ml of Krebs-Ringer solution containing 124 mM NaCl, 3 mM KCl, 1.25 mM KH_2PO_4, 2.5 mM $MgSO_4$, 3.4 mM $CaCl_2$, 26 mM $NaHCO_3$, and 10 mM glucose, oxygenated with O_2/CO_2 (95:5). The homogenate was passed through four layers of nylon material (100 mesh), followed by filtration through a Millipore 10-μm filter (LCWP-047). The filtered solution was centrifuged at 1,000 × *g* for 15 min and the resultant pellet was resuspended by gentle homogenization in Krebs-Ringer solution.

Chemical modification of membranes. For chemical modification, membrane suspensions (or synaptoneurosomes) were incubated with the indicated concentrations of reagents directed at free sulfhydryl groups or disulfide bonds for 30 min at 35°. The membranes were then pelleted by centrifugation, washed, and resuspended in Tris/acetate buffer. In some cases they were further treated with a different reagent and washed again. In addition, treatment of membranes was also performed immediately before the binding assays.

Binding assays. Binding assays were performed in a final volume of 50 μl (approximately 20–40 μg protein/assay) in Tris/acetate buffer containing the indicated concentrations of ³H-ligands. Incubations were at 0° for 30–50 min and were terminated by dilution with 3 ml of ice-cold buffer and filtration through nitrocellulose filters (Millipore, 0.45 μm pore size) for [³H]glutamate, [³H]kainate, and [³H]glycine or GF/C filters for [³H]AMPA. Filters were washed with 3 ml of buffer and the radioactivity retained on the filters was measured with liquid scintillation counting. Nonspecific binding was determined in the presence of 1 mM glutamate, 40 μM kainate, 100 μM glycine, and 200 μM quisqualate, respectively.

For [³H]AMPA binding, 100 mM KSCN was added to the assay buffer. To minimize loss due to dissociation, KSCN was also included in the stop solution and care was taken to perform filtration and filter washing as rapidly as possible (completed in less than 4 sec); under these conditions, binding losses were in general below 20%. In saturation experiments, concentrations above 250 nM were obtained by diluting [³H]AMPA with unlabeled AMPA. In some [³H]AMPA binding experiments, assays were terminated by centrifuging the samples at 48,000 × *g* for 15 min, superficially washing the pellets with buffer containing 100 mM KSCN, resuspending the pellet in 0.1 N NaOH, and determining the radioactivity by liquid scintillation counting.

In the detergent-treated membranes used for these experiments, about 80% of [³H]glutamate binding represents NMDA receptors, with the remaining sites being mostly of the kainate type (5, 6, 25) (binding to AMPA/quisqualate sites is negligibly small in the absence of thiocyanate ions). In the glutamate binding assays reported here, 5 μM kainate was included in the assay buffers to eliminate the latter component. Thus, [³H]glutamate binding represents almost exclusively NMDA receptors (more than 90% displaceable by AP5 or NMDA). It should also be pointed out that previously studied Cl-dependent glutamate 'binding sites,' which have now been shown to represent uptake into resealed vesicles (26, 28), are eliminated by the detergent treatment. In binding experiments with [³H]glutamate and [³H]glycine, similar precautions were taken as for AMPA binding to minimize binding loss during filtration.

The binding data were analyzed using the Ligand program (29) run on an AT&T computer. Protein content was determined according to the method of Bradford (30) with bovine serum albumin as standard.

Chemicals. [³H]Glutamate (specific activity, 50 Ci/mmol) and [³H]glycine (specific activity, 20 Ci/mmol) were purchased from ICN (Irvine, CA), [³H]kainate (specific activity, 60 Ci/mmol) and [³H]AMPA (specific activity, 25 Ci/mmol) from NEN (Boston, MA). All other drugs and reagents were obtained from commercially available sources.

Results

Effects of chemical modifications of telencephalic membranes on [³H]AMPA binding. Membranes were prein-

TABLE 4

Effect of PCMBS treatment on the characteristics of [³H]AMPA binding to rat telencephalon membranes

The data from Figs. 3 and 4 were analyzed by the Ligand program. For the filtration assay, results are mean ± standard error of four experiments. For the centrifugation assay, results are means of two experiments, which differed by less than 15%. *, $p < 0.01$ (Student's *t* test).

Method	Control		PCMBS	
	K_d	B_{max}	K_d	B_{max}
	nM	*pmol/mg of protein*	*nM*	*pmol/mg of protein*
Filtration				
High affinity	12.9 ± 3.5	0.32 ± 0.01*	25.6 ± 6.3	4.16 ± 0.72*
Low affinity	514 ± 67	15.1 ± 1.5	215 ± 43	10.8 ± 0.5
Centrifugation				
High affinity	11	0.67	Not resolvable	
Low affinity	543	25.9	73	20.6

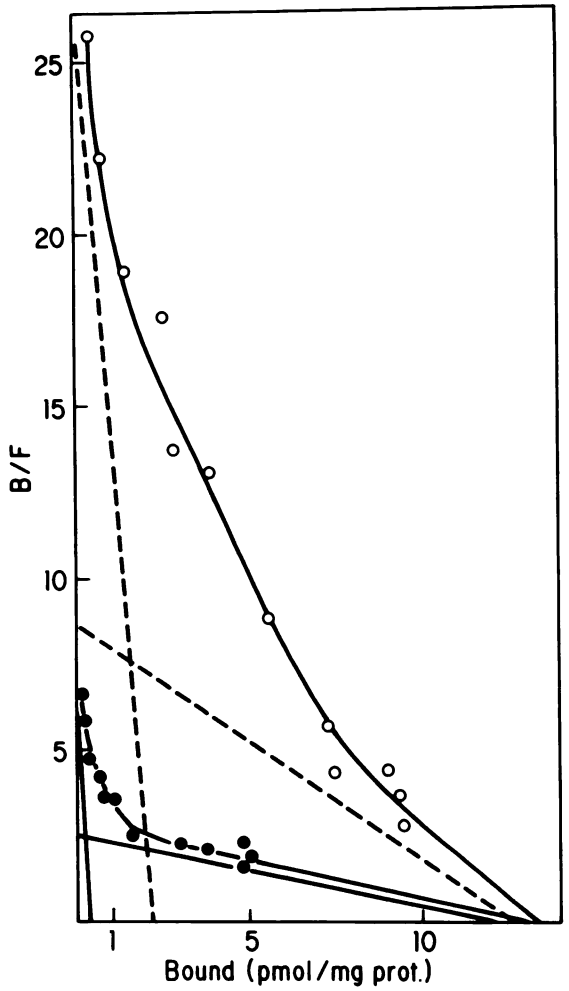

Fig. 3. Effect of PCMBS treatment measured by filtration assay on the equilibrium kinetics of [^{3}H]AMPA binding. Synaptic membranes were pretreated with (○) and without (●) 2 mM PCMBS and were washed by two cycles of centrifugation and resuspension in Tris/acetate buffer. The binding of increasing concentrations of [^{3}H]AMPA (ranging from 1 nM to 5 μM) was then performed using a filtration assay. Results were expressed as pmol/mg of protein and are represented as a Scatchard plot of the data. The same experiment was repeated three more times with similar results (see Table 4). The *straight lines* were generated by the LIGAND analysis of the averaged data.

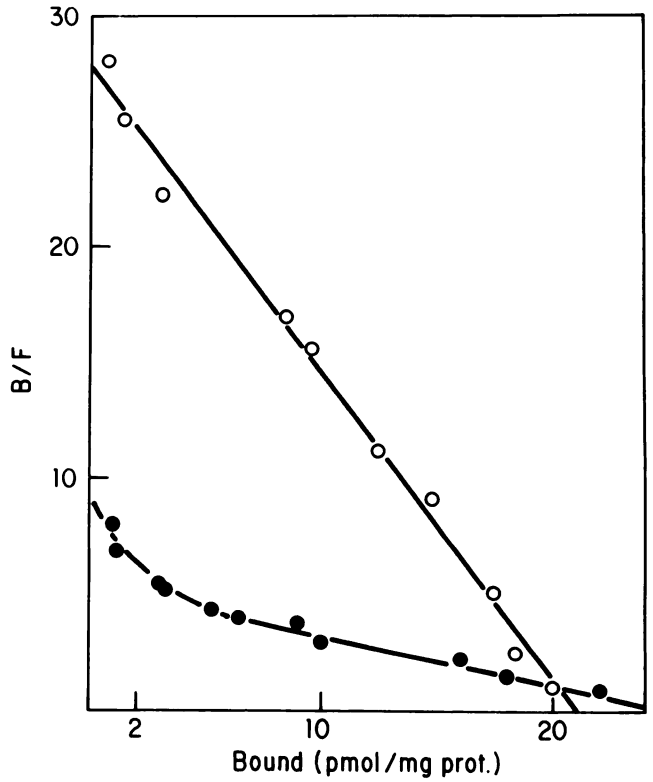

Fig. 4. Effect of PCMBS treatment, measured by centrifugation assay, on the equilibrium kinetics of [^{3}H]AMPA binding. Same as in legend to Fig. 3 except that the binding of [^{3}H]AMPA was performed using a centrifugation assay. The experiment was replicated twice with similar results. (Although not shown in the figure, the Ligand program provided a better fit of the data with a two-site model than a one-site model. See Table 4.)

cubated with various reagents known to react with sulfhydryl groups or disulfide bonds and washed by centrifugation and the binding of [^{3}H]AMPA was measured by the filtration assay (Fig. 1). The three mercurials, $HgCl_2$, PCMB, and its sulfonate analog PCMBS produced a 2- to 4-fold increase in AMPA binding, PCMBS being slightly more potent than the others. Half-maximal activation was achieved at about 60 μM, and maximal activation at 200 μM (Fig. 2). All the other reagents, including the reducing agent DTT, the oxidizing agent DTNB, and the alkylating reagent NEM, were ineffective at concentrations up to 2 or 5 mM.

In order to test whether the mercurials produced irreversible alterations in the structure or composition of the binding protein, we attempted to restore the free sulfhydryl groups by incubating mercurial-treated and washed membranes with the reducing agent DTT. [^{3}H]AMPA binding was restored by DTT treatment to about the same level as in untreated control membranes (Table 1), suggesting that both the sulfur-mercury bond and the changes induced by the SH-group derivatization are fully reversible.

We then sought to obtain further information on the role and location of the sulfhydryl groups whose modifications are associated with the increase in binding. In one group of experiments, saturating concentrations (50 μM) of L-glutamate or quisqualate were added before PCMBS treatment (Table 2) in order to protect the amino acids located at the agonist binding site from the SH-reagent. The total lack of protection indicates that the reactive SH-groups must be located outside the AMPA or quisqualate recognition site. In a second set of experiments, the effectiveness of PCMB was compared with that of the much less hydrophobic sulfonate analog PCMBS. Previous studies on other membrane proteins had demonstrated that the difference in the membrane permeability of these reagents can be used to localize reactive SH-groups (31). These experiments were performed with synaptoneurosomes, the cellular entities in which a significant proportion of both the synaptic terminals and the attached postsynaptic sacs are thought to be resealed. As in the membrane preparation, both reagents proved to be equally effective in increasing AMPA binding (data not shown).

Pretreating membranes with the oxidizing agent DTNB or the alkylating agent NEM had minimal impact on AMPA binding, but it almost completely eliminated the increase in binding produced by PCMBS (Table 3). Because other oxidizing agents such as diamide, a membrane-permeant oxidizing reagent, and phenylarsine oxide, a membrane-permeant trivalent arsenical specific for dithiol groups (32) did not provide

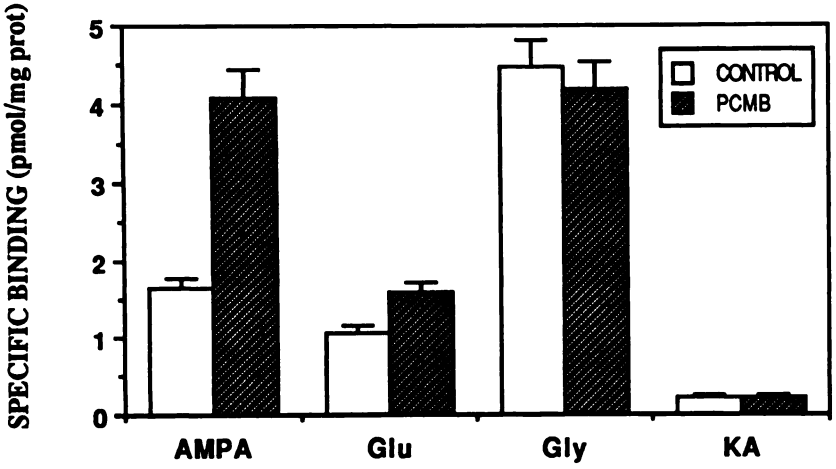

Fig. 5. Effect of PCMB treatment on the binding of various ligands for different types of glutamate receptors. Synaptic membranes were pretreated with 2 mM PCMB at 35° for 20 min. After two cycles of centrifugation and resuspension in Tris/acetate buffer the binding of different ligands was determined as described under Materials and Methods: [3H]AMPA (50 nM); [3H]glutamate (*Glu*) (100 nM), [3H]glycine (*Gly*) (250 nM), and [3H]kainate (*KA*) (20 nM). Results are expressed as pmol/mg of protein and are mean ± standard error of four to six experiments. Only the effects on [3H]AMPA and [3H]glutamate binding are statistically significant. ($p < 0.001$, Student's *t* test).

comparable protection, it appears more likely that the DTNB and NEM effects were due to an SH-derivatization than that they were due to an oxidation of vicinal SH-groups to a disulfide bond. Whatever the mechanism may be, derivatization alone of the free SH-groups does not seem sufficient to cause the shift in AMPA binding.

Effects of PCMBS on the binding characteristics of [3H]AMPA. Saturation kinetics were performed in control membranes and in membranes treated with 2 mM PCMBS. [3H]AMPA binding was measured both by filtration and by centrifugation and the data were analyzed by the Ligand program (Table 4; Figs. 3 and 4). In control membranes the analysis systematically provided a better fit of the data with a two-site model, in good agreement with results reported by other groups (9, 10). A small number of sites have a high affinity for [3H]AMPA whereas a large number of sites have a 40-fold lower affinity. This was true with both types of binding assays, although the centrifugation assay provided a larger maximal number of sites. After treatment with PCMBS, analysis of the data obtained by filtration indicated a large increase in the number of high affinity sites and a decrease in the number of low affinity sites, as well as an increase in the affinity of the low affinity sites. However, analysis of the data obtained by centrifugation could not resolve two sites and indicated the presence of a single site with a large number of maximal sites and an affinity intermediate between the high and low affinity.

Effects of PCMB treatment on other glutamate receptors. Membranes were treated with PCMB and the binding of different glutamate receptor-related ligands was determined (Fig. 5). In addition to an increase in [3H]AMPA binding, the binding of [3H]glutamate to the NMDA receptors was also increased under these conditions. The binding of [3H]glycine and of [3H]kainate was *not* affected under these conditions. Preliminary experiments indicated that PCMB treatment also increased the affinity of [3H]glutamate without modifying the maximal number of sites (data not shown).

Discussion

The present results indicate that certain chemical modifications of free sulfhydryl groups produce an allosteric increase in the affinity of quisqualate receptor binding sites for [3H]AMPA. In good agreement with reports by other groups (9, 10), we found that [3H]AMPA binds to two types of sites in untreated membranes, a high affinity site with a K_d of about 10 nM and a lower affinity site with a K_d of about 500 nM, which accounts for 90% or more of all the AMPA sites. Analysis of the data obtained by the filtration technique suggests that sulfhydryl group modification produces different effects on these two types of sites; high affinity sites are increased in their number, whereas low affinity sites exhibit both a decrease in number and an increase in affinity. This interpretation is partly supported by the data obtained with the centrifugation assay, which also indicate a decrease in number and an increase in affinity of the low affinity component. However, under those conditions, the data could not be resolved into two components, presumably because the two components have affinities too similar to be separated by the computer program. It is not surprising that the centrifugation assay results in a larger maximal number of sites compared with the filtration assay, as the dissociation rate of the low affinity component is fast enough to cause a significant loss of binding during the filtration process (data not shown). Whether sulfhydryl reagents produce a redistribution of AMPA sites between two different affinity states as indicated by the filtration assay data or whether low and high affinity AMPA sites represent two different receptor types cannot be resolved with the present data.

Independent of these questions, it remains valid to conclude that the free sulfhydryl groups regulate the affinity of [3H]AMPA for the lower affinity component of the binding sites. This conclusion is based on the specificity of PCMB, its sulfonate derivative PCMBS, and $HgCl_2$ and the reversibility of the effect by DTT reduction treatment. The fact that saturating concentrations of quisqualate or L-glutamate do not protect AMPA binding from PCMBS modification suggests that the SH-groups involved are not located directly at the binding site but at a site that allosterically modulates the properties of the binding site. Moreover, these free sulfhydryl groups might be localized in the extracellular domain of the receptor inasmuch as treatment of synaptoneurosomes, structures that exhibit a large number of resealed postsynaptic membranes, with PCMBS is as effective as treatment with the more lipophilic and thus more membrane-permeant PCMB. As is the case for the nicotinic acetylcholine receptor (33), only certain SH-reagents produce an alteration of receptor configuration resulting in a change in [3H]AMPA binding. In particular, only the formation of thio-mercurial bonds appears to increase the binding whereas other types of thiol-derivatizations do not; this lack of effect of other SH-reagents does not appear to be due

to limitations in their accessibility to the critical SH-groups, as some of the same reagents do prevent the increase in binding produced by PCMBS. However, it is also possible that there exist two types of SH-groups, one type accessible only to certain compounds that are critical for the change in AMPA binding and a second type that is accessible to a wider number of SH-reagents and modulates the properties of the first type.

Reduction of disulfide bonds by DTT treatment did not modify the properties of [^{3}H]AMPA binding, indicating that disulfide bonds are not critical for determining the affinity of the ligand to the recognition site of the receptor/channel complex. This result is in contrast to the numerous reports indicating that disulfide bonds are critical for ligand binding and treatment with DTT results in a decrease in ligand binding (17, 19, 20, 23). It is, however, still conceivable that other aspects of the quisqualate receptor function depend on the integrity of disulfide bonds.

It has been proposed that the existence of intramolecular disulfide bridges regulating the affinity of the agonist is a characteristic of G protein-coupled receptors (34). If this proved to be true, our data would suggest that quisqualate receptors (or at least the AMPA receptors) are not coupled to a G protein. In this regard it is interesting to note that although quisqualate appears to stimulate phosphatidylinostiol turnover (35), the receptor involved in this effect is probably not labeled by [^{3}H] AMPA.[1]

The functional effect of the increase in affinity of AMPA for the quisqualate receptor produced by some SH-reagents is difficult to assess at the present time. Kiskin *et al.* (24) showed that physiological responses to kainate and glutamate were markedly depressed after chemical modification of free SH-groups in hippocampal pyramidal neurons. However, the lack of specific quisqualate or kainate receptor antagonists has so far prevented a clear distinction between the kainate and quisqualate receptors, and it is therefore difficult to evaluate which receptors Kiskin *et al.* (24) were stimulating. Moreover, the effects of SH-reagents they observed appear to be noncompetitive with respect to kainate and would suggest that the SH-reagents were thus not acting at the kainate recognition site. This is consistent with the lack of effect of SH-reagents on [^{3}H]kainate binding, although DTT treatment markedly reduced the binding.[2] Moreover, it remains to be shown that the binding sites studied in membrane preparations correspond to the functional state of receptors. The development of new techniques allowing the comparison of the functional properties of the receptors, as well as their binding characteristics, on the same membrane fractions (36) should provide interesting answers to these questions.

Independent of these issues, it remains that the quisqualate receptors share with other receptors what might represent a general feature of receptor structure and function, namely the presence of free sulfhydryl groups that play critical roles in the regulation of receptor function. In this regard it is interesting to note that different types of glutamate receptors may be differentially affected by chemical modification of free sulfhydryl or disulfide groups. Finally, the existence of a regulation of glutamate receptors at the level of sulfhydryl groups raises the issue of a potential physiological or pathological role of this regulation.

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