Molecular Neurobiology

ISSN0893-7648/98/17(1–3): 33–58/$14.50

The Regulation of AMPA Receptor-Binding Sites

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Abstract

A wide variety of mechanisms have been identified that can regulate the α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA)-receptor complex. Modulation has been shown to occur at the nucleic acid level via RNA editing and alternative splicing. At the posttranslational level, processes such as phosphorylation, glycosylation, chemical modification of reactive groups on the receptor proteins, interaction with a putative receptor-associated modulatory protein, and changes in the lipid environment have been reported to regulate receptor binding and function. In this review, we discuss general aspects of the cell biology, pharmacology, and function of AMPA receptors. In particular, we focus on some factors shown to modulate agonist binding and discuss possible molecular mechanisms underlying the regulation observed.

Index Entries: AMPA; glutamate receptors; regulation; radiation inactivation; solubilization; thiol reagents; potassium thiocyanate; phospholipase A_2.

Introduction

The excitatory effects of L-glutamate were first observed more than four decades ago (Curtis and Watkins, 1961). Since that time and especially over the last 20 yr, it has been conclusively demonstrated that L-glutamate is the predominant excitatory neurotransmitter in the central nervous system (CNS), mediating neurotransmission at an overwhelming majority of synapses.

Both ligand-gated ion channel (ionotropic) and G protein coupled (metabotropic) types of glutamate receptors have been characterized. We shall not deal here with the metabotropic receptors, but excellent recent reviews are available (Pin and Duvoisin, 1995; Nakanishi, 1994; Watkins and Collingridge, 1994). In the mammalian CNS there are three distinct classes of ionotropic glutamate receptors initially named according to their functional sensitivity to the selective agonists N-methyl-

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Table 1
Pharmacology

Site	Compound
Agonists	*L-glutamate, Acromelate, Quisqualate, AMPA, FW[a], ATPA[a]* 2-amino-3-(3-hydroxy-5-*tert*-butylisoxazol-4-yl)propionate (ATPA)
Antagonists	*CNQX, NBQX[a], YM90K[a]* 6-(1H-imidazol-1-yl)-7-nitro-2,3-(1H,4H)-quinoxalinedione.HCl (YM90K; S. Sasamata, 1996)
Channel block	*Joro spider toxin, Argiotoxin-636[a]* Joro spider toxin (JSTX) and Argiotoxin do not block channel if receptor is edited at Q/R sites
Modulators	*GYKI 52466a; GYKI 53655[a]* atypical 2,3, benzodiazepine, block AMPA-receptor-gated currents, act at a different site from cyclothiazide (Donevan, 1993; Partin, 1996)
	Cyclothiazide[a], Aniracetama, 1-BCP[a] Cyclothiazide inhibits desensitization at flip variants (Partin, 1995); Aniracetam inhibits desensitization at flop (Johansen, 1995); 1(1,3-benzodioxol-5-yl carbonyl)piperidine (1-BCP), blocks desensitization (Desai, 1995).

[a] Denotes high selectivity towards AMPA receptors compared to kainate receptors (Fig. 1).

D-aspartate (NMDA), kainate (KA) and α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA). There are a number of reviews dealing with the molecular biology, pharmacology and physiology of AMPA receptors (Bettler and Mulle, 1995; Henley, 1994; Hollmann and Heinemann, 1994; McBain and Mayer, 1994; Mori and Mishina, 1995; Barnes and Henley, 1992; Seeburg, 1993; Fletcher and Lodge, 1996). Here we shall focus specifically on the modulation of AMPA receptor binding sites.

Pharmacology

The rank order of potency for ligands binding to AMPA receptors is generally defined as quisqualate > FW > AMPA > CNQX = glutamate >> kainate > domoate (Fletcher and Lodge, 1996; Honoré, et al., 1982; Hampson et al., 1992; Smith and McIlhinney, 1992; Monaghan, 1989). Few compounds are available that provide a complete pharmacological distinction between kainate and AMPA receptors (Table 1 and Fig. 1), and, whereas recombinant AMPA receptors (GluR1–GluR4) bind [^{3}H]AMPA with high affinity they also have a relatively low measurable affinity for kainate (Keinänen et al., 1990). It is noteworthy that kainate and AMPA-receptor subunits do not coassemble within the same receptor complex (Wenthold et al., 1992; 1994; Puchalski et al., 1994). Furthermore, at low nanomolar concentrations, [^{3}H]kainate and [^{3}H]AMPA display different autoradiographic binding patterns (Young and Fagg, 1990).

Agonists

The pharmacological characterization of AMPA receptors has been greatly advanced by the synthesis and subsequent radiolabeling of the defining agonist AMPA (Honoré et al.,

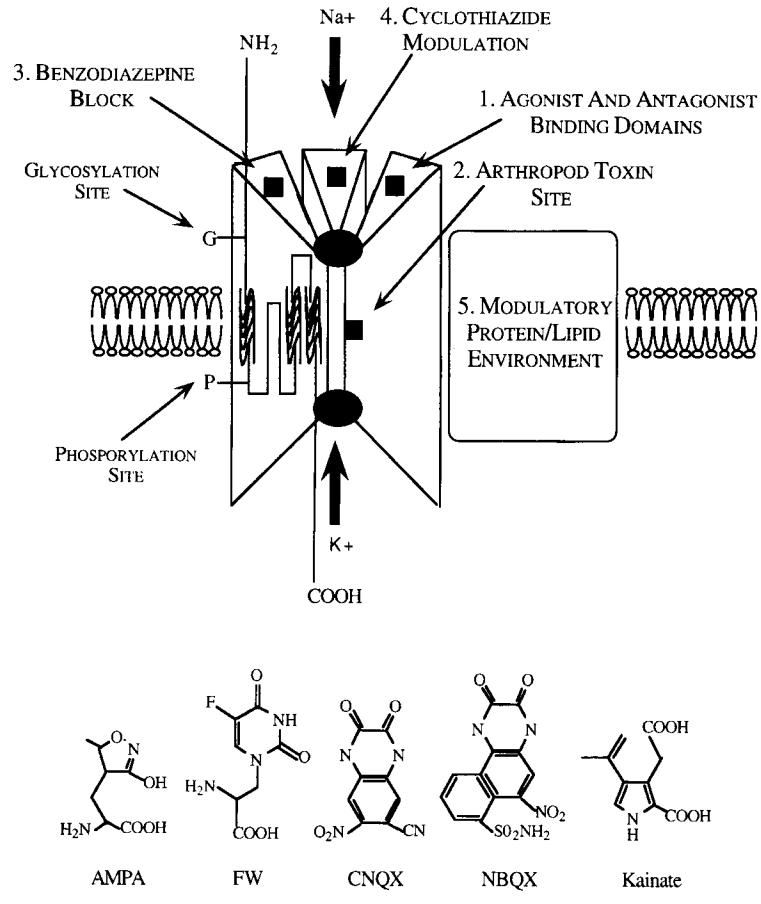

Fig. 1. The AMPA/(Kainate) Receptor: Multiple distinct binding sites on the AMPA receptor occur and include (Table 1) :

1. Overlapping agonist and antagonist sites.
2. Noncompetitive toxin channel block.
3. Noncompetitive benzodiazepine blockade.
4. Cyclothiazide modulation.
5. Action on the modulatory component or microlipid environment.

1982; Krogsgaard-Larsen et al., 1980). AMPA is a structural derivative of glutamate that is most potent in its (S)-enantiomer form (Krogsgaard-Larsen et al., 1980; Hawkins et al., 1995; Hansen et al., 1983). A more recent development has been the synthesis of derivatives to the amino acid willardiine (Wong et al., 1994). Characterization of a new ligand, (s)-5-fluorowillardiine (FW), in functional and binding studies shows a high selective potency towards AMPA receptors with little affinity for kainate binding sites (Wong et al., 1994; Hawkins et al., 1995; Dev et al., 1996).

Antagonists

Cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 6-nitro-7-sulphamoylbenzo(f) quinoxaline-2-3-dione (NBQX) are the two most commonly used competitive antagonists at AMPA and kainate receptors. Compared to the five-fold selectivity of CNQX, NBQX is 30-fold more selective at displacing [^{3}H]AMPA over

[^{3}H]kainate in radioligand-binding studies (Honoré et al., 1988; 1989; Sheardown et al., 1990; Dev et al., 1996). These quinoxalinedione derivatives also act as weak antagonists at the glycine site of NMDA receptors (Monaghan et al., 1989).

Allosteric Regulation

To date, the best available compounds that distinguish between AMPA and kainate receptors are those working allosterically to the AMPA ligand-binding site. For example the benzothiadiazide diuretic, cyclothiazide, inhibits AMPA-receptor desensitization without affecting kainate receptors (Partin et al., 1993). Another set of compounds providing clear distinction between kainate and AMPA receptors are the 2,3-benzodiazepines. These GYKI compounds are noncompetitive antagonists that act at a different site from both AMPA and cyclothiazide (Donevan and Rogawski, 1993; Johansen et al., 1995; Kessler et al., 1996; Partin and Mayer, 1996).

Function and Clinical Importance

Patch-clamp recordings of single-cell cerebellar and hippocampal neurons show discrete single-channel Na^+/K^+ currents through AMPA receptors usually below 20 pS (Cull-Candy and Usowicz, 1987). These channels have open times of approx 1 ms and are responsible for the fast component of synaptic excitatory neurotransmission. Recombinant GluR1–GluR4 subunits can form homomeric ligand-gated ion channels and have properties similar to native receptors (Hollman et al., 1989; Boulter et al., 1990). In functional studies, GluR1–GluR4 are rapidly desensitized by AMPA but not by kainate (Partin et al., 1993).

AMPA receptors are of widespread interest because of their fundamental role in a range of normal and pathological neuronal processes. These include fast excitatory neurotransmission, synaptic plasticity and neuronal cell death (*see* reviews by Choi, 1992; Bliss and Collingridge, 1993. Meldrum and Garthwaits, 1990). The involvement of AMPA receptors has been proposed in many neurodegenerative diseases including Parkinson's disease (Klockgether and Turski, 1989), epilepsy (Dingeldine et al., 1990), and motor neuron disease (Shaw et al., 1994). Glutamate receptors have also been linked to conditions such as cerebral ischemia (Sheardown et al., 1990), schizophrenia (Wachtel and Turski, 1990), Huntington's chorea (Albin et al., 1990), and Alzheimer's disease (Lipton, 1993). Compounds thus far developed show little ability to distinguish dysfunctional receptors from normal ones. Attention is increasingly being focused on the regulation of AMPA receptors in order to further develop possible clinical therapies.

AMPA Receptor Diversity

Subunits

Ionotropic glutamate receptors comprise a family of distinct subunits allowing the possibility of multiple receptor combinations (Hollman and Heinemann, 1994; Sommer and Seeburg, 1992; Nakanishi and Masu, 1994). Within this group, AMPA receptors are encoded by four subunits, GluR1–GluR4 (Keinänen et al., 1990; Hollman et al., 1989; Boulter et al., 1990). Each of the GluR1–GluR4 subunits is made up of approx 900 amino acid residues and have molecular weights of approx 100 kDa. Sequence identity is approx 70% within the GluR1–GluR4 subgroup and approx 30% with other glutamate receptor subunits (Bettler and Mulle, 1995).

Distributions of the mRNAs encoding GluR1–GluR4 (Pellegrini-Giampietro et al., 1991) and of the subunits themselves (Petralia and Wenthold, 1992; Molnár et al., 1993) have shown a widespread expression throughout the CNS. Receptor autoradiography using [^{3}H]AMPA and [^{3}H]FW demonstrated similar distribution patterns to those found by *in situ* hybridization, suggesting that proteins GluR1–GluR4 are responsible for AMPA receptor-ligand binding (Hawkins et al., 1995; Dev et al., 1996; Dev et al., 1996; Wenthold et al., 1990). Dense [^{3}H]AMPA binding occurs in the CA1

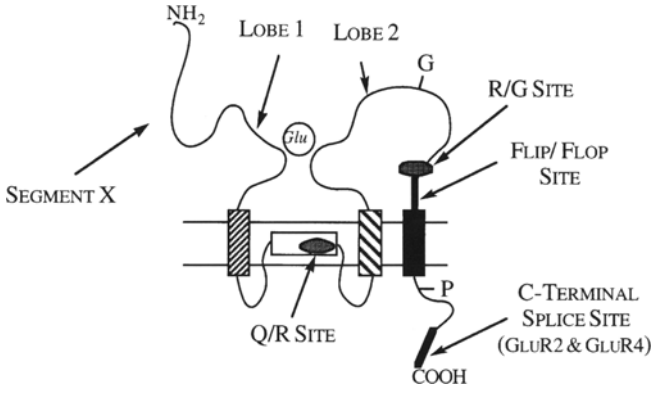

Fig. 2. Topology of ionotropic subunits: Hydrophobic T-MD positions for a three T-MD model are shown (Wo and Oswald, 1994). Abbreviations: P, phosphorylation sites; G, glycosylation sites.

stratum radiatum of the hippocampus, I–III outer cortical layers, dorsal lateral septum, molecular layer of the cerebellum, and striatal areas of adult rat CNS (Monaghan et al., 1984; Nielsen et al., 1990). AMPA receptor subunits colocalize within cell bodies, dendrites, and dendritic spines and are clustered at postsynaptic sites (Molnár et al., 1993; Craig et al., 1993). A proportion of AMPA receptors are thought to be located intracellularly (Molnár et al., 1993; Standley et al., 1994; Henley, 1995).

Alternative Splicing

In GluR1–GluR4, alternative splicing of a mRNA exon sequence encoding 38 amino acids just preceding the last membrane domain-IV (MD-IV) produces 'flip' and 'flop' isomers (Sommer et al., 1990 and Fig. 2). Compared to flop, the flip versions exhibit slower desensitization (Mosbacher et al., 1994), have larger whole-cell currents and are modulated by cyclothiazide (Partin et al., 1995). The flip and flop splice variants are also spatially and developmentally regulated (Sommer et al., 1990; Monyer, 1991). A splice cassette has also been detected in the C-terminals of both GluR2 and GluR4 subunits (Gallo et al., 1992; 1992; Köhler et al., 1994). These reports have suggested short and long types of all GluR1–GluR4 subunits, however C-terminal splice variants of GluR1 and GluR3 are still yet to be found (Hollmann and Heinemann, 1994; Gallo et al., 1992; Köhler et al., 1994). Differential splice variation in the C-terminal region of GluR1–GluR4 may provide great implications in protein–protein interactions as recently revealed by the two-hybrid system (Dong et al., 1997).

RNA Editing

Conversion of adenosine to guanosine in the cDNA sequences is thought to be responsible for GluR subunit editing (*see* review by Seeburg, 1996). A so-called Q/R "switch" exists in GluR2 and, at this site, a single residue in position 586 of MD-II is edited from glutamine to arginine (Sommer et al., 1991 and Fig. 2). AMPA receptor complexes that express edited GluR2 are Ca^{2+} impermeable, whereas unedited GluR2 allow Ca^{2+} flow. Unedited GluR2 is not expressed in vivo (Lomeli et al., 1994), whereas most principal neurons express edited GluR2 (Boulter et al., 1990; Sommer et al., 1991). Cells that lack GluR2 and gate Ca^{2+}, include inhibitory GABAergic interneurons of neocortex and of hippocampal CA3 region and the Bergmann glial cells of cerebellum (Burnashev et al., 1992).

Another site termed R/G is located in lobe 2 just before the flip/flop module (Fig. 2). At this position, the unedited form contains argi-

nine, whereas the edited pattern codes for glycine. GluR1–GluR4 can exist with arginine but only GluR2, GluR3, and GluR4 are edited at the R/G position (Lomeli et al., 1994). Similar to flip/flop, the R/G site is involved in altering subtle kinetic properties of AMPA receptors, particularly in AMPA-receptor desensitization events. The R/G edited version recovers from desensitization faster and normally it desensitizes to a lesser extent than the unedited version (Lomeli et al., 1994). In general, R/G editing increases to approx 85% in adulthood, although development may depend on subunit and flip/flop characteristics (Lomeli et al., 1994).

Biochemistry

Subunit Topology

The classical four transmembrane domain (T-MD) model characterized previously for nicotinic acetylcholine and γ-amino-n-butyric acid ($GABA_A$) receptor subunits has proved inconsistent for glutamate ionotropic receptors (Keinänen et al., 1990; Hollmann et al., 1989). The now-favored three T-MD model proposes a MD-II that does not to cross the membrane but rather is located inside the membrane placing the Q/R site close to the intracellular surface (Wo and Oswald, 1994; Sutcliffe, et al., 1996). This segment has significant homology to the corresponding P-region of voltage-gated K^+ channels and probably determines ion flow by similar mechanisms (Sutcliffe et al., 1996).

Importantly, an odd number of transmembrane crossings places the C-terminus intracellularly (Fig. 2). This has been confirmed by immunocytochemical studies using antiserum directed against the C-terminus of GluR1 (Molnár et al., 1993) and in vitro translation-translocation of GluR1 using canine pancreatic microsomes (Seal et al., 1995). Additionally, the C-terminal domains of GluR1–GluR4 subunits are shown to be phosphorylated by diacylglycerol-stimulated protein kinase (PKC) and Ca^{2+} calmodulin-dependent kinase (CaM-KII), strongly suggesting an intracellular localization (McGlade-McCulloch et al., 1993).

Ligand Binding Domains

Sequence analyses have suggested that GluRs may have evolved from bacterial binding proteins (O'Hara et al., 1993). Using GluR4 subunits, it was found that lobe 2 and the extracellular N-terminus close to the membrane (lobe 1) can form a ligand-binding site that has good homology with bacteria lysine-arginine-ornithine (LAO-BP) and glutamine (Q-BP) binding proteins (Kuusinen et al., 1995). In addition, GluR1 subunit-truncated mutants, only expressing the N-terminal and MD-I, indicate that binding requires lobe 1–lobe 2 and that N-terminal surface expression requires MD-I (McIlhinney and Molnár, 1996). Furthermore, in GluR1 the substitution of Arginine-481, an N-terminal-conserved charged residue that aligns with bacterial-binding proteins, affects [^{3}H]AMPA receptor binding (Uchino et al., 1992).

AMPA Receptor Assembly

Ionotropic glutamatergic receptors are oligomeric membrane proteins assembled around a central ion-conducting pore (Bettler and Mulle, 1995). A study using subunit-specific antibodies suggests that AMPA receptors in rat cortex are heteromeric combinations of GluR1–GluR4 (Brose et al., 1994). Furthermore, heteromeric combinations more closely display properties of the native receptor than that of AMPA-receptors composed of homomeric subunits. Although the exact stoichiometry is not yet clear, a nicotinic receptor-like pentameric arrangement has been proposed. For example, specific subunit antibodies co-immunoprecipitated more than one AMPA-receptor subunit from solubilized membranes, suggesting a heteromeric structure of native AMPA receptors (Wenthold et al., 1992). In the same study, chemical crosslinking experiments also suggested a pentameric nature.

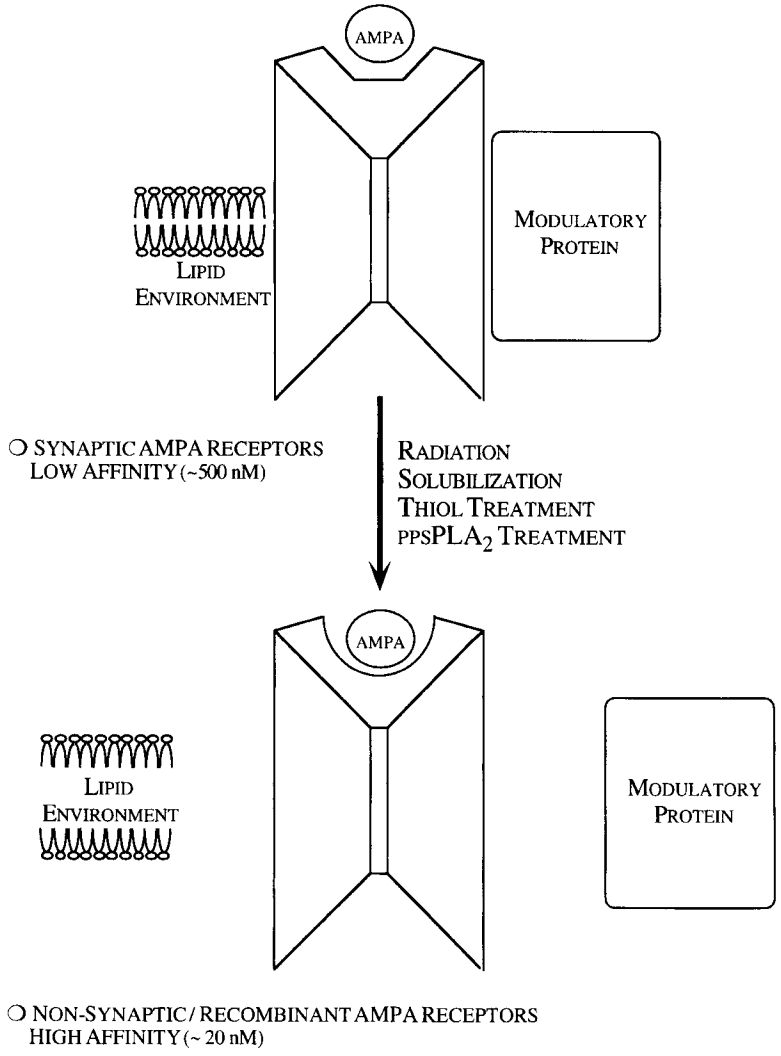

Fig. 3. The high and low binding states of [^{3}H]AMPA: The treatments indicated are thought to convert low-affinity receptors into higher-affinity states. The direction of conversion by each treatment is indicated in the figure. Notice that KSCN affects both high- and low-affinity receptors (Fig. 4). Reasons for the presence and interconversion between high- and low-affinity states may be severalfold, two of the main possibilities include:

1. Attachment to the modulatory component.
2. Changes in the microlipid environment.

The Two Binding Sites of [^{3}H]AMPA

The poor specificity of [^{3}H]glutamate towards AMPA receptors prevented their initial discovery with this endogenous ligand. However, the regulation of [^{3}H]AMPA receptor binding has been of intense interest since its first synthesis over a decade ago by Honoré et. al., (Honoré et al., 1982; Krogsgaard-Larsen et al., 1980). The further development of ligands

[^{3}H]CNQX (Honoré et al., 1989; Nielson et al., 1990) and more recently [^{3}H]NBQX (Dev et al., 1996) and [^{3}H]FW (Hawkins et al., 1995) have also helped define pharmacological and biochemical aspects of AMPA receptors. Nevertheless, most reports have concentrated on the use of [^{3}H]AMPA when determining AMPA-receptor regulation by a number of chemical processes, including solubilization (Henley and Barnard, 1991; Hunter et al., 1990; Hall, Kessler, and Lynch, 1992) , high energy irradiation (Honoré and Nielsen, 1985; Hall et al., 1996), chaotropic ions (Olsen et al., 1987; Honoré and Drejer, 1988), thiol reagents (Hall et al., 1996; Terramani et al., 1988) and phospholipase A_2 (Massicotte and Baudry, 1990). *See* Fig. 3.

The High and Low Binding Sites of [^{3}H] AMPA

In some early reports, kinetic properties of [^{3}H]AMPA binding were suggested to confirm the presence of two binding sites that represent multiple affinity states of the same receptor rather than different receptors (Honoré et al., 1982; Olsen et al., 1987; Honoré and Drejer, 1988). The heterogeneous nature of the AMPA receptor family raises questions as to why [^{3}H]AMPA binds multiple affinity states. Indeed, unlike [^{3}H]kainate binding where high (K_d approx 10 n*M*) and low (K_d approx 50 n*M*) affinity sites represent KA1–KA2 and GluR5–GluR7 kainate-receptor subunits respectively (Young and Fagg, 1990; Ogita et al., 1994; Honoré et al., 1986; Bettler et al., 1990; 1992; Werner et al., 1991; Lomeli et al., 1992), the different AMPA-receptor subunits do not display differences in [^{3}H]AMPA-binding affinities (Honoré et al. 1986, but *see* Porter and Greenamyre, 1994). Furthermore, whereas [^{3}H]AMPA was shown to detect high- and low-affinity sites (Honoré et al., 1982), the antagonist [^{3}H]CNQX bound with equal affinity to both these states (Honoré et al., 1989; Nielsen et al., 1990). In general several reports show that in the presence of potassium thiocyanate (KSCN), approx 80% of [^{3}H]AMPA binds to a high number of low-affinity sites (K_d approx 500 n*M*) and approx 20% binds to a low number of high-affinity sites (K_d approx 20 n*M*) (Olsen et al., 1987; Honoré and Drejer, 1988). For [^{3}H]FW, similar observations were made, showing higher amounts of binding toward the low-affinity site compared with the higher-affinity state (Hawkins et al., 1995).

The Reasons and Roles for Two Affinity Binding Sites

Using autoradiography, studies show a differential distribution of high and low affinity sites (Monaghan et al., 1989; Young and Fagg, 1990; Dev et al., 1996; Nielsen et al., 1990; Olsen et al., 1987). For example, a high density of low-affinity sites occur in the strata oriens and radiatum dendritic layers of the CA1 region of the hippocampus. In comparison, a low number of high-affinity sites are predominant in the cell-body layer of the CA1, the stratum pyramidale (Young and Fagg, 1990; Dev et al., 1996). Differential patterns in [^{3}H]AMPA binding using concentrations of 5 n*M*, where mainly high affinity AMPA-binding sites are detected, and 500 n*M*, at which high and low AMPA-binding sites are labeled, gives support to the existence of high and low AMPA-receptor-binding sites (Catania et al., 1993). When using the AMPA-receptor antagonists [^{3}H]CNQX or [^{3}H]NBQX, which equally detect high- and low-affinity states, only the number of sites (B_{max}) are relevant to the pattern of binding. Here it is shown that a high number of low affinity sites occur whereas a low number of high affinity sites exist (Sheardown et al., 1990; Dev et al., 1996).

In the case of Porter and Greenamyre (1994), who showed regional variations in the ability of NBQX and (S)AMPA to displace [^{3}H]AMPA from rat cerebellum and forebrain, it was suggested that these properties occur because of a regional imbalance of AMPA-receptor subunit expression. At first instance such differences in displacement affinities suggest that alter-

native combinations of regionally expressed subunits represent high and low [^{3}H]AMPA-binding-affinity sites. Although, it is more likely that the varying ability of NBQX and (S)AMPA to displace [^{3}H]AMPA is caused by a disparity of a pharmacological nature for each individual subunit.

It has been suggested that high-affinity [^{3}H]AMPA-binding sites, when translocated to the plasma membrane, become low-affinity states (Hall et al., 1992). The physiological role of a reduction in the affinity of AMPA receptors as they are formed in the cell body, trafficked and then eventually inserted into the synaptic membrane may be several-fold (Fig. 3). Electrophysiological studies on excised patches from cultured hippocampal neurons show two separate AMPA receptor responses—low-affinity/high-conductance and high-affinity/low-conductance (Tang et al., 1991). Similar to the excitatory postsynaptic currents (EPSCs) in hippocampal slices, the low-affinity/high-conductance is rapidly desensitizing. Desensitizing currents are slowed by aniracetam and cyclothiazide (Kessler et al., 1996; Tang et al., 1991; Arai et al., 1995). Although these low-affinity/high-conducting currents fit the role of synaptic AMPA-receptors, the role of high affinity/low conducting channels within the cell is unclear. Nevertheless, AMPA receptor desensitization is accompanied by an increase in affinity for the agonist (Kessler et al., 1996; Arai et al., 1995). This phenomenon may provide a link between low-affinity/highconducting receptors and high-affinity/low-conducting complexes. Furthermore the different expression levels of high- or low-affinity AMPA receptors, at any one time, may have important implications in synaptic plasticity events such as long term potentiation (LTP) and in the regulation of silent synapses (Bliss and Collingridge, 1993; Isacc et al., 1995).

Other evidence of intracellular AMPA receptors is available from subcellular fractionation techniques, in which [^{3}H]AMPA binding was shown to occur in both microsomal and synaptosomal fractions of rat brain hippocampal membranes (Standley et al., 1994; Henley, 1995). In the latter study, however, [^{3}H]AMPA binding did not occur to two distinct binding sites (Henley, 1995). Instead a single class of sites, with similar affinities, were obtained in both the microsomal and synaptosomal fractions. As discussed later, the detection of high and low affinities are often dependent upon the methods applied (*see* KSCN).

Clearly, multiple binding site models remain very complex and several possible reasons for high- and low-binding sites have been suggested. The treatments discussed below have described possible reasons for the exhibited properties of [^{3}H]AMPA binding to its receptor. In general, these treatments do not affect the ligand binding of other receptors in the same way as AMPA receptors. Two possible reasons given for the reduction of AMPA-receptor affinity as they are inserted into the synapse are: the attachment of a putative inhibitory AMPA receptor-associated modulatory component present in the plasma membrane (Honoré and Nielsen, 1985) and changes in the lipid environment and in membrane fluidity occurring between synaptic and extrasynaptic membranes (Massicotte and Baudry, 1990). *See* Fig. 3.

Interconversion of High and Low [^{3}H] AMPA-Binding Sites

Radiation Inactivation and the Modulatory Component

Radiation inactivation has been applied successfully to determine the molecular weights of several proteins, enzymes, and receptors in situ (Chang et al., 1981; Nielsen and Braestrop, 1988; Nielsen et al., 1984; Schlegel, 1983). In accordance with classical target-size theory, the high-energy particles inactivate larger-molecular-weight proteins before smaller-sized molecules (Kepner and Macey, 1968). This technique was used to estimate the molecular weights of glutamate receptors prior to their cloning.

Exposure of rat cortex to increasing radiation doses showed a curvilinear profile of [^{3}H]AMPA binding (Honoré and Nielsen, 1985; Hall et al., 1996; Henley, 1993). It was concluded that the rising phase of this curve represented destruction of a 110-kDa AMPA receptor-associated inhibitory protein which upon removal/destruction caused an increase in the affinity of [^{3}H]AMPA binding (Figs. 1 and 3). The declining phase of the curve represented the destruction of AMPA receptors themselves. Both [^{3}H]AMPA and [^{3}H]CNQX bound a molecular target site of approx 50 kDa (Honoré et al., 1989). It should be noted that the predicted target size of approx 50 kDa for AMPA-receptor subunits (Hall et al., 1996) is inconsistent with the approx 100 kDa molecular weights found by molecular biology analysis (Nakanishi et al., 1990).

Nevertheless, recently a 53-kDa glutamate-related antigen (GR53), recognized by both GluR1 and GluR4 antibodies directed towards N- and C-terminals respectively, has been identified (Bahr et al., 1996). Previously unitary kainate/AMPA binding proteins of 40–50 kDa have been proposed in nonmammalians (Henley et al., 1992). Species that possess such small kainate-binding proteins (KBP) include the toad (*Xenopus laevis*), frog (*rana pipiens*), goldfish, and chick (*see* review by Henley, 1994). Compared with GluR1–GluR4 subunits, KBPs are truncated at the N-terminal by approx 250 amino acids and generally do not form functional channels (Henley, 1994; Ishmaru et al., 1996). *Xenopus* complexes have also been suggested to contain NMDA-binding sites (Henley et al., 1992). Although the mammalian GR53 protein did not directly copurify with [^{3}H]AMPA binding, it was suggested that weak association may occur with AMPA receptors (Bahr et al., 1996). Alternatively, GR53 may represent a novel [^{3}H]AMPA-binding protein that is analogous to invertebrate kainate-binding proteins (Henley, 1994) and requires further investigation (Bahr et al., 1996).

In an attempt to define the regional effects of irradiation on [^{3}H]AMPA binding, we have recently performed quantitative autoradiography on irradiated rat brain sections (Dev et al., 1996). In our hands, irradiation produces a dramatic increase in [^{3}H]AMPA binding in the hippocampal dendritic fields of CA1 region, the stratum radiatum, and oriens. In relation to both the high- and low-affinity components of [^{3}H]AMPA binding, it seems that irradiation treatment only increases binding to the low-affinity AMPA receptor component. This observation supports the idea that low-affinity sites are attached to the modulatory component, whereas high-affinity sites are not (Fig. 3).

Detergent Solubilization

Several studies have demonstrated the solubilization of AMPA receptors where the native phospholipid environment around the receptor has been replaced by detergent micelles. Studies agree that solubilization causes a rise in [^{3}H]AMPA binding, however explanations offered vary from increases in B_{max} values by exposure of cryptic sites (Henley and Barnard, 1991) to increases in the affinity of binding sites (Hunter, Wheaton, and Wenthold, 1990). Recently the relationship and changes between high and low components of [^{3}H]AMPA binding during solubilization have been examined (Hall et al., 1992).

Solubilization of rat brain membranes, with Triton X-100, showed an increase in the amount of [^{3}H]AMPA binding toward the high-affinity sites with a matching decrease of binding in the low-affinity component (Hall et al., 1992). From this result it appears that solubilization converts low-affinity AMPA receptors into high-affinity states. In the same study it was found that synaptic plasma membranes contained low-affinity sites only. A possible explanation was offered suggesting that high-affinity sites represent binding to extrasynaptic AMPA receptors, whereas low-affinity sites represent AMPA receptors that are inserted into the synaptic plasma membrane (Hall et al., 1992). In addition, it was suggested that solubilization released low-affinity synaptic AMPA receptors into an extrasynaptic envi-

ronment that rendered them in a native high-affinity state (Fig. 3).

Although it is appealing that the insertion of an AMPA receptor complex into the lipid bilayer and its attachment to an inhibitory modulatory unit is a mechanism for converting high-affinity sites into lower affinity states, the biochemical isolation and functional relevance of such a protein has not yet been established (Fig. 3). In addition, studies have shown that the modulatory component is still attached to solubilized AMPA receptors (*see* review 105). Although, in that study, a milder detergent (octylglucoside) was used that may have been insufficient at detaching the component from its associated AMPA receptor. Nevertheless, it cannot be dismissed that solubilization effects on AMPA receptors could be caused by changes in the microlipid environment as a whole, rather than by to the detachment of a single inhibitory unit.

Recombinant AMPA Receptors

In cell lines or baculovirus systems expressing recombinant GluR1–GluR4 subunits, another set of AMPA receptors that may be deemed nonneuronal, [^{3}H]AMPA binding in the presence of KSCN generally occurs to a single high-affinity site with a K_d approx 20 nM (Keinänen et al., 1990; Nakanishi et al., 1990; Hattori et al., 1994; Hall and Soderling, 1997).

The expression of high-affinity recombinant AMPA receptors in cell lines may be caused by the loss of expression in a modulatory unit. However, differences between the lipid environments of brain tissue and cell line systems cannot be excluded as a contributing factor for high affinity recombinant AMPA receptors (Fig. 3). Additionally, expression of homomeric rather than heteromeric AMPA receptors, may help reason why [^{3}H]AMPA binding occurs only to a single high-affinity site. It is noteworthy, that heteromeric and homomeric AMPA receptors are trafficked to the surface of the cell with equal rates (Hall et al., 1997). Furthermore, unlike NMDA receptors where NR1 subunit requires coexpression of NR2A before insertion into the membrane (Hall and Soderling, 1997; McIlhinney et al., 1996), no individual AMPA receptor subunit is necessary for the transfer of another subunit to the membrane (Hall et al., 1997). This result suggests that differences in the surface expression of homomeric and heteromeric AMPA receptors cannot, in part, explain a mechanism for existence of high- and low-affinity AMPA receptors.

Nevertheless, in the same study, glycosylation was shown to be associated with the trafficking of AMPA-receptor subunits to the cell surface (Hall et al., 1997). This postranslational modification and indeed other similar modifications, such as phosphorylation (Hall and Soderling, 1997; Raymond et al., 1993), palmitoylation (Pickering et al., 1995), and/or calpain-induced proteolysis (Bi et al., 1996) may represent models for introducing the two binding states of AMPA receptors. Although several reasons do exist, the exact mechanism responsible for high- and low-affinity [^{3}H]AMPA binding sites and also differences in affinity between native, solubilized, and recombinant AMPA receptors, to date, remains unclear.

Thiol Reagents

Binding Studies

The regulation of nicotinic and muscarinic receptors by modification of disulfide and sulfhydryl groups, respectively, has been previously established (Heollund and Bartfai, 1997; Schwartz and Kellar, 1983). In addition, mercuric compounds have been shown to reduce binding of several other receptor ligands including opiate (Simon and Groth, 1975), β_1-adrenergic (Moxham and Malbon, 1985) and dopamine D_1 receptors (Braestrup and Andersen, 1987). Studies suggest the involvement of cysteine residues that occur either within or distant from the receptor-binding site, modification of which causes a conformational change in the ligand-binding loop and thus a reduction in ligand binding (Hedlund and Bartfai, 1997; Schwartz and Kellar, 1983).

In direct contrast to other types of receptors, mercuric compounds such as $HgCl_2$, p-chloromercuribenzoate (PCMB) and its sulfonate analog p-chloromercuriphenyl-sulfonate (PCMBS) were shown to enhance [^{3}H]AMPA binding (Hall et al., 1996; Terramani et al., 1988). In addition, whereas the reducing agent dithiothreitol (DTT) itself had no effect on [^{3}H]AMPA binding, the reduction of Hg-S bonds back to -SH groups by DTT was shown to revert the ctions of Hg^{2+}-containing agents on [^{3}H]AMPA binding (Terramani et al., 1988). The difference in AMPA-receptor structure may explain why mercuric reagents enhance AMPA receptors compared to their inhibitory effects on others. For instance, AMPA receptors do not comply with the four T-MD structure of the ionotropic superfamily and neither are the conserved cysteine residues found in many other receptors present in AMPA receptors.

In a recent study, the conversion of low-affinity [^{3}H]AMPA binding sites to higher affinity states has been proposed as an explanation for the mercury-evoked effects on [^{3}H]AMPA binding kinetics (Hall et al., 1996). Although suggestions were made, an exact mechanism behind the conversion of low- to high-affinity [^{3}H]AMPA binding sites was not investigated. Nevertheless, we have also observed that treatment with PCMB causes a differential effect on the two binding sites of [^{3}H]AMPA: high-affinity states showed an increase in the number of sites, whereas the B_{max} for low-affinity sites were reduced and their affinity enhanced (Chittajallu et al., 1997).

We have also investigated the effects of PCMB on [^{3}H]NBQX. Like [^{3}H]CNQX, this antagonist fails to differentiate between the high- and low- affinity sites of AMPA receptors. Accordingly, the conversion of low-affinity [^{3}H]AMPA binding sites to higher-affinity states is not recognized by [^{3}H]NBQX and thus the affinity of [^{3}H]NBQX binding is relatively unaffected by PCMB (Chittajallu et al., 1997). Furthermore we have studied the effects of thiol reagents on the *Xenopus laevis* unitary non-NMDA receptor subunit (unpublished observations). Surprisingly, whereas [^{3}H]kainate binding to this receptor was unaltered by thiol reagents, an increase in [^{3}H]AMPA binding was detected. Reasons for these results remain unclear.

Functional Studies

In several studies, Ben-Ari and coworkers have suggested functional consequences of thiol modification on the redox site of NMDA receptors but not of AMPA receptors (Gozlan et al., 1994; 1995). In particular, they show that 5,5 O-dithiobis-2-nitrobenzoic acid (DTNB), a thiol oxidizing agent, produces an irreversible block of NMDA-receptor excitatory postsynaptic potentials (EPSPs). Furthermore, this agent inhibits CA1 hippocampal LTP. On the other hand, the disulfide reducing agent, tris (carboxyethyl) phosphine (TCEP) reverses the effects of DTNB. TCEP shows no effects of its own but restores NMDA receptor EPSPs and LTP to original levels before DTNB application. Interestingly, these workers showed little or no effect of either compound on AMPA receptors or indeed AMPA-receptor-mediated synaptic neurotransmission (Gozlan et al., 1994; 1995). In agreement, a similar investigation shows specific inhibitory effects of glutathione on the Ca^{2+} influx through NMDA receptors of rat cortical and retinal ganglion cell cultures (Lipton, 1993; Sucher and Lipton, 1991). Oxidized glutathione (GSSG) but not reduced glutathione (GSH) was shown to work in a similar fashion to DTNB. GSSG was specific for NMDA receptors alone and its inhibitory effects were reversed by DTT (Sucher and Lipton, 1991).

Although these studies suggest little functional relevance of AMPA modulation by thiol agents, it should be noted that DTNB has previously been shown to have little effect on [^{3}H]AMPA binding (Terramani et al., 1988). The application of PCMB, a thiol agent that significantly modulates [^{3}H]AMPA-receptor binding, may yet suggest functional roles for the regulation of AMPA receptors via their SH-groups. In fact, the chemical modification of sulfhydryl groups on glutamate receptors in hippocampal pyramidal neurons have shown

a reduction of glutamate and kainate response (Kiskin et al., 1986).

Mechanism of Action

The absence of any direct action by DTT on [^{3}H]AMPA-binding lends support to the idea that the thiol-evoked increase in [^{3}H]AMPA binding is caused by modification of SH- rather than disulfide-groups. In addition, the inadequate protection from a Hg^{2+}-evoked increase in [^{3}H]AMPA binding by saturating concentrations of glutamate suggested that the site of regulation was allosteric to the ligand recognition domain (Terramani et al., 1988). One possibility is that thiol reagents act to reduce putative disulfide bonds between conserved cysteine residues that occur in the extracellular loop just after MD-III and before MD-IV. Upon reduction, these residues may separate lobes 1 and 2 thus providing easier access for [^{3}H]AMPA to its binding site and so enhance the affinity of [^{3}H]AMPA binding (Fig. 2). Assuming this hypothesis to be correct, then the differences in high- and low-affinity [^{3}H]AMPA binding sites may occur because of complete and incomplete reduction of cysteine residues respectively.

Alternatively, the reduction of SH-groups may cause a dissociation of the inhibitory modulatory unit from the AMPA receptor resulting in an increase of [^{3}H]AMPA binding. In an attempt to bring together the mechanisms lying behind irradiation, solubilization, and thiol treatments, a study has recently examined all three modulations in combination (Hall et al., 1996). Exposure of rat brain membranes to ultraviolet (UV) light, PCMBS and detergent all showed an enhancement of [^{3}H]AMPA binding. Treatment with one method reduced or nullified the effect of the other. These results were taken to suggest that UV, PCMBS and solubilization effects all modify AMPA receptors via a similar mechanism (Hall et al., 1996). It was proposed that the interconversion of low-affinity [^{3}H]AMPA binding sites into higher-affinity states could be the possible mechanism for the effects of all three treatments on [^{3}H]AMPA binding. Furthermore, during such treatments the detachment of a modulatory component from the AMPA receptor was given as one possibility to explain the conversion of low- to high-affinity [^{3}H]AMPA binding sites (Fig. 3).

Potassium Thiocyanate

Binding Studies

The chaotrope anion potassium thiocyanate (KSCN) is a potent modulator of agonist binding to AMPA receptors (Honoré and Nielsen, 1985; Olsen et al., 1987; Honoré and Drejer, 1988; Hall et al., 1993; Nielsen et al., 1988). In the absence of KSCN [^{3}H]AMPA binds to rat brain membranes with an apparent single class of binding sites which are difficult to detect using standard radioligand-binding protocols (Honoré and Drejer, 1988). Furthermore, removal of the inactive R-enantiomer form of AMPA does not appear to increase the signal of [^{3}H]AMPA binding (Hawkins et al., 1995). On the other hand, in the presence of 50–100 m*M* KSCN, specific [^{3}H]AMPA binding is enhanced almost 10-fold in rat brain membranes and autoradiographic sections (Dev et al., 1996; Honoré and Drejer, 1988). Therefore, [^{3}H]AMPA binding is routinely carried out in the presence of KSCN.

In some studies in which KSCN has been used during binding, [^{3}H]AMPA has been reported to recognize two classes of binding sites (Hunter et al., 1990; Honoré and Drejer, 1988; Hall et al., 1993). Other groups, however, have detected only a single binding site (Smith and McIlhinney, 1992; Henley, 1995). The detection of one or two sites may depend upon analysis methods (Dev et al., 1995) and in most cases the discrepancies are normally issues of methodology. More specifically, studies that employ filtration procedures for the separation of bound and free radioligand seem to lose the lower affinity sites, whereas those using centrifugation techniques are able to distinguish both high- and low-affinity states (Honoré et al., 1982; Smith and McIlhinney, 1992; Honoré and Drejer, 1988). Nonetheless, it generally

appears that KSCN increases the affinity of [^{3}H]AMPA binding (Honoré and Drejer, 1988; Dev et al., 1995).

In addition, an increase in the number of [^{3}H]AMPA-binding sites have also been reported (Smith and Ilhinney, 1992; Olsen et al., 1987; Honoré and Drejer, 1988; Nielsen et al. 1988). At that time, it remained unclear whether this change in B_{max} was a direct effect of KSCN on AMPA receptors. Alternatively, a consequence of a KSCN-evoked increase of low-affinity binding sites can result in a higher number of detectable AMPA receptors. With the recent use of FW, where high and low AMPA receptor-binding sites are detected in the presence or absence of KSCN, it has been shown that KSCN enhances the affinity of both binding states without affecting B_{max} values (Hawkins et al., 1995; Hall et al., 1993).

The effects of KSCN are made more complex when examining its actions on other AMPA-receptor ligands. For example the ability of FW to displace [^{3}H]AMPA or [^{3}H]FW itself remains relatively unchanged in the presence or absence of KSCN (Hawkins et al., 1995; Dev et al., 1996). In another study, thiocyanate increases the [^{3}H]AMPA displacement abilities of acromelic acid A and domoate without affecting the displacement value of kainate (Smith and McIlhinney, 1992). For AMPA-receptor antagonists, KSCN has been shown to have a small inhibitory effect on [^{3}H]CNQX (Honoré and Drejer, 1988) without significantly altering the binding properties of [^{3}H]NBQX (Dev et al., 1996).

Functional Studies

Functional studies by ionophoretic application of KSCN in the CA1 region of the hippocampus caused an increase in amplitude and initial slope of synaptic responses (Shahi and Baudry, 1992). This implies a positive correlation between the affinity of AMPA receptors and functional response. In contrast, perfused application of KSCN to excised patches of hippocampal pyramidal cells and in hippocampal slices (Arai et al., 1995) and also exposure of KSCN to cultured cerebellar neurons and *Xenopus* oocytes expressing AMPA receptors (Bowie and Smart, 1993), caused a reduction in AMPA-mediated currents. The reduction in current was accompanied by an increase in the rate of AMPA receptor current desensitization. It was concluded that KSCN promotes the conversion of AMPA receptors into a stabilized desensitized state, reducing ligand dissociation, and thus also explaining an increase in binding affinity (Fig. 4). In agreement, desensitization of the AMPA receptor has been linked to an increase in agonist affinity (Arai et al., 1995; Trussell and Fischbach, 1989).

Electrophysiological and binding data suggest that cyclothiazide can hold the receptor in its active state where upon the open state can then be entered. This maintains longer access to a nondepolarized channel (Kessler et al., 1996). The effects of KSCN are thus in contrast to cyclothiazide, which slows desensitization, enhances AMPA receptor currents (Wong and Mayer, 1993; Yamada and Tang, 1993) and in the presence of KSCN inhibits [^{3}H]AMPA binding (Kessler et al., 1996; Hall et al., 1993). For [^{3}H]CNQX binding, in the presence of KSCN, cyclothiazide reduced the ability of AMPA to displace [^{3}H]CNQX. In the absence of KSCN, cyclothiazide showed no effect on the ability of AMPA to displace [^{3}H]CNQX binding (Kessler et al., 1996).

Mechanism of Action

Chaotropes are classified as ions that promote the transfer of polar groups into water. Early kinetic studies have suggested that KSCN may increase the affinity of [^{3}H]AMPA binding by interconversion of low-affinity sites to higher-affinity states (Honoré and Drejer, 1988). Recently, however, KSCN has been reported to equally increase the binding of both high and low affinity sites without affecting the densities of either component (Hawkins et al., 1995; Hall et al., 1993 and Fig. 4). To date, the mechanism of KSCN action on [^{3}H]AMPA-binding remains unclear:

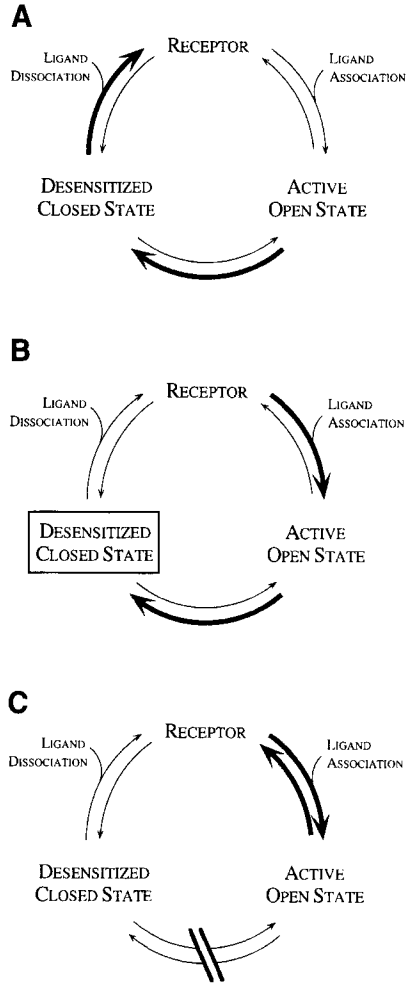

Fig. 4. Effects of KSCN and cyclothiazide: This figure represents a simplistic model of the effects of KSCN and cyclothiazide on AMPA-receptor function. For a more detailed picture please refer to (Kessler et al., 1996; Honoré and Drejer, 1988; Arai et al., 1995). **(A)** Normal cycle of equilibrium binding: Rapid conversion of active state to desensitized state causes rapid desensitizing AMPA-receptor currents. Slow ligand association and fast dissociation results in low basal affinities of both high (approx 200 n*M*) and low (approx 2000 n*M*) binding sites. **(B)** KSCN holds AMPA receptors in desensitized states for longer time intervals: Reduced AMPA currents are caused by increased conversion rates from active to desensitized states. Fast ligand association and slow dissociation from desensitized receptor increases high (approx 20 n*M*) and low (approx 500 n*M*) affinities. **(C)** Cyclothiazide reduces AMPA receptor entry to desensitized state: Enhanced AMPA receptor currents are caused by prolonged time periods within the active state. Reduced or loss of time in desensitized state results in reduction of ligand affinities. The effect on binding is more apparent in the presence of KSCN.

its opposing effects on agonist and antagonist binding do however suggest a preference for CNQX toward the active state and for AMPA toward the desensitized state. If KSCN does put AMPA receptors into a desensitized state, then its effects on other AMPA receptor ligands may be explained by correlating their attraction for either desensitized or active-AMPA receptor states. Furthermore, it is clear that the effects of KSCN appear to be directly on the AMPA receptor rather than via the lipid environment or a modulatory unit as KSCN can modulate [^{3}H]AMPA binding to both solubilized (Hall, Kessler, and Lynch, 1992) and irradiated AMPA receptors (Honoré and Nielsen, 1985).

Phospholipase A_2

The family of phospholipase A_2 (PLA_2) enzymes are a distinct and ubiquitous class of Ca^{2+}-dependent lipolytic esterases that specifically hydrolyze the sn-2-acyl ester bond in glycerophospholipids to liberate free fatty acids and lysophospholipids (Hanasaki and Arita, 1992). The PLA_2 family is divided into two classes, secretary ($sPLA_2$) and cytosolic ($cPLA_2$) (*see* reviews by Dennis, 1994; Mukherjee et al., 1994; Clark et al., 1995; Murakami et al., 1995). $cPLA_2$ have molecular weights of approx 85 kDa, they are found exclusively on the intracellular side of cell membranes and can be regulated by receptor G protein-coupled pathways. $sPLA_2$ enzymes have molecular weights between 11–18 kDa and have been divided into two subclasses. Type-I includes the mammalian pancreatic $sPLA_2$, whereas type-II are those found in snake venom (Murakami, Kudo, and Inoue, 1995; Heinrikson, Krueger, and Keim, 1977; DeHass et al., 1968). Pancreatic $sPLA_2$ also plays a role in processes such as smooth muscle constriction

(Nakajima et al., 1992) and cell proliferation (Arita et al., 1991).

There is increasing evidence to suggest important physiological roles of both $sPLA_2$ and its metabolites in synaptic plasticity events regulating membrane transport, neurotransmitter release, and channel activation (Catania et al., 1993; Massicotte et al., 1991; Tocco et al., 1992; Linden et al., 1987; Lynch and Voss, 1990; Clements, Bliss, and Lynch, 1991; Bramham, Alkon, and Lester, 1994). Clinically elevated levels of $sPLA_2$ activity have been reported in a wide range of diseases (Glaser et al., 1993; Vadas et al., 1993). Of most relevance to this review, porcine pancreatic secretary phospholipase A_2 ($ppsPLA_2$) has been shown to modulate [^{3}H]AMPA binding (Massicotte and Baudry, 1990; Dev, Honoré, and Henley, 1995; Massicotte et al., 1991; Tocco et al., 1992). Although, still controversial, its mechanism of action on AMPA receptors may provide further insight to reasons for the existence of high- and low- affinity [^{3}H]AMPA receptor binding sites.

Binding Studies

In one of the original studies, $ppsPLA_2$ was shown to specifically increase [^{3}H]AMPA binding (Massicotte and Baudry, 1990). In the presence of KSCN, a single binding site was observed, and $ppsPLA_2$ was shown to specifically increase the affinity of [^{3}H]AMPA binding without changing the number of binding sites. Binding of [^{3}H]kainate, [^{3}H]glutamate, or [^{3}H]glycine have been shown to be unaltered by $ppsPLA_2$ (Massicotte and Baudry, 1990; Dev, Honoré, and Henley, 1995; Cruickshank and Henley, 1994). However, in a later study, binding to metabotropic receptors has been shown to be enhanced by $ppsPLA_2$ treatment (Catania et al., 1993). The effects of $ppsPLA_2$ were Ca^{2+} dependent and blocked by bromophenacyl bromide (BPB), a potent PLA_2 inhibitor. Neither bovine serum albumin (BSA) blocked the effects of $ppsPLA_2$ nor did arachidonic acid mimic the increase in [^{3}H]AMPA binding. These results suggested that the actions of $ppsPLA_2$ were direct rather than being dependent on $ppsPLA_2$ metabolites (Massicotte and Baudry, 1990).

The effects of $ppsPLA_2$ are developmentally regulated. The $ppsPLA_2$ treatment of rat telencephalic membranes of postnatal day (PND) 5 and 10 caused a decrease in [^{3}H]AMPA binding (Baudry et al., 1991; Massicotte et al., 1992). However, the same $ppsPLA_2$ treatment of PND 20, 25 and adult membranes resulted in an increase in [^{3}H]AMPA binding (Massicotte et al., 1992). Interestingly those workers showed that in contrast to [^{3}H]AMPA binding, [^{3}H]CNQX binding was reduced by $ppsPLA_2$ treatment at all ages investigated.

Quantitative autoradiography indicates that $ppsPLA_2$ produces a specific regional increase in [^{3}H]AMPA binding, and causing a decrease in [^{3}H]CNQX binding throughout the brain (Tocco et al., 1992). More specifically, for [^{3}H]AMPA, an increase in binding was detected in the outer layers of the cortex and in the hippocampal dendritic fields of the CA1 region. In a more recent study, where both high- and low-affinity components of [^{3}H]AMPA binding were identified, $ppsPLA_2$ was shown only to increase [^{3}H]AMPA binding to low-affinity AMPA receptor components present in the stratum radiatum and oriens of the CA1. Binding to the high-affinity [^{3}H]AMPA binding site in the stratum pyramidale of the hippocampus was not markedly affected by $ppsPLA_2$. This observation lends further support to the hypothesis that low-affinity AMPA receptors are of physiological relevance (Catania et al., 1993). In addition, the regional effect of $ppsPLA_2$ on [^{3}H]AMPA binding are very similar to those of irradiation treatments (Dev et al., 1996). Collectively, these results suggest a common mechanism for irradiation and $ppsPLA_2$ effects on [^{3}H]AMPA binding.

Opposingly, we have shown that $ppsPLA_2$ elicits a Ca^{2+}- and concentration-dependent decrease in [^{3}H]AMPA binding to adult rat cortical membranes comprising a twofold decrease in affinity (Dev et al., 1995). Depending on the data analysis employed, binding fitted either a single- or a two-site model. In the two-

site fit, ppsPLA$_2$ modulated only the low affinity binding site. The decrease in affinity caused by ppsPLA$_2$ was attributed to its action as an enzyme rather than any competitive interaction at the [^{3}H]AMPA-binding site (Dev et al., 1995). In support, we have recently shown that the binding properties of [^{125}I]ppsPLA$_2$ do not fully correlate with ppsPLA$_2$ properties exhibited during the modulation of [^{3}H]AMPA binding (Dev et al., 1997). In the same study, it is shown that AMPA does not competitively inhibit [^{125}I]ppsPLA$_2$ binding to rat brain membranes. Interestingly, experiments to determine the effect of ppsPLA$_2$-treatment on [^{3}H]CNQX binding demonstrated an increase in specific binding compared to controls (Dev et al., 1995).

Several possible explanations for the discrepancies that we have observed in ppsPLA$_2$ actions may exist. However, experimental protocol variations with studies reporting an ppsPLA$_2$-evoked increase in [^{3}H]AMPA binding were deemed not to account for the decrease in [^{3}H]AMPA binding observed by our study. Indeed, using identical experimental conditions, we have observed a ppsPLA$_2$ evoked increase in [^{3}H]AMPA binding to several other tissue preparations including the proposed "unitary" non-NMDA receptor in *Xenopus* CNS (unpublished observations); rat spinal cord (Cruickshank and Henley, 1994); rat hippocampal membranes; and rat brain autoradiographic sections (Dev et al., 1996). Furthermore, in our study, cortical membranes were prepared from adult rats ranging from 5 wk and older, therefore the down regulation of [^{3}H]AMPA binding does not relate simply to the age of the animals used (Dev et al., 1995).

Functional Studies

Several studies suggest the involvement of sPLA$_2$ in synaptic plasticity (Massicotte et al., 1992; Massicotte et al., 1990). NMDA receptor activation and Ca^{2+} ion influx are critical in producing AMPA-receptor currents that maintain LTP (Bliss and Collingridge, 1993). Processes that require Ca^{2+} to modulate AMPA receptors are thus important in mechanisms underlying long-lasting changes in synaptic function. Appropriately sPLA$_2$ has been suggested to be involved in the formation of LTP (Massicotte et al., 1990). More specifically, changes in the lipid environment around synaptic AMPA receptors could be involved in long-lasting changes of synaptic efficiency (Gagne et al., 1996). The induction of LTP, triggered by NMDA-receptor activation is prevented by sPLA$_2$ inhibitors. The sPLA$_2$ inhibitor, BPB, reduced the magnitude of LTP in hippocampal slices (Massicotte et al., 1990). Neither paired-pulse facilitation, short-term potentiation (STP) or established LTP were affected. This suggests that sPLA$_2$ plays a role in the induction but not maintenance of LTP (Massicotte et al., 1990).

Studies have also found that LTP and the ppsPLA$_2$ evoked increase in [^{3}H]AMPA binding are both reduced after kainate-induced epileptic seizures (Massicotte et al., 1991). The chemical link between KA-induced epilepsy and its inhibitory effects on ppsPLA$_2$ was suggested to be the calcium-dependent protease, calpain. This enzyme is specifically activated after KA administration (Fitzpatrick et al., 1992; Bi et al., 1996). Calpains have been suggested to be involved in the regulation of AMPA receptors, such that calpain partially proteolyses the C-terminal domain of GluR1-receptor subunits (Bi et al., 1996). Both KA-induced seizures and pretreatment of membranes with calpain have shown to reduce the effects of ppsPLA$_2$ on [^{3}H]AMPA binding (Massicotte et al., 1991). The membrane soluble bee venom peptide, melittin, is a potent activator of endogenous sPLA$_2$ and has been shown to enhance both glutamate release and increase sensitivity of AMPA receptors. This indicates both pre- and post synaptic roles of sPLA$_2$ in LTP (Aronica et al., 1992). In another study, BPB was used to investigate the roles of sPLA$_2$ in long-term depression (LTD) occurring in the schaffer-commissural pathway of juvenile rats. Again both pre- and postsynaptic effects of sPLA$_2$ in LTD were suggested (Fitzpatrick and Baudry, 1994).

In accordance with the effects of ppsPLA$_2$ on [^{3}H]AMPA binding it was implied that in adult

rat brains the increase in [^{3}H]AMPA binding evoked by endogenous sPLA$_2$ may be involved in LTP, whereas the evoked decrease in neonates could represent LTD (Gagne et al., 1996; Fitzpatrick and Baudry, 1994). Although studies show that direct actions of ppsPLA$_2$ are responsible for modulating [^{3}H]AMPA binding, it is of some controversy whether direct effects of endogenous sPLA$_2$ are involved in synaptic function. For instance, in functional studies, the liberation of unsaturated fatty acids by sPLA$_2$ are thought to play a part in synaptic changes. Furthermore, studies suggest that release of fatty acids by sPLA$_2$ contribute to a synergistic activation of PKC, the full activation of which may occur with PLC to result in LTD induction (Linden et al., 1987; Linden, 1995). Additionally, exogenous fatty acids have also been reported to help induce or convert STP to LTP (Linden et al., 1987; Bramham et al., 1994). For example, levels of arachidonic acid have been shown to increase in LTP (Clements et al., 1991). This molecule has been implicated to serve as a retrograde messenger that increases glutamate release (Lynch and Voss, 1990).

Mechanism of Action

The exact mechanisms of ppsPLA$_2$ action on AMPA receptors are somewhat unclear (Fig. 3). Possible mechanisms include: a ppsPLA$_2$-evoked disturbance in the microlipid environment surrounding the AMPA receptor (Massicotte et al., 1991; Gagne et al., 1996); ppsPLA$_2$ effects on an associated modulatory unit (Honoré and Nielsen, 1985), and although unlikely activation of a specific high-affinity 180-kDa sPLA$_2$ receptor (Dev et al., 1997; O'Hara et al., 1995).

It has been proposed that ppsPLA$_2$ treatment results in a regionally specific modification of the AMPA receptors that is probably caused by different AMPA-receptor subunit compositions. It is clear that GluR1–GluR4 receptor subunits are independently expressed in different brain regions and at different stages during development. Perhaps these reasons may also explain the opposing effects of ppsPLA$_2$ during different stages of development. Alternatively, different lipid compositions in specific parts of the neuron and/or in regions of the brain, at defined stages of development, may also affect the direction of ppsPLA$_2$ effects on [^{3}H]AMPA binding (Tocco et al., 1992). Although reasons remain unclear, discrepancies between our results and previous reports may in fact be caused by changes in the phospholipid environment surrounding the individual AMPA-receptor preparations. For converse effects to occur between agonist and antagonist binding, a possible explanation is that ppsPLA$_2$ alters the AMPA receptor in a similar fashion to KSCN, such that binding of the agonist is more favorable than that of the antagonist.

We have conducted studies to address the question of subunit-selective effects by ppsPLA$_2$ (Dev, Honoré, and Henley, 1997). Using homomeric GluR1, GluR2, and GluR4 subunits expressed in baby hamster kidney cells we determined the effects of ppsPLA$_2$. In agreement with previous studies, control affinity values were approx 25 n*M* (Keinänen et al., 1990; Nakanishi et al., 1990; Hattori et al., 1994; Hall and Soderling, 1997) and were similar to the higher affinity state of native AMPA receptors found in extrasynaptic membranes (Hall et al., 1992). Similar to our rat brain membrane studies, in which the high-affinity sites were not modulated by ppsPLA$_2$ (Dev et al., 1995), our results on homomeric AMPA receptors show that neither flip nor flop version of any subunit responded to ppsPLA$_2$ treatments (manuscript in preparation). AMPA receptors contain fatty acid moieties, however from these results, it appears that ppsPLA$_2$ does not directly attack AMPA receptors. Instead our studies suggest that cell lines, like intracellular AMPA receptors, lack in something that allows sensitivity towards ppsPLA$_2$. At this time it is difficult to determine whether ppsPLA$_2$ has a requirement for the modulatory component that may not be expressed in cell lines or for the correct phospholipid environment. In addition heteromeric combinations may also be a possible

requirement for ppsPLA$_2$ effects. However, we have previously shown a ppsPLA$_2$-evoked modulation of putative homomeric GluR2 receptors in rat spinal cord (Cruickshank and Henley, 1994), suggesting that requirement of heteromeric assemblies is not a necessity for ppsPLA$_2$ effects on [^{3}H]AMPA binding.

Recently, calpain proteolysis of the C-terminal region of GluR1 receptor subunits has been identified (Bi et al., 1996). Calpain has been shown to reduce ppsPLA$_2$ effects on [^{3}H]AMPA binding (Massicotte et al., 1991). Collectively these results suggest that the C-terminal region of AMPA receptor subunits may be important for ppsPLA$_2$ regulation. In another study, the C-terminal portion of GluR2 has been shown to interact with a newly termed protein called glutamate receptor interacting protein (GRIP) (Dong et al., 1997). In addition we have identified protein–protein interaction between N-ethylmaleimide-sensitive fusion protein (NSF) and the C-terminal portion of GluR2 (manuscript in preparation and Nishimune et al., 1996). To speculate, perhaps the C-terminal region also recognizes a putative modulatory protein, interaction of which is modulated by a host of enzymes (ppsPLA$_2$ and calpain), chemicals (thiol reagents), and other treatments (solubilization and irradiation).

Concluding Remarks

Clearly [^{3}H]AMPA binding to high- and low-affinity binding sites is a very complex topic. In many cases the regulation of [^{3}H]AMPA binding using different protocols has brought increased difficulties to explaining [^{3}H]AMPA binding. Biological complexity may occur because of different mechanisms working in conjunction during the treatment of AMPA receptors by the regulators described above. After the large amount of literature cited, it still remains to be resolved exactly why differences in high- and low-affinity [^{3}H]AMPA binding sites occur. Moreover, the physiological relevance of the high-affinity site is also still a mystery. If indeed the high affinity site has a role in synaptic function that is different than the low-affinity site, then selective therapeutic targeting towards either low- or high-affinity [^{3}H]AMPA binding sites may be of clinical importance. Furthermore, drug development against novel target sites, such as the putative modulatory component, may also serve as useful approaches to helping AMPA receptor associated diseases. Unfortunately, these possible implications remain unchallenged until it becomes clear why [^{3}H]AMPA binds to two sites.

Acknowledgments

We are grateful to the MRC and the Wellcome Trust for financial support. KKD holds a Wellcome Trust Prize Travelling Research Fellowship.

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