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Multiple States of Rat Brain (*RS*)-α-Amino-3-hydroxy-5-methylisoxazole-4-propionic Acid Receptors as Revealed by Quantitative Autoradiography

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SUMMARY

The binding of (*RS*)-α-[^{3}H]amino-3-hydroxy-5-methylisoxazole-4-propionic acid ([^{3}H]AMPA), a selective ligand for non-*N*-methyl-D-aspartate excitatory amino acid receptors, was investigated in rat brain using an autoradiographic receptor binding technique. [^{3}H]AMPA binding sites were widely distributed throughout the rat central nervous system, and the rank order of potency of displacers of [^{3}H]AMPA binding was quisqualate > AMPA > 6,7-dinitroquinoxaline-2,3-dione = 6-cyano-7-nitroquinoxaline-2,3-dione > β-*N*-oxalylamino-L-alanine > glutamate > kainate. Potassium thiocyanate (0–100 mM) exerted a 4-fold stimulation of [^{3}H]AMPA binding, without changing the relative regional distribution of [^{3}H]AMPA binding densities among rat brain regions. Scatchard analysis of equilibrium saturation binding revealed high affinity and low affinity components of [^{3}H]AMPA binding, even in the absence of potassium thiocyanate. Addition of potassium thiocyanate increased the number of high affinity [^{3}H]AMPA binding sites without a change in affinity. In addition, the number of low affinity [^{3}H]AMPA binding sites was unchanged in the presence of potassium thiocyanate, but the affinity of low affinity [^{3}H]AMPA binding was greatly increased. [^{3}H]AMPA thus binds specifically to two affinity conformations of postsynaptic binding sites that appear to be interconverted with potassium thiocyanate. The pharmacologic profile of these sites is consistent with that of the ion channel-linked ("ionotropic") quisqualate/AMPA class of excitatory amino acid receptor in the rat central nervous system.

The essential amino acid glutamate is believed to be the predominant neurotransmitter carrying excitatory signals within the mammalian central nervous system (1–3). The excitatory effects of glutamate are mediated by a variety of excitatory amino acid receptors (3–5). Excitatory amino acid receptors, whose activation is linked to changes in ionic conductances ("ionotropic" receptors), are divided into the NMDA-preferring receptors (NMDA receptors) and non-NMDA receptors (1, 3–5). Ionotropic non-NMDA receptors have traditionally been further classified into quisqualate-preferring and kainate-preferring receptors. A separate quisqualate-preferring receptor linked to phosphoinositide metabolism, the "metabotropic" quisqualate receptor, has also been described (6). The most well characterized of these receptors is the NMDA receptor. NMDA receptors have been implicated in learning and memory, synaptic development, and seizures, as well as being postulated as playing an etiologic role in neurodegenerative diseases and the neurologic damage resulting from hypoxia-ischemia (7). However, because NMDA receptor responses are voltage dependent, activation of these receptors seems often to require prior depolarization of the neuronal membrane via non-NMDA receptors.

The bulk of fast excitatory synaptic transmission thus likely occurs through ionotropic non-NMDA receptors. Many of the fundamental pharmacologic properties of these receptors, however, have yet to be elucidated. AMPA, a structural analogue of ibotenic acid, is a specific agonist of ionotropic non-NMDA receptors and has a low affinity for NMDA and metabotropic receptors. We have used [^{3}H]AMPA in an autoradiographic binding assay to investigate the pharmacologic properties of ionotropic quisqualate/AMPA receptors in the rat brain. Specifically, we have investigated the possibility that multiple types of [^{3}H]AMPA binding sites exist, as well as the nature of the relationship between these sites.

Experimental Procedures

Materials. [^{3}H]AMPA (27.6 Ci/mmol) was obtained from New England Nuclear (Boston, MA). DNQX and CNQX were obtained

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ABBREVIATIONS: NMDA, *N*-methyl-D-aspartate; AMPA, (*RS*)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; DNQX, 6,7-dinitroquinoxaline-2,3-dione; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; BOAA, β-*N*-oxalylamino-L-alanine; KSCN, potassium thiocyanate; γDGG, γ-D-glutamylglycine; GDEE, glutamic acid diethyl ester; ANOVA, analysis of variance; APB, L-aminobutyric acid.

from Tocris Neuramin (Essex, UK). Quisqualate, BOAA, and nonradioactive AMPA were obtained from Cambridge Research Biochemicals (Valley Stream, NY). KSCN was obtained from Fisher Scientific (Fairlawn, NJ). All other compounds were purchased from Sigma (St. Louis, MO).

Tissue preparation. Male Sprague-Dawley rats were decapitated, and the brains were quickly removed, mounted with Lipshaw embedding matrix on a cryotome pedestal, and frozen under powdered dry ice. Sections (20 μm) were cut on a Lipshaw cryostat and thaw-mounted onto gelatin-coated slides. Sections were stored for <24 hr, at −20°. All sections underwent a wash for 30 min at 2°, in 50 mM Tris·HCl buffer containing 2.5 mM $CaCl_2$, pH 7.20, either with or without KSCN. Sections were blown dry under a stream of room temperature air.

Receptor autoradiography. A detailed description of the method for [^{3}H]glutamate binding autoradiography has been published (8). A modification of this method has been developed for [^{3}H]AMPA binding autoradiography (9). Briefly, in competition studies tissues were incubated for 45 min at 2° in the presence of 37 nM [^{3}H]AMPA. All solutions were adjusted to pH 7.2, with either Tris base or hydrochloric acid, before use. Nonspecific binding was defined as that [^{3}H]AMPA binding occurring in the presence of 1 mM unlabeled glutamate and was <5% of total [^{3}H]AMPA binding.

After the incubation, sections were rinsed quickly three times with cold buffer and then rinsed with cold 2.5% (v/v) glutaraldehyde in acetone. Sections were blown dry with warm air. The rinse/drying procedure took no more than 10 sec. Dried sections were placed in X-ray cassettes with appropriate radioactive standards (10) and apposed to Amersham Hyperfilm. The film was exposed to the tissue sections for 14–21 days at 4°, developed, fixed, and dried. The optical density of the resultant film images was determined using a commercially available computer-based image analysis system (Imaging Research, Inc., St. Catherine's, Ontario, Canada). Ten to 25 readings were averaged from each region of interest. The radioactivity was determined by a computer-generated polynomial regression analysis, which compared film densities produced by the tissue sections with those produced by the radioactive standards. All data presented were analyzed densitometrically from autoradiographic images.

KSCN dose-response studies. To measure the effects of KSCN, a dose-response curve with KSCN was performed. All sections were prewashed in Tris-HCl buffer containing 2.5 mM $CaCl_2$ but no KSCN. Incubation with 37 nM [^{3}H]AMPA was performed in the presence of 0–1000 mM KSCN. All rinses were performed with cold buffer containing no KSCN.

Saturation studies. [^{3}H]AMPA saturation studies were performed in Tris·HCl buffer with 2.5 mM $CaCl_2$, in the presence of four concentrations of KSCN (0, 1, 10, and 100 mM). Appropriate concentrations of KSCN were included in the prewash, the incubation with [^{3}H]AMPA, and the rinses. [^{3}H]AMPA concentrations ranged from 1 nM to 37 μM. [^{3}H]AMPA was diluted isotopically with known amounts of unlabeled AMPA. Nonspecific binding was determined in the presence of at least 100-fold excess unlabeled glutamate and was determined at each concentration of [^{3}H]AMPA. For saturation studies, each determination (n) represents an individual animal in an independent experiment. K_d and B_{max} values were generated by the nonlinear regression program LIGAND (11).

Results

Regional distribution of [^{3}H]AMPA binding. [^{3}H]AMPA binding was nonuniformly distributed throughout the rat brain. The highest levels of [^{3}H]AMPA binding were found within the hippocampal formation, the molecular layer of the cerebellum, and the lateral septum. Cortical areas had intermediate amounts of [^{3}H]AMPA binding, with a marked gradient of distribution, such that outer layers of cortex (layers I and II) consistently had higher levels of [^{3}H]AMPA binding than did inner layers (layers V and VI). The thalamus, granule cell layer of cerebellum, and inferior colliculus had relatively low levels of [^{3}H]AMPA binding.

Competition studies. Quisqualate and AMPA were the most potent displacers of [^{3}H]AMPA binding, followed by DNQX, CNQX, BOAA, glutamate, and kainate (Table 1). All of these compounds produced complete displacement of [^{3}H]AMPA binding. BOAA, kainate, and glutamate had Hill coefficients of 0.6 ± 0.1, 0.7 ± 0.1, and 0.8 ± 0.1, respectively. For quisqualate, AMPA, CNQX, and DNQX, the Hill coefficients ranged from 0.95 to 1.1. Ibotenate, aspartate, GDEE, APB, kynurenate, and γDGG were ineffective displacers of [^{3}H]AMPA binding, with IC_{50} values greater than 100 μM.

Stimulatory effects of KSCN. KSCN increased [^{3}H]AMPA binding, with stimulatory effects of KSCN observed at concentrations as low as 0.3 mM (Fig. 1). The maximal amount of stimulation occurred between 10 and 100 mM KSCN, resulting in a 4-fold stimulation of [^{3}H]AMPA binding in the molecular layer of cerebellum. At 100 mM KSCN, there was a near-maximal stimulation of total [^{3}H]AMPA binding, without a

TABLE 1
Displacers of [^{3}H]AMPA binding
Values are mean ± standard error.

Displacer	IC_{50}	n_H
	μM	
Quisqualate	0.08 ± 0.01	1.0 ± 0.04
AMPA	0.25 ± 0.02	1.1 ± 0.04
DNQX	0.34 ± 0.01	1.0 ± 0.1
CNQX	0.36 ± 0.03	0.9 ± 0.1
BOAA	0.83 ± 0.15	0.6 ± 0.1
Glutamate	1.8 ± 0.49	0.8 ± 0.1
Kainate	6.7 ± 0.93	0.7 ± 0.1

Displacer	Binding in the presence of 100 μM displacer
	% of control
Kynurenate	72 ± 4.9
Ibotenate	75 ± 4.9
γDGG	86 ± 3.0
Aspartate	91 ± 0.6
GDEE	95 ± 4.6
APB	109 ± 2.7

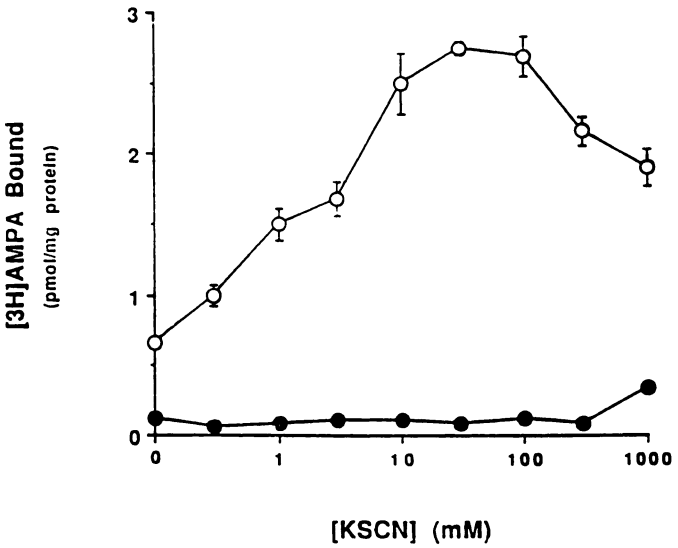


Fig. 1. KSCN dose-response curve. ○, Total [^{3}H]AMPA binding; ●, nonspecific [^{3}H]AMPA binding. Data are from the molecular layer of cerebellum. *Points* represent mean ± standard error (n = 3 animals).

marked increase in the amount of nonspecific binding. Fig. 1 shows autoradiographic images of [^{3}H]AMPA binding to horizontal rat brain sections in the presence and absence of KSCN and illustrates the stimulatory effects of KSCN.

The regional distribution of [^{3}H]AMPA binding sites in the presence of KSCN correlated very highly with the distribution of binding in the absence of KSCN, indicating that KSCN stimulation of [^{3}H]AMPA binding occurs to an equal proportion in all brain areas (Figs. 2 and 3).

[^{3}H]AMPA saturation binding with different concentrations of KSCN. In order to characterize the mechanism by which KSCN increased [^{3}H]AMPA binding, the effects of varying concentrations of KSCN on [^{3}H]AMPA saturation isotherms were investigated. Scatchard plots were obtained for [^{3}H]AMPA binding in the molecular layer of cerebellum, measured in the presence of four different concentrations of KSCN (0, 1, 10, and 100 mM) (Fig. 4). For all concentrations of KSCN, the Scatchard plots were curvilinear and best fitted by a two-site model versus a one-site model (F test, $p = 0$ for all four concentrations of KSCN tested). As the concentration of KSCN present increased from 0 to 100 mM, the presence of two binding sites was more apparent.

Using a one-way ANOVA to compare binding parameters derived for each concentration of KSCN, there was no significant difference in the B_{max} of low affinity sites ($p > 0.05$), although there was a trend towards lower low affinity B_{max} values with increasing concentrations of KSCN (Table 2). The K_d values for the low affinity sites were significantly different from each other, however, with increasing affinity (smaller K_d values) in the presence of increasing concentrations of KSCN ($p < 0.01$). Significant differences between groups were also observed for the high affinity B_{max} values, with the number of high affinity sites increasing with higher concentrations of KSCN ($p < 0.01$). Although ANOVA indicated that the high affinity K_d values were significantly different from each other ($p < 0.01$), there was no clear directional trend.

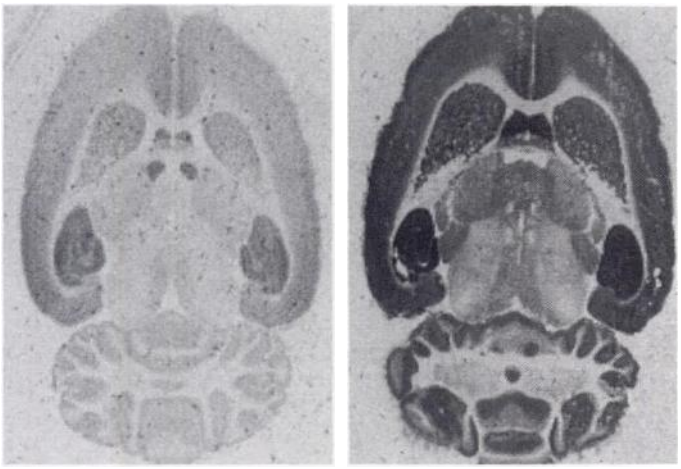


Fig. 2. Computer-enhanced autoradiographs of [^{3}H]AMPA binding to horizontal sections of rat brain, in the absence (*left*) and presence (*right*) of 100 mM KSCN. Both sections were incubated with 37 nM [^{3}H]AMPA.

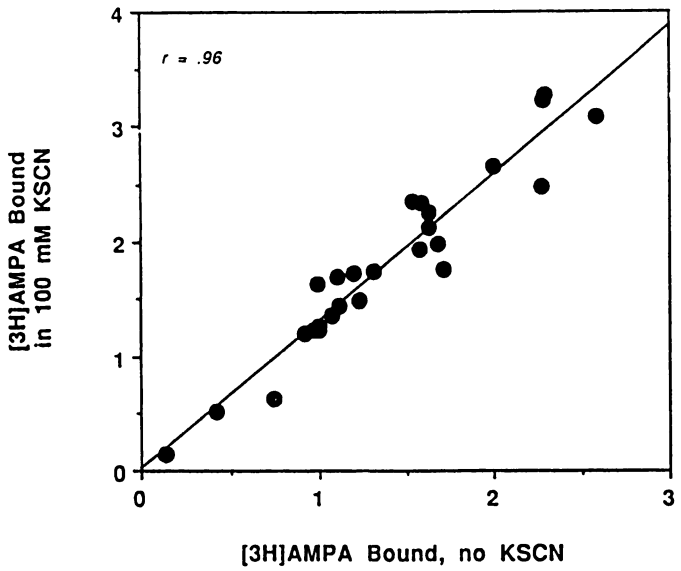


Fig. 3. Correlation of regional distribution of [^{3}H]AMPA binding to rat brain and presence of 100 mM KSCN. Each *point* represents a distinct brain region. *Abscissa*, amount of [^{3}H]AMPA binding (in pmol/mg of protein) in the absence of KSCN; *ordinate*, amounts of [^{3}H]AMPA bound in the presence of 100 mM KSCN. The concentration of [^{3}H]AMPA was 60 nM in the absence of KSCN and 37 nM in the presence of 100 mM KSCN.

Discussion

AMPA was synthesized by Krogsgaard-Larsen *et al.* (12) as a derivative of the excitatory amino acid ibotenate. AMPA was found to be a potent excitant and, on the basis of antagonism of AMPA-induced responses by GDEE, was presumed to act at the quisqualate subclass of excitatory amino acid receptors (9, 12–14). Electrophysiologic studies have shown AMPA to be a potent agonist for the quisqualate receptors linked to a cationic membrane channel (12–17), and the currently preferred name for this receptor is the "AMPA receptor." Initial [^{3}H]AMPA binding studies, using brain homogenate preparations, supported the notion that AMPA interacted with an excitatory glutamate receptor (14, 18, 19). Quisqualate, glutamate, and unlabeled AMPA were the most potent displacers of [^{3}H]AMPA binding. Kainate also competed for [^{3}H]AMPA binding. Kainate also competed for [^{3}H]AMPA binding. The present observations that quisqualate, AMPA, and kainate are effective displacers of [^{3}H]AMPA binding are consistent with recent studies showing parallel antagonism of kainate- and quisqualate- or AMPA-induced responses (20–23) and suggest that kainate, quisqualate, and AMPA share a common site of action (16, 17, 23–27). If, in the presence of KSCN, low affinity (R_2) receptor conformations are converted to high affinity (R_1) conformations, one would expect to see a corresponding decrease in the B_{max} of low affinity conformations accompanying the increased B_{max} of high affinity forms. In the current study, there was a concomitant increase in the high affinity B_{max} and a decrease in the low affinity B_{max}. The magnitudes of the changes in the B_{max} values were not equal, however. This discrepancy probably reflects the difficulty of accurately measuring numbers of low affinity sites. Finally, the increase in B_{max} of high affinity sites was small, relative to the number of low affinity sites; such a small decrease in the B_{max} of low affinity sites is difficult to measure reliably.

[^{3}H]AMPA binding demonstrated a marked dependence on thiocyanate ions (9, 28–31), which were presumed to increase the binding of [^{3}H]AMPA via their chaotropic action (28, 29, 32). In addition, several researchers have observed that two binding sites exist for [^{3}H]AMPA, depending on the absence or presence of thiocyanate ions (28–31). In the absence of

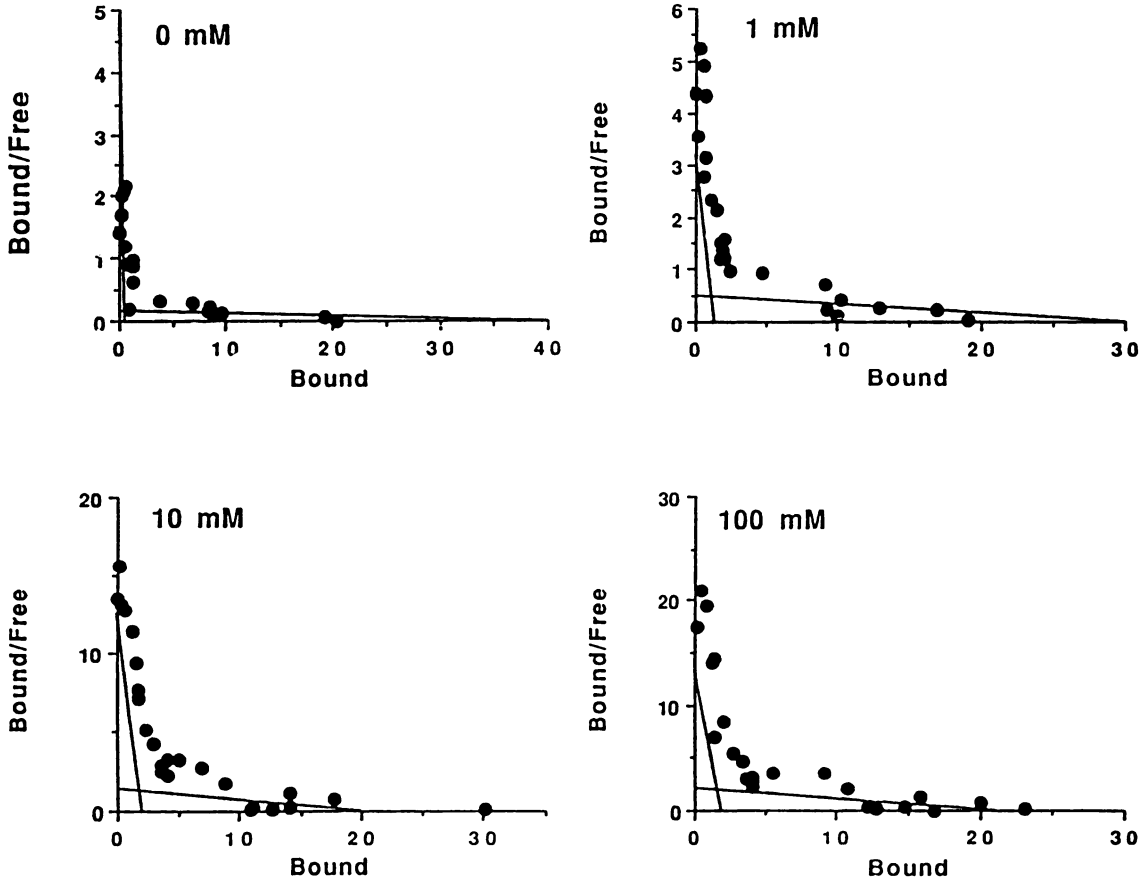


Fig. 4. Representative Scatchard plots for [³H]AMPA binding measured with 0, 1, 10, and 100 mM KSCN. Data are from the molecular layer of cerebellum. *Lines* represent fitted parameters for two-site model fits for pooled data.

TABLE 2

Scatchard analyses of [³H]AMPA saturation binding experiments

Data are from the molecular layer of cerebellum, using 1 nM to 37 μM [³H]AMPA. Statistical comparisons between two-site and one-site fits were by *F* test. Statistical comparison between groups was performed by one-way ANOVA.

[KSCN]	*n*	Two-site fit better than one-site fit?	High affinity		Low affinity	
			K_d	B_{max}	K_d	B_{max}
mM			*nM*	*pmol/mg of protein*	*μM*	*pmol/mg of protein*
0	5	Yes	10 ± 4.4	0.5 ± 0.1	27 ± 19	40 ± 22
1	4	Yes	45 ± 12	1.4 ± 0.32	6.3 ± 2.2	30 ± 6
10	3	Yes	16 ± 6.7	2.0 ± 0.6	1.5 ± 0.6	20 ± 3
100	5	Yes	14 ± 5.0	1.9 ± 0.5	1.0 ± 0.3	21 ± 2
		$ANOVA_{3,13}$	20.0	11.7	6.0	2.5
		p value	<0.01	<0.01	<0.01	>0.05

thiocyanate ions [³H]AMPA binding produces a linear Scatchard plot, whereas in the presence of thiocyanate a biphasic Scatchard plot is obtained (28). The mechanism of the ability of thiocyanate to recruit additional binding sites is unclear. Thiocyanate could actually uncover a novel population of binding sites. Alternately, thiocyanate could increase the affinity of a very low affinity site (so low as to be unmeasurable in the absence of thiocyanate). The present results favor this latter hypothesis.

KSCN has been shown to increase [³H]AMPA binding in homogenate preparations (28–31) and autoradiographic paradigms (9). KSCN also increases the ability of AMPA to compete for [³H]glutamate binding (33). In the present study, concentrations of KSCN as low as 0.3 mM stimulated [³H]AMPA binding, whereas maximal stimulation of binding occurred at KSCN concentrations of 10–100 mM. The stimulatory effects are consistent with the actions of KSCN as a chaotropic anion (28, 32). Chaotropic ions increase lipid membrane fluidity; the addition of the detergent Triton X-100 also stimulates [³H]AMPA binding, presumably via an increase in membrane fluidity (30). Calcium chloride is also a chaotropic agent and may stimulate binding. Compared with KSCN, however, these effects are small (9). The calcium chloride concentration was constant in all the experiments presented here, and its stimulatory effect is unlikely to account for the observed differences.

Although AMPA binds to a subset of quisqualate-sensitive [³H]glutamate recognition sites, KSCN does not affect the overall binding of [³H]glutamate (9, 33). Thus, KSCN is not

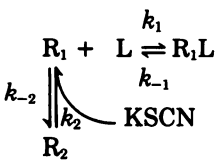
acting by increasing the total amount of [^{3}H]glutamate binding sites. Rather, KSCN appears to "convert" certain of the [^{3}H]glutamate binding sites to an AMPA-preferring form; that is AMPA inhibits more [^{3}H]glutamate binding in the presence of KSCN than in the absence of KSCN.

In order to investigate the mechanism by which KSCN increased [^{3}H]AMPA binding, [^{3}H]AMPA saturation experiments were performed in the presence of increasing concentrations of KSCN. If KSCN were increasing [^{3}H]AMPA binding by uncovering a second receptor, one would expect, as the concentration of KSCN increased, an increase in the B_{max} value measured for that second site. In contrast, if the stimulatory effects of KSCN were from increasing the affinity of a low affinity site, one would observe a gradual increase in the affinity, as reflected in a decrease of the measured K_d values, of the second binding site for [^{3}H]AMPA.

The results of the [^{3}H]AMPA saturation experiments performed at 0, 1, 10, and 100 mM KSCN favor the second of these two possibilities (Fig. 4; Table 2). That is, as the concentration of KSCN increased, so did the affinity of the low affinity site. No increase in the number of low affinity sites was observed; in fact, the data suggest that the B_{max} of the low affinity sites may decrease. Honoré and Drejer (28) and Murphy *et al.* (30) found that AMPA bound to a single population of binding sites in the absence of KSCN, but the highest concentration of [^{3}H]AMPA used in their saturation studies was 2 μM. As the present study shows, in the absence of KSCN the K_d of the low affinity [^{3}H]AMPA binding site was 27 μM. Such a low affinity site would easily go undetected in saturation experiments using only low micromolar concentrations of [^{3}H]AMPA.

However, based on kinetic studies, Honoré and Drejer (28) reached similar conclusions, that two states of the AMPA-preferring quisqualate receptor exist, one with high and one with low affinity for [^{3}H]AMPA. Drejer and Honoré speculated that [^{3}H]AMPA does indeed have a low affinity for a second site, although their study could not resolve its actual affinity. The low affinity K_d of 27 μM confirms this supposition.

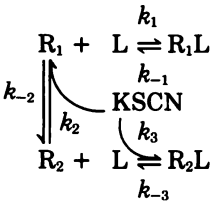
In addition to increasing the affinity of the low affinity site, KSCN also increased the density of high affinity sites, with no observed change in affinity. Honoré and Drejer (28) speculated that AMPA receptors exist in two conformations and that KSCN influences the equilibrium between them, such that the proportion of high affinity sites is increased. They proposed the following model:



(I)

R_1 represents the high affinity site and R_2 the low affinity site. K_I, the relaxation constant, is defined as the equilibrium constant between high affinity sites, k_{-2}/k_2, which in the absence of ligand is R_1/R_2. They postulate that KSCN increases K_I, thus increasing the number of high affinity sites. Model I predicts an increase in high affinity [^{3}H]AMPA binding, but it does not account for [^{3}H]AMPA binding to a low affinity site. Honoré and Drejer calculated that K_I would be 0.24×10^{-2} in the absence of KSCN and 2.7×10^{-2} in the presence of 100 mM KSCN. Using the parameters derived from the current experiments, K_I would be 1.2×10^{-2} in the absence of KSCN and 8.9×10^{-2} in the presence of 100 mM KSCN. Although these theoretical calculations differ by about a factor of 4, the predicted trends are similar, in that K_I increases in the presence of KSCN. Given that Honoré and Drejer used homogenate preparations and derived their values from kinetic data, whereas the current experiments employed an autoradiographic technique and equilibrium binding data, the agreement is reasonable.

The present results suggest that model I can be expanded to account for [^{3}H]AMPA binding to low affinity sites:



(II)

In this model, not only does KSCN increase K_I (favoring the conversion of R_2 to R_1), it also increases the binding affinity of the R_2 conformation. The quinoxalinedione antagonist compounds CNQX and DNQX have also been found to inhibit [^{3}H]AMPA binding (28), as confirmed in the present study. The plant neurotoxin BOAA (also known as β-*N*-oxalyl-L-α,β-diaminopropionic acid) was also a potent displacer of [^{3}H]AMPA binding.

One prediction that derives from model II is that, as the concentration of KSCN increases, the overall amount of [^{3}H]AMPA bound will increase. Further, the percentage of binding that is bound to the high affinity site will be increased.

Using the formula

$$B_{tot} = \frac{B_{max_1} \cdot F}{K_{D_1} + F} + \frac{B_{max_2} \cdot F}{K_{D_2} + F}$$

where B_{tot} is the total amount of ligand bound and F is the concentration of free ligand, one can calculate the percentage contribution of high affinity and low affinity binding. K_d and B_{max} values from Table 2 are used. In the absence of KSCN, with [AMPA] = 37 nM, B_{tot} would be 0.45 pmol/mg of protein, with 87.6% bound to high affinity sites and 12.4% bound to low affinity sites. In the presence of 100 mM KSCN, B_{tot} increases to 2.09 pmol/mg of protein, with 64.7% bound to high affinity sites and 35.3% bound to low affinty sites. Thus, KSCN increases not only high affinity AMPA binding but also low affinity AMPA binding. These results favor model II over model I, largely because model II accounts for the contribution of low affinity binding.

These data argue for interconvertibility of conformation of the [^{3}H]AMPA receptor. First, the regional distribution of [^{3}H]AMPA binding in the absence and presence of KSCN appears identical. If the two affinity states represented independent receptors, one would not expect this to be the case; the KSCN-stimulated [^{3}H]AMPA binding would have a distinct distribution from the unstimulated [^{3}H]AMPA binding. Next, KSCN stimulates overall [^{3}H]AMPA binding and the ability of unlabeled AMPA to compete for [^{3}H]glutamate binding, but it does not increase [^{3}H]glutamate binding (9, 33). Taken together, these data suggest that some sites are converted by KSCN to an AMPA-preferring form. It is likely, as suggested by Honoré and Drejer (28), that low affinity sites are converted to high affinity states, as evidenced by an observed increase in high

affinity binding site density (Table 2). However, some measure of noninterconvertibility must exist. If all the binding states were freely interconvertible on the time scale of the assay, a linear Scatchard plot would result (34, 35). In order to produce biphasic Scatchard plots, conversion between high and low affinity states must be slow, compared with the rate of ligand binding. This appears to be the case with [^{3}H]AMPA binding to homogenates (28). The conversion rate between high and low affinity states could be studied by investigating the time course of action of KSCN. For example, measuring increases in [^{3}H]AMPA binding as a function of the time at which KSCN is added to the incubation would yield direct information regarding the rates of conversion between conformations with different affinities. The present experiments also do not rule out the possibility that independent multiple [^{3}H]AMPA binding sites exist. However, the high regional correlation of [^{3}H]AMPA binding determined in the presence and absence of KSCN favor the interpretation that interconvertible conformations of a single receptor exist.

The functional significance of the existence of two affinity states of AMPA receptors is unclear. One possibility is that different affinity states of the receptor correspond to different types of agonist-gated channels opening. Kainate, AMPA, and quisqualate appear to open the same receptor (16, 17, 23–27), although types of responses induced by these agonists differ. Quisqualate and AMPA typically produce rapidly desensitizing responses, whereas kainate produces nondesensitizing responses. AMPA and quisqualate appear to act as partial agonists, whereas kainate is a full agonist (17, 36). One possibility is that one of the affinity states of the receptor corresponds to a nondesensitizing response, whereas the other affinity state corresponds to a desensitizing form. The present observation that BOAA, kainate, and glutamate (all of which are toxic to neurons in culture) have Hill numbers less than 1 raises the possibility that these agonists favor a conformation of the AMPA receptor that is neurotoxic. Quisqualate and AMPA, which are relatively less toxic, have Hill numbers closer to one.

Experiments with chemically lesioned and neurologically mutant mice indicate that low affinity [^{3}H]AMPA binding sites are localized on cerebellar Purkinje neurons (37). In mice lacking Purkinje cells, there is a marked decrease in low affinity [^{3}H]AMPA binding sites, indicating that, with respect to Purkinje cells, the low affinity AMPA receptors may be the physiologically relevant entities. The present studies indicate that low affinity [^{3}H]AMPA binding sites predominate in number. AMPA receptors are thought to be involved in rapid excitatory neurotransmission. In order to transmit discrete signals on a millisecond time scale, ligand-receptor association and dissociation must occur rapidly. Ligand association and dissociation rates tend to be faster at low affinity receptors. Thus, the possibility is raised that the low affinity forms of the AMPA receptor are the entities that participate in the bulk of rapid excitatory signalling within the mammalian central nervous system.

Recently, a family of AMPA-selective glutamate receptors has been cloned (38, 39). The functional properties of the receptor clones are being elucidated. There are at least four cloned sequences, designated GluR-A, GluR-B, GluR-C, and GluR-D. When expressed in a non-neuronal mammalian cell line, each of the cloned sequences confers responsiveness to AMPA, quisqualate, kainate, and glutamate (38). Although the native receptors almost certainly are composed of heteromers of subunits, each of the individual clones retain some functional capacity. In *Xenopus* oocytes injected with the clone for the GluR1 (GluR-A) subunit (39), quisqualate and AMPA act as partial agonists, whereas kainate, which binds with lower affinity than quisqualate or AMPA, produces full responses (40, 41). Thus, even at the level of individual receptor subunits, kainate, AMPA, and quisqualate share a common site of action, while producing disparate responses. *In vivo*, different stoichiometries of assembled subunits are likely to produce the different affinity states observed in the present study. Further, each of the AMPA receptor gene sequences can undergo alternate splicing to produce one of two different splice products, designated "flip" and "flop" (42). Flip and flop versions of the same DNA sequence impart different pharmacologic and kinetic properties to AMPA-induced but not kainate-induced currents. With at least four AMPA receptor genes found thus far, each of which can be processed into either a flip or a flop form, there exist numerous bases for the observed heterogeneity of [^{3}H]AMPA binding affinities.

These results add to the evidence that the ionotropic quisqualate/AMPA receptor has a complex structure (17, 29, 43–45). Binding sites for [^{3}H]AMPA exist in at least two affinity states. Both affinity states are influenced by thiocyanate ions. These affinity states may account for the complex binding behavior observed for the ionotropic quisqualate/AMPA ligands. The molecular substrate for multiple affinity states may ultimately be found to depend on the relative expression of different AMPA receptor genes, stoichiometry of assembled subunits, or alternate splicing of gene products into flip or flop forms. The exact relationship between expressed gene products and observed [^{3}H]AMPA binding affinities remains unclear at this point, but it is not surprising that the main excitatory neurotransmitter receptors in the mammalian central nervous system have multiple sites of potential regulation and diversity.

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