Effects of acromelic acid A on the binding of [³H]-kainic acid and [³H]-AMPA to rat brain synaptic plasma membranes

¹Adam L. Smith & R.A. Jeffrey McIlhinney

MRC Anatomical Neuropharmacology Unit, Mansfield Road, Oxford OX1 3TH

1 The ability of acromelic acid A to inhibit [³H]-kainic acid and [³H]-(RS)- α -amino-3-hydroxy-5-methyloxazole-4-propionic acid ([³H]-AMPA) binding to rat brain synaptic plasma membranes was investigated by equilibrium radioligand binding assay.

2 Kinetic analysis of [³H]-kainic acid binding demonstrated the existence of two kainate binding sites in this tissue preparation and yielded equilibrium dissociation constants for [³H]-kainic acid of $K_D = 0.4$ nM and $K_D = 20.8$ nM.

3 Kainic acid and domoic acid both appeared to displace [³H]-kainic acid from a single binding site with equilibrium binding constants of $K_D = 19.4$ nM and $K_i = 14.5$ nM respectively. Acromelic acid A exhibited a biphasic inhibition of [³H]-kainic acid binding to synaptic membranes with binding affinities of $K_i = 15.1$ nM and $K_i = 1.49$ μ M.

4 In the absence of chaotropic ions, the order of potency of inhibition of [³H]-AMPA binding was acromelic acid A ($K_i = 26$ nM) > AMPA ($K_D = 184$ nM) > domoic acid ($K_i = 499$ nM).

5 The inclusion of 100 mM thiocyanate ion in the [³H]-AMPA binding assay resulted in a change in the order of potency to: AMPA ($K_D = 160$ nM) > acromelic acid A ($K_i = 289$ nM) > domoic acid ($K_i = 9.02$ μ M).

6 These results show that acromelic acid A distinguishes two kainate binding sites in rat brain synaptic plasma membranes and in addition, that in the absence of chaotropic ions, acromelic acid A is the most potent displacer of [³H]-AMPA binding yet described.

Keywords: Kainate; AMPA; thiocyanate; excitatory amino acid; acromelic acid A; domoic acid

Introduction

Excitatory amino acids are thought to be the major excitatory transmitters in mammalian brain (Cotman *et al.*, 1987) and have been shown by electrophysiological and pharmacological means to act at least four classes of glutamate receptor termed the N-methyl-D-aspartate (NMDA), kainate, α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), and metabotropic quisqualate receptors (Watkins *et al.*, 1990). Of all the agonists for these receptors, the most potent are the neurotoxins which are thought to act specifically at the kainate receptor and are used in neurobiology as cytotoxins (McGeer *et al.*, 1978). Two of these agonists, kainic acid and its more potent analogue domoic acid, have been known for some time but recently another kainic acid analogue, acromelic acid, was extracted from a poisonous mushroom (Konno *et al.*, 1983). Natural supplies of the two isomers of this compound, acromelic acids A and B, were extremely limited but syntheses of acromelic acid A (Konno *et al.*, 1986; Baldwin & Li, 1988) have enabled its electrophysiological characterization in the isolated spinal cord of the rat revealing it to be the most potent excitatory amino acid yet described (Ishida & Shinozaki, 1988).

To date, there has been only limited investigation of the pharmacology of domoic acid and acromelic acid A by radioligand binding studies. Domoic acid has been reported to bind with high affinity ($K_i = 13$ nM) to a kainate binding site in rat cerebellar membranes (Slevin *et al.*, 1983) and the only study on acromelic acid A was conducted on synaptic membranes from frog spinal cord where it recognized a single kainate binding site with an inhibition constant of $IC_{50} = 3$ nM (Maruyama & Takeda, 1989). Since both acromelic acid A and domoic acid are potentially of some importance as affinity chromatography ligands for the purification of the kainate

receptor and as tools for the selective lesioning of brain, the binding characteristics of these compounds to rat brain synaptic membranes have been investigated. The results show that acromelic acid A binds to two different rat brain kainate binding sites and that both acromelic acid A and domoic acid have high affinity for the AMPA binding site in the rat brain.

Methods

Membrane preparation

The method was essentially that of Jones & Matus (1974). Female Wistar rats (200–250 g) were stunned and killed by decapitation and the whole brains rapidly removed and stored on ice. The following isolation was performed at 4°C unless otherwise stated. The tissue was homogenized in 9 volumes of 0.32 M sucrose in 10 mM Na-phosphate buffer (pH 7.1) in a glass homogenizer with a motorized teflon pestle. The homogenate was centrifuged (2000 g_{av} , 10 min), the pellet discarded and the supernatant spun (39,000 g_{av} , 20 min). The pellet was resuspended in distilled water with a hand-held homogenizer and the suspension incubated at 37°C for 30 min. After centrifugation (39,000 g_{av} , 20 min) the pellet was resuspended in 0.32 M sucrose and overlaid upon 0.8 M sucrose above 1.2 M sucrose for density gradient centrifugation. Centrifugation (60,000 g_{av} , 120 min) yielded a layer of synaptic plasma membrane at the interface of the 1.2 M and 0.8 M sucrose layers which was then washed three times by suspension in 50 mM Tris-acetate buffer (pH 7.0) and centrifugation (39,000 g_{av} , 20 min). After resuspension in the minimum volume of 50 mM Tris-acetate the membrane suspension was rapidly frozen in liquid nitrogen and stored at –70°C for up to two months before use. After thawing, the membranes were washed three times by resuspension in 50 mM Tris-acetate (pH 7.0) and centrifugation (39,000 g_{av} , 20 min). Finally the pellet was

¹ Author for correspondence at: University Laboratory of Physiology, Parks Road, Oxford OX1 3PT.

resuspended in 50 mM Tris-acetate (pH 7.0) to give a protein concentration of 2.5–3.5 mg ml⁻¹.

Protein determination

Protein determinations were performed by BCA protein assay (Pierce Chemical Corporation, Rockford, Illinois, U.S.A.), based on the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Binding experiments

Membrane suspensions (0.25–0.35 mg protein) were incubated in triplicate with [³H]-kainic acid (5 nM) or [³H]-AMPA (15 nM) in the presence or absence of competitive ligands. The incubations were conducted in Tris-acetate (pH 7.0) in a final volume of 0.2 ml for 30 min at 4°C. Bound radioactivity was separated from free radioactivity either by rapid filtration through Whatman GF/C glass fibre filters followed by 2 × 2 ml washes with ice-cold distilled water, or by centrifugation (12,000 *g*_{av}, 3 min) followed by superficial rinsing of the pellets twice with ice-cold distilled water (0.2 ml). The pellets were dissolved in SDS (1%, 1 ml) and the radioactivity in the solutions, or that retained by the filters in the case of filtration assays, was determined by liquid scintillation spectrometry after equilibration (3 h) in Lisciscint (National Diagnostic). Specific [³H]-kainic acid binding (approximately 8000 d.p.m. mg⁻¹) or [³H]-AMPA binding (approximately 1700 d.p.m. mg⁻¹) was obtained by subtracting from the total bound radioactivity the amount not displaced by an excess (0.1 mM) of the appropriate unlabelled ligand: kainic acid or AMPA. Both filtration and centrifugation procedures gave the same results, although non-specific binding values obtained by the centrifugation method were typically 40% of the total, compared to 10% non-specific binding found with the filtration method. In some [³H]-AMPA binding experiments sodium thiocyanate was added to the incubation to give a final concentration of 100 mM and this caused the specific binding to rise to approximately 6000 d.p.m. mg⁻¹.

Kinetic analysis of binding

[³H]-kainic acid (7.5 nM) was incubated with membrane suspensions as described above and association rates were determined by termination of the assay either by rapid filtration or centrifugation. Dissociation rates were obtained by adding 0.1 ml of 0.3 mM kainic acid to membrane suspensions that had been incubated with 7.5 nM [³H]-kainic acid for 30 min and the incubations were terminated either by filtration or centrifugation.

Data analysis

Data from the equilibrium binding assays and kinetic experiments were analysed by the non-linear curve fitting programmes Ligand (Munson & Rodbard, 1980) and Kinetic respectively. These programmes form part of the package Kinetic, EBDA, Ligand, Lowry (Biosoft, Cambridge).

Materials

Samples of synthetic (3S,4S,5S)-5-[5-carboxy-4-(carboxymethyl)-3-pyrrolidinyl]-1,6-dihydro-6-oxo-pyridine-carboxylic acid (acromelic acid A) were obtained as gifts from Prof. J.E. Baldwin and Prof. H. Shinozaki. [Vinylidene-³H]-kainic acid (specific activity 58.0 Ci mol⁻¹) and [³H]-(RS)- α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid ([³H]-AMPA) (specific activity 29.2 Ci mmol⁻¹) were obtained from New England Nuclear (Boston, MA, U.S.A.). (RS)- α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) was purchased from Tocris Neuramin (Buckhurst Hill, Essex). All

other compounds were obtained from Sigma (St. Louis, MO, U.S.A.).

Results

A detailed kinetic analysis of [³H]-kainic acid binding to synaptic plasma membranes (SPMs) was conducted. The dissociation curve is as shown in Figure 1(a) and computer-assisted fitting revealed a single dissociation rate constant (k_{-1}) of 0.00136 ± 0.00052 s⁻¹ (s.e.mean, $n = 3$). In contrast, the association curve (Figure 1b) appears biphasic with observed association rates (k_{OBS}) of 0.0245 ± 0.0083 s⁻¹ and 0.00185 ± 0.0008 s⁻¹ ($n = 3$) at two sites occurring in the ratio $28 \pm 9\%:72 \pm 9\%$ ($n = 3$) respectively. From these data, association rates (K_{+1}) can be calculated using the relationship $K_{+1} = (K_{\text{OBS}} - K_{-1})/L$ where L represents the radioligand concentration (7.5 nM). Thus K_d values for the two sites of 0.44 nM and 20.8 nM were derived from the relationship $K_D = K_{-1}/K_{+1}$.

However, equilibrium binding data obtained on the binding of [³H]-kainic acid to SPMs, using a radioligand concentration of 5 nM, demonstrated a single population of kainate binding sites with $K_D = 19.4 \pm 1.7$ nM ($n = 13$) and $B_{\text{max}} = 288 \pm 42$ fmol mg⁻¹ ($n = 13$) (see also Figure 2). The kainic acid analogue domoic acid also bound to a single site with $K_i = 14.5 \pm 0.7$ nM (Hill slope = 0.86 ± 0.06) ($n = 3$) (Figure 2).

Figure 1 (a) [³H]-kainic acid dissociation from and (b) [³H]-kainic acid association to rat brain synaptic plasma membranes. The insets display linear transformations of the data, which are from single experiments, each representative of three separate experiments.

Figure 2 Inhibition of [^3H]-kainic acid binding to rat brain synaptic membranes by kainic acid ($\square$), domoic acid ($\circ$) and acromelic acid A ($\triangle$). The data shown are from single experiments representative of at least three separate experiments, each performed in triplicate.

However when acromelic acid A was used to inhibit the binding of [^3H]-kainic acid the shallow binding curve obtained (Hill slope = 0.61 ± 0.02) was biphasic (Figure 2) showing that acromelic acid A recognizes two kainate binding sites with K_i s of $15.1 \pm 4.6 \text{ nM}$ and $1.49 \pm 0.20 \mu\text{M}$ ($n = 5$) representing $28.6 \pm 6\%$ and $72 \pm 6\%$ ($n = 5$) of the total kainate binding sites respectively.

The ability of acromelic acid A and domoic acid to displace [^3H]-AMPA from SPMs was also investigated. In the absence of chaotropic ions, AMPA bound to a single site in SPMs with a $K_D = 184.5 \pm 21 \text{ nM}$ ($n = 7$) and $B_{\text{max}} = 265 \pm 32 \text{ fmol mg}^{-1}$ ($n = 7$). Under the same conditions, acromelic acid A displaced [^3H]-AMPA with a $K_i = 15.6 \pm 5.6 \text{ nM}$ ($n = 4$) (Figure 3). [^3H]-AMPA binding to SPMs was inhibited by domoic acid with a $K_i = 499 \pm 64 \text{ nM}$ ($n = 3$) (Figure 3) and by kainic acid with a $K_i = 11.5 \pm 2.8 \mu\text{M}$ ($n = 4$) (data not shown).

Since [^3H]-AMPA binding assays are normally performed in the presence of chaotropic ions to enhance binding (Young & Fagg, 1990) the effect of the inclusion of $100 \mu\text{M}$ potassium thiocyanate was determined. This caused no change in the affinity of AMPA for the [^3H]-AMPA binding site, $K_D = 160.5 \pm 28 \text{ nM}$ ($n = 7$), but caused the number of sites to rise dramatically to $B_{\text{max}} = 995 \pm 130 \text{ fmol mg}^{-1}$ ($n = 7$). However, the addition of 100 mM potassium thiocyanate also

Figure 3 Inhibition of [^3H]-AMPA binding to rat brain synaptic plasma membranes by domoic acid (squares) and acromelic acid A (circles) in the presence (open symbols) and absence (closed symbols) of 100 mM potassium thiocyanate. The data are from single experiments, representative of at least three separate experiments.

had a profound effect on the binding of acromelic acid A and domoic acid to the [^3H]-AMPA binding site. The affinity of acromelic acid A fell to $K_i = 289 \pm 34 \text{ nM}$ ($n = 3$) and that of domoic acid to $K_i = 9.02 \pm 1.07 \mu\text{M}$ ($n = 3$) (Figure 3). The affinity of kainic acid for the [^3H]-AMPA binding site appeared to be unaffected by the inclusion of thiocyanate.

Discussion

The results of our experiments demonstrate that acromelic acid A exhibits a complex pattern of binding to rat brain synaptic plasma membrane (SPMs). Furthermore, the inclusion of chaotropic ions in [^3H]-AMPA binding assays can drastically alter the binding affinities of certain glutamate receptor agonists.

A kinetic analysis of [^3H]-kainic acid binding to SPMs revealed the presence of a high affinity site with $K_D = 0.44 \text{ nM}$ and a lower affinity site of $K_D = 20.8 \text{ nM}$ in the ratio of 28%:72% respectively. Previous studies on the binding of kainic acid to rat brain have shown the existence of two binding sites in rat brain membrane preparations (London & Coyle, 1979; Honoré *et al.*, 1986) but these were of lower affinity than the sites found in this study. The discrepancy might be accounted for by the fact that an enriched synaptosomal membrane preparation was used in our binding studies, rather than the crude P2 membrane preparation used elsewhere. However, Scatchard analysis of [^3H]-kainic acid equilibrium binding data indicated that kainic acid recognized only a single binding site in SPMs and it was only when acromelic acid A was used to displace [^3H]-kainic acid from synaptosomal membranes that a biphasic displacement curve was obtained and the presence of two kainate binding sites, with differing affinities for acromelic acid A, was confirmed. The proportion of high and low affinity kainate binding sites recognized by acromelic acid A matches that found by kinetic analysis of [^3H]-kainic acid binding while the higher of the kinetically-derived K_D s is almost the same as that derived from the equilibrium binding assays.

It has been suggested that acromelic acid might exert its depolarizing action in immature rat spinal cord mainly via AMPA receptors (Watkins *et al.*, 1990) and so the ability of this compound and other 'kainate agonists' to displace specifically bound [^3H]-AMPA was investigated. The finding that in the absence of thiocyanate, acromelic acid A bound to the AMPA binding site with a dissociation constant of $K_i = 15.6 \text{ nM}$ means that acromelic acid A has a higher affinity for this site than any compound yet described, more than ten times higher than AMPA itself. Domoic acid, with a dissociation constant of $K_i = 499 \text{ nM}$, also exhibited a surprisingly high affinity for the AMPA binding site for a compound which has hitherto been thought to be a pure kainate receptor agonist. Domoic acid has also been reported to display high affinity for an AMPA binding site recently solubilized from frog brain (Henley *et al.*, 1989) but kainic acid had an even higher affinity for this site. Kainic acid itself exhibited only micromolar affinity for the AMPA binding site, but it has been pointed out (Young & Fagg, 1990) that micromolar concentrations of kainic acid are generally required to elicit an electrophysiological response, in marked contrast to the low nanomolar dissociation constant kainic acid exhibits at the kainate binding site. Indeed the electrophysiological potencies of acromelic acid A, domoic acid and kainic acid in rat spinal cord (Ishida & Shinozaki, 1988) are much more in keeping with their relative affinities for the AMPA binding site than for the kainate binding site. The recent evidence that kainic acid is able to evoke responses from functionally expressed AMPA receptors derived from cloned DNAs (Keinanen *et al.*, 1990) supports the idea that AMPA receptors are capable of mediating the effects of kainic acid. While at least one AMPA binding site has been isolated which does not recognize kainic acid (Wentholt *et al.*, 1990) it seems possible that all these

compounds with a high affinity for the kainate binding site may sometimes elicit their electrophysiological responses through the AMPA receptor. The inclusion of thiocyanate ion in [^3H]-AMPA binding assays is now commonplace and in a recent review the practice was described as being essential (Young & Fagg, 1990). Although in this study the assay counts obtained in the absence of thiocyanate were quite adequate for routine measurements, we have found that the presence of thiocyanate ion enhances the specific binding of [^3H]-AMPA by more than three times, apparently by unmasking occluded AMPA binding sites as previously suggested (Olsen *et al.*, 1987). However, the reduction in the affinity of acromelic acid A and domoic acid for the AMPA binding site that was observed in this study due to the presence of 100 mM thiocyanate ion was eighteen fold. It is worth noting that electrophysiological studies on AMPA receptors are generally conducted in the absence of thiocyanate, in marked contrast to membrane binding studies on the [^3H]-AMPA binding site.

The finding that acromelic acid A and domoic acid exhibit sub-micromolar affinities for both the kainate and AMPA binding sites would suggest that affinity columns based on these compounds could be used to purify both sites. In this context it is interesting to note that in a recent study the use of a domoic acid affinity resin to purify kainate binding sites from *Xenopus* brain did indeed result in the co-purification of both the kainate and the AMPA binding sites (Ambrosini *et*

al., 1990). Since they were purified in a 1:1 ratio it is probable that in this tissue the kainate and AMPA binding sites are present on a single protein. The affinity-purified kainate binding site from frog (Wada *et al.*, 1989) and chick brain (Gregor *et al.*, 1989) is reported to be a protein of around $M_r = 48$ kD, but that cloned from rat brain has a molecular weight of 100 kD (Hollmann *et al.*, 1989). Thus if kainate and AMPA binding sites are present on the same protein in non-mammalian brains this may not be the case in mammalian brain. The use of affinity columns based on acromelic acid A, domoic acid and other kainate agonists such as those recently described (Smith & McIlhinney, 1990) may help to resolve the nature of the kainate and AMPA binding sites in rat brain.

In conclusion we have shown that acromelic acid A is a high affinity ligand for both the kainate and AMPA binding sites in rat brain SPMs. The existence of two kainate binding sites has been demonstrated and it has been shown that acromelic acid A exhibits markedly different affinities for the two sites. Finally we offer a caveat to those conducting [^3H]-AMPA binding assays that the inclusion of thiocyanate ion can significantly affect the binding affinities of some compounds and may therefore result in a misinterpretation of the rank order of potency obtained in binding studies.

We would like to thank Prof. J.E. Baldwin and Prof. H. Shinozaki for their very generous gifts of acromelic acid A and Prof. J.C. Watkins for his advice and encouragement.

References

- AMBROSINI, A., HENLEY, J.H. & BARNARD, E.A. (1990). Kainate and quisqualate binding sites are co-purified from *Xenopus* central nervous system. *Biochem. Soc. Trans.*, **18**, 401–402.
- BALDWIN, J.E. & LI, C.-S. (1988). Enantiospecific synthesis of acromelic acid A via a cobalt-mediated cyclisation reaction. *J. Chem. Soc., Chem. Commun.*, 261–263.
- COTMAN, C.W., MONAGHAN, D.T., OTTERSON, O.P. & STORM-MATHISEN, J. (1987). Anatomical organization of excitatory amino acid receptors and their pathways. *Trends Neurosci.*, **10**, 273–280.
- GREGOR, P., MANO, I., MAOZ, I., MCKEOWN, M. & TEICHBERG, V.I. (1989). Molecular structure of the chick cerebellar kainate-binding subunit of a putative glutamate receptor. *Nature*, **342**, 689–692.
- HENLEY, J.M., AMBROSINI, A., KROGSGAARD-LARSEN, P. & BARNARD, E.A. (1989). Evidence for a single glutamate receptor of the ionotropic kainate/quisqualate type. *New Biol.*, **1**, 153–158.
- HOLLMANN, M., O'SHEA-GREENFIELD, A., ROGERS, S.W. & HEINEMANN, S. (1989). Cloning by functional expression of a member of the glutamate receptor family. *Nature*, **342**, 643–648.
- HONORÉ, T., DREJER, J. & NIELSEN, M. (1986). Calcium discriminates two [^3H]kainate binding sites with different molecular target sizes in rat cortex. *Neurosci. Lett.*, **65**, 47–52.
- ISHIDA, M. & SHINOZAKI, H. (1988). Acromelic acid is a much more potent excitant than kainic acid or domoic acid in the isolated rat spinal cord. *Brain Res.*, **474**, 386–389.
- JONES, D.H. & MATUS, A.I. (1974). Isolation of synaptic plasma membrane from brain by combined flotation-sedimentation density gradient centrifugation. *Biochim. Biophys. Acta*, **356**, 276–287.
- KEINANEN, K., WISDEN, W., SOMMER, B., WERNER, P., HERB, A., VERDOORN, T.A., SAKMANN, B. & SEEBURG, P.H. (1990). A family of AMPA-selective glutamate receptors. *Science*, **249**, 556–560.
- KONNO, K., SHIRAHAMA, H. & MATSUMOTO, T. (1983). Isolation and structure of acromelic acid A and B. New kainoids of *Clitocybe acromelalga*. *Tet. Lett.*, **24**, 939–942.
- KONNO, K., HASHIMOTO, K., OHFUNE, Y., SHIRAHAMA, H. & MATSUMOTO, T. (1986). Synthesis of acromelic acid A, a toxic principle of *clitocybe acromelalga*. *Tet. Lett.*, **27**, 607–610.
- LONDON, E.D. & COYLE, J.T. (1979). Specific binding of [^3H]kainic acid to receptor sites in rat brain. *Mol. Pharmacol.*, **15**, 492–505.
- LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.
- MCGEER, E.G., OLNEY, J.W. & MCGEER, P.L. (1978). *Kainic Acid as a Tool in Neurobiology*. New York: Raven Press.
- MARUYAMA, M. & TAKEDA, K. (1989). Effects of acromelic acid A on the binding of [^3H]glutamic acid and [^3H]kainic acid to synaptic membranes and on the depolarization at the frog spinal cord. *Brain Res.*, **504**, 328–331.
- MUNSON, P.J. & RODBARD, D. (1980). LIGAND: a versatile computerized approach for the characterization of ligand binding systems. *Anal. Biochem.*, **107**, 220–239.
- OLSEN, R.W., SZAMRAJ, O. & HOUSER, C.R. (1987). [^3H]AMPA binding to glutamate receptor subpopulations in rat brain. *Brain Res.*, **402**, 243–254.
- SLEVIN, J.T., COLLINS, J.F. & COYLE, J.T. (1983). Analogue interactions with the brain receptor labeled by [^3H]kainic acid. *Brain Res.*, **265**, 169–172.
- SMITH, A.L. & McILHINNEY, R.A.J. (1990). Synthesis and characterization of two novel agonists for the kainate receptor. *Br. J. Pharmacol.*, **100**, 386P.
- WADA, K., DESCHESNE, C.J., SHIMASAKI, S., KING, R.G., KUSANO, K., BUONANNO, A., HAMPSON, D.R., BANNER, C., WENTHOLD, R.J. & NAKATANI, Y. (1989). Sequence and expression of a frog brain complementary DNA encoding a kainate-binding protein. *Nature*, **342**, 684–689.
- WATKINS, J.C., KROGSGAARD-LARSEN, P. & HONORÉ, T. (1990). Structure-activity relationships in the development of excitatory amino acid receptor agonists and competitive antagonists. *Trends Pharmacol. Sci.*, **11**, 25–33.
- WENTHOLD, R.J., HUNTER, C., WADA, K. & DECHESNE, C.J. (1990). Antibodies to a c-terminal peptide of the rat brain glutamate receptor subunit, GluR-A, recognize a subpopulation of AMPA binding sites but not kainate sites. *FEBS Lett.*, **276**, 147–150.
- YOUNG, A.B. & FAGG, G.E. (1990). Excitatory amino acid receptors in the brain: membrane binding and autoradiographic approaches. *Trends Pharmacol. Sci.*, **11**, 126–133.

(Received June 7, 1991
Revised September 11, 1991
Accepted September 13, 1991)