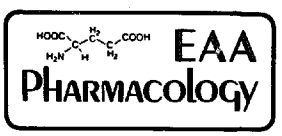
Regardless of the long-term usefulness of such models, over the past few years a variety of putative sleep-promoting factors have been described and it is clear that they are linked to each other. These advances should allow the design of more effective and safer somnogenic agents.

References

1 Toth, L. A. and Krueger, J. M. (1988) *Infect. Immun.* 56, 1785–1791
2 Johannsen, L. *et al. Am. J. Physiol.* (in press)
3 Pappenheimer, J. R., Koski, G., Fencl, V., Karnovsky, M. L. and Krueger, J. M. (1975) *J. Neurophysiol.* 38, 1299–1311
4 Krueger, J. M., Pappenheimer, J. R. and Karnovsky, M. L. (1982) *Proc. Natl Acad. Sci. USA* 79, 6102–6106
5 Yamamoto, K., Miwa, T., Ueno, R. and Hayaishi, O. (1988) *Biochem. Biophys. Res. Commun.* 156, 882–888
6 Sapolsky, R., Rivier, C., Yomamoto, G., Plotsky, P. and Vale, W. (1987) *Science* 238, 522–524
7 Krueger, J. M. (1985) *Trends Pharmacol. Sci.* 6, 218–221
8 Johannsen, L. *et al.* (1989) *Infect. Immun.* 57, 2726–2739
9 Chedid, C. (1983) *Prog. Immunol.* 5, 1349–1358
10 Krueger, J. M., Kubillus, S., Shoham, S. and Davenne, D. (1986) *Am. J. Physiol.* 251, R591–R597
11 Cady, A. B., Kotani, S., Shiba, T., Kusumoto, S. and Krueger, J. M. (1989) *Infect. Immun.* 57, 396–403
12 Munford, R. S. and Hall, C. L. (1986) *Science* 234, 203–205
13 Carter, W. A. and DeClecq, E. (1974) *Science* 186, 1172–1178
14 Krueger, J. M. *et al.* (1988) *Am. J. Physiol.* 255, R748–R755
15 Majde, J. A., Cady, A. B., Brown, R. K., Jones, M. W. and Krueger, J. M. (1989) *Cytokine* 1, 112
16 Krueger, J. M., Walter, J., Dinarello, C. A., Wolff, S. M. and Chedid, L. (1984) *Am. J. Physiol.* 246, R994–R999
17 Krueger, J. M. and Karnovsky, M. L. (1987) *Ann. NY Acad. Sci.* 496, 510–516
18 Opp, M., Obal, F., Jr, Cady, A. B., Johannsen, L. and Krueger, J. M. (1989) *Physiol. Behav.* 45, 1069–1072
19 Lumkin, M. D. (1987) *Science* 238, 452–454
20 Hadden, J. W., Masek, K. and Nistico, G., eds (1989) in *Interactions Among Central Nervous System, Neuroendocrine and Immune Systems*, Pythagora Press
21 Moldofsky, H., Lue, F. A., Eisen, J., Keystone, E. and Gorczynski, R. M. (1986) *Psychosomatic Med.* 48, 309–318
22 Lue, F. A. *et al.* (1988) *Int. J. Neurosci.* 42, 179–183
23 Moldofsky, H., Lue, F. A., Davidson, J. and Gorczynski, R. (1989) *FASEB J.* 3, 1972–1977
24 Fontana, A., Kristensen, F., Dubs, R., Gemsa, D. and Weber, E. (1982) *J. Immunol.* 129, 2413–2419
25 Breder, C. D., Dinarello, C. A. and Saper, C. B. (1988) *Science* 240, 321–324
26 Farrar, W. L., Hill, J. M., Harel-Bellan, A. and Vinocour, M. (1987) *Immunol. Rev.* 100, 361–378
27 Krueger, J. M. *et al. Yale J. Biol. Med.* (in press)
28 Dinarello, C. A. (1984) *Rev. Infect. Dis.* 6, 51–95
29 Hayaishi, O. (1988) *J. Biol. Chem.* 263, 14593–14596
30 Opp, M., Obal, F., Jr and Krueger, J. M. (1989) *Am. J. Physiol.* 257, R528–R535
31 Opp, M. R., Obal, F., Jr and Krueger, J. M. (1989) *Am. J. Physiol.* 255, R914–R922
32 Obal, F., Jr *et al.* (1988) *Am. J. Physiol.* 255, R310–R316
33 Danguir, J. and Nicolaidis, S. (1984) *Brain Res.* 306, 97–103
34 Walter, J., Davenne, D., Shoham, S., Dinarello, C. A. and Krueger, J. M. (1986) *Am. J. Physiol.* 250, R96–R103

Dap: diaminopimelic acid

Excitatory amino acid receptors in the brain: membrane binding and receptor autoradiographic approaches



Anne B. Young and Graham E. Fagg

In last month's article in this series, Lodge and Johnson discussed the contribution of noncompetitive excitatory amino acid antagonists to understanding of these receptors. In this third article, Anne Young and Graham Fagg describe how radioligand binding experiments have helped to fuel the recent burst of progress in understanding excitatory amino acid receptors in the brain. New and selective radioligands have facilitated mapping the distributions of the major excitatory receptor subtypes in normal and diseased brain, examining allosteric interactions within the NMDA receptor, searching for novel therapeutic agents and determining drug mechanisms, and making first steps along the path to defining receptor structure at the molecular level.

Glutamate, aspartate and possibly other excitatory acidic amino acids are thought to be neurotransmitters at the majority of excitatory synapses in the vertebrate CNS. The synaptic responses elicited by excitatory amino acids are mediated by at least four (probably five) different receptor subtypes. Three of these receptors were defined in the mid to late 1970s by electrophysiological analyses of the actions of a large number of excitatory amino acid analogues. These were initially known as the *N*-methyl-D-aspartate (NMDA), quisqualate and kainate receptor subtypes, since these three exogenous agonists evoke pharmacologically distinct excitatory responses when applied to neurons in the CNS (Refs 1–5). The quisqualate receptor was renamed the AMPA receptor because AMPA has greater selectivity than quisqualate. In the early 1980s, the existence of a fourth receptor subtype was proposed on the basis of the potent antagonist properties of AP4 at subpopulations of identified brain and spinal cord excitatory synapses[3,6,7]. More recently a fifth receptor subtype (metabotropic) has been discovered that is linked to phosphoinositol (PI) metabolism[8,9].

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Radioligand binding methods: historical development

At about the time that early physiological investigations were suggesting the existence of multiple excitatory amino acid receptors, several groups were attempting to label these sites in isolated brain membranes using [^{3}H]glutamate as a radioligand[3]. In parallel with these studies were attempts to label subtypes of glutamate receptor using the selective ligands [^{3}H]kainate and

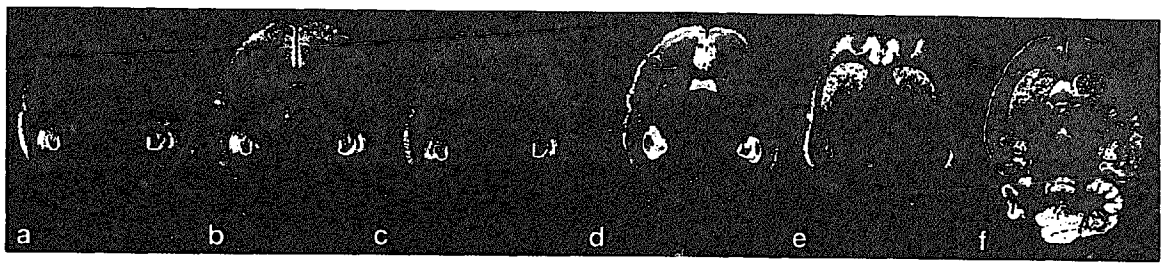
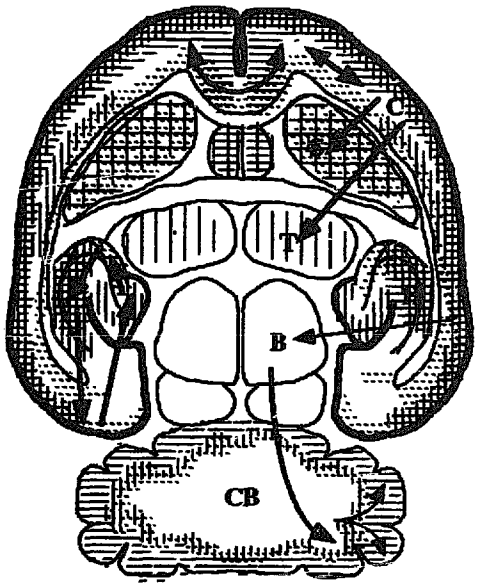


Fig. 1. **Above**: *Autoradiograms showing the distributions of excitatory amino acid receptor binding sites in horizontal sections of rat brain.* **a**: *Distribution of NMDA receptor recognition site as assessed using [^{3}H]glutamate* (see Ref. 20); **b**: *distribution of glycine modulatory site using [^{3}H]glycine [McDonald, J. W.* et al. *(1988)* Soc. Neurosci. Abstr. *14, 1022];* **c**: *distribution of PCP binding site using [^{3}H]MK801 [Sakurai, S. Y.* et al. *(1988)* Soc. Neurosci. Abstr. *14, 1022];* **d**: *distribution of AMPA receptor using [^{3}H]AMPA* (see Ref. 25); **e**: *distribution of high affinity kainate site using [^{3}H]kainate [Monaghan, D. T. and Cotman, C. W. (1982)* Brain Res. *252, 91–100];* **f**: *distribution of metabotropic receptor using [^{3}H]glutamate* (see Ref. 28).

Right: *Major excitatory amino acid transmitter pathways (→) and the distribution of excitatory amino acid receptor subtypes in rodent brain. The correspondence between pathway and receptor distributions is striking, with the exception that diencephalic, midbrain and spinal cord structures have relatively sparse receptor binding despite substantial excitatory amino acid inputs.* **C**, *cerebral cortex;* **S**, *striatum;* **T**, *thalamus;* **H**, *hippocampus;* **B**, *brainstem;* **CB**, *cerebellum.*



[^{3}H]AMPA (for review see Ref. 7). Unfortunately, differences between the sites labelled by the various ligands led to some uncertainty as to which ligand binding site was more physiologically relevant. At that time, receptor binding methodology had been employed successfully to label and to characterize several membrane receptors and was gaining popularity as a way of examining the molecular properties of receptors, without the influence of intracellular events.

In the case of the excitatory amino acids, however, such studies were plagued by their ubiquitous involvement in cellular metabolism (and the likely existence of numerous glutamate binding sites), and by the dearth of specific receptor radioligands. The identification of excitatory amino acid receptor subtypes using these methods therefore lagged significantly behind the electrophysiological advances.

Initial studies suggested that [^{3}H]glutamate did bind to sites in isolated brain membranes and that such binding could be inhibited by several amino acid analogues with neuroexcitatory or antagonist properties[3]. However, the pharmacological profile of these sites was unlike those of the receptors defined electrophysiologically. The sites did not appear to represent Na^+-coupled excitatory amino acid transport (Na^+ was excluded from the assay buffers), but neither were they affected significantly by selective receptor agonists or antagonists such as NMDA, AMPA, kainate or AP5. It was subsequently shown that most of this binding was dependent on the presence of Cl^- (Tris-Cl buffers were employed in early experiments), that it was eliminated by freezing and detergent treatment, and that it was absent from postsynaptic densities, where synaptic receptors are believed to be located[3,10]. Experiments conducted by several groups have now suggested that in fresh membrane preparations Cl^- and Ca^{2+}-dependent [^{3}H]-glutamate binding is primarily composed of Cl^--linked transport of the amino acid into resealed membrane vesicles[7].

Major steps forward in identifying excitatory amino acid receptors using radioligand binding techniques came in the years 1983–1985. Investigations involving both the membrane binding and receptor autoradiographic approaches succeeded in defining non-overlapping populations of [^{3}H]glutamate binding sites that exhibited high affinity for the agonists NMDA, AMPA and kainate; these sites had pharmacological profiles consistent with the hypothesis that they were the receptors characterized physiologically[10–12]. The autoradiographic approach additionally demonstrated that their receptor subtypes were differentially located within the brain. Moreover, their regional distributions were in good agreement with known target areas of excitatory amino acid pathways and with their pathophysiological functions as predicted from electrophysiological and neurotoxicological studies. It thus became possible to use radioligand binding assays

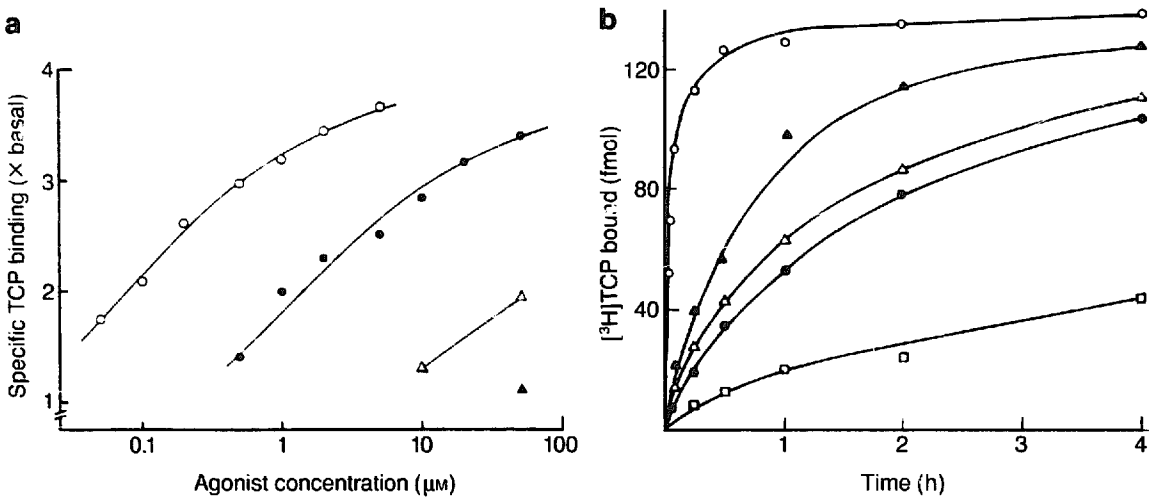


Fig. 2. Effects of NMDA receptor agonists and glycine on radioligand binding to the PCP-type site. **a**: *Glutamate (○), NMDA (●), quisqualate (△) and kainate (▲) increase [^{3}H]TCP binding to rat brain postsynaptic densities with potencies paralleling their affinities for the NMDA receptor recognition site.* (Taken, with permission, from Ref. 14.) **b**: *NMDA receptor agonists and the allosteric modulator glycine enhance the association rate of [^{3}H]TCP binding whereas the NMDA antagonist AP5 slows the association rate of ligand binding.* Glu *(▲), Glu + Gly (○), NMDA (△), Glu + Gly + AP5 (□), no agonist (●).* (Taken, with permission, from Ref. 16.)

with confidence to examine questions such as the detailed anatomical and subcellular localization of the receptors and their plasticity during development and in disease, and also to search rationally for novel therapeutic agents acting at excitatory amino acid receptors.

Recent years have seen the introduction of more selective radioligands for the transmitter recognition site on each receptor subtype. For the NMDA receptor complex, radioligands for modulatory sites have become available, and studies of allosteric mechanisms within the receptor complement the electrophysiological data derived from patch- and voltage-clamp experiments. Recent preliminary data also suggest that it may be feasible to use radioligand binding methods to study the metabotropic receptor linked to phosphoinositol metabolism.

The NMDA receptor: multiple interacting domains

The most well-characterized excitatory amino acid receptor subtype is the NMDA receptor. Antagonists for this receptor were described in the late 1970s, but it only became popular to examine its roles in CNS function when the potent and selective ω-phosphonic acid homologues AP5 and AP7 became available (see Ref. 1). Recent evidence using these and related antagonists indicates that the NMDA receptor serves as an input-sensitive amplifier of excitatory synaptic responses (i.e. a small input in the right circumstances can lead to a large response). There is also evidence that the NMDA receptor participates in CNS development and is involved in the pathophysiology of neurological disorders ranging from epilepsy to ischaemic brain damage and perhaps even to degenerative conditions such as Huntington's and Alzheimer's diseases[6,13].

Radioligand binding studies have shown that the NMDA receptor is localized subcellularly at the postsynaptic density (consistent with a synaptic, rather than an extrasynaptic function[11]), and is present in high density in the cerebral cortex, hippocampus, striatum, septum and amygdala[12,13]. Its location and electrophysiological properties suggest that it plays a critical role in learned behaviours and in synaptic plasticity. Its high density in the principal regions affected in degenerative conditions has also led to speculation that NMDA receptor mechanisms may underlie some forms of neuronal injury. Indeed, autoradiographic studies indicate a loss of NMDA receptors accompanying the neuronal loss that occurs in patients with Huntington's and Alzheimer's diseases.

In characterizing the molecular properties of the NMDA receptor, electrophysiological and radioligand binding studies have each played essential and complementary roles. For example, physiological investigations demonstrated that NMDA receptor agonists activate an ion channel that allows the passage of Ca^{2+} and Na^{+} into the neuron, and that this response is blocked in a voltage-dependent manner by Mg^{2+} and by dissociative anaesthetics such as PCP, ketamine and MK801 (Refs 2, 4 and 5). Radioligand binding experiments, however, provided the first evidence that the receptor recognition site and the PCP binding site were physically coupled.

Autoradiographic experiments showed a high correlation between the anatomical distributions of NMDA-sensitive [^{3}H]-glutamate and [^{3}H]TCP binding sites in the brain (Fig. 1). Moreover, although dissociative anaesthetics had no effect on the binding of [^{3}H]glutamate or [^{3}H]CPP, NMDA receptor agonists increased (Fig. 2), and antagonists decreased [^{3}H]TCP or [^{3}H]MK801 binding, suggesting that PCP and related substances bind to an activated state of the NMDA receptor

complex[7,14]. Such interactions also occurred in solubilized receptor preparations, thereby excluding the possibility that the PCP binding site was not part of the receptor[15].

More recently, the activating effect of NMDA receptor agonists on [^{3}H]TCP and [^{3}H]MK801 binding has been demonstrated to result from an increase in the association rate of the radioligands (Fig. 2); antagonists such as AP5 block the dissociation of prebound [^{3}H]TCP (Ref. 16). The radioligand binding data thus demonstrate that dissociative anaesthetics bind to a site within the NMDA receptor complex, that this site is maximally exposed when the receptor is in the activated (open) state, and that it is occluded in the resting (closed) state.

In addition to dissociative anaesthetics, recent investigations have unmasked a host of substances that alter the activity of the NMDA receptor (Fig. 3). These include Zn^{2+}, tricyclic antidepressants, polyamines, ifenprodil and the simple amino acid glycine (see also Lodge and Johnson, in last month's *TiPS*[2]). Of these, available data suggest that some (tricyclic antidepressants) are acting as weak channel blockers, while the precise function or mode of action of others remains unclear. The most well-characterized modulator of the NMDA receptor is glycine.

Using the patch-clamp technique, Johnson and Ascher[17] observed that glycine increases the frequency of NMDA receptor-induced channel opening in isolated membrane fragments; this action is distinct from the well-known strychnine-sensitive inhibitory action of glycine. This distinction was further emphasized by receptor autoradiographic investigations which showed that the distribution of [^{3}H]glycine binding sites in the CNS is quite unlike that of [^{3}H]-strychnine binding sites, but very similar to those of binding sites for NMDA and PCP-type radioligands (Fig. 1). Membrane binding experiments have shown that glycine does not influence NMDA-sensitive [^{3}H]glutamate binding, or [^{3}H]TCP or [^{3}H]MK801 binding competitively and therefore must exert its actions at a locus distinct from the transmitter binding site and the channel. Many of the interactions that have been observed between these sites are analogous to the allosterism described for the GABA–benzodiazepine–picrotoxin receptor complex[18,19]. The NMDA receptor may indeed be organizationally related to other members of the channel-linked receptor superfamily, such as the nicotinic cholinoceptors and $GABA_A$ receptors.

An issue of considerable interest is the existence of subtypes of NMDA receptor. Autoradiographic studies have suggested that, although NMDA, glycine and PCP binding sites are generally present in a constant ratio throughout the CNS, clear exceptions are apparent. Particularly striking discrepancies have been observed in the cerebellar granule cell layer, where the density of glycine and NMDA binding sites is relatively high but that of PCP binding sites is low[7,20]. Other areas of the brain exhibit definite but more subtle disparities.

Such variations may of course reflect regional differences in NMDA receptor subtypes; alternatively each binding site may exist in multiple affinity states which are regulated differently in different regions[5,7]. Indeed, several groups have postulated the existence of agonist- and antagonist-preferring forms of the NMDA receptor on the basis of dissimilar pharmacological profiles of [^{3}H]glutamate and [^{3}H]CPP binding; Monaghan *et al.*[21] have recently shown that these sites are differentially distributed and reciprocally regulated by glycine. However, even in the presence of glycine or a glycine antagonist, antagonists such as AP5 display regional variations in potency, suggesting that two distinct binding sites exist. One possibility therefore is that, as in the case of nicotinic cholinoceptors and

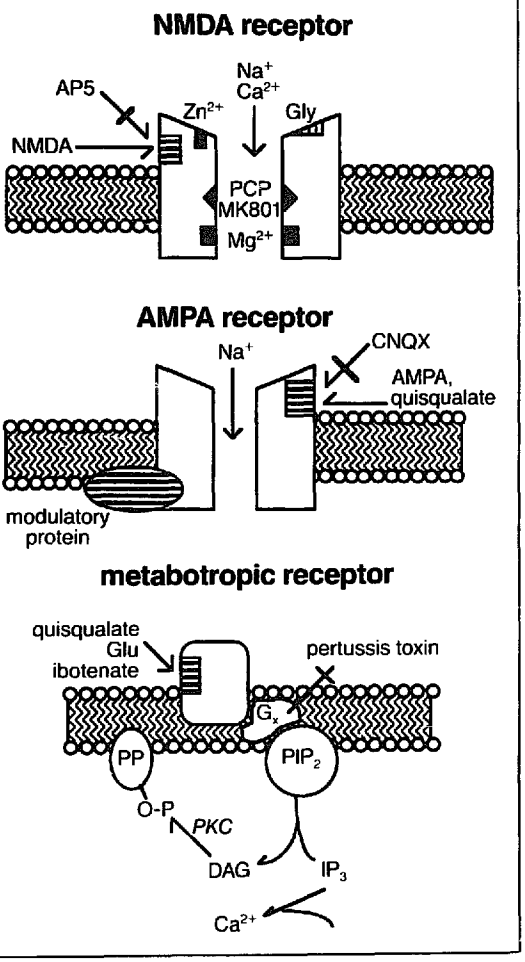


Fig. 3. Three excitatory amino acid receptor subtypes. The **NMDA receptor** *gates a cation channel that is permeable to Ca^{2+} and Na^+ and is gated by Mg^{2+} in a voltage-dependent fashion; K^+ is the counterion. The NMDA receptor channel is blocked by PCP and MK801 and the complex is regulated at two modulatory sites by glycine and Zn^{2+}; AP5 is an antagonist. The* **AMPA receptor** *gates cation conductances that underlie fast depolarizing responses at most excitatory synapses; K^+ is the counterion. The receptor appears to be influenced by a modulatory protein and chaotropic anions; AMPA and quisqualate are agonists and CNQX is an antagonist. The metabotropic receptor has a distinct but limited pharmacology and is likely to play an important role in long-term effects mediated by excitatory amino acids. Quisqualate, ibotenate and glutamate are agonists; the G protein (G_x) is sensitive to pertussis toxin.* **PKC**, *protein kinase C.*

$GABA_A$ receptors, multiple genes may exist for NMDA receptor subunits with similar but non-identical structures.

The AMPA receptor: a 'general-purpose' excitatory amino acid receptor

The AMPA receptor was originally termed the 'quisqualate' receptor. In retrospect this was unfortunate, since it is now clear that quisqualate is not a selective ligand. Although a highly potent neuroexcitant, acting in submicromolar concentrations at a receptor that is insensitive to NMDA receptor antagonists, quisqualate also exhibits high affinity for other excitatory amino acid receptors (the kainate and metabotropic receptors), transport sites (Cl^--dependent AP4-sensitive site), and an enzyme that degrades the dipeptide *N*-acetylaspartylglutamate (NAALADase) (see Ref. 22). High affinity quisqualate-sensitive [^{3}H]glutamate binding (K_i for quisqualate, 30–300 nM) appears to measure both the AMPA receptor and the metabotropic receptor. The discovery of AMPA has helped to characterize the fast-acting 'quisqualate' receptor and to distinguish it from other sites at which quisqualate acts. Thus the currently preferred term is the 'AMPA receptor'[1,22,23]. No specific antagonists for this receptor have been described, although two analogues of kynurenic acid, CNQX and DNQX, show some selectivity and relatively high affinity for [^{3}H]AMPA binding sites and block AMPA- and quisqualate-evoked excitation[1,23,24].

Receptor autoradiographic studies using [^{3}H]AMPA (or [^{3}H]-glutamate in conjunction with kainate and NMDA to prevent binding to other receptor types) show that AMPA receptors are localized primarily in telencephalic regions, with high levels in hippocampus, cortex, lateral septum, striatum and the molecular layer of cerebellum[7,25]. This distribution corresponds closely to that of NMDA receptors, and suggests that these two receptor subtypes may act in concert to activate the postsynaptic neuron. Electrophysiological findings that fast synaptic responses are often blocked by non-NMDA-receptor antagonists, and that NMDA receptor responses are apparent only under certain circumstances (e.g. high-frequency activation), support this view[4,7]. Thus, AMPA receptors may be the 'general-purpose' depolarizing receptor at many excitatory synapses in the brain.

Molecular studies of the AMPA receptor have been pursued by Honoré and his colleagues. Target size analysis using the irradiation inactivation technique indicates that the [^{3}H]AMPA binding protein has a mass of 52 kDa, and that it may be associated with a modulatory protein of about 130 kDa (Ref. 26). The binding of [^{3}H]-AMPA is markedly enhanced by chaotropic ions (ions that change the hydration state of the membrane) such as thiocyanate, probably due to a shift to a high affinity binding state of the receptor[25,27].

The kainate receptor: a binding site searching for a function?

Although the first excitatory amino acid binding site to be labelled selectively using radioligands[3], the relationship of the [^{3}H]kainate binding site to the receptor mediating its neuroexcitatory actions remains unclear. Indeed, several investigators have recently suggested that the neurophysiological effects of kainate may be mediated via the AMPA receptor. Evidence in favour of this view includes physiological and radioligand binding observations. For example, [^{3}H]kainate binds to high and low affinity sites (K_d values in the low nanomolar range) in brain membranes, whereas low micromolar concentrations (at which kainate also interacts with the [^{3}H]AMPA binding site) are generally required to elicit an excitatory response[4,7]. Similarly, the antagonists CNQX and DNQX are some fivefold more potent as inhibitors of [^{3}H]AMPA binding than of [^{3}H]kainate binding, but equipotent as antagonists of kainate, AMPA and quisqualate-evoked increases in neuronal firing[23]. Hence, all three agonists may act at the same neuronal receptor to elicit excitatory responses.

Despite these observations, kainate does appear to induce at least some neuronal responses independent of the AMPA receptor. In cultured neurons, kainate and quisqualate activate channels with dissimilar conductance and desensitization properties[4,27]. Moreover, spinal C-fibre afferents are depolarized by kainate and other excitatory amino acid analogues with the same order of potency as found for high affinity [^{3}H]kainate binding sites in isolated brain membranes (and this is distinct from the pharmacological profile of AMPA receptors)[3,7]. Finally, in autoradiographic experiments, the regional distribution of high affinity [^{3}H]-kainate binding sites is unlike those of either AMPA or NMDA receptors (Fig. 1), but corresponds well to those brain locations (e.g. hippocampal area CA3, cortex, lateral septum) that are highly vulnerable to the neurotoxic actions of kainate[3,7]. Like the depolarizing effects of kainate on C-fibres, kainate neurotoxicity appears to be mediated by a receptor that displays the same agonist selectivity as the high affinity [^{3}H]kainate binding site, suggesting that this site is involved in the kainate-induced neurodegenerative changes[7].

The L-AP4 receptor: a function searching for a binding site?

In contrast to the NMDA, AMPA and kainate receptor subtypes, which were defined through the actions of exogenous excitatory amino acids, the L-AP4 receptor was discovered because L-AP4 was a potent antagonist of subpopulations of synaptically evoked excitatory responses[3,7]. Despite the clear physiological significance of this site, however, it has proved difficult to elucidate the precise mechanism or membrane binding site through which L-AP4 exerts its antagonist action. Electrophysiological studies demonstrated that, at the low micromolar concentrations at which it is pharmacologically active, L-AP4 does not block the excitatory responses evoked by NMDA, quisqualate or kainate; indeed, in the retina, L-AP4 appears to mimic the action of the natural transmitter (probably glutamate) at retinal ON-bipolar cells. Recent experiments indicate that L-AP4 may act presynaptically to inhibit synaptic responses[7].

A membrane binding site at which L-AP4 acts has not been identified. A Cl^--dependent [^{3}H]-

Radioligand binding assays for excitatory amino acid receptors

This Table is intended as a guide for the selection and implementation of radioligand binding assays in CNS membranes and tissue sections. For details of assay conditions, it is recommended that readers refer to the research articles cited (see Ref. 1 for a review). The authors will be pleased to answer queries from investigators interested in establishing excitatory amino acid receptor assays in their own laboratories.

The radioligand binding assays listed here are those that have stood the test of time and, given care, can be confidently employed to determine the properties of the receptors and subsites indicated. For almost all investigations, it is preferable to use the radioligand of highest affinity and selectivity; on this basis, the radioligands preferred currently are [^{3}H]CGP39653 for the NMDA receptor recognition site, [^{3}H]MK801 for the NMDA receptor channel site, [^{3}H]AMPA for the AMPA receptor recognition site and [^{3}H]kainate for the kainate receptor recognition site. [^{3}H]Glutamate is the only ^{3}H-labelled agonist of practical value for labelling the NMDA receptor recognition site (and the putative metabotropic site), and [^{3}H]CNQX the only antagonist for the AMPA receptor. [^{3}H]Glycine is currently the only radioligand available for examining the strychnine-insensitive modulatory site associated with the NMDA receptor.

The use of non-selective radioligands such as [^{3}H]glutamate and [^{3}H]glycine demands special attention to assay conditions, and it may be necessary to include high concentrations of other receptor ligands in the assay buffer to improve the selectivity of labelling. For example, inclusion of kainate and quisqualate have been employed to restrict the binding of [^{3}H]glutamate to NMDA receptors, and strychnine may be used to prevent the binding of [^{3}H]glycine to inhibitory glycine receptors. In addition, the ionic composition of the assay buffer may markedly influence the sites labelled; Na^+ may promote labelling of amino acid transport sites in brain membranes, and Cl^- ions augment the binding and transport of [^{3}H]glutamate at a number of sites unrelated to excitatory amino acid receptors. The safest approach to ensure selective labelling of receptor-associated sites is to use buffers (e.g. Tris-acetate, HEPES-KOH) that do not contain these ions. The addition of thiocyanate to the buffer is essential for assay of AMPA receptors.

Most of the radioligands for excitatory amino acid receptors are not of sufficiently high affinity to enable filtration assays to be employed, and centrifugation (membrane assays) or very rapid rinses (autoradiography) must be employed to prevent dissociation and loss of receptor-bound ligand. Filtration assays are feasible, however, when the radioligand is [^{3}H]CGP39653, [^{3}H]CGS19755, [^{3}H]AMPA, [^{3}H]MK801 or [^{3}H]kainate (high affinity site).

Reference

1 Foster, A. C. and Fagg, G. E. (1984) *Brain Res. Rev.* 7, 103–164

TABLE. Radioligands for analysing excitatory amino acid receptors

Ligand	Agonists	Antagonists	Comments	Ref.
NMDA receptor–channel complex				
NMDA binding site				
[^{3}H]Glutamate	Glu > Asp > NMDA = ibotenate	CGP37849 > CGS19755 = CPP > D-AP5	inclusion of quisqualate and kainate improves selectivity	a–e
[^{3}H]CGP39653*, [^{3}H]CGS19755, [^{3}H]CPP	same as above	same as above	filtration assays possible with [^{3}H]CGP39653	f–h
Glycine binding site				
[^{3}H]Glycine	Gly > DSer > DAla >> LVal = LSer-LAla >> DVal	7-Cl-Kyn > Kyn > HA966	–	i, j
Channel site				
[^{3}H]TCP, [^{3}H]MK801	MK801 > TCP > dexoxadrol > PCP >> (+)-SKF10047 > ketamine	–	ligands bind to activated state; Glu, Gly, Zn^{2+} and Mg^{2+} all affect rate of ligand association and dissociation	b,k,l
AMPA receptor				
[^{3}H]Glutamate	AMPA = quisqualate > Glu > kainate >>> ibotenate	CNQX = DNQX	must use AMPA or CNQX to define nonspecific binding; inclusion of NMDA improves selectivity of labelling	m
[^{3}H]AMPA	same as above	CNQX = DNQX	–	n,o
[^{3}H]CNQX	same as above	CNQX = DNQX	CNQX also binds to glycine site and possibly NMDA site	p
Kainate receptor				
[^{3}H]Kainate	domoate > kainate > quisqualate > Glu	CNQX = DNQX	–	q,r

Agonists and antagonists are listed in order of potency not selectivity. *Not yet commercially available. [a]Fagg, G. E. and Baud, J. (1988) in *Excitatory Amino Acids in Health and Disease* (Lodge, D., ed.), pp. 63–90, Wiley; [b]Maragos, W. F. *et al.* (1988) *J. Neurosci.* 8, 493–501; [c]Monaghan, D. T. and Cotman, C. W. (1986) *Proc. Natl Acad. Sci. USA* 83, 7532–7536; [d]Monahan, J. B. and Michel, J. (1987) *J. Neurochem.* 48, 1699–1708; [e]Monaghan, D. T. and Cotman, C. W. (1985) *J. Neurosci.* 5, 2909–2919; [f]Sills, M. A. *et al.* (1989) *Soc. Neurosci. Abstr.* 15, 1165; [g]Murphy, D. E. *et al.* (1988) *Br. J. Pharmacol.* 95, 932–938; [h]Murphy, D. E. *et al.* (1987) *J. Pharmacol. Exp. Ther.* 240, 778–784; [i]Kessler, M. *et al.* (1989) *J. Neurochem.* 52, 1319–1328; [j]Bristow, D. R. *et al.* (1986) *Eur. J. Pharmacol.* 126, 303–307; [k]Kloog, Y. *et al.* (1988) *Biochemistry* 27, 843–848; [l]Foster, A. C. and Wong, E. H. (1987) *Br. J. Pharmacol.* 91, 403–410; [m]Nielsen, E. O. *et al.* (1988) *Eur. J. Pharmacol.* 157, 197–203; [n]Murphy, E. D. *et al.* (1987) *Neurochem. Res.* 12, 775–782; [o]Honoré, T. and Drejer, J. (1988) *J. Neurochem.* 51, 457–461; [p]Honoré, T. *et al.* (1988) in *Frontiers in Excitatory Amino Acid Research* (Cavalheiro, E. A. *et al.*, eds), pp. 39–46, Liss; [q]Ferkany, J. *et al.* (1984) *Neurosci. Lett.* 44, 281–286; [r]Monaghan, D. T. and Cotman, C. W. (1982) *Brain Res.* 252, 91–100.

glutamate binding site, which (on the basis of the limited number of AP4 analogues initially available) appeared to represent the L-AP4 receptor, has been shown through more detailed pharmacological and biochemical analyses not to correspond to the synaptic receptor but rather to a Cl^--dependent transport system in isolated membranes[3,7].

The metabotropic receptor

Recent investigations by a number of groups indicate that, in addition to channel-linked receptors mediating fast depolarizing responses in CNS neurons, an excitatory amino acid receptor exists that is coupled to PI metabolism[7–9] (Fig. 3). This receptor has been identified through studies of PI turnover in brain slices and (following intracellular injection of rat brain mRNA) of Cl^- currents induced by 1,4,5-inositol trisphosphate (IP_3) in *Xenopus* oocytes[7,8]. Its pharmacological properties indicate that it is distinct from previously described receptors, in that quisqualate, ibotenate and glutamate are potent agonists and NMDA, AMPA and kainate are not. No competitive antagonists at this receptor have been described (although AP4 may be an indirect antagonist[9]). Its physiological significance also remains to be clarified, although it may involve the regulation of synaptic development and regrowth[8].

The availability of a radioligand binding assay for this receptor would greatly facilitate its further characterization and the development of potent and selective competitive antagonists. Preliminary data indicate that a subpopulation of quisqualate-sensitive, AMPA-insensitive [^{3}H]glutamate binding sites may represent the recognition site of this PI-linked receptor[28] (Fig. 1). The agonists have higher affinity for this binding site than they do for evoking the physiological responses. (Similar observations have been made for other transmitter binding sites.) Like the receptor mediating IP_3 formation, however, this binding site is characterized by its high affinity for quisqualate, glutamate, ibotenate and *trans*-ACPD, whereas aspartate, kainate and NMDA are weak or inactive.

□ □ □

Substantial progress has been made in recent years in defining the types and roles of excitatory amino acid receptors in the brain. Receptor binding approaches, coupled with new and selective radioligands, have defined the regional localizations of the NMDA, AMPA and kainate receptors in the brain, their alterations in disease, properties of their recognition sites and, in the case of the NMDA receptor complex, have helped elucidate the organization of and allosteric interactions between pharmacological domains. Autoradiographic investigations have suggested that AMPA and NMDA receptors may act in concert at many excitatory synapses and, on the basis of studies using different NMDA receptor radioligands, have indicated that subtypes of NMDA receptor may exist. Two additional receptors (the L-AP4 and metabotropic receptors), which have been identified in physiological experiments, have been little studied using radioligand binding methodology, although preliminary data indicate that it may be possible to label the metabotropic receptor.

Radioligand binding approaches can be expected to play a prominent role in further clarifying allosteric mechanisms within the NMDA receptor complex, in developing similar models of the AMPA and kainate receptors, and in isolating and characterizing the receptor molecules. Channel ligands such as [^{3}H]MK801 offer potential for expanding the range of questions normally asked using receptor binding methodology, in that their binding provides an indicator of receptor 'function' (channel opening). Recently, for example, [^{3}H]MK801 binding *in vivo* has been employed to monitor regions of high NMDA receptor activation in the ischaemic rat brain[29]. At the therapeutic level, receptor binding procedures will undoubtedly be at the forefront of new drug discovery. Significant recent advances in this direction include the identification of the first orally active competitive NMDA receptor antagonists (CGP37849 and CGP39653) which have potential value as anti-epileptic agents[30], of MK801 as a noncompetitive NMDA receptor antagonist to be applied to treatment of ischaemic brain injury[31], and of D-cycloserine, a partial agonist at the allosteric glycine site which improves learning performance in animals[32].

References

1 Watkins, J., Honoré, T. and Krogsgaard-Larsen, P. (1990) *Trends Pharmacol. Sci.* 11, 25–33
2 Lodge, D. and Johnson, K. M. (1990) *Trends Pharmacol. Sci.* 11, 81–86
3 Foster, A. C. and Fagg, G. E. (1984) *Brain Res. Rev.* 7, 103–164
4 Mayer, M. L. and Westbrook, G. L. (1987) *Prog. Neurobiol.* 28, 197–276
5 Stone, T. W. and Burton, N. R. (1988) *Prog. Neurobiol.* 30, 333–368
6 Cotman, C. W. and Iversen, L. L., eds (1987) *Excitatory Amino Acids in the Brain: Focus on NMDA Receptors* (Special Issue) *Trends Neurosci.* 10, 263–302
7 Monaghan, D. T., Bridges, R. J. and Cotman, C. W. (1989) *Annu. Rev. Pharmacol. Toxicol.* 29, 365–402
8 Sladeczek, F., Recasens, M. and Bockaert, J. (1988) *Trends Neurosci.* 11, 546–549
9 Schoepp, D. E. and Johnson, B. G. (1989) *J. Neurochem.* 53, 273–278
10 Fagg, G. E. and Matus, A. (1984) *Proc. Natl Acad. Sci. USA* 81, 6876–6880
11 Monaghan, D. T., Holets, V. R., Toy, D. W. and Cotman, C. W. (1983) *Nature* 306, 176–179
12 Greenamyre, J. T., Olson, J. M. M., Penney, J. B. and Young, A. B. (1985) *J. Pharmacol. Exp. Ther.* 233, 254–263
13 Choi, D. W. (1988) *Neuron* 1, 623–634
14 Fagg, G. E. (1987) *Neurosci. Lett.* 76, 221–227
15 McKernan, R. M., Castro, S., Poat, J. A. and Wong, E. H. F. (1989) *J. Neurochem.* 52, 777–785
16 Kloog, Y., Haring, R. and Sokolovsky, M. (1988) *Biochemistry* 27, 843–848
17 Johnson, J. W. and Ascher, P. (1987) *Nature* 325, 529–531
18 Kessler, M., Tarramani, T., Lynch, G. and Baudry, M. (1989) *J. Neurochem.* 52, 1319–1328
19 Snell, L. D., Morter, R. S. and Johnson, K. M. (1988) *Eur. J. Pharmacol.* 156, 105–110
20 Maragos, W. F., Penney, J. B. and Young, A. B. (1988) *J. Neurosci.* 8, 493–501
21 Monaghan, D. T., Olverman, H. J., Nguyen, L., Watkins, J. C. and Cotman, C. W. (1988) *Proc. Natl Acad. Sci. USA* 85, 9836–9840
22 Cha, J. J., Greenamyre, J. T., Nielsen, E. O., Penney, J. B. and Young, A. B. (1988) *J. Neurochem.* 51, 469–478
23 Honoré, T. *et al.* (1988) *Science* 241, 701–703
24 Blake, F., Yates, R. G., Brown, M. W. and Collingridge, G. L. (1989) *Br. J. Pharmacol.* 97, 71–76
25 Nielsen, E. O., Cha, J. J. Honoré, T., Penney, J. B. and Young, A. B. (1988) *Eur. J. Pharmacol.* 157, 197–203
26 Honoré, T. and Drejer, J. (1988) in *Excitatory Amino Acids in Health and Disease* (Lodge, D., ed.), pp. 91–106, Wiley
27 Mayer, M. L. and Vyklicky, L. (1989) *Proc. Natl Acad. Sci. USA* 86, 1411–1415
28 Cha, J. J., Makowiec, R. L., Penney, J. B.

and Young, A. B. *Neurosci. Lett.* (in press)
29 Wallace, M. C., Teasdale, G. M. and McCulloch, J. (1989) *J. Cereb. Blood Flow Metab.* 9, S745
30 Fagg, G. E. *et al.* in *Current and Future Trends in Anticonvulsant, Anxiety and Stroke Therapy* (Williams, M., ed.), Liss (in press)
31 Iversen, L. L. *et al.* (1989) in *Pharmacology of Cerebral Ischaemia* (Krieglstein, J., ed.), pp. 165–171, CRC Press
32 Monahan, J. B., Handelmann, G. E., Hood, W. F. and Cordi, A. A. (1989) *Pharmacol. Biochem. Behav.* 34, 649–653

AMPA: α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid
AP4: 2-amino-4-phosphonobutanoate
AP5: 2-amino-5-phosphonopentanoate
AP7: 2-amino-7-phosphonoheptanoate
***trans*-ACPD**: *trans*-1-aminocyclopentyl-1,3-dicarboxylate
CGP37849: DL-(*E*)-2-amino-4-methyl-5-phosphono-3-pentenoic acid
CGP39551: DL-(*E*)-2-amino-4-methyl-5-phosphono-3-pentenoic acid ethyl ester
CGP39653: (*E*)-2-amino-4-phosphonomethyl-3-heptanoic acid
CGS19755: 4-phosphonomethyl-2-piperidinecarboxylic acid
CNQX: 6-cyano-7-nitroquinoxaline-2,3-dione
CPP: (±)-2-carboxypiperazine-4-yl-propyl-1-phosphonic acid
DNQX: 6,7-dinitroquinoxalone-2,3-dione
MK801: (+)-5-methyl-10,11-dihydrc-5*H*-dibenzo [*a*,*d*]cyclohepten-5,10-imine maleate
PCP: phencyclidine
SKF10047: (±)-*N*-allylnormetazocine
TCP: 1-[1-(2-thienyl)-cyclohexyl]piperidine

● Ion channels coupled to NMDA receptors differ from those coupled to kainate and AMPA receptors in that they have multiple sites of pharmacological regulation. In next month's *TiPS*, the series continues with an article discussing the characteristics of the voltage dependence of drugs and ions that bind at these sites: 'Mechanisms of blockade of excitatory amino acid receptor channels' *by John MacDonald and Linda Nowak.*

Books

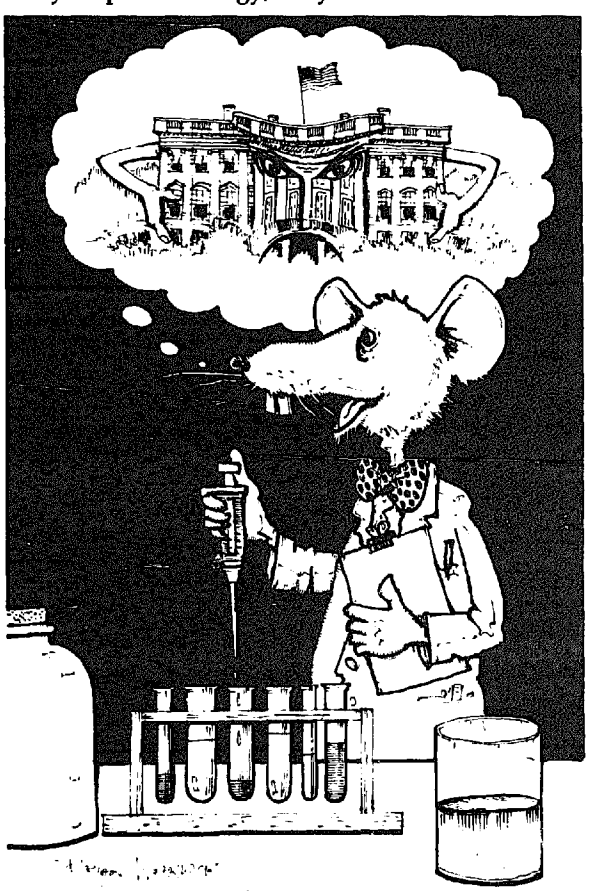
An addictive read

Brainstorming: The Science and Politics of Opiate Research

by Solomon H. Snyder, Harvard University Press, 1989. $22.50 (208 pages) ISBN 0 674 08048 3

Brainstorming is an absorbing account of an extraordinarily productive decade of work by Snyder and his colleagues that gave new definition to a host of the receptors that mediate the actions of drugs in the CNS. The development of a direct binding assay that could be used with crude membrane preparations freed pharmacological research on receptors from the difficulties of whole animal and tissue preparations. The speed, simplicity and low cost of these binding assays revolutionized drug screening, gave new precision to the elucidation of receptor subtypes and provided assays for the detection of new biological mediators in the brain. When coupled with autoradiography, the new methods provided powerful tools for the analysis of the role of specific receptors in neural mechanisms in the CNS.

The story starts with the opiate receptor, which attracted Snyder's attention because of its sociopolitical importance, its relevance for psychiatry and, according to the account given here, because money was available via a center grant from the NIMH. In a success story that is known by every first year student of neuroscience and pharmacology, Snyder and his colleague Candace Pert developed a direct binding assay for the opiate receptor, giving it biochemical respectability and providing an indispensable tool for its study. As is so often the case in science, they were successful because they connected two lines of previous experiments: Avram Goldstein's attempts to identify the opiate receptor by exploiting its specificity for stereoisomers; and assay methods developed by Cuatrecasas and his colleagues for isolation of the insulin receptor. Both debts are generously acknowledged.



The same techniques proved amenable to other receptors, and Snyder and his colleagues quickly characterized a whole panoply of receptors from the CNS, including those for acetylcholine, dopamine, 5-HT and histamine. The most exciting payoff of the opiate receptor experiments was the identification of the endogenous opiates, the enkephalins and endorphin.

The book is written in an engaging and easy style and gives a quick and deft account of the history and sociology of opiate use. The personal history of Snyder's involvement in receptor research, and the account of the contributions of various collaborators and colleagues are also of interest. The book is disappointing, however, in two ways. First, in spite of the title and the