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Central Nervous System Receptors for Glutamic Acid

Graham A. R. Johnston

Department of Pharmacology, John Curtin School of Medical Research, Australian National University, Canberra City, ACT 2601 Australia

L-Glutamic acid, and structurally related acidic amino acids such as L-aspartic acid, excite most neurones in the mammalian CNS (16). A variety of membrane receptors seem to be involved in such excitation, and it is the purpose of this review to discuss evidence for the multiplicity of CNS receptors for L-glutamic acid from *in vivo* studies on excitant amino acid agonists and antagonists, and from *in vitro* studies of ligand binding to isolated receptors.

There is increasing neurochemical evidence that both L-glutamic acid and L-aspartic acid may function as excitatory synaptic transmitters in the mammalian CNS. For example, in the rat cerebellum, L-glutamic acid is associated with granule cells (29) and L-aspartic acid with climbing fibers (28); and in the cat spinal cord, L-glutamic acid appears to be associated with some primary afferent fibers (32) and L-aspartic acid with interneurones destroyed by temporary aortic occlusion (6). This review presents evidence that L-glutamic acid and L-aspartic acid activate different but overlapping populations of excitatory receptors.

EXCITANT AMINO ACID AGONISTS

Many substances appear to act as L-glutamic acid agonists when administered microelectrophoretically near CNS neurones. Most of these excitants are structurally related to L-glutamic acid (16) and show a remarkable variety of moieties that can mimic the ω-carboxylic acid group of L-glutamic acid (4). The naturally occurring L-isomers of glutamic and aspartic acids are only marginally more potent excitants of CNS neurones than the corresponding D-isomers (5,16). Some agonists are more potent excitants than L-glutamic acid, e.g. kainic, quisqualic, and D-homocysteic acids. The potency of microelectrophoretically administered excitants, however, can be modified by sodium-dependent active transport systems that remove certain substances from the extracellular synaptic environment, both "high-" and "low-affinity" systems being involved (5), and this must be considered when attempting to assess the relative potencies of excitants. Kainic acid (20) and D-homocysteic acid (5) are not actively transported, and this could account

for their apparent high potency when compared to L-glutamic acid, which is actively transported. The high excitant potency of substances that are not actively transported supports other evidence that excitation and acidic amino acid transport are separate processes (1).

L-Glutamic acid can exist in a variety of low-energy conformations in aqueous solution as judged by proton magnetic resonance spectroscopy (13). In order to gain insight into the likely active conformation(s) of L-glutamic acid during interaction with excitatory receptors, it is necessary to study conformationally restricted analogs of L-glutamic acid, e.g., kainic acid, where the flexibility of that part of the molecule equivalent to L-glutamic acid is restricted by incorporation into a pyrrolidine ring structure. Such studies have led us to propose that there are at least two types of receptors for excitant amino acids in the cat spinal cord: "L-glutamic acid-preferring" and "L-aspartic acid-preferring" receptors (18). L-Glutamic acid was proposed to interact with L-glutamic acid-preferring receptors in partially extended conformations, and also with L-aspartic acid-preferring receptors in partially folded conformations. On the other hand, L-aspartic acid was proposed to interact only poorly with L-glutamic acid-preferring receptors, because the carbon chain of L-aspartic acid is one atom shorter than that of L-glutamic acid and thus could not stretch out sufficiently to interact efficiently with receptors requiring the extended conformations of L-glutamic acid. Kainic acid was proposed as a selective agonist for L-glutamic acid-preferring receptors since it is an analog of L-glutamic acid in an extended conformation, and N-methyl-D-aspartic acid was proposed as a selective agonist with high affinity for L-aspartic acid-preferring receptors. Experiments on the differential sensitivity of cat spinal neurones to kainic and N-methyl-D-aspartic acids provide support for these proposals (25), as do the results of ligand-binding studies with [^{3}H] kainic acid that will be discussed later (20,34).

Kainic acid is of particular interest as a probable selective agonist of L-glutamic acid. This anthelmintic, found in marine algae, is a potent excitant of CNS neurones (4,18,33) and, like L-glutamic acid, can act as a neurotoxin (30). On direct injection into certain brain regions, kainic acid appears to be specifically neurotoxic to neurones having cell bodies in the vicinity of the injection site while sparing axons of passage and nerve terminals arising from neurones distant from the injection site. The structurally related domoic acid is also a potent excitant (4), but dihydrokainic acid and *α-allo*-kainic acid are much less potent than kainic acid (4,18).

ANTAGONISTS OF AMINO ACID-INDUCED EXCITATION

If different populations of excitatory receptors exist for L-glutamic and L-aspartic acids, it seems reasonable to expect that certain antagonists may be able to antagonize selectively the excitation mediated by one or other of these receptor populations. Most of the known antagonists of amino acid-induced excitation, e.g., 1-hydroxy-3-aminopyrrolidone-2 and nuciferine, show little, if any, selectivity for particular amino acids (16,23). Recent studies using magnesium ions (7), D-α-ami-

noadipic acid (12), and L-glutamic acid diethylester (GDEE) (14) have revealed, however, relatively selective antagonistic actions, which should be of great value in distinguishing synaptic excitation mediated by L-glutamic or L-aspartic acid when used in appropriate conjunction with other evidence.

Magnesium ions antagonize the L-aspartic acid-induced excitation of cat spinal interneurones more effectively than L-glutamic acid excitation (7). Excitations induced by N-methyl-D-aspartic acid and by acetylcholine were antagonized by magnesium ions. Kainic acid-induced excitation was not influenced by magnesium ions on any of the spinal neurones tested. It seems likely that magnesium ions interfere with some ionic events common to the activation of L-aspartic acid-preferring receptors and certain acetylcholine receptors. Differences in the ionic events associated with the actions of various amino acid excitants have been noted in other types of experiments (9,21).

The prediction that D-α-aminoadipic acid "might be a useful antagonist of the action of the excitatory amino acids" (12) has been amply justified by three groups of workers, who have shown that D-α-aminoadipic acid is a significantly more effective antagonist of excitation induced by L-aspartic acid and N-methyl-D-aspartic acid than of excitation induced by L-glutamic acid and kainic acid (3,22,24). D-α-Aminoadipic acid is particularly useful when used in conjunction with GDEE, since the order of susceptibility of excitant amino acids differs for these two antagonists (14). α,ϵ-Diaminopimelic acid also shows selectivity as an excitant amino acid antagonist (2). Experiments indicate that D-α-aminoadipic acid and α,ϵ-diaminopimelic acid act in a like manner, whereas magnesium ions act at a different site as antagonists of excitation induced by N-methyl-D-aspartic acid (10).

LIGAND-BINDING STUDIES

Until relatively recently, the investigation of CNS receptors for neurotransmitters has had to rely almost exclusively on electrophysiological procedures. Following the development of methods for studying the binding of insulin to isolated receptors (15), and the dramatic advances resulting from the application of similar methods to the binding of opiates (35), ligand-binding studies are becoming an ever-increasing part of amino acid neuropharmacology (17), complementing and extending the results available from electrophysiological studies. Ligand-binding studies can be used to aid in the purification and characterization of receptor macromolecules, in the development of new drugs that act specifically on receptors, in the investigation of abnormal brain function, in the study of receptor regulation and turnover, in the discovery of endogenous ligands that might modulate receptor activity, and to gain a better understanding of binding dynamics with respect to cooperativity and multiple-site interactions.

Appropriate ligands for binding studies are substances that act as potent and specific agonists or antagonists with respect to the receptor(s) under investigation. The ligands need to be labeled to high specific activity, and numerous criteria need

to be met before the observed binding can be considered as physiologically relevant. The specificity, affinity, and number of binding sites must be evaluated by careful and detailed comparison with the *in vivo* activity of the ligand (15,35). Absolute correspondence between *in vivo* and *in vitro* data can be achieved rarely, and it is probably the differences between the two sets of data that will provide the most interesting advances in our understanding of receptors.

Ligand-binding studies of excitant amino acid agonists are only at a very early stage of development and studies on the binding of amino acid antagonists have yet to be reported. The probable overlapping populations of receptors binding both L-glutamic acid and L-aspartic acid, together with receptors specific for either L-glutamic acid or L-aspartic acid, complicate binding studies with either of these ligands to relatively crude membrane preparations from CNS tissue. Selective agonists or antagonists for each type of CNS receptor for excitant amino acids need to be developed. To date, only kainic acid seems to exhibit appropriate selectivity, binding to some 8 to 10% of the total sites that bind L-glutamic acid in a crude preparation of rat brain membranes, such binding being relatively insensitive to inhibition by L-aspartic acid (20,34).

Since uptake processes for excitant amino acids are sodium dependent, ligand-binding studies are usually carried out in the absence of sodium ions in order to avoid confusing sodium-independent binding to receptors and sodium-dependent binding to transport carriers (1,35).

L-GLUTAMIC ACID BINDING

Michaelis et al. (27) have described sodium-independent binding of L-glutamic acid to a synaptic membrane subfraction from rat brain. Binding was biphasic to a high-affinity site having a dissociation constant (K_d) of 0.2 μM and a maximal binding capacity (B) of 0.002 nmoles/mg protein, and a low affinity site, $Kd = 4$ μM, $B = 0.009$ nmoles/mg. Binding was stereospecific in that 0.2 mM D-glutamic acid did not influence high-affinity binding. L-Aspartic acid was a competitive inhibitor ($Ki = 1$ μM) of high-affinity L-glutamic acid binding, consistent with L-aspartic acid binding to the high-affinity sites with about 20% of the affinity of L-glutamic acid binding to these sites. The excitant amino acid antagonist GDEE was a relatively weak inhibitor (24% at 1 μM), whereas GABA was a relatively potent inhibitor (86% at 1 μM), acting in a noncompetitive manner ($Ki = 1$ μM). The L-glutamic acid binding system could be solubilized by treatment with 0.5 to 1% Triton X-100 detergent at a briefly elevated pH (9.5 for 1 min) at 0 to 4° C and purified some 200-fold to afford a small (MW 14,000) acidic glycoprotein fraction (26). Binding of L-glutamic acid to this soluble receptor preparation was not influenced by D-glutamic acid or GABA (1 μM) and appeared to be associated with a single class of binding sites, $Kd = 0.8$ μM, $B = 66$ nmoles/mg. This binding was inhibited by L-aspartic acid (36%) and GDEE (30%) at 1 μM.

Roberts (31), using a synaptic membrane fraction from rat brain, found that

L-glutamic acid bound to a single population of binding sites, $Kd = 8$ μM, B approximately 0.03 nmoles/mg, in a sodium-independent manner. This binding was inhibited only weakly by D-glutamic acid (11%) but strongly by L-aspartic acid (63%) and GDEE (60%) at 1 mM.

DeRobertis and Fiszer de Plazas (8) have purified a proteolipid fraction from rat cerebral cortex, by extraction with chloroform-methanol and Sephadex column chromatography, that bound L-glutamic acid in a sodium-independent manner. Three binding sites appear to be involved: high affinity, $Kd = 0.3$ μM, $B = 0.5$ nmoles/mg; medium affinity, $Kd = 5$ μM, B = 32 nmoles/mg; and low affinity, Kd 55 μM, $B = 166$ nmoles/mg. D-Glutamic acid (0.1 mM) did not inhibit the high-affinity site, but greatly inhibited the medium-affinity site. GDEE (65%), L-aspartic acid (55%), and nuciferine (54%) inhibited the high-affinity site at 20 μM.

The above studies show that L-glutamic acid can bind to receptors isolated from rat brain in a manner that may represent the interaction of L-glutamic acid with excitatory receptors on neurones. The observed binding is stereoselective in that D-glutamic acid has little influence on L-glutamic acid binding. This is particularly interesting in view of the fact that L- and D-glutamic acid have very similar excitant actions on CNS neurones *in vivo* (5,16). The binding studies indicate that L- and D-glutamic acid act on different excitatory receptors, or perhaps the medium-affinity sites described by DeRobertis and Fiszer de Plazas (8). In most of the above studies, L-aspartic acid and GDEE inhibit the binding of L-glutamic acid to a similar extent, suggesting that these three substances can act on the same binding sites, L-glutamic acid binding with a 5- to 10-fold higher affinity than do L-aspartic acid and GDEE. Since L-glutamic acid and L-aspartic acid have very similar excitant actions *in vivo* (5,16), L-aspartic acid appears likely to be able to bind to sites other than those preferentially binding L-glutamic acid.

L-ASPARTIC ACID BINDING

Fiszer de Plazas and DeRobertis (11) have studied the sodium-independent binding of L-aspartic acid to the proteolipid fraction that bound L-glutamic acid as described above (8). As with L-glutamic acid binding, the binding of L-aspartic acid appeared to involve three kinetically distinct sites: high affinity, $Kd = 0.2$ μM, $B = 3$ nmoles/mg; medium affinity, $Kd = 10$ μM, $B = 132$ nmoles/mg; and low affinity, Kd 50 μM, $B = 617$ nmoles/mg. Extrapolating back to fresh tissue, it was estimated that there were some eight times more high-affinity L-aspartic acid binding sites (8 nmoles/g fresh tissue) than high-affinity L-glutamic acid binding sites. D-Aspartic acid did not inhibit the high-affinity binding of L-aspartic acid, but strongly inhibited medium-affinity binding. L-Glutamic acid was only a relatively weak inhibitor of high-affinity L-aspartic acid binding (28% at 40 μM), as were GDEE (27%) and nuciferine (18%), whereas N-methyl-D-aspartic acid (45%) was about 50% as potent as was L-aspartic acid itself (92%). Kainic acid (40 μM) did not influence L-aspartic acid binding. The following can be suggested on the basis of

these results: (a) D- and L-aspartic acids bind to different sites, though they excite CNS neurones in a similar fashion (5); (b) there are sites preferring L-aspartic acid to L-glutamic acid, and vice versa; and (c) kainic acid and L-aspartic acid bind to different sites.

KAINIC ACID BINDING

Simon et al. (34) described the sodium-independent binding of kainic acid to synaptic membranes from rat brain, with a *Kd* of 0.06 μM. From the apparent maximal number of kainic acid binding sites (B = 0.001 nmoles/mg) and cross-inhibition studies with L-glutamic acid, they estimated that there was a definite difference between the kainic acid binding sites and the bulk of the L-glutamic acid binding sites in their preparations, with the density of the L-glutamic acid binding sites being 8 to 10 times greater than the density of the kainic acid binding sites. This indicates that kainic acid binds to only a relatively small population of L-glutamic acid binding sites. We (20) are in general agreement with most of the observations made by Simon et al. (34). L-Glutamic acid was an order of magnitude weaker than kainic acid in displacing bound radioactive kainic acid, and L-aspartic acid was at least 500 times weaker than L-glutamic acid. D-Glutamic acid and D-aspartic acid appeared to be weaker than L-aspartic acid in displacing kainic acid. N-Methyl-D-aspartic acid did not inhibit kainic acid binding. Kainic acid binding was not inhibited by GDEE, magnesium ions, or a variety of other known antagonists of excitant amino acid action (1-hydroxy-3-aminopyrrolidone-2, LSD, L-methionine-DL-sulfoximine, 9-methoxyaporphine, nuciferine), but DL-α-aminoadipic acid was a weak inhibitor (20,34). Specific kainic acid binding appeared to be localized to CNS tissues and showed a fivefold regional variation in the brain, with the highest binding being associated with the striatum and the lowest with the midbrain and pons medulla (34). Subcellular fractionation indicated that most of the binding activity was associated with synaptosomal membrane fractions.

The affinity of kainic acid for the above binding sites appeared to be some four orders of magnitude higher than its affinity for the sodium-dependent, high-affinity L-glutamic acid transport carrier, kainic acid being a weak competitive inhibitor (Ki = 250 μM) of this carrier (20). Dihydrokainic acid was about 500 times less potent than kainic acid as an inhibitor of kainic acid binding, but was approximately equipotent with kainic acid as an inhibitor of L-glutamic acid transport.

D-ASPARTIC ACID BINDING

We have done some preliminary experiments on the sodium-independent binding of D-aspartic acid to a Triton-extracted membrane preparation from rat brain (19). Bound radioactive D-aspartic acid could be displaced most potently by N-methyl-D-aspartic acid followed by D-aspartic acid itself, L-aspartic acid, L-glutamic acid, and DL-α-aminoadipic acid, and less potently by kainic acid. D-Aspartic acid appears to bind to a relatively nonspecific population of sites, which show

little selectivity for L-glutamic acid or D- or L-aspartic acid, but interact strongly with N-methyl-D-aspartic acid.

MULTIPLICITY OF BINDING SITES FOR EXCITANT AMINO ACIDS

The above binding studies on various membrane preparations from rat brain with L-glutamic acid, L-aspartic acid, kainic acid, and D-aspartic acid as ligands suggested that several binding sites are involved in the binding of each of these ligands. Certainly, they support the concept of L-glutamic acid-preferring and L-aspartic acid-preferring populations of receptors developed on the basis of *in vivo* studies of amino acid-induced excitation of CNS neurones. Binding studies provide information on the relative affinity of the interaction of ligands with binding sites, but it needs to be emphasized that binding studies do not provide information on the efficacy of such interactions with respect to changes induced in membrane permeability. Indeed, not all binding need be functional. Furthermore, on the basis of binding studies per se it is difficult to distinguish between agonist and antagonist binding phenomena. With these reservations in mind, it is possible to propose that at least four populations of excitant amino acid agonist *binding sites* are associated with rat brain membranes, the classification being based on the naturally occurring excitants L-glutamic and L-aspartic acids:

1. L-Glutamic acid-preferring (extended) binding sites. These sites show a preference for kainic acid over L-glutamic acid and interact poorly with L-aspartic acid, D-aspartic acid, and N-methyl-D-aspartic acid. They may represent sites that preferentially bind extended conformations of L-glutamic acid. These are the sites studied using kainic acid as the binding ligand.
2. L-Glutamic acid-preferring (partially folded) binding sites. These sites prefer L-glutamic acid to either kainic acid or L-aspartic acid and may represent sites which preferentially bind partially folded conformations of L-glutamic acid. These are the bulk of the sites studied using L-glutamic acid as the binding ligand.
3. L-Aspartic acid-preferring binding sites. These sites show a strong preference for L-aspartic acid over L-glutamic acid and show little interaction with kainic acid or D-aspartic acid. These are the sites studied using L-aspartic acid as the binding ligand.
4. L-Glutamic and L-aspartic acid binding sites. These sites show little selectivity between L-glutamic, or D- or L-aspartic acids and may be studied using D-aspartic as the binding ligand. N-Methyl-D-aspartic acid interacts very strongly, and kainic acid interacts only poorly with these sites.

It is probably not coincidental that four populations of binding sites are proposed on the basis of studies with four ligands! Examination of more ligands would no doubt produce further classifications of binding sites, and it will be difficult to relate the various populations of binding sites to functional receptors mediating neuronal excitation until more selective agonists and antagonists are developed. The numbers of binding sites on rat brain membranes associated with these four proposed

populations appears to decrease in the following order 3 > 2 > 1 > 4. Of the known excitant amino acid antagonists, there appears to be some selectivity with respect to their interaction with the various proposed populations of binding sites. GDEE appears to interact most strongly with population 2, to a lesser extent with population 3, and not at all with populations 1 and 4. DL-α-Aminoadipic acid appears to interact strongly with population 4 and very weakly with population 1, but further tests are necessary using the pure D-α-aminoadipic acid on all the binding site populations. With respect to ligands that merit future study, D-α-aminoadipic acid, GDEE, D-glutamic acid, and N-methyl-D-aspartic acid are obvious choices.

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