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EXCITATORY AMINO ACID TRANSMITTERS

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Since the more recent reviews on excitatory amino acid actions in the vertebrate central nervous system (1–5) there has been considerable progress along two particular investigative fronts, leading to firm conclusions about amino acid–mediated synaptic excitation. These two approaches are, first, the development of new agonists and antagonists for determining the pharmacological properties of different excitatory amino acid receptors, and, second, the use of lesioning techniques to determine the effects of selective neuronal and/or afferent input loss upon regional levels, uptake, and release of suspected transmitters. A third technique in widespread use is the study of membrane binding sites for excitatory amino acids by the use of radioactive ligands. This review correlates the findings of these three different lines of research, and, in particular, highlights gaps in the evidence for amino acid–mediated synaptic excitation in specific neuronal pathways. Prior to this, however, some recent neurochemical and pharmacological findings of more general relevance are discussed.

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Putative Excitatory Amino Acid Transmitters

Although endogenous excitatory amino acids other than L-glutamate and L-aspartate are known to occur, or might well occur, in central nervous tissue the possibility that they could function as transmitters (2, 6–8) has been essentially ignored. Among known endogenous excitants are L-cysteate (9, 10) and L-cysteine sulfinate (11), while the higher homologues L-homocysteate and L-homocysteine sulfinate (12, 13) and the thio analogue S-sulfocysteine (14) occur in other tissues under certain nutritional or pathological conditions. D-Aspartate has been reported to occur in relatively high concentrations in the cephalopod nervous system (15) and human lens (16), and D-aspartate oxidase has long been known to be present in the mammalian central nervous system (17–19), though its function is obscure (2). The common objection that L-glutamate and L-aspartate are too intimately involved in general metabolic pathways (and particularly in energy metabolism) to be likely to function in the same manner as the "classical" peripheral transmitters acetylcholine and norepinephrine, and the necessity for considering distinct "transmitter" and "metabolic" pools of the substances in neurochemical studies (1, 20, 21) would not apply to the other endogenous excitants mentioned above. It is interesting to draw an analogy between possible brain levels of these amino acids and levels of dopamine, 5-hydroxytryptamine, norepinephrine (22), and acetylcholine (23) which occur in concentrations of up to 5 nmol/g in rat brain. Thus "low" concentrations of approximately 100 nmol/g reported for L-cysteate in rat brain (9, 10) should not be discounted. This analogy also serves to emphasize the analytical difficulty of separating and estimating levels of amino acids which have chemically similar properties to L-glutamate and L-aspartate from brain extracts containing up to 1000 or more times those levels of the latter amino acids. This problem would be particularly difficult in a search for D-glutamate and D-aspartate in mammalian central nervous tissue. However, the observations to be discussed in this review strongly support the idea that both L-glutamate and L-aspartate do function as transmitters and, in the absence of any convincing evidence in support of alternative candidates, no other substances are further considered.

Regional Distribution of Amino Acids and Enzymes

Regional levels of L-glutamate and L-aspartate in the mammalian central nervous system have been compared in earlier reviews (1, 7). Such data have not provided any compelling indications of specific regions of transmitter function for either of the two amino acids mainly because of their general metabolic function and the ubiquity of their probable transmitter role. An

exception is, however, the greater concentration of L-glutamate in dorsal compared with ventral roots (24) and of L-aspartate in auditory nerve compared with most other white matter studied (25–27). The concentration of L-aspartate in the cochlear nucleus parallels the distribution of the auditory nerve endings (25–27). A high concentration of aspartate in the tip of the ventral horn (four times higher than tip of dorsal horn, and higher than any other region of the central nervous system) has been reported in the rabbit spinal cord (28).

Histochemical methods suggest that glutamate dehydrogenase (GDH), one of several enzymes possibly involved in glutamate synthesis, appears to be particularly highly concentrated in chick cerebellar glomeruli (29) and biochemical assays show a relative enrichment of this enzyme in the auditory and optic nerves (30) compared with other nerves. The significance of such observations would seem to be reduced, however, by the apparent abundance of GDH in glial cells. Glutaminase, another enzyme possibly involved in the synthesis of excitatory amino acid transmitters (20, 31–33), is also relatively concentrated in the auditory nerve (30). Aspartate amino transferase (AAT) is even more differentially concentrated in the auditory nerve (30), and immunocytochemical localization of this enzyme at auditory nerve endings within the cochlear nucleus has been demonstrated very recently (34). An enrichment of AAT in fibers within the chick cerebellar granular layer was suggested by a lead precipitation histochemical method (29), which, however, also indicated a localization of the enzyme within inhibitory basket cell endings. It will be important to see whether such findings are confirmed by the immunocytochemical method (34).

Evidence for vesicular storage of transmitter amino acids has long been sought without success. Factors influencing vesicular uptake of L-glutamate resemble those for acetylcholine and norepinephrine (35). An enrichment of glutamate and aspartate, along with acetylcholine and GABA, was recently reported in the vesicular fraction from bovine cerebral cortex (36). However, in another study, no such concentration was found in vesicles from several different brain regions (37).

Uptake/Inactivation

It is generally accepted that reuptake from the extracellular space is the process whereby transmitter amino acids are removed from the vicinity of their receptors and thereby inactivated [see (1, 7, 21, 38, 39) for reviews]. Depending on the concentrations of the transmitters in the synaptic cleft, both the so-called high and low affinity uptake systems (40) could participate in such inactivation. Low affinity uptake is probably an important factor in the inactivation of iontophoretically administered excitatory amino acids in the mammalian spinal cord (41, 42). Both glia and neurons

possess the two types of uptake systems for excitatory amino acids [see Hertz (21)]. It is possible that high affinity uptake into synaptic terminals, and even into the postsynaptic cell bodies near the receptor sites (43, 44) follows release of "normal" concentrations of transmitter amino acids during synaptic excitation, but that glial and intersynaptic perikaryal uptake prevents the spread of "overspill" transmitter to other receptors such as may occur during hyperexcitation (2). The finding that satellite glial cells can prevent exogenous glutamate from reaching neurons in dorsal root ganglia (45) would support this possibility. Schousboe & Divac (46) have suggested that the efficiency of glial uptake systems for amino acids varies with the concentration of the amino acid in particular regions, being greater for glycine in the spinal cord and for glutamate in the striatum, and that a comparison of the uptakes into cultured glia from different regions may give indications as to the relevant importance of the amino acids as transmitters in those regions.

Of particular significance with respect to both the mechanism of action of excitatory amino acids and their inactivation is the probable cotransport of monovalent cations during uptake, two sodium ions accompanying such uptake of L-glutamate into synaptosomes (47) and a cerebellar neuronal cell line (48), whereas only one sodium ion is transported with L-glutamate into glial cells (49). In view of the well-known ability of glial cells also to accumulate K^+ and the enhancement of L-glutamate transport by increases in extracellular K^+ concentration (49, 50), co-transport of both Na^+ and K^+ with L-glutamate into glia might be envisaged. The concomitant accumulation of K^+ with L-glutamate and L-aspartate in brain slices has long been known (51, 52). Uptake of Na^+ with excitatory amino acids into neurons could well be associated with depolarization and it may be significant that those amino acids that have been shown to be taken up with sodium ions into cultured cerebellar cells (e.g. L-glutamate, L-aspartate, D-aspartate, L-cysteate, L-cysteine sulfinate) have been shown to activate a different population of receptors (discussed below) from those amino acids that are not good substrates for this uptake process (e.g. D-glutamate, L-homocysteate, *N*-methyl-DL-aspartate and kainate) (48). It is noteworthy also that responses of frog spinal neurons to excitatory amino acids are differentially affected by variations in the extracellular monovalent cation concentration (53) and these effects have been correlated with alterations in rates of uptake of the amino acids by the tissue (50).

Inhibitors of uptake are potentially important not only for characterizing the transmitter released at particular synapses but conceivably also as new types of centrally active drugs. For transmitter identification, uptake blockers could facilitate collection of a synaptically released transmitter. Also, specific uptake blockers would be expected to enhance synaptic excitation

mediated by an excitatory amino acid, so supporting a proposed transmitter role of such substances at particular synapses. Difficulties of interpretation would exist, however, where a glutamate/aspartate uptake blocker was itself a substrate for the uptake system, or where it was an agonist for the excitatory receptors. Release of glutamate and aspartate from synaptic endings by hetereoexchange (see 38) might be expected where the uptake inhibitor was transported into the endings, and any agonist activity of the uptake inhibitor could result in transmitter release from excited cells. Both actions might complicate interpretation of the effects of the substance on transmitter accumulation or action resulting from stimulating specific pathways. Thus, the origin of L-glutamate released from guinea pig olfactory cortex slices on stimulating the lateral olfactory tract in the presence of L-cysteate (54) must be interpreted with caution, L-cysteate being probably a substrate for uptake on the glutamate carrier (40) as well as an excitatory agonist (2).

Two uptake inhibitors that have recently been investigated for their possible pharmacological usefulness are threo-3-hydroxyaspartate and dihydrokainate (42, 55). Both D and L isomers of threo-3-hydroxyaspartate are probably substrates for the excitatory amino acid uptake system(s) (56) and are also agonists of moderate potency (55). Dihydrokainate is probably not a substrate for the high affinity L-glutamate uptake system (57) and has been reported to be only a weak agonist (58). Our unpublished observations indicate that dihydrokainate causes slow depolarization of motoneurons in the isolated frog spinal cord, which may be due to accumulation of spontaneously released transmitter. However, although threo-3-hydroxyaspartate (55) and dihydrokainate (42) both enhance responses of cat spinal neurons to L-glutamate and L-aspartate, no enhancement of synaptic excitation by these agents has yet been reported.

It is of interest that responses to the potent excitant quisqualate are also enhanced by dihydrokainate and threo-3-hydroxyaspartate (42), suggesting that quisqualate may be taken up into CNS tissue in accord with its relatively fast offset of action. Two excitatory amino acid antagonists to be discussed below, D-α-aminoadipate (DAA) (59–61) and 3-amino-1-hydroxy-2-pyrrolidone (HAP) (59, 60), also enhance quisqualate responses, but have no effect on the uptake of glutamate or aspartate (60). Therefore, quisqualate may be transported by a separate system from that involved in the uptake of L-glutamate and L-aspartate. The baclofen analogue, (±)-3-(*p*-chlorophenyl) glutamic acid, selectively enhanced responses of frog motoneurons to L-homocysteate and depressed the uptake of [^{35}S]L-homocysteate into the frog spinal cord, the actions and uptake of L-glutamate and L-aspartate being little affected by this glutamate derivative (R. H. Evans, A. A. Francis, D. J. Oakes, and J. C. Watkins, unpublished

observations). Such observations suggest that uptake systems for excitatory amino acids may not be as nonselective as usually believed (40).

Vincent & McGeer (62) have described the structural specificity for inhibition of uptake of [^{3}H]L-glutamate into striatal homogenates. In general, a similar pattern of inhibition to that originally described by Balcar & Johnston (40) for chopped rat brain slices was obtained with the exception that some inhibition by *N*-methyl-aspartic acid (presumably the DL form) was also observed.

Selective reduction following axotomy in high affinity uptake of radiolabeled glutamate, compared with other putative transmitters, in regions where specific tracts terminate (e.g. 63, 64) suggest that axon terminals are a major site of such uptake. This is supported by autoradiographic studies (64a). High affinity uptake may thus be a useful biochemical marker for synaptic terminals which release an acidic amino acid as transmitter. The use of this technique for transmitter characterization and a modification of the technique utilizing the metabolically more stable [^{3}H]D-aspartate (65) are discussed below in the section on lesion studies.

Release

Release of excitatory amino acids from central nervous tissue is commonly investigated in slice or synaptosome preparations or by the use of depolarizing agents such as high K^+ concentration or veratridine. Neuronal is considered to be distinguishable from glial release by the Ca^{2+}-dependency of the former (66) though Ca^{2+}-dependent release of amino acids from glia has also been reported (67). General aspects of this widely studied phenomenon have been recently reviewed (1, 21, 38). Some more recent data of a general nature are emphasized here, while investigations utilizing release measurements in relation to transmitter identification studies involving specific synaptic pathways is reserved for the final section on lesion studies.

Electrophysiological investigations have suggested that a major action of the antispasticity drug baclofen [3-(*p*-chlorophenyl)GABA] may be to inhibit release of excitatory transmitters (68–71). In conformity with this possibility, baclofen-induced decreases in the electrically or potassium-evoked release of excitant amino acids from brain slice preparations have been reported (72, 73). That this effect may not be specific for excitatory amino acid transmitters, however, is suggested by reports that baclofen depressed K^+-evoked release of norepinephrine, dopamine, and 5-hydroxytryptamine from slices of rat cerebellar cortex, striatum, and cerebral cortex, respectively, and of norepinephrine from rat atria, and that the drug also depressed transmission in vas deferens and superior cervical ganglion preparations (74, 75). These actions were stereoselective, the (–) form being the more active isomer in both cases. However, depression of peripheral

transmission by baclofen has been disputed (76–78). Moreover K^+-evoked release of amines from brain slices may be mediated at least in part by activation of neuronal pathways involving excitatory amino acid transmitters (79–80). It is important that further experiments be carried out to determine the site of action of baclofen, since if this drug does prove to have a selective action on presynaptic release of excitant amino acids it will be extremely valuable in the neurophysiological identification of excitatory pathways.

Dopamine receptor agonists also have been shown to depress the release of L-glutamate from striatal preparations, and it has been postulated that this effect reflects the presence of dopamine receptors on corticostriatal terminals (81). Release of excitatory amino acids from brain slices and synaptosomes is stimulated by the tremorgenic mycotoxins, veruculogen and Penetrem A (82), and by the scorpion toxin, tityustoxin (83). The action of the latter toxin is blocked by tetrodotoxin (83) and morphine (84).

Bradford et al (85) have reported that the K^+-and veratrine-induced release of L-glutamate and L-aspartate varies in different regions of the striatum, L-glutamate release being evident in the caudate nucleus, putamen, and substantia nigra (but not the globus pallidus), whereas L-aspartate release was observed only in the putamen, and even here, was lower in magnitude than that of glutamate.

PHARMACOLOGY OF EXCITATORY AMINO ACID RECEPTORS

Interpretation of neurochemical evidence in terms of the proposed transmitter function for glutamate or aspartate, unlike that for acetylcholine, amines, or peptides, is complicated by the general metabolic role of the amino acids. Thus, specific pharmacological antagonists for excitatory amino acids have long been recognized as essential for definitive characterization of amino acid–mediated synaptic excitation. The recent development of such antagonists constitutes a major breakthrough in the study of excitatory transmission in the mammalian central nervous system [for reviews see (2, 8, 86–90)]. Not only are many of these substances selective for excitatory amino acid–induced responses, having little or no effect on responses to cholinergic, aminergic, and peptidergic agonists, but these substances are also selective among amino acid excitants themselves, some agonists being much more susceptible than others. This has led to the recognition of different types of excitatory amino acid receptors which have been described in different terms by different authors. An attempt to unify these concepts is made below.

NMDA Receptors

The most clearly defined receptors are those which are activated by *N*-methyl-D-aspartate (NMDA) and blocked by a variety of organic and inorganic antagonists (59, 60, 94, 95, 98–102, 105, 106, 112). Some authors (61, 103, 108–111) have referred to these receptors as D-α-aminoadipate (DAA)-sensitive receptors, DAA having been among the first organic antagonists to be shown to have pronounced and selective activity as an excitatory amino acid antagonist (59, 99–101, 103, 108). DAA has been superseded by considerably more powerful and, in some cases, even more selective antagonists, and if an antagonist is to be used to describe these receptors, then *APV-sensitive receptors* would seem to be a more appropriate term, APV standing for the highly specific NMDA antagonist 2-amino-5-phosphonovalerate (8, 88–93). However, we prefer the term *NMDA receptors* since NMDA has been found to be a very selective agonist at these receptors (Table 2), and this is the agonist of choice to be used, in conjunction with any of several selective antagonists, to demonstrate the presence of these receptors. A large number of such antagonists are now known, though their degree of specificity varies. The actions of some of these antagonists are summarized in Table 1.

Quisqualate and Kainate Receptors

The actions of selective NMDA antagonists as described above suggested that some agonists act predominantly at different receptors from those activated by NMDA. The least susceptible agonists include quisqualate and kainate, two highly potent excitants of natural origin (2, 58, 113–115). McLennan & Lodge (61) demonstrated that L-glutamic acid diethyl ester (GDEE) depressed quisqualate-induced responses, but not responses to kainate in the cat spinal cord. Similar results were obtained in a separate study (94). Such observations suggest that the two agonists activate different receptors. GDEE also depresses responses to L-glutamate and a range of other excitants (61, 94, 103, 108, 109, 116) and the term *GDEE-sensitive* has been used to describe the site of action of the antagonist in depressing these responses. However, GDEE is not a highly specific antagonist for amino acid receptors, since it also blocks certain responses to acetylcholine (61, 94). Moreover, subdivisions may later be recognized between amino acid receptors sensitive to GDEE; thus we prefer the more specific terms of quisqualate and kainate receptors, rather than GDEE-sensitive receptors, to describe the predominant sites of action of these two agonists. Additional support for the idea of different quisqualate and kainate receptors has come from the use of the NMDA antagonist, γ-D-glutamylglycine (DGG), which in the cat spinal cord markedly depresses kainate-induced responses (in

Table 1 Specificity of excitatory amino acid antagonists[a, b]

Antagonist	K_D μM	Effectiveness against responses induced by NMDA	KA	Q	Ch	NE	sP	References
APV	2	VH	NS	NS	NS	NS	NS	8, 88–93
DAS	16	H	L	L	NS		NS	60, 86, 94, 95
DGG	21	H	M	M/L[c]	NS	NS		8, 88–90, 93, 96, 97, 112
βDAβA	22	H	M–L	L	NS			8, 89, 90
Co^{2+}		H	L	L				8, 86, 98
DAA	42	H	L	—[d]	NS	NS	NS	59, 61, 99–103
CPG		H	M	L				—[g]
PDA[f]	54	H	M	M	—[e]		NS	8, 88–90, 93, 104
P_zDA	104	M	M–L	M–L				8, g
HAP		M	VL	VL	NS/M–L[c]	NS	NS	60, 99, 101, 102
DAP[f]	120	M	L	L	NS/L[c]	NS	NS	60, 99, 101, 102
Mg^{2+}		M	L	VL	NS/M[c]	L	VL	98, 105, 106
APB[f]	>200	M–L	M–L	L	NS			60, 87, 94
GDEE	>2000	NS	NS	M	M			61, 94, 103

[a] Abbreviations: K_D, apparent dissociation constant for NMDA-receptor complex (89, 90); NMDA, N-methyl-D-aspartate; KA, kainate; Q, quisqualate; Ch, cholinergic agonists; NE, norepinephrine; sP, substance P; APV, (±)-2-amino-5-phosphonovalerate; DAS, D-α-aminosuberate; DGG, γ-D-glutamylglycine; βDAβA, β-D-aspartyl-β-alanine; DAA, D-α-aminoadipate; CPG, (±)-*m*-carboxyphenylglycine; PDA, *cis*-2,3-piperidine dicarboxylate; P_zDA, (±)-*cis*-2,3-piperazine dicarboxylate; HAP, 3-amino-1-hydroxy-2-pyrrolidinone; DAP, (±)-α,ε-diaminopimelic acid; APB, (±)-2-amino-4-phosphonobutyrate; GDEE, L-glutamic acid diethyl ester; VH, very high; H, high; M, medium; L, low; VL, very low; NS, no significant effect observed.

[b] Other compounds which have been shown to have NMDA antagonist actions but which have not been fully investigated with respect to their actions at other receptors include D-5-amino-hex-2-enedioic acid (107); D-α-aminopimelate, (±)-α,δ-diaminoadipate, (±)-2,6-diaminosuberate, (±)-2,7-diaminoazelaate, (±)-α-aminoazelaate (60); γ-D-glutamylphenylalanine, γ-D-glutamyl-*p*-chlorophenylalanine, γ-D-glutamyl-β-alanine, γ-D-glutamyl-GABA, γ-D-glutamylleucine, β-D-aspartylglycine (8); (±)-2-amino-6-phosphonohexanoate, (±)-2-amino-7-phosphonoheptanoate, (±)-2-amino-8-phosphonooctanoate, β-D-aspartyl-GABA, 4-(β-D-aspartyl)-2,4-diaminobutyric acid, β-D-aspartyltaurine, β-D-aspartylaminomethylphosphonate, β-D-aspartylaminoethylphosphonate.g

[c] Differing assessments, relating to bath/iontophoretic methods of administration.

[d] Responses potentiated (59, 61), possibly due to inhibition of uptake of quisqualate (see Uptake/Inactivation section).

[e] Responses potentiated, probably due to the partial NMDA agonist activity of PDA (88, 89, 93, 104).

[f] The (–) forms are the more active isomers (8, 60).

[g] R. H. Evans, A. A. Francis, A. W. Jones, and J. C. Watkins, unpublished observations.

addition to responses induced by NMDA) whereas responses to quisqualate are relatively insensitive to the dipeptide (93, 96, 97).

What appears to be a relatively pure population of kainate receptors, which may or may not be similar to those within the spinal cord, is present on fibers in dorsal roots of neonatal rats (87, 117). Quisqualate is a much weaker agonist than kainate at these receptors, whereas in the spinal cord itself, quisqualate is the more potent agonist. Such nonsynaptic kainate receptors are effectively blocked by *cis*-2,3-piperidine dicarboxylate (PDA) (88), and less effectively by relatively high concentrations of 2-amino-4-phosphonobutyrate (APB) and GDEE (87).

The Mixed Agonist Action of L-Glutamate and L-Aspartate

L-Glutamate and L-aspartate are mixed agonists in that their actions are depressed by all the antagonists of Table 1, though usually only partially (90). However, responses to L-aspartate are frequently much more sensitive to NMDA blockers, particularly in the spinal cord, than are responses to L-glutamate (59, 60, 86–90, 94, 95, 98–102, 105, 106), whereas L-glutamate is somewhat the more susceptible agonist to the blocking action of GDEE (61, 103, 118, 119). This suggests that, at least for some cells within the spinal cord, NMDA receptors make a greater contribution to the composite responses induced by L-aspartate than to the composite responses induced by L-glutamate, while quisqualate receptors contribute more to the L-glutamate than to the L-aspartate-induced responses. This deduction finds support from the action of DGG in the mammalian spinal cord in vivo, where responses to L-glutamate parallel those to quisqualate in being relatively insensitive to DGG, whereas responses to L-aspartate, like those to NMDA, are easily blocked by the dipeptide (88, 93, 97). L-Glutamate is also an effective agonist at kainate fiber receptors, whereas L-aspartate is a poor agonist at these receptors (87). This finding again points to different efficacies of the two putative transmitters at different receptors.

It must be emphasized that the relative antagonism of responses to L-glutamate and L-aspartate has to be interpreted with caution, as such differential effects will depend not only on the relative efficacies of the agonists at the different receptors mediating the composite response, but also on the relative abundance of the different receptors and the specificity of the antagonist used (90). Moreover, comparison of excitation induced by exogenous amino acids with synaptic excitation is complicated because the two types of excitation may be mediated by different populations of receptors. This would be particularly important if extrasynaptic receptors (e.g. of the kainate fiber type) contribute significantly to the effects of exogenous excitants. For similar reasons, comparison of the relative potencies of L-glutamate and L-aspartate on different groups of cells (120–122) are unlikely to be as informative as using the more selective receptor agonists,

NMDA, kainate, and quisqualate. Large differences in the relative potencies of kainate and NMDA have been found in different groups of spinal neurons (123), suggesting a regional heterogeneity in the distribution of NMDA and kainate receptors in the spinal cord.

The Action of Other Excitants

Most amino acid excitants resemble L-glutamate and L-aspartate in having mixed actions on different receptors. Approximate rank orders for the susceptibilities of a range of agonists to NMDA antagonists and GDEE (61, 87, 94, 103, 110, 124) are shown in Table 2. With the exception of kainate, GDEE shows generally the reverse pattern of activity to NMDA antagonists though its potency is very much lower. Relative agonist potencies for depolarizing the nonsynaptic receptors on dorsal root fibers in the baby rat (87, 117) are also indicated in Table 2.

Attempts have been made to define the structural requirements for interaction with the different receptors by investigating the actions of compounds with conformationally restricted structures. The predominant

Table 2 Relative susceptibilities of excitants to antagonism by NMDA receptor blockers and GDEE, and relative potencies of excitants at depolarizing dorsal root fiber receptors[a]

Relative effect	Susceptibility to		Agonist potency at fiber receptors[b]
	NMDA antagonists	GDEE	
Very high to high	NMDA		Domoate
	NMLA		
	2,3-*trans*-PDA		
	2,4-*trans*-PDA		
	Ibotenate		Kainate
	L-Homocysteate		
	D-Glutamate		
	cis-ADCP		
Moderate	L-HCS	L-Cysteate	L-Glutamate
	D-Homocysteate	L-Glutamate	
	L-Aspartate	Quisqualate	Quisqualate
	D-Aspartate	L-Aspartate	
	L-CSA	D-Glutamate	
	L-Cysteate	Ibotenate	
	L-Glutamate	L-Homocysteate	
Low to undetected	Quisqualate	Kainate	L-Aspartate
	(±)-Willardiine	ADCP	(±)-Willardiine
	Kainate	NMDA/NMLA	NMDA

[a] Abbreviations: NMDA, NMLA, N-methyl-D- and L-aspartate; 2,3- and 2,4-*trans*-PDA, 2,3- and 2,4-*trans*-piperidine dicarboxylate; *cis*-ADCP, *cis*-1-amino-1,3-dicarboxycyclopentane; L-HCS, L-homocysteine sulfinate; L-CSA, L-cysteine sulfinate.
[b] Dorsal root fibers of the neonatal rat.

activation of NMDA receptors by such glutamate analogues as *trans*-2,4-PDA (8, 88–90), ibotenate (60, 61, 87, 94, 103, 110), ibotenate relatives (116) and *cis*-ADCP (110), in addition to the aspartate analogue, *trans*-2,3-PDA (8, 88, 89) (Table 2), and the antagonist actions of the aspartate analogues PDA and P_zDA (Table 1), may be explained by a preference of the NMDA receptor for a relatively extended aspartate conformation and a slightly folded glutamate conformation (8, 88). On the other hand, longer-chain agonists such as L-α-aminoadipate (125), 4-bromohomoibotenate (116), and α-amino-3-hydroxy-5-methyl-isoxazolepropionic acid (AMPA) (116) interact more effectively with non-NMDA (DAA-insensitive) receptors that are sensitive to blockade by GDEE. Whether this should be interpreted in terms of a greater or lesser intercarboxyl separation in the effective conformation of the agonists compared with that preferred by the NMDA receptor is debatable. McLennan (125) has argued in favor of reduced minimum intercarboxyl distances, permitted by the greater flexibility of the longer-chain molecules, as being a factor in the predominant action of these compounds on non-NMDA receptors. No conclusions have yet been drawn with respect to kainate fiber receptors, but an unsaturated side chain may be important for high agonist activity. The ability of *cis*-2,3-piperidine dicarboxylate to antagonize all three classes of receptors (88, 89, 93, 104) suggests that the conformational specificities of the receptors may be very subtle.

Other Excitatory Amino Acid Antagonists

Various other agents have recently been proposed as excitatory amino acid antagonists, but in general their specificity is poor compared with most of the substances described above or has not been fully investigated.

Nuciferine, an alkaloid structurally related to 9-methoxyaporphine, has been reported to block responses to kainate more effectively than those to NMDA (126) but subsequent experiments have failed to confirm this conclusion (127, 128). Its spectrum of action (128) resembles that of GDEE rather than NMDA antagonists. Nuciferine is not selective for amino acid–induced responses and appears to have some local anesthetic properties (129).

SP 111, a tetrahydrocannabinol derivative, was reported by Segal (130) to block L-aspartate-induced excitation of hippocampal cells, and also to potentiate depressant effects of norepinephrine and GABA in the cerebellum. Acetylcholine-induced excitation of hippocampal neurons was less sensitive to the drug.

Thyrotropin-releasing hormone (TRH) has been reported to block L-glutamate-induced responses more effectively than those induced by acetylcholine or L-aspartate (131). However, excitatory effects of this compound have also been reported (132).

Chlorpromazine, diazepam, and amitriptyline all have differential actions on amino acid–induced responses in the frog spinal cord (133), which in general resemble those of the more recently discovered selective NMDA antagonists described in the preceding section. However, the well-known local anesthetic and other effects of these drugs on CNS activity would render them unsuitable as pharmacological tools for the study of amino acid–induced excitation.

Pentobarbitone depresses excitatory amino acid–induced responses in a relatively nonselective manner (133–136). However, this effect is not specific to amino acids since pentobarbitone also depresses acetylcholine-induced responses in the cerebral cortex (137), and cholinoceptor-mediated excitation in sympathetic ganglia (138). The GABA-mimetic actions of pentobarbitone (134, 139–141) would also contribute to its depressant effects.

Depressant Actions of Excitatory Amino Acids

Certain inhibitory effects of acidic amino acids have sometimes been observed [for review, see (3)]. It has been suggested that the ability of ibotenate, unique among a range of excitatory amino acids, to produce long-lasting depression of cat spinal neurons is due to a high affinity of this substance for a specific class of inhibitory receptors (142). An alternative explanation of this phenomenon, however, is that it is caused by the breakdown of microelectrophoretically administered ibotenate to the potent depressant, muscimol (143).

Binding Studies

Following the pioneering work in this area by Roberts (144) and Michaelis (145) using [^{3}H]L-glutamate, and by Simon et al (146) using [^{3}H]kainate, numerous studies have been carried out on the binding of these and other ligands to brain membranes. Such studies have been reviewed by Coyle (147), Johnston (148), Nistri & Constanti (3), and Roberts & Sharif (149). Recent investigations in various areas of the central nervous system include the use of [^{3}H]L-glutamate in cerebral cortex (144), striatum (150), cerebellum (149, 151, 152), hippocampus (153–156), multiple regions (157); [^{3}H]kainate in striatum (158–160), retina (161), cerebellum (162), multiple regions (163), different tissues and organisms (164); [^{3}H]L-aspartate (149) and [^{3}H]N-methyl-D-aspartate (165) in cerebellum; and [^{3}H]D-aspartate in cerebral cortex (148). In general, the results have not yet led to a clarification of the types of excitatory amino acid receptors and their functional significance. Also it must be emphasized that the presence of receptor sites does not necessarily indicate the presence of a transmission system. For instance, kainate (87, 117) and GABA receptors (166) are known to occur at extrajunctional sites. Here we summarize some of the main conclusions to emerge from this work.

[^{3}H]L-GLUTAMATE BINDING Two types of binding sites can be differentiated. Na-dependent binding appears to be associated with sites of uptake of excitatory amino acids (144, 150, 156), while Na-independent sites may be associated with one or more types of pharmacological receptors (144, 147, 149, 151, 156, 157). A detailed investigation of the distribution of the two types of binding sites has been made, the hypothalamus and cerebral cortex showing the highest concentrations of the Na-independent and Na-dependent binding sites, respectively (157). Inhibition studies suggest differences in the structural specificities of Na-independent sites in different regions (144, 147, 149, 151, 156, 157), possibly as a consequence of variation in the proportions of the pharmacologically distinct types of excitatory amino receptor discussed above but possibly also because of methodological differences in membrane preparation (166). However, while quisqualate sometimes inhibits L-glutamate binding (151, 157), inhibition by NMDA or kainate has never been observed. L-Aspartate has usually been found to be a moderate to very effective inhibitor, though D-aspartate and D-glutamate [which are similarly potent to the L forms as excitants, ref. (2)] are usually very weak. Among excitatory amino acid antagonists, DL-α-aminoadipate and APB show moderate activity as inhibitors of L-glutamate binding, but GDEE is very weak (151, 156, 157).

Baudry and colleagues (153, 154) have shown that the divalent cations Ca^{2+} and Mn^{2+} enhance binding of L-glutamate to hippocampal membranes and that this effect is associated with activation of a protease enzyme (153). Ca-dependent binding is very much more pronounced in the hippocampus than in the cerebellum (153). Sodium ions inhibit binding of L-glutamate to hippocampal membranes at low concentrations and potentiate binding at higher concentrations (154, 156), the latter effect presumably being a reflection of binding to uptake sites. Potassium ions enhanced binding at low (< 5 mM) and inhibited binding at higher concentrations. The relationship between these K^+ ion effects on glutamate binding and the effects of K^+ ions on uptake of the amino acid (see section on Uptake/Inactivation) remains to be elucidated.

Using low concentrations of the ligand, Biziere et al (157) recognized additional Na-independent binding sites for L-glutamate with K_D values of 11 and 80 nM. The inhibitor pattern for this very high affinity glutamate binding appeared not to be markedly different from that of the "usual" high affinity mode (K_m 0.1–1 μM). Marked regional variation was observed, but in general the pattern of very high affinity binding resembled that of Na-dependent rather than Na-independent binding observed in other studies (156).

Michaelis (169) isolated a glutamate-binding glycoprotein from whole brain synaptosomal membranes which shows similar kinetic and inhibitor

characteristics to those of binding sites for glutamate in the original membranes. This glycoprotein contains Fe which may be involved in coordination complexes with excitatory amino acid ligands. Both the native binding sites in synaptic membranes and the isolated glycoprotein were inhibited by iron-bonding agents such as azide. However, Sharif & Roberts (152) have shown that pretreatment of cerebellar membranes with azide potentiated rather than depressed the binding of L-glutamate. It is interesting that 6-hydroxyDOPA, which is an excitatory amino acid (113), inhibits the binding of glutamate to the glycoprotein, which raises the possibility that both effects may be related to the ability of this substance to coordinate with iron (169, 170).

Michaelis et al (171) have shown that ethanol and chlorpromazine both cause enhancement of L-glutamate binding to brain synaptic membranes. They suggest that this enhancement may reflect effects of these drugs on receptor-ionophore coupling. A proteoplipid isolated from brain by De Robertis & Fiszer de Plazas (172, 173), which stereoselectively bound L-glutamate and had considerably lower affinity for L-aspartate, appears not to have been further studied.

[^{3}H]L-ASPARTATE BINDING While the kinetic parameters for the Na-independent binding of this ligand to cerebellar membranes are not greatly different from those for L-glutamate, marked differences occur in the inhibitor pattern for the two ligands (149). In particular the NMDA antagonists DL-α-aminosuberate and HAP were effective and selective inhibitors of the aspartate binding. However, another NMDA antagonist, DL-α-aminoadipate, was less effective on L-aspartate than on L-glutamate binding while NMDA itself was devoid of inhibitory activity. Thus it seems unlikely that this binding site is the NMDA receptor, although some of the above anomalies may be due to the use of racemates rather than pure optical isomers. Moreover, Ramirez et al (174) have reported that while NMDA had little effect on [^{3}H]glutamate binding to chick retinal membranes, it had different effects on [^{3}H]L-aspartate binding in this preparation according to the protein concentration in the assay system, marked inhibition of aspartate binding occurring at low protein concentrations, and enhancement of binding occurring at high protein concentrations. The important implication of this phenomenon with respect to the constitution and stability of the L-aspartate binding sites warrants detailed investigation.

The inhibitor pattern for L-aspartate binding sites in brain membranes shows similarities to and differences from the aspartate-binding proteolipid previously extracted from rat cerebral cortex by De Robertis and colleagues (172) and the relation between these two entities remains to be established.

[^{3}H]KAINATE BINDING Sodium-independent binding sites for this ligand are distinct from those for L-glutamate or L-aspartate since kainate is not an inhibitor of the binding of either of the latter two amino acids (149, 151, 157). This accords with the difference between pharmacologically defined receptors for kainate and those for glutamate or aspartate as described above. A difference between binding sites for kainate and L-glutamate is further emphasized by the finding that kainate binding was depressed by 50% in membranes treated with Na cholate whereas L-glutamate binding was moderately increased (175). It has been estimated that the number of binding sites for L-glutamate exceeds those for kainate by approximately 10-fold (57, 146). L-Glutamate and quisqualate can apparently interact with kainate binding sites to a moderate extent, but L-aspartate is a very weak inhibitor and NMDA is inactive (57, 146, 157, 158, 162, 163). A number of ions inhibit kainate binding in the striatum, Ca^{2+} and Mn^{2+} being the most potent with IC_{50} values of around 2 mM (159).

London & Coyle (163) have recognized two binding sites for kainate with K_D values of approximately 10 and 40 nM, inhibitor patterns being generally similar for both sites but with some exceptions. The higher affinity sites are most concentrated in striatum and the lower affinity sites in the cerebellum and medulla/pons.

A physiological role of kainate receptors is suggested by the discovery that high affinity kainate binding to striatal membranes increased dramatically during maturation of animals from birth (176).

[^{3}H]D-ASPARTATE BINDING Preliminary studies on the Na-independent binding of this ligand to triton-extracted rat brain membranes suggest that these binding sites have an affinity for NMDA, L-aspartate, L-glutamate, and DL-α-aminoadipate; these substances inhibit [^{3}H]D-aspartate binding with that descending order of potency (148). Such an order of inhibitory action, particularly the potency of NMDA, which has been found to interact with amino acid binding sites in only two other studies (165, 172), suggests the possibility that D-aspartate binding sites are NMDA receptor sites. However, except for the action of Mg^{2+}, which has been reported to inhibit the excitatory responses of D-aspartate (105), this amino acid generally resembles L-glutamate rather than NMDA with respect to the pharmacological profiles of their excitatory actions (60, 94, 103).

[^{3}H]NMDA BINDING This ligand has been reported to bind to cerebellar membranes with an inhibitor spectrum similar to that which would be expected if the binding sites and NMDA receptors were the same (165). However, other studies have failed to confirm these effects (H. J. Olverman

and J. C. Watkins, unpublished observations), and in view of the observation of Ramirez et al (174) described above, certain difficulties may be encountered in the study of membrane binding sites for this substance.

In conclusion, it is difficult to compare excitant potency of agonists with chemical binding data because excitant responses are a function of efficacy and affinity whereas binding reflects affinity only. This is not a complication in the case of antagonists where chemical binding constants should compare directly with pharmacological values calculated from dose ratios (see 177). It is therefore to be hoped that future studies with radiolabeled antagonists will resolve some of these problems.

MECHANISMS OF ACTION

Agonists

MEMBRANE CONDUCTANCE There is considerable evidence supporting the concept that excitatory amino acids cause neuronal depolarization by increasing the membrane permeability to sodium ions (2–4, 39). However, this concept implies that amino acid–induced depolarization should be accompanied by an increase in membrane conductance, as shown for synaptic activation of cat spinal neurons (178). While this is true for some amino acids, particularly kainate (179, 180), membrane conductance may be either increased or decreased by L-glutamate or L-aspartate (179–184). The effect of L-glutamate is usually to increase membrane conductance at high concentrations of the amino acid whereas low concentrations may cause a decrease (181, 182). Only a decrease in membrane conductance has been observed with NMDA (179, 180). D-Homocysteate likewise causes a decrease in membrane conductance, but L-homocysteate produces a conductance increase (135, 182). The increases in membrane conductance observed with L-glutamate, L-aspartate, and L-homocysteate may be associated with uptake of these amino acids (41, 182); however, such an explanation is unlikely to apply to kainate, which is not taken up by central nervous tissue (57). It is therefore probable that these effects involve not only differential ionic concomitants of uptake but also different ionic mechanisms of receptor activation. In particular, NMDA receptor activation may be associated with a decrease in membrane K^+ conductance as suggested for DL-homocysteate (182), which acts substantially on NMDA receptors (60). Shapovalov et al (184) attribute the conductance changes induced by L-glutamate in amphibian motoneurons to an increase in Na^+ conductance and a decrease in K^+ conductance, while Engberg et al (182) have also suggested this mechanism for the excitatory postsynaptic potentials of cat motoneurons. Application of L-glutamate or synaptic excitation

of lamprey reticulospinal neurons increases permeability to both Na^+ and K^+ (185). It is possible that the extent of the Na^+ and K^+ conductance changes produced by different excitatory amino acids varies with the type of receptor activated, or even with the mode of interaction of different amino acids with the same receptors. Another possibility is that different amino acids (or activation of different receptors) may produce different effects on Ca^{2+} fluxes (186), which could indirectly affect monovalent ion transport (66).

However, such conductance measurements are usually recorded through electrodes placed in the somata of motoneurons, whereas the electrophoretically applied agonists can act at sites near to or distant from the somatic recording site. Thus if, for example, NMDA receptors were distributed only at excitatory synaptic locations [which are considered to be mainly dendritic (187)], but receptors with which L-glutamate interacted were distributed more widely, such a differential receptor distribution would be expected to complicate the measurement, at somatic sites, of conductance changes induced by these two amino acids. It may be relevant that decreased membrane conductance of leech neurons following periods of intense activity has been attributed to electrogenic pump action (188). Possibly NMDA, by acting discretely at dendrites, mimics such activity and the decreased conductance observed at motoneurons (179) may reflect an electrogenic response at the soma to ionic changes, possibly Na^+ entry, at dendrites.

Suggested decreases in neuronal K^+ conductance produced by excitatory amino acids (182, 184) are hard to correlate with the marked increases in extracellular K^+ concentration that occur in association with excitatory amino acid action (189, 191, 192) irrespective of the type(s) of receptors activated, although extracellular K^+ accumulation could well be due, at least partially, to passive redistribution of these ions in response to the neuronal depolarization, as occurs with the late phase of synaptically evoked primary afferent depolarization (193, 194). Extracellular K^+ accumulation has been postulated to explain excitatory amino acid–induced depolarization of primary afferent terminals (189) as against a direct action of the amino acids on presynaptic receptors (190). Such excitant-evoked increases in extracellular K^+ may also be relevant to the variable effects of excitants on membrane conductance (179, 180, 182). For instance, the initial stages of potassium depolarization might be expected to be associated with decreased membrane conductance through the mechanism of anomalous rectification. Lambert et al (182) claim that excitant-induced decreases in conductance cannot be entirely accounted for by anomalous rectification, but very recent results from their laboratory have indicated large differences between "effective" transmembrane potential changes and potential

changes measured conventionally with a distant indifferent electrode (195, 196). This phenomenon introduces a serious problem in the measurement of membrane current/voltage relationships. Maintained depolarization has been shown to increase conductance in the squid giant axon (197) and in spinal motoneurons (198), and high levels of increased external K^+ have been shown to cause a large increase in membrane conductance of snail neurons (199). Thus a possible role of K^+-induced potential and conductance changes in the observed action of excitant amino acids should not be discounted.

EFFECTS OF EXCITANTS ON CYCLIC NUCLEOTIDE SYNTHESIS

Stimulation of both cyclic AMP and cyclic GMP synthesis by excitatory amino acids has been observed in brain slice preparations (200–208). These effects are Ca^{2+}-dependent (200) and since other depolarizing agents have similar actions (204, 207) it is likely that the observed stimulation by excitatory amino acids is secondary to depolarization, possibly involving Ca^{2+} influx. Although it has been argued that similar stimulation of cyclic nucleotide synthesis by high concentrations of K^+ in the extracellular fluid may be due to release of excitatory amino acids and adenosine (207), the reverse interpretation would seem equally possible, i.e. that the effects of the amino acids are due to K^+ release (189, 191, 192). Stimulation of cyclic GMP production in cerebellar slices (200, 209–214) (for review see 212) depends on the morphological integrity of the tissue, which again suggests that such effects are secondary to depolarization, rather than involved in the depolarization mechanism itself. However, it is noteworthy that low concentrations of guanine nucleotides inhibit the binding of [^{3}H]L-glutamate to cerebellar membranes (213), suggesting a possible association between guanyl cyclase and the receptor.

Although probably secondary to depolarization such stimulation of cyclic nucleotide production by excitatory amino acids provides a useful chemical means of studying differential receptor activation. In the immature cerebellum, NMDA is the most potent excitant for stimulating cGMP production while kainate is relatively weak. In the adult animal, however, kainate is the more potent stimulator, the difference being attributable to the development with age of a new population of discrete kainate receptors, an effect reminiscent of the increase in striatal binding sites for kainate during development (176). Kainate-induced cGMP formation in the adult is resistant to NMDA blocking agents, while in the immature animal the effects of both NMDA and kainate are blocked by such antagonists, suggesting that, in the immature animal either both agonists act at the same (NMDA) receptors (though with markedly different efficacies), or the effect of kainate may be relayed transynaptically via a transmitter acting at

NMDA receptors. L-Glutamate- and L-aspartate-induced cGMP production is not affected in either the adult or immature cerebellum by NMDA blockers, suggesting that the putative transmitters interact predominantly with receptors that are distinct from both kainate and NMDA receptors. It will be important to determine in future studies whether any correlation exists between these receptors and the quisqualate-type receptors distinguishable pharmacologically and discussed above. A possible indication of such a relationship is the sensitivity of the L-glutamate-induced stimulation of cGMP production to blockade by GDEE (214).

Similar results have been obtained with cerebellar cultures with the exception that L-glutamate and L-aspartate were markedly more effective (100- and 20-fold respectively) in this preparation than in slices. Such enhanced effects are probably attributable to the avid uptake of the amino acids in slices, which would be expected to limit their access to receptors (41, 57, 215).

Antagonists

The action of monoamino and diamino dicarboxylic acids related to D-α-aminoadipate and α,ε-diaminopimelate appears to be competitive with agonists for the NMDA receptor (60). Intracellular recording in cultured mouse spinal neurons showed no effect of DL-α-aminoadipate on membrane potentials or passive membrane properties (216). A similar lack of effect on membrane parameters has been observed in hippocampal neurons with DGG (217). Thus it appears that the organic NMDA antagonists act simply by blocking access of the agonists to the receptors and do not themselves induce potential or conductance changes.

Mg^{2+} and the organic antagonists act at different membrane sites (218). The divalent cations probably act by decreasing the affinity of the agonist for the receptor or by interfering with receptor-ionophore coupling (86). Alterations in extracellular Mg^{2+} concentrations associated with synaptic activity may regulate the sensitivity of the NMDA receptor to an excitatory amino acid transmitter (86).

ROLE OF AMINO ACID RECEPTORS IN SYNAPTIC EXCITATION

There is little doubt that NMDA receptors are involved in spinal synaptic excitation (8, 88). Thus, for series of selective NMDA antagonists, depression of dorsal root–evoked excitation of motoneurons in the frog spinal cord parallels depression of amino acid–induced depolarization (60). Again, all known NMDA antagonists, irrespective of their chemical specificity, have the ability to depress synaptic excitation in the mammalian and amphibian

spinal cords (60, 88–94, 96, 97, 104, 111, 112). Synaptic excitation has also been antagonized by these agents in other regions of the central nervous system: cerebral cortex, cuneate nucleus (219), caudate nucleus (220); lateral geniculate body (221); hippocampus (217, 222); cochlear nucleus (223, 224); cerebellum (225). The role of these studies in transmitter identification at specific synapses is described below.

It is less certain that either kainate or quisqualate receptors are also involved in synaptic excitation. Although the quisqualate antagonist GDEE has been found to depress synaptic excitation in several regions [cerebral cortex (226); thalamus (118); striatum (220, 227); lateral geniculate body (221); hippocampus (217, 222, 228, 229)], its lack of specificity as an amino acid antagonist (61, 94, 221), its relatively low potency, and its effect on spike height at higher concentrations (103) make this substance of somewhat doubtful value for transmitter receptor characterization. Intracellular investigation (230, 231) has raised additional questions as to the mode of action of GDEE. Preliminary studies with DGG and PDA have, however, provided supporting evidence that quisqualate and/or kainate receptors may be involved in synaptic excitation in addition to NMDA receptors. Thus APV- or DAA-insensitive synaptic excitation in the spinal cord (89, 93) or caudal trigeminal nucleus (232, 233) has been shown to be sensitive to DGG and/or PDA.

TRANSMITTER IDENTIFICATION IN SPECIFIC PATHWAYS

Problems associated with transmitter identification within the vertebrate central nervous system have been discussed by Curtis (4). Among the many criteria that would need to be fulfilled, a transmitter candidate would need to be shown to be released from the endings of afferent fibers and, when added exogenously, to exert an identical action at postsynaptic sites to that produced by afferent stimulation.

The release criterion would seem extremely difficult to fulfil with present techniques in the case of short axon interneurons that cannot be stimulated selectively. Less difficulty would be expected in demonstrating release of putative transmitter candidates from the central endings of peripheral afferent fibers or from the terminals of well-defined tracts within the central nervous system, particularly if specific inhibitors of uptake can be found. Dihydrokainate may prove useful in this respect (see Uptake/Inactivation section).

The identity of action criterion is also difficult to meet, especially where an exogenous transmitter may activate different receptors from those activated by the endogenous transmitter released synaptically. Thus, the vari-

able increase or decrease in membrane conductance produced by exogenous L-glutamate (discussed above) may reflect actions at different receptors in differing proportions according to concentration and/or locus of action. Nevertheless, this criterion may be at least partially satisfied by the use of receptor-specific antagonists. Several NMDA antagonists (especially APV, which has a specificity and potency for NMDA receptors probably higher than that of bicuculline for GABA receptors) would appear to be satisfactory for characterizing the action of a transmitter at specific synapses as being similar to or different from that produced by NMDA. Similarly specific antagonists are likewise needed for exogenous agonists such as quisqualate and kainate in order to determine whether any endogenous transmitter acts at these types of receptors. It must be emphasized, however, that similarity of receptor activation by exogenous candidate and endogenous transmitter identifies the receptor rather than the transmitter. Discrimination between mixed agonists such as L-glutamate and L-aspartate is difficult if not impossible by pharmacological means alone and such techniques are best employed in conjunction with lesion studies in order to deduce which of these two amino acids is the more likely transmitter released at specific synapses.

Lesion Studies

A major technique employed in neurochemical studies is to destroy discrete regions of central nervous tissue and to compare chemical parameters before and after this procedure. The lesions may be of various types, for instance, the surgical sectioning of specific nerves or tracts, ablation of whole areas or nuclei, neuronal destruction with potent "excitotoxic" amino acids such as kainate, which spares axons of passage and synaptic endings of extrinsic neurons, the use of X rays to destroy specific populations of cells in the cerebellum, viral destruction of discrete cerebellar cell types, 3-acetylpyridine–induced destruction of climbing fibers in the cerebellum and controlled ischemia to selectively destroy interneurons in the spinal cord; in addition, the neurochemistry of mutant animals with well-defined neurological deficits has been compared with that of normal animals (for references, see Table 3).

The aim of such studies—whatever the precise nature of the lesions employed (or of the neuropathology in mutants)—is to correlate the neurochemical differences that occur between normal and lesioned (or mutant) tissue, with the reduction of specific cell types or afferent fibers and endings. Thus, if presynaptic terminals are caused to degenerate, their transmitter stores might be expected to be depleted along with enzymes of synthesis. Moreover, where reuptake into terminals is a major factor in transmitter inactivation and/or conservation, uptake into the synaptosomal fraction prepared from the area of terminal degeneration should be depleted. This

is sometimes investigated using [^{3}H]D-aspartate (234) which is taken up similarly to L-glutamate and L-aspartate but which is metabolically more stable after uptake (65). Finally, Ca^{2+}-dependent release of the putative transmitter evoked by depolarizing stimuli should be diminished. Where lesions cause postsynaptic degeneration without loss of afferent terminals the main change might be expected to be a decrease in transmitter receptor sites—hopefully reflected in a decrease of specific binding of radioactively labeled transmitter agonists or antagonists. The main difficulties in interpretation of the results of such studies include the following:

1. L-Glutamate and L-aspartate are so intimately involved in cellular metabolism that neurochemical changes occurring as a result of lesions could be due simply to a metabolic response to injury in the short term and to altered densities of cell types, particularly glia, in the longer term.

2. Not only presynaptic but also postsynaptic lesions would alter uptake and release of putative transmitters, in so far as cell bodies are able to participate in such phenomena (43–45) and/or where a significant density of short axon cells are contained within the lesioned area.

3. Ca^{2+}-dependent release, which is usually regarded as having a synaptic terminal origin (21), has been observed from glia in dorsal root ganglia (67) and such release may therefore not be confined to terminals or even to neurons.

4. The identity of kinetic and/or structural parameters of binding sites in degenerating or regenerated tissue with those of unlesioned tissue cannot be assumed; for instance, desensitization or changes leading to supersensitivity may have taken place, or even changes in the concentrations of modulatory components of the receptors.

5. Where radioactive L-glutamate, L-aspartate, or D-aspartate are used to study changes in uptake following lesions, no deductions can be drawn about whether the transmitter used by the degenerated terminals is more likely to be L-glutamate or L-aspartate, since all three amino acids are apparently transported by the same systems (40, 65).

6. The specificity of the lesions, be they produced by surgical section of specific tracts, or by different types of physical, chemical, or biological damage, may not be known with certainty. For instance, the extent of kainate-induced lesions has been found to depend, in certain cases, on the integrity of afferent systems to the lesioned area (160, 235–237) thus implicating presynaptic mechanisms in the excitotoxic action. Furthermore, lesions in the limbic system can occur at sites distal to the kainate injection (237). In some cases distant damage may relate to diffusion of kainate from the original injection site (238). Thus kainate should be used with caution as a selective lesioning agent. For a detailed account of problems in the use of kainate as a selective lesioning tool see reference (239).

These difficulties notwithstanding, considerable information pertinent to

the special chemical properties of relatively discrete cell populations and/or synaptic pathways has been adduced from such studies. When collated with pharmacological data, a strong case can be made in favor of L-glutamate- or L-aspartate-mediated synaptic excitation in many regions of the central nervous system. A compilation of such data is presented in Table 3. [For references to the excitation of neurons by L-glutamate and L-aspartate in these and other regions of the central nervous system see (7).]

CONCLUDING REMARKS

With the advent of selective antagonists for amino acid–induced excitation, the long postulated role of acidic amino acids (or very similar substances) as transmitters in synaptic excitation in the vertebrate central nervous system can no longer be seriously doubted. As shown by Table 3, blockade of synaptic excitation by specific amino acid antagonists has now been demonstrated in a number of specific pathways. This adds compelling support to a wealth of neurochemical evidence, which by itself could not be regarded as definitive.

In future studies, it should not prove difficult to demonstrate whether or not particular receptors mediating synaptic excitation are of the NMDA or non-NMDA type. The involvement of NMDA receptors in spinal synaptic excitation is already well documented and it will be important to investigate their role elsewhere in the central nervous system. To this end the NMDA antagonist of choice is APV. Pending the discovery of selective antagonists for non-NMDA receptors, excitation that is resistant to APV but depressed by DGG and/or PDA can probably be assumed to be mediated by the kainate or quisqualate type of receptors.

Identification of the transmitters acting at different excitatory amino acid receptors is likely to be a more difficult problem. This may ultimately depend on whether consistent correlations can be obtained between strong neurochemical evidence favoring particular transmitters in defined pathways and discrete pharmacological profiles for synaptically evoked excitation in those pathways. The strongest neurochemical evidence for an acidic amino acid transmitter is probably that obtained by Collins (259, 280) for aspartate as the transmitter released by terminals of the lateral olfactory tract (see Table 3). The pharmacology of this pathway is currently under investigation. High sensitivity to APV of monosynaptic excitation evoked by stimulation of the lateral olfactory tract would add support to the suggestion (100) that L-aspartate may be the transmitter acting at NMDA receptors.

Problems associated with the binding of L-glutamate and L-aspartate to brain membranes may be diminished by the likely development of highly

Table 3 Evidence supporting transmitter roles for aspartate or glutamate in specific pathways

CNS region or system	Proposed transmitter	Synaptic release	Lesion study				Antagonism of synaptic excitation[a]
			Type of lesion	Reduction in			
				Endogenous level	Stimulated release	Uptake	
Cerebral cortex							
Afferent pathways	GLU	240, 241					220 (DAA), 226 (GDEE)
Efferent pathways	GLU		Cortical ablation	234 (thalamus) 244 (striatum) 234, 245 (lateral geniculate body and superior colliculus) 246 (inferior colliculus)	242, 243 (striatum)	63, 158, 235, 247 (striatum[b]) 234 (amygdala, (substantia nigra & nucleus accumbens) 234 (thalamus) 234, 245 (lateral geniculate body and superior colliculus)	227 (GDEE, striatum) 226 (GDEE, cortex) 219, 220 (HAP, DAA, cortex, thalamus, cuneate nucleus)
Basal ganglia							
Striatum, intrinsic	GLU		Kainate		248	235, 248	
Hippocampus							
Pyramidal cell projection to lateral septum	GLU	249[c]	Kainate or axotomy	250, 251		250, 251, 293	
Perforant path	GLU	252, 292	Ablation of entorhinal cortex	253	32, 254	64, 253	222, 229 (GDEE) 255, 256 (APB, HAP)

Table 3 *(Continued)*

CNS region or system	Proposed transmitter	Synaptic release	Lesion study: Type of lesion	Lesion study: Reduction in: Endogenous level	Lesion study: Reduction in: Stimulated release	Lesion study: Reduction in: Uptake	Antagonism of synaptic excitation[a]
Hippocampus *(cont'd)*							
Schaffer collateral-commissural path	ASP/GLU	257, 258[c]	Ablation of contralateral hippocampus or axotomy		254	64	217 (APV, DGG) 222 (DAA) 228 (GDEE) 256 (APB, HAP)
Olfactory							
Lateral olfactory tract	ASP	259[d]	Olfactory bulbectomy	259, 280	259		260 (APB, GDEE, DAA)
Visual[e–h]							
Retinal path to optic tectum[i]	ASP/GLU		Retinal ablation			261	262 (GDEE, nuciferine)
Auditory[j]							
Primary afferent to cochlear nucleus	ASP[k]/GLU		Cochlear nerve section	263–265			223, 224 (DAA, DAS, HAP)
			Cochlear ablation[l]	27	289		
			Mutant	266			
Cerebellum							
Granule cell parallel fibers	GLU		X ray	267–269	270	268	225 (DAA)
			Virus	271		271	
			Kainate	272, 273		272	
			Mutant	274			

Cerebellum *(cont'd)*				
Climbing fibers	ASP	3-Acetyl pyridine	275–277	
Spinal cord[m]				
Descending tracts	ASP[n]	Transection	278	
Excitatory interneurons	ASP	Ischemia	279, 291	100, 111 (DAA); 94 (DAS, APB); 91, 92 (APV); 96, 97, 112 (DGG); 60, 104 (PDA); see also 88, 93

[a] For antagonist abbreviations see Table 1.

[b] Development of high affinity uptake system for glutamate correlates with ontogeny of corticostriatal pathway (176).

[c] Preloaded D-aspartate.

[d] Glutamate release from olfactory cortex following stimulation of lateral olfactory tract (54, 281) has been attributed by Collins et al (290) to release from interneurons rather than terminals of afferent fibers.

[e] Visual pathway stimulation of rat lateral geniculate neurons is blocked by excitant amino acid antagonists (221).

[f] APB selectively blocks "ON" responses in mudpuppy retina (M. M. Slaughter and R. F. Miller, manuscript submitted).

[g] Light stimulation of retina depresses L-aspartate, but not L-glutamate, release suggesting that L-aspartate is a retinal transmitter (282).

[h] DAA blocks photoreceptor transmission to horizontal cells in carp retina (283).

[i] Pigeon.

[j] Glutamate released specifically from toad skin containing lateral line organs following water-jet stimulation suggests that glutamate may be the vestibular hair cell transmitter (284, 285).

[k] Aspartate concentrations parallel cochlear nerve terminal distribution (25, 26).

[l] Glutaminase and aspartate aminotransferase are concentrated in cochlear nerve and are decreased after cochlear ablation (30).

[m] No evidence is presented for the primary afferent transmitter to spinal neurons. Although regional variation in spinal cord glutamate levels parallels the distribution of primary afferent terminals (24), synaptically evoked release of glutamate (287) and antagonism of synaptic excitation (102) fail to differentiate convincingly between the activity of primary afferent terminals and excitatory interneurons. A lesion study has provided anomalous data in that a reduction in glutamate uptake following axotomy was accompanied by increased levels of the amino acid (288). However, reduction in glutamate uptake following brain stem lesions suggests that glutamate or aspartate is the transmitter of baroreceptor afferents (294).

[n] Stimulation of descending tracts caused increased release of glutamate (286).

potent, receptor-specific antagonists in radioactive form. Such agents would be useful not only to demonstrate regional variation in excitatory amino acid receptor sites but conceivably also for the development of new drugs acting at these sites, and for investigating the extent to which known drugs may exert their effects by competing with excitatory amino acid transmitters for receptor occupancy.

It is more than two decades since excitatory amino acids first emerged as possible transmitters in the vertebrate central nervous system. With the question of whether or not such substances do indeed exercise this function now apparently answered beyond reasonable doubt, and with an armory of new pharmacological and neurochemical tools increasingly available, our knowledge and understanding of this type of central synaptic excitation seems likely to be entering a phase of rapid growth.

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