GLUTAMATE TRANSMITTER IN THE CENTRAL NERVOUS SYSTEM

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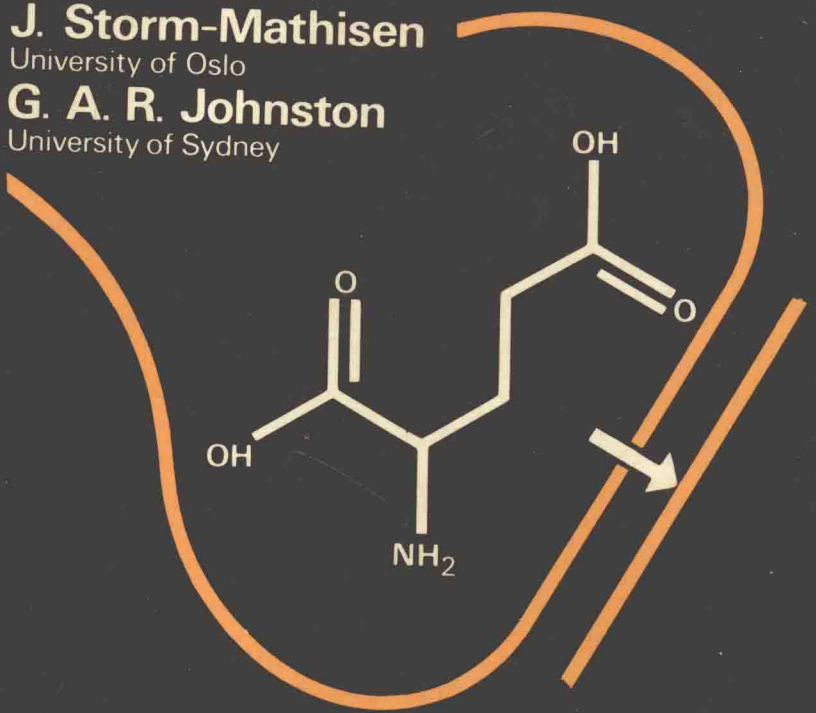

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Preface

It is now 20 years since Curtis *et al.* (1960) first demonstrated the excitation of single neurons in the CNS by L-glutamate. One might imagine that these potent and clear-cut excitatory properties would long have made this substance a favoured neurotransmitter candidate. However, its ability to affect almost all neurons, as a relatively non-specific excitant, has in the past been invoked as strong evidence to the contrary, rather than suggesting that glutamate fibres might constitute the main afferent and efferent pathways within the mammalian central nervous system.

Neurochemical studies aimed at delineating a possible transmitter role for glutamate have been fraught with difficulties because of the multitude of roles this amino acid performs. For example, it is involved in energy metabolism, fatty acid synthesis, the regulation of ammonia levels; it is incorporated into protein and peptides, it is a precursor for certain cofactors such as folic acid and glutathione, and, in addition to its own likely transmitter role, is the major precursor for GABA in the CNS. Not surprisingly therefore, the concentrations of glutamate found in nervous tissue are higher than those of any other amino acid in adult brain, and rather evenly distributed throughout the different areas. This clearly presents major difficulties with regard to investigating the role of glutamate as it relates specifically to synaptic function, and it is only very recently that convincing evidence has been forthcoming, defining certain specific pathways in the CNS.

The current impetus to the increased interest in glutamate as a transmitter, may perhaps be attributed to three major factors. Firstly, the discovery and development of a number of relatively potent and selective excitatory amino acid antagonists (although better drugs are still wanted) dispel the notion of non-specific excitatory actions. Secondly, kainic acid (a conformationally-restricted cyclic analogue of glutamate) has been introduced and widely used as a tool for the selective lesioning of neurons by virtue of an 'excitotoxic' action allegedly mediated through a subpopulation of receptors for glutamate. Thirdly, the neurochemical and autoradiographic detection of glutamate neurons has been made possible by the introduction of high-affinity membrane transport of L-glutamate and D-aspartate as a marker, combined with denervation procedures. Subsequently, results with the latter approach have been substantiated by the application of *in vitro* techniques for the study of synaptic release of exogenous, *in situ* synthesized, and endogenous glutamate.

The finding that the intrastriatal injection of kainic acid (and glutamate itself at high doses) in rats, evokes biochemical changes analogous to those observed in caudate nuclei from post-mortem brains of Huntington's chorea patients, has led to consideration of the possibility that abnormal function of glutamate neurons, or the associated postsynaptic receptors, might produce the cell death which occurs in this condition. Indeed, the striatum receives a massive afferent input from the frontal cortex, and there is good evidence that this releases glutamate from the axon terminals. In addition to neuropharmacological evidence with the newer antagonists, convincing neurochemical evidence based on lesioning experiments supports this proposed role for glutamate. Hemidecortication results in a profound reduction of high affinity striatal synaptosomal glutamate uptake and a smaller, but completely specific decrease in the calcium-dependent release of either radiolabelled, or endogenous glutamate from striatal slices. Similar studies have been performed in several other regions of the central nervous system.

An elegant approach recently introduced by Cuénod and his colleagues is the utilization of the phenomenon of fast axonal transport of ^{3}H-labelled D-aspartate, a metabolically inert substrate for the glutamate membrane transport carrier. After microinjections in the terminal areas, perikarya of glutamate neurons are labelled by retrograde axonal transport. Conversely, terminals are labelled by anterograde transport after injections in the cell body areas, and can be brought to release their D-aspartate content on stimulation. These data have implications also for the way in which the transmitter amino acid is stored in the neurons, since fast axonal transport probably requires it to be sequestered in subcellular organelles, e.g. synaptic vesicles.

An important question to be answered is whether the release of glutamate from nervous tissue can be correlated with physiological neuronal activity. *In vivo* release studies are notoriously difficult to execute, but one of the most convincing findings has been that of a selective release of glutamate from the visual cortical surface during photic stimulation. Other approaches have involved *in vitro* systems, in particular hippocampal and olfactory bulb slice preparations, which remain electrically and biochemically viable for many hours, and where release studies can be carried out with concurrent determination of synaptic activity.

At the present time, our knowledge is still not sufficient to say with any certainty what the main determinants are in the synthesis of transmitter glutamate, despite a wealth of studies on the compartmentation of glutamate and its associated metabolic enzymes. It does seem clear that functional glutaminase occurs preferentially in neurons, and that there is, perhaps, a net transfer of glutamine from a glial to a neuronal compartment, whereupon conversion to glutamate rapidly occurs. Whether this is associated specifically

with the transmitter pool has yet to be resolved; however, incubation of brain slices with labelled glutamine, and their subsequent depolarization, results in a calcium-dependent release of glutamate. The situation may be rather different *in vivo* however, and glucose may play a much more central role as precursor than has been implied from *in vitro* studies.

Neurochemical approaches are also being used for investigating the post-synaptic glutamate receptors. For example, binding assays have been developed for glutamate and kainic acid, and indeed a glutamate binding protein has now been isolated, purified, and partly reconstituted in artificial membranes. Glutamate is also a potent activator of cyclic GMP synthesis, and while this effect may not be related directly to the fast neuronal response associated with glutamate, it might be involved in modulation of the physiological response.

Today, glutamate research has reached the point occupied by GABA, approximately 10 years ago. Progress has been hampered by a combination of factors: lack of suitable techniques and pharmacological tools, and the sheer complexity of glutamate's role in the nervous system. More refined data are needed to prove that the synaptically released transmitter is, indeed, identical with glutamate. Rapid advances are now likely to be made in the demonstration of many more pathways which may utilize glutamate as their transmitter, and the elucidation of interactions with other neuronal systems.

At present, we still know very little about the overall functioning of glutamate in the CNS, and the behavioural manifestations of abnormalities in its function. The blood–brain barrier effectively isolates the sensitive neurons from circulating glutamate, and extraneuronal concentrations are maintained at low levels by avid uptake systems. Defects in these mechanisms might be expected to result in excessive neuronal discharge, and ultimately cell death. Many neuropathological states may be consequent upon such fundamental alterations in function.

Our intention in this book is to bring together the results of a number of research workers who have considerable experience in the glutamate field, in order to provide for those considering entering the glutamate arena, a broad overview of the current status of glutamate as a transmitter in the CNS, reviewing the different approaches that can be used and their associated problems and pitfalls.

Acknowledgement

In September 1979, at the Seventh Meeting of the International Society for Neurochemistry (ISN) held in Jerusalem, we organized a short symposium entitled 'Glutamate and the Central Nervous System'. This attracted enormous interest and indeed provided the main impetus for this book. We

therefore are especially grateful to the ISN for encouraging us to organize, for the first time, a symposium concerned exclusively with the role of glutamate in the CNS.

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Contents

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Pharmacology of Excitatory Amino Acid Receptors

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Although it has been known for more than 20 years that L-glutamate and L-aspartate, together with structurally related acidic amino acids, are able to depolarize and excite neurons in the mammalian central nervous system (Curtis, Phillis, and Watkins, 1960; Curtis and Watkins, 1960; for review, see Watkins, 1978) compelling evidence to support the putative transmitter role of these substances has been difficult to obtain. One of the main difficulties has been that, until recently, no specific antagonists were known which could be used to compare the actions of excitatory amino acids with those of a natural transmitter released synaptically at discrete populations of neurons. In consequence, the weight of the evidence favouring a transmitter role for such substances has come mainly from results of neurochemical studies. This evidence has been reviewed by Johnson (1978), and more recent findings are discussed by other authors in this volume. Additional aspects of the problem have also been discussed in other recent symposia devoted to this topic (Curtis, 1979; Johnston, 1979). A breakthrough in the pharmacology of excitatory amino acids has been the recognition of different types of receptors for these substances, and the demonstration that at least one type of receptor is involved in synaptic excitation in certain regions of the central nervous system. This encouraging development has been the result of a continuing study of structure–activity relations of amino acid excitants, culminating in the recent discovery of selective antagonists that are able not only to distinguish between excitation produced by amino acid and non-amino acid agonists, but also between different amino acid agonists.

EXCITATORY AMINO ACID AGONISTS

Approximately 100 excitatory amino acids are now known (Watkins, 1978). With rare exception these each have one cationic and two anionic groups in the molecule. The latter two groups are optimally situated α,β (as in aspartate) or α,γ (as in glutamate) in relation to one another. One of these anionic groups is a carboxylate group in all known excitatory amino acids and the cationic group is a protonated primary or secondary amino group usually attached to the same (α) carbon atom as this carboxylate group (Fig. 1). The

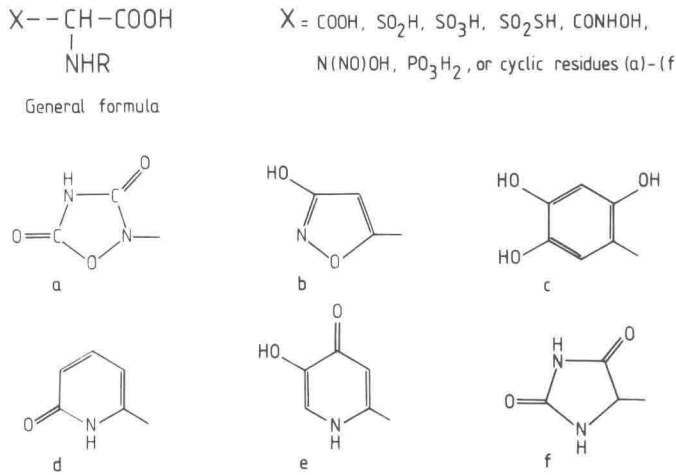


Fig. 1 General structure of excitatory amino acids, showing the diversity of the terminal (ω) acidic residues

other (ω) anionic group can take a number of different forms (Watkins, 1978 and unpublished observations), as shown in Fig. 1. The chain linking this ω-acidic group to the α-amino carboxylate terminal is usually (but not always) composed of one or two methylene or substituted methylene groups, but where the amino carboxylate terminal is of the L configuration, the chain can be extended to as much as five methylene groups with retention of significant excitatory activity (Evans *et al.*, 1979). Among the most potent excitatory amino acids are kainic acid, quisqualic acid and *N*-methyl-D-aspartic acid, the structures of which, together with those of aspartic and glutamic acids, are shown in Fig. 2. These compounds have been used extensively in the characterization of different receptors as described below.

ANTAGONISTS

The search for antagonists capable of differentiating between amino-acid-induced excitation and excitation induced by other putative transmitters such

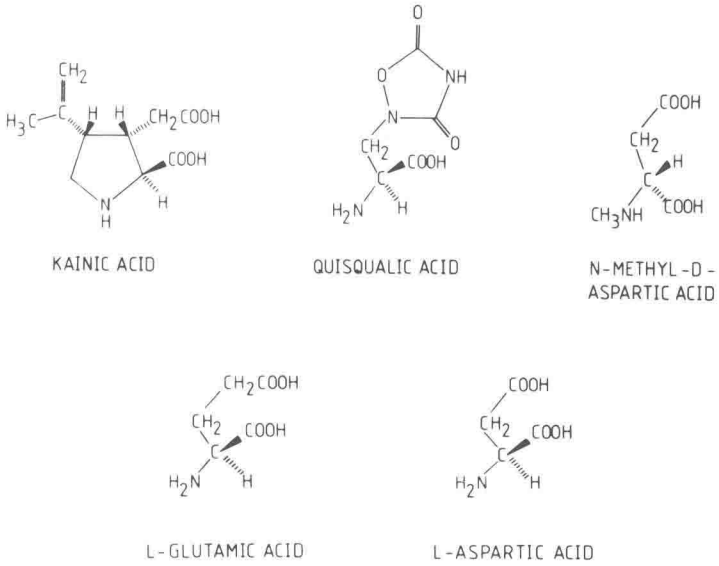


Fig. 2 Structures of amino acid agonists used in studies of receptor types

as acetylcholine was for many years quite discouraging. Amino acid antagonists were indeed discovered, but their potency and selectivity were relatively low (Curtis and Johnston, 1974). Among the few compounds that have subsequently proved to be of some value are L-glutamic acid diethyl ester (GDEE) (Haldemann *et al.*, 1972) and 3-amino-1-hydroxy-2-pyrrolidinone (HA-966) (Davies and Watkins, 1973). Neither of these compounds was

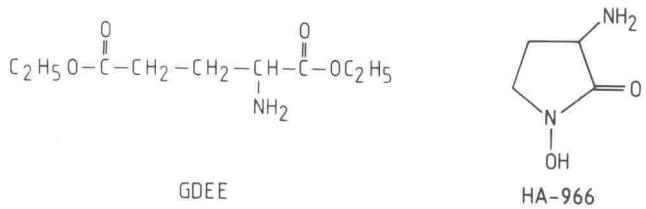


Fig. 3 Structures of L-glutamic acid diethyl ester (GDEE) and 3-amino-1-hydroxy-2-pyrrolidone (HA-966)

originally considered specific enough to find general acceptance as a tool for identifying sites of amino-acid-mediated synaptic excitation (Curtis *et al.*, 1972; Curtis *et al.*, 1973; Aieglgänsberger and Puil, 1973; Altmann, Ten Bruggencate and Steinberg, 1976; McLennan and Wheal, 1976) but both have subsequently played an important role in the characterization of different types of excitatory amino acid receptor sites. Their structures are shown in Fig. 3.

NMDA and Non-NMDA Receptors

Following the suggestion by Duggan (1974) that the differential sensitivity of discrete groups of spinal neurons to L-glutamate and L-aspartate may be explained by the existence of separate receptors for these amino acids, it was pointed out by Johnston *et al.* (1974) that, if this were so, a conformationally restricted glutamate analogue, such as kainate, may be a more specific agonist for glutamate receptors than glutamate itself, which could adopt a multiplicity of conformations and thus possibly interact with more than one type of receptor. In particular, these latter authors suggested that kainate may not be able to adopt a conformation suitable for interaction with the same receptors as those activated by the aspartate analogue, *N*-methyl-D-aspartate (NMDA). In support of this idea of different kainate and NMDA receptors it was found that the potency of kainate relative to NMDA varied in similar directions to, but more markedly than, the potency of glutamate relative to aspartate when tested on different groups of spinal neurons (McCulloch *et al.*, 1974). These findings stimulated the incorporation of a range of different excitatory amino acids in screening programmes for potential antagonists.

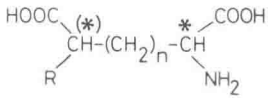
The first agent found to differentiate clearly between different amino acid excitants was, somewhat unexpectedly, Mg^{2+}. On the tetrodotoxin (or procaine)-blocked isolated frog spinal cord, low concentrations of these ions (threshold about 10 μM) depressed responses to NMDA while concentrations as high as 20 mM had little or no effect on responses to quisqualate. Responses to kainate and L-glutamate were also relatively resistant to Mg^{2+}, while responses to other agonists were depressed to intermediate degrees (Evans, Francis, and Watkins, 1977). Similar results were obtained in the cat spinal cord *in vivo* (Davies and Watkins, 1977). These findings suggested that there were at least two types of excitatory amino acid receptors, Mg^{2+}-sensitive and Mg^{2+}-insensitive types, and that the former may be 'aspartate-preferring' and the latter 'glutamate-preferring'. Co^{2+} and Ni^{2+} had qualitatively similar and yet more potent actions than Mg^{2+}, while Mn^{2+} was somewhat less potent than Mg^{2+} in producing the same spectrum of actions (Table 1). These actions were not antagonized by raising $[Ca^{2+}]$; in fact, Ca^{2+} (and to a lesser extent, Sr^{2+} and Ba^{2+}) produced similar though very much weaker effects (Ault *et al.*, 1980).

It was soon found that certain organic agents produced a practically identical pattern of depressant effects on the actions of a range of amino acid excitants. These organic agents included the previously reported amino acid antagonist, HA-966 (Davies and Watkins, 1973), and a range of monoamino and diamino dicarboxylic acids of chain length greater than that present in the glutamate molecule and having the 'unnatural' D configuration at the aminated carbon atom(s) (Fig. 4). When an extensive series of excitants were compared in both the amphibian and mammalian spinal cords, responses

Table 1 Antagonism of frog motoneuron depolarization induced by different excitant amino acids and by synaptic excitation

Antagonist	Per cent control response induced by						
Divalent metal ions	NMDA	L-HCA	L-ASP	L-GLU	KA	QA	SYN
Mg^{2+} (0.5 mM)[1]	22 ± 1	34 ± 3	82 ± 2	89 ± 2	96 ± 3	95 ± 1	70 ± 9
Mn^{2+} (0.5 mM)[2]	57 ± 1	64 ± 3	81 ± 5	91 ± 3	95 ± 1	98 ± 1	9, 27
Co^{2+} (0.5 mM)[2]	17 ± 3	40 ± 5	64 ± 11	93 ± 5	96 ± 7	105 ± 2	27, 53
Long chain amino dicarboxylic acids							
DαAA (0.25 mM)[1]	12 ± 1	26 ± 4	72 ± 3	83 ± 2	82 ± 3	128 ± 6	55 ± 9
DαAS (0.25 mM)[3]	6 ± 1	23 ± 1	81 ± 2	87 ± 4	75 ± 5	90 ± 2	37 ± 1
α,ε-DAPA (0.5 mM)[1]	15 ± 1	34 ± 3	71 ± 3	83 ± 3	98 ± 2	101 ± 4	52 ± 9
Dipeptides							
γDGG (0.25 mM)[4]	9 ± 2	18 ± 4	64 ± 10	85 ± 5	61 ± 5	72 ± 6	51 ± 1
γLGG (0.5 mM)[4]	29 ± 2	42 ± 1	72 ± 3	86 ± 3	87 ± 3	90 ± 4	51 ± 8
Phosphono amino acids							
2APB (1.0 mM)[3]	57 ± 3	63 ± 5	91 ± 2	95 ± 3	50 ± 3	89 ± 4	49 ± 6
2APV (50 μM)[4]	5 ± 2	25 ± 3	79 ± 2	93 ± 2	105 ± 2	95 ± 2	45 ± 2
Cyclic compounds							
cis-2,3-PDA (0.5 mM)[4]	18 ± 2	33 ± 3	63 ± 5	73 ± 3	28 ± 2	59 ± 6	40 ± 2
cis-2,3-PZDA (0.5 mM)[4]	35 ± 3	42 ± 4	83 ± 1	92 ± 5	73 ± 3	84 ± 3	58 ± 5
HA-966 (0.5 mM)[1]	13 ± 2	25 ± 4	55 ± 4	67 ± 3	83 ± 5	115 ± 11	24 ± 4

The values given are the magnitudes of the depolarizations measured in tetrodotoxin(TTX)-containing medium in the presence of an antagonist expressed as a percentage (mean ± SE mean, 3–7 experiments) of matched control responses measured in the absence of the antagonist. Depression of synaptic responses, measured in TTX-free medium, was estimated 10–20 min after addition of the antagonist, during which period a new plateau level had usually been reached. Abbreviations: NMDA, *N*-methyl-D-aspartate; L-HCA, L-homocysteate; L-Asp, L-aspartate; L-Glu, L-glutamate; KA, Kainate; QA, quisqualate; syn, synaptic response evoked by dorsal root stimulation (1/min); DαAA, D-α-aminoadipate; DαAS, D-α-aminosuberate; α,ε-DAPA, (±)-α,ε-diaminopimelate; γDGG, γ-D-glutamylglycine; γLGG, γ-L-glutamylglycine; 2APB, DL-2-amino-4-phosphonobutyrate; 2APV, DL-2-amino-5-phosphonovalerate; *cis*-2,3-PDA, (±)-*cis*-2,3-piperidine dicarboxylate; *cis*-2,3-PZDA, (±)-*cis*-2,3-piperazine dicarboxylate; HA-966, (±)-1-hydroxy-3-aminopyrrolid-2-one. (1) Evans *et al.*, 1978; (2) Davies *et al.*, 1979a; (3) Evans *et al.*, 1979; (4) Evans, Francis, Jones, and Watkins, unpublished observations.



n	R	*	(*)	Compound	Abbreviation
2	H	D	-	D-α-AMINOADIPIC ACID	D-α-AA
3	H	D	-	D-α-AMINOPIMELIC ACID	D-α-AP
4	H	D	-	D-α-AMINOSUBERIC ACID	D-α-AS
3	NH_2	D	D or L	α,ε-DIAMINOPIMELIC ACID	α,ε-DAP

Fig. 4 Structures of long chain monoamino and diamino dicarboxylic acids

induced by NMDA were the most susceptible to antagonism by these organic agents and responses to kainate and L-glutamate among the least susceptible, while quisqualate-induced responses were either unaffected, slightly depressed, or, in the case of HA-966 and D-α-aminoadipate, actually enhanced (Biscoe *et al.*, 1977a; Biscoe *et al.*, 1977b; Biscoe *et al.*, 1978; Evans, Francis, and Watkins, 1978; Evans and Watkins, 1978b; Evans *et al.*, 1979; Davies and Watkins, 1979; Lodge, Headley, and Curtis, 1978; McLennan and Lodge, 1979). Similar effects were observed in the thalamus of the cat (McLennan and Hall, 1978; Hall, Hicks, and McLennan, 1978; Hicks, Hall, and McLennan, 1978; Hall *et al.*, 1979). The effects of Mg^{2+}, other divalent metal ions, and several organic NMDA antagonists on responses of frog motoneurons to different excitant amino acids are compared in Tables 1 and 2. Preliminary

Table 2 Dose ratios for antagonism of NMDA-induced frog motoneuron depolarization

Group	Antagonist (conc)	Dose ratio*
Divalent metal ions	Mg^{2+} (1 mM)[1]	5.7 ± 0.4 (7)
	Mn^{2+} (1 mM)[2]	3.8 ± 0.5 (3)
	Co^{2+} (1 mM)[2]	13.3 (2)
	Ni^{2+} (1 mM)[4]	> 20 (3)†
Long chain amino dicarboxylic acids	DαAA (0.25 mM)[1]	6.9 ± 0.1 (4)
	DαAS (0.25 mM)[3]	16.7 ± 1.2 (4)
	DLαAS (0.5 mM)[3]	14.9 ± 1.1 (3)
	α,ε-DAPA (1 mM)[3]	9.3 ± 0.4 (5)
Dipeptides	γDGG (0.25 mM)[4]	12.7 ± 1.0 (3)
	γLGG (0.5 mM)[4]	4.4 ± 0.5 (4)
Phosphono amino acids	2APV (0.5 mM)[4]	205 ± 19 (3)
Cyclic compounds	*cis*-2,3-PDA (0.5 mM)[4]	10.3 ± 1.2 (3)
	cis-PZDA (0.5 mM)[4]	5.8 ± 0.5 (3)

* Ratio of concentration of agonist in presence of the antagonist to the concentration of the agonist in the absence of the antagonist required to produce the same motoneuron depolarization (approx. 2 mV) in TTX-containing medium. Values (mean ± SE mean for the number of preparations shown) were obtained approximately 30 min after addition of antagonist.

† Progressively increased with time. (1) Evans *et al.*, 1978; (2) Davies *et al.*, 1979a; (3) Evans *et al.*, 1979; (4) Evans, Francis, Jones and Watkins, unpublished observations. Abbreviations as for Table 1.

experiments suggested that Mg^{2+} and the organic agents acted at different sites in the receptor or receptor–ionophore complex (Evans and Watkins, 1978a; Davies *et al.*, 1979a).

These results supported the concept of at least two types of excitatory amino acid receptors, one type (NMDA receptors) activated by NMDA and blocked by both the inorganic and organic NMDA antagonists, while other receptors, activated preferentially by such agonists as L-glutamate, kainate and quisqualate were relatively resistant to blockade by either class of NMDA antagonists.

The Action of GDEE: Separate Quisqualate and Kainate Receptors?

The question then arises as to whether all the non-NMDA receptors—that is, those receptors that are relatively insensitive to the action of organic and inorganic NMDA antagonists—represent a single, or more than a single population. Certain evidence strongly indicates that non-NMDA receptors can be divided into two sub-types, for which quisqualate and kainate are preferential agonists. Thus, iontophoretically applied GDEE blocks quisqualate- but not kainate-induced responses of cat spinal neurons (McLennan and Lodge, 1979; Davies and Watkins, 1979). Some caution should be exercised in the interpretation of this finding, however (Davies *et al.*, 1979b; Davies and Watkins, 1979), since GDEE is a relatively weak antagonist that seems to act predominantly on those responses which are produced by amino acids that are subject to rapid uptake, and whose actions would thus be confined to near the tip of the micropipettes administering them. Amino acids that are not rapidly taken up, such as NMDA and kainate (Balcar and Johnston, 1972; Johnston, Kennedy, and Twitchin, 1979) may act on receptors which are out of range of the relatively weak antagonist action of GDEE. Although no uptake studies have yet been done on quisqualic acid, the fact that responses induced by this amino acid can be enhanced by DαAA and HA-966 (Evans *et al.*, 1978, 1979) could be explained if these agents inhibit the uptake of quisqualate. Moreover, GDEE has little if any effect on amino-acid-induced responses when tested by bath administration in the isolated frog spinal cord (Evans *et al.*, 1979). Nevertheless, other evidence does support the concept of different quisqualate and kainate receptors. Thus, in the frog spinal cord (Table 1), kainate-induced responses are antagonized by 2-amino-4-phosphonobutyrate (2APB) considerably more effectively than are responses induced by quisqualate (Davies *et al.*, 1979b). Also, the relative potencies of quisqualate and kainate vary in different preparations. Thus, on spinal neurons (Biscoe *et al.*, 1976) quisqualate is considerably more potent than kainate, whereas on isolated dorsal roots of the immature rat (extrasynaptic receptors), this order is reversed (Davies *et al.*, 1979b). Finally, a dipeptide analogue of D-α-aminosuberate, γ-D-