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STRUCTURE-ACTIVITY RELATIONS OF EXCITATORY AMINO ACIDS ON FROG AND RAT SPINAL NEURONES

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1 A series of compounds structurally related to glutamic acid has been tested on frog and rat spinal neurones. The substances were added to procaine-containing medium bathing the isolated hemisected spinal cord of the frog, and their potencies in depolarizing motoneurones were assessed by the magnitude of the potential produced in the ventral root. The electrophoretic technique was used to administer the substances around single interneurones of the rat spinal cord and the relative potencies of the compounds as excitants assessed by the magnitude of the currents required to produce similar rates of neuronal firing.

2 Parallel structure-activity relations were observed in the two series of experiments, suggesting that the receptors for excitatory amino acids on frog and rat spinal neurones are similar.

3 Quisqualate, domoate and kainate were the strongest excitants in both animals, with potencies around two orders of magnitude higher than that of L-glutamate.

4 2,4,5-Trihydroxyphenylalanine (6-OH-DOPA) was a stronger excitant and L-3,4-dihydroxyphenylalanine (L-DOPA) a weaker excitant than L-glutamate on frog spinal motoneurones. The former compound was also a more potent convulsant than L-glutamate on intraventricular injection into mouse brain. The lack of activity of 6-OH-DOPA on electrophoretic administration was attributed to oxidation.

5 Unlike the majority of amino acid excitants, several of the compounds shown in the present work to have moderate excitatory activity are not anionic at physiological pH. This indicates either that two negatively charged groups are not essential for interaction with a common excitatory receptor, or that more than one type of receptor is involved in the actions demonstrated.

Introduction

There is mounting evidence that the anions of some acidic amino acids may function as excitatory transmitter substances in the mammalian central nervous system. Although L-glutamate and L-aspartate are the two compounds most generally favoured to perform such a role, structure-activity studies (Curtis, Phillis & Watkins, 1961; Curtis & Watkins, 1963) have revealed several other compounds that must also be considered as possible transmitters (Curtis & Johnston, 1974). A continuing study of structure-activity relations is important not only to extend the list of transmitter candidates, but also to gain insight into the mechanisms underlying the membrane depolarizing effects of these compounds on central neurones.

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It was previously shown that the actions of amino acids on neurones of the amphibian isolated hemisected spinal cord, as manifested by changes in the level of ventral root polarization when the substances were added to the perfusion medium (Curtis *et al.*, 1961), were generally consistent with the effects of the substances when applied by micro-electrophoresis around single neurones of the mammalian spinal cord (Curtis & Watkins, 1960, 1963). However, interpretation of the amphibian results was complicated by the fact that the ventral root responses were compounded of both direct actions of the added substances on the motoneurones and indirect effects on neurones forming part of synaptic pathways terminating on motoneurones. This difficulty has recently been overcome by the inclusion in the perfusion medium of procaine, tetrodotoxin or a high Mg^{2+} concentration, all of which prevent synaptically relayed effects, so restricting the

responses of the ventral roots to the direct actions of the amino acids on motoneurones (Barker & Nicoll, 1973; Konishi & Otsuka, 1974; Barker, Nicoll & Padjen, 1975; Evans & Watkins, 1975a). Using the procaine-containing system we have tested an extensive series of excitants covering a wide range of related structures and established an order of potency for the compounds on frog motoneurones. In view of the convenience of the preparation for studying amino acid actions, it was important to investigate if such results might be meaningful in relation to the mammalian central nervous system. Accordingly, a selected series of the same compounds was also tested on rat spinal neurones by the microelectrophoretic technique, and the relative potencies compared with those obtained on the frog. Preliminary reports of this work have been published (Biscoe, Evans, Headley, Martin & Watkins, 1975; Evans & Watkins, 1975b).

Methods

Amino acids were tested for excitatory activity on the isolated hemisected cord preparation of the frog (Curtis *et al.*, 1961) modified by the inclusion of 1 mM procaine in the perfusion medium (Evans & Watkins, 1975a). The other components of the perfusion medium were (mM): NaCl 111, KCl 2, $CaCl_2$ 2, glucose 12 and tris base 10; the pH was adjusted to 7.5 with 11.3 N HCl. The solution was dripped directly on to the tissue at a rate of 1.5 ml per min, with the temperature maintained at 12.5 ± 0.2°C. Ag/AgCl electrodes were used to record the potentials occurring between the distal end of ventral roots 8 or 9 and the bathing solution; the potential so recorded reflects the level of motoneurone transmembrane polarization. Solutions of the amino acids in the perfusion medium were tested in 1 ml volumes at two concentrations within the range 0.25 μM to 5 mM (pH adjusted to 7.5 with NaOH where necessary) on at least three preparations. The depolarizations produced were matched with those obtained with L-glutamate (0.05–2 mM). Relative potencies were obtained from the ratios of the concentrations producing equal depolarizations. Recovery times, following washout of the substances, were also measured and compared with those observed for L-glutamate.

Many of the compounds were also tested on rat lumbar spinal interneurones by microelectrophoresis (Biscoe, Duggan & Lodge, 1972). Extracellular recordings of the discharge rates of neurones were made with the 4 M NaCl-filled centre barrel of seven barrel micropipettes, and were displayed on a potentiometric pen recorder by conventional techniques. The remaining barrels contained solutions of the mono-sodium salts of the amino acids to be tested; these were prepared by dissolving the free acids in one equivalent of NaOH solution of the desired molarity. Estimates of the excitatory potency of the various amino acids were made by comparing the ejecting currents required to produce approximately equal frequencies of spike discharge. In an attempt to relate the potencies of the strong excitants (such as the anthelmintics kainate, domoate and quisqualate) to that of L-glutamate, comparisons were made on the same cells first of the potencies of the strong excitants with DL-homocysteate (all at 2.5 or 5 mM in 47.5 or 45 mM NaCl, respectively) and then of DL-homocysteate with L-glutamate (both at 200 mM in water). The use of these dilute solutions of the strong excitants was necessary in order to minimize the effects of diffusion from the electrode tips. All other amino acids were used as 200 mM solutions of the mono-sodium salts in water.

In order to gain information about the purity and also about the ionization of the substances, all the amino acids used in this study were subjected to high-voltage paper electrophoresis using buffers of various pH values between 3 and 10 (2000 V/15 min; Whatman No. 1 paper). In the case of 2,4,5-trihydroxyphenylalanine (6-OH-DOPA) and L-3,4-dihydroxyphenylalanine (L-DOPA), 50 mM ascorbic acid was included in the buffers to protect against oxidation. Spots were detected with ninhydrin.

The compounds used, and their sources, were as follows: D- and L-glutamic acid, D- and L-aspartic acid, D-asparagine, L-serine (BDH); kainic acid, L-DOPA, 6-OH-DOPA, D- and L-malic acid, D-asparagine, D-serine (Sigma); DL-homocysteic acid (Calbiochem); quisqualic acid, domoic acid, α-allokainic acid (gifts from Professor T. Takemoto, Tohoku University); DL-2-amino-4-thiosulphonylbutyric acid and L-2-amino-3-thiosulphonylpropionic acid (gifts from Dr C. de Marco, Cagliari); 6-hydroxy-2-pyridylalanine (gift from Dr P.T. Sullivan, Ann Arbor); *O*-sulpho-L-serine (gift from Dr R.A. John, Cardiff); mimosine (gift from Professor K-H. Ling, Taipei); *N*-methyl-D-aspartic acid (prepared by the method of Watkins, 1962).

Results

Anthelmintics

Quisqualic acid, domoic acid and kainic acid (Table 1, compounds 1, 2 and 3, respectively) are naturally occurring substances which have powerful anthelmintic properties; quisqualic acid occurs in the seeds of the plant genus *Quisqualis* (Takemoto, Takagi, Nakajima & Koiki, 1975; Takemoto, Koike, Nakajima & Arihara, 1975; Takemoto, Nakajima, Arihara & Koiki, 1975), while kainic and domoic acids are found in the marine algae *Digenea simplex* and *Chondria armata* respectively (Ueno, Nawa, Ueganagi, Morimoto, Nakamori & Matsuoka, 1955;

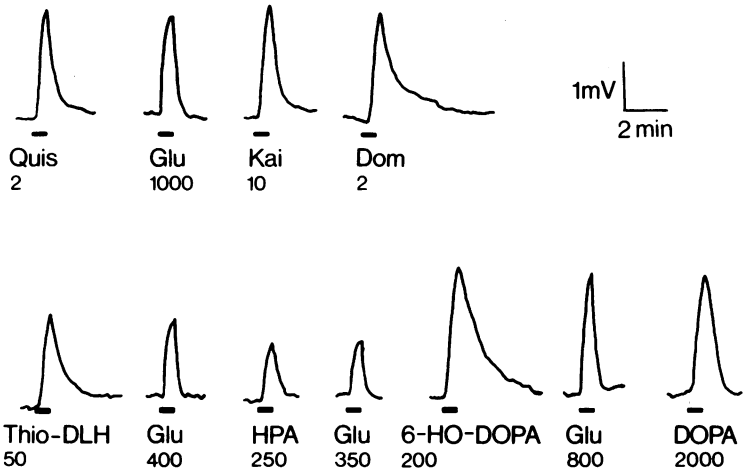


Figure 1 Ventral root depolarizations produced by excitatory amino acids on the isolated hemisected spinal cord of the frog in the presence of 1 mM procaine. Period of contact of the amino acids with the tissue shown by the horizontal bars. Figures below each compound refer to concentration in μM. Quis, quisqualate; Glu, L-glutamate; Kai, kainate; Dom, domoate; Thio-DLH, DL-2-amino-4-thiosulphonyl-*n*-butyrate; HPA, 1,2-dihydro-2-oxo-6-pyridylalanine; 6-OH-DOPA, 2,4,5-trihydroxyphenylalanine; DOPA, L-3,4-dihydroxyphenylalanine.

Takemoto & Draigo, 1960; Takemoto, Draigo, Kondo & Kondo, 1966). Quisqualate is a potent glutamate-like excitant at the crayfish neuromuscular junction (Shinozaki & Shibuya, 1974) and kainate strongly excites rat cortical (Shinozaki & Konishi, 1970) and cat spinal (Johnston, Curtis, Davies & McCulloch, 1974) neurones. Both compounds are considerably more potent than L-glutamate in these actions.

In the present study, all three amino acids were found to be remarkably potent as excitants of spinal cord neurones in both the frog and rat (Table 1). Concentrations in the range of 0.25–10 μM gave readily measurable depolarizations in the frog ventral root. The actions of these amino acids were more prolonged than that of L-glutamate, persisting for up to 5 min after washout in the case of domoate (Figure 1). The action of α-allo-kainate (Table 1, 13), a stereoisomer of kainate, was considerably weaker than that of kainate in the frog cord, but was similarly prolonged; this compound has also been shown to be less potent than kainate on rat cerebral neurones (Shinozaki & Konishi, 1970).

When tested in the rat spinal cord the three anthelmintics and also α-allo-kainate showed similar relative potencies to those observed in the frog. The excitatory action of domoate and kainate was sometimes prolonged, occasionally lasting for several minutes after termination of the ejection currents. This phenomenon, coupled with the effects of the high retaining currents necessary to prevent diffusional escape of the substances, caused difficulties in the assessment of the potencies of these compounds relative to other amino acid excitants. Two methods were used in an attempt to evaluate relative potencies. With the first method, substances were applied repeatedly for relatively short periods (0.5–1.5 min) until successive applications gave similar responses. This procedure occasionally resulted in maintained low frequency excitation between applications of the longer-acting substances (e.g. kainate and domoate in Figure 2a). Such an effect might be expected to give spuriously high estimates for the relative potencies of these substances; however, equally high or higher potencies relative to DL-homocysteic acid were sometimes observed even in the absence of maintained baseline excitation (Figure 2b). With the second method (not illustrated), substances were applied continuously over a period of 1–3 min, the ejection currents being adjusted as necessary to achieve a plateau of firing frequency as close as possible to that obtained by similarly prolonged administration of DL-homocysteate. The two methods yielded similar ranges of potency ratios, and results from both are included in the ranges given in Table 1.

Sulphur-containing amino acids

The high activity of DL-2-amino-4-thiosulphonyl-butyric acid (De Marco, 1972) (Table 1, 6), observed in both the frog (Figure 1) and rat (Figure 2c) was similar to that of the sulphonic acid analogue DL-homocysteic acid (Table 1, 5). L-2-Amino-3-thio-sulphonylpropionic acid (Table 1, 14), which is the L-

Table 1 Compounds tested on frog and rat spinal neurones

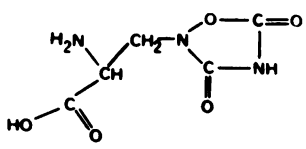
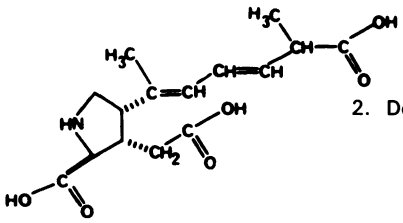
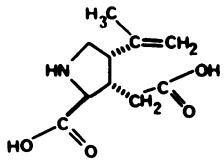
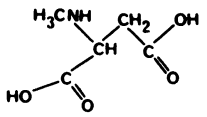
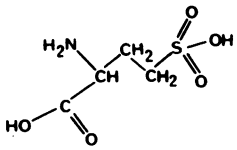
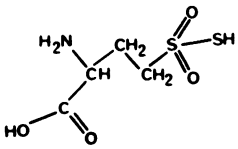
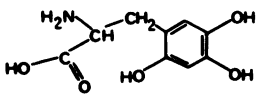
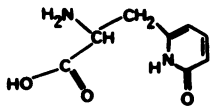
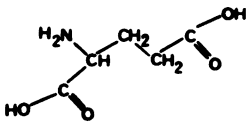
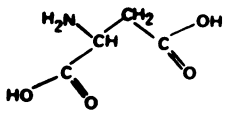
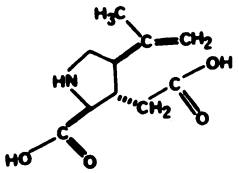
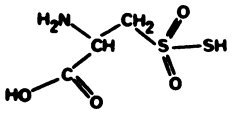
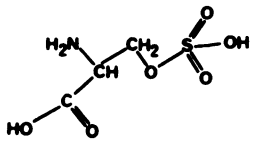
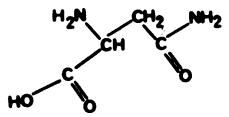
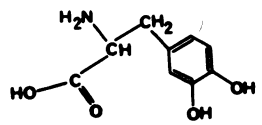
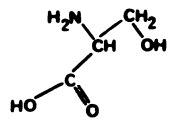
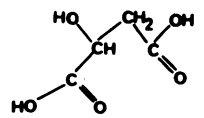
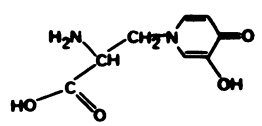
Structure	No.	Name	Frog		Rat
			*Potency**	*Recov.* †	*Potency range**
	1.	Quisqualic acid	416 ± 68	3.7 ± 0.4	22–90
	2.	Domoic acid	280 ± 49	8.8 ± 0.8	36–190
	3.	Kainic acid	101 ± 18	4.7 ± 1.1	18–54
	4.	*N*-Methyl-D-aspartic acid	12.4 ± 1.9	4.5 ± 0.8	4–12
	5.	DL-Homocysteic acid	11.4 ± 1.2	3.5 ± 0.8	2–5
	6.	DL-2-Amino-4-thiosulphonyl butyric acid	8.3 ± 0.7	4.1 ± 0.2	4–11
	7.	2,4,5-Trihydroxy-phenylalanine (6-OH-DOPA)	4.3 ± 0.3	10.3 ± 2.2	
	8.	1,2-Dihydro-2-oxo-6-pyridylalanine	0.96 ± 0.15	2.3 ± 0.4	0.5–2
	9.	L-Glutamic acid	1.0	1.0	1.0
	10.	D-Glutamic acid	2.1 ± 0.6	1.3 ± 0.1	

Table 1—*continued*

Structure	No.	Name	Frog		Rat
			Potency*	Recov.†	Potency range*
	11.	L-Aspartic acid	0.49 ± 0.06	1.3 ± 0.2	
	12.	D-Aspartic acid	0.75 ± 0.13	1.0 ± 0.1	
	13.	α-*allo*-Kainic acid	0.25 ± 0.05	6.3 ± 0.8	0.5–2
	14.	L-2-Amino-3-thiosulphonyl-propionic acid	0.4		0.1–0.2
	15.	*O*-Sulpho-L-serine	0.46 ± 0.07	1.4 ± 0.2	
	16.	L-Asparagine	0.13 ± 0.3	2.1 ± 0.1	
	17.	D-Asparagine	0.33 ± 0.05	2.3 ± 0.2	
	18.	L-DOPA	0.26 ± 0.06	2.3 ± 0.2	0–0.2
	19.	L-Serine	0.03 ± 0.005	2.6 ± 0.6	
	20.	D-Serine	0.24 ± 0.02	2.4 ± 0.7	**
	21.	L-Malic acid	0		
	22.	D-Malic acid	0.14 ± 0.03	2.8 ± 0.2	
	23.	Mimosine	0.09 ± 0.01	3.9 ± 0.5	

* Relative to L-Glutamate = 1.0. † Time from peak response to 90% of full recovery expressed as a ratio to the recovery from L-glutamate-induced depolarizations of equal magnitude. ** Paroxysmal excitation, presumably due to co-administered H^+ ions.

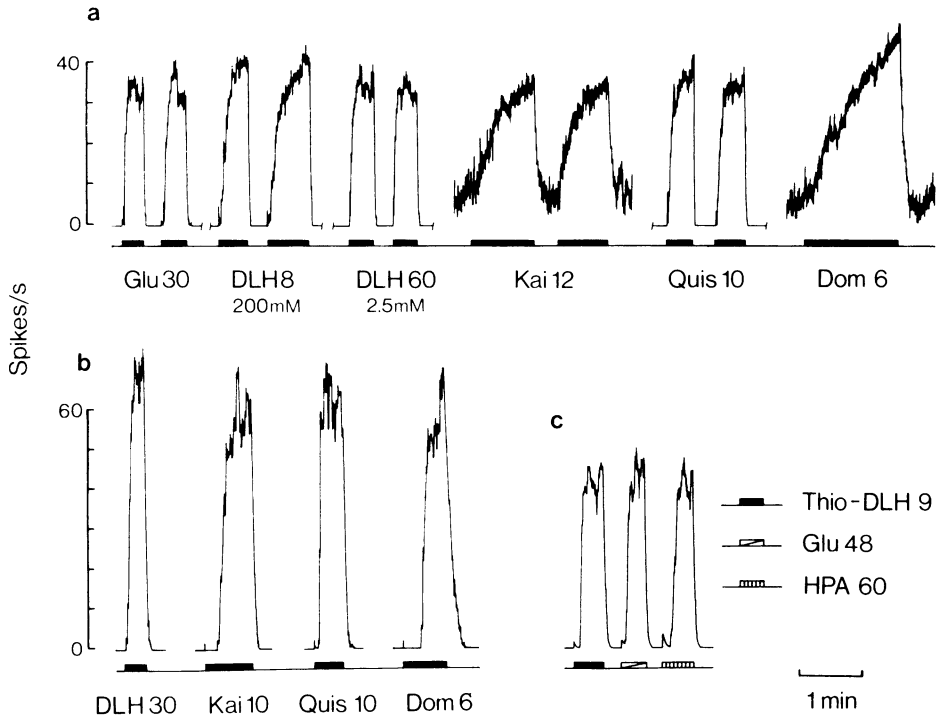


Figure 2 Chart recordings of the excitation of three different rat spinal interneurones by electrophoretically administered amino acids. Ordinates, spike frequency; abscissae, time (min). Horizontal bars show duration of administration. The compounds and the electrophoretic current (nA) are indicated below each bar. (a) Showing method of matching responses of the anthelmintics, kainate, quisqualate, and domoate (all 2.5 mM in 147.5 mM NaCl) with DL-homocysteate (DLH, 2.5 mM in 147.5 mM NaCl and 200 mM in H_2O) and with L-glutamate (200 mM in H_2O). Note continued base line excitation produced by kainate and domoate. (b) Similar to (a), but no base line excitation produced in this case. (DLH solution, 200 mM in H_2O, anthelmintic solutions as in (a).) (c) Matched responses for two other amino acids and L-glutamate (all 200 mM in H_2O). Abbreviations as in Figure 1.

form of the lower homologue of compound 6, and the thio-acid analogue of the moderately strong excitant L-cysteic acid (Curtis & Watkins, 1960; 1963; Curtis, Phillis & Watkins, 1960; 1961) was a much weaker excitant than compound 6 in both species; it was not compared with L-cysteic acid. *O*-Sulpho-L-serine (Table 1, 15), an irreversible inhibitor of aspartate aminotransferase (John & Fasella, 1969), was also a weak excitant in the frog cord, corresponding to the weak action of this compound on cat spinal neurones (Curtis, Duggan, Felix, Johnston, Tebēcis & Watkins, 1972); it was not tested on rat neurones.

Aromatic amino acids

A number of polyhydroxyphenyl and hydroxypyridyl amino acids showed a range of excitatory activity. 6-OH-DOPA (Table 1, 7) was the strongest excitant of this type when tested on the frog cord where it had a very prolonged action (Figure 1). No activity could be demonstrated on rat spinal neurones when the substance was tested electrophoretically. This lack of effect in the rat was probably due to oxidation of the compound prior to or during its electrophoretic administration from alkaline solutions (pH 8–9) of the monosodium salt, since stock solutions of similar pH were shown to lose activity at the rate of about 4% per min when tested on the frog spinal cord. This assumption was supported by the effects of fresh solutions of 6-OH-DOPA which were intraventricularly injected into mice by the method of Crawford (1963). The compound (10 μmol/ml) was dissolved in 150 mM NaCl containing 10 mM NaOH, the solution was adjusted to pH 7.4 with HCl, and 10- or 20-μl volumes were injected immediately. Convulsions occurred within a few seconds. When tested concurrently by the same method, DL-homocysteate was more potent and L-glutamate less potent than 6-OH-DOPA.

Three other compounds in this group showed activity both in the superfusion experiments on the frog spinal cord and by the electrophoretic method in the rat spinal cord. L-DOPA (Table 1, 18; Figure 1), which has been shown to be a weak excitant of mammalian central neurones (Krnjević & Phillis, 1963) had weak activity in both species in the present studies. Two pyridine-related compounds, 1,2-dihydro-2-oxo-6-pyridylalanine (Table 1, 8; Figure 1 and 2c), which is the stable (keto) form of 6-hydroxy-2-pyridylalanine, and mimosine (Table 1, 23), were also excitants. The former compound, a synthetic substance (Sullivan, Sullivan & Norton, 1971), was approximately equi-effective with L-glutamate on both frog and rat spinal neurones. In the rat, the substance gave similar results when administered electrophoretically either with anionic currents from solutions of the sodium salt at pH 9 or with cationic currents from solutions of the amino acid adjusted to pH 3 with HCl. Mimosine, a toxic substance which occurs in leguminous plants (De Wreede & Wayman, 1970) was not tested in the rat, but has been reported to be an excitant of cat spinal neurones (Curtis *et al.*, 1972). It was considerably weaker than L-glutamate in depolarizing frog motoneurones.

Other compounds

(a) *N*-Methyl-D-aspartate. This compound (Table 1; 4) was more potent and had a longer lasting effect than L-glutamate on both frog and rat neurones, in accordance with previous studies (Curtis *et al.*, 1961; Curtis & Watkins, 1963; Crawford & Curtis, 1964). However, its potency relative to L-glutamate was considerably lower than that observed in non-procaine treated (unblocked) amphibian spinal cord preparations (Curtis *et al.*, 1961).

(b) D-Glutamate, L- and D-aspartate. These compounds (Table 1; 10, 11 and 12) were tested only on frog spinal neurones in the present study; their relative potencies were within the ranges of those observed in other investigations in both mammals (Curtis & Watkins, 1960; 1963; Crawford & Curtis, 1964) and amphibia (Curtis *et al.*, 1961) though only in the latter study was the mean potency of D-glutamate higher than that of the L-isomer as also found in the present work.

(c) L- and D-Asparagine. The L form (Table 1; 16) had a very weak depolarizing action on the frog cord but the D form (Table 1; 17) was somewhat stronger, being about one third as potent as L-glutamate. This finding conforms with those of previous studies on unblocked amphibian spinal cord (Curtis *et al.*, 1961). Excitatory actions have also been observed in electrophoretic studies in the cat (Krnjević & Phillis, 1963; Crawford & Curtis, 1964) when the substances were administered as cations from acidic solutions, but the actions in those cases were dissimilar to that of L-glutamate and may have involved wholly or in part the effects of raised extracellular pH. Hydrogen ions are co-administered from acidic solutions by electrophoresis, and are also liberated from the cationic form of neutral amino acids when these enter the extracellular fluid (Curtis & Watkins, 1960). The two forms of asparagine were not tested in the present electrophoretic studies on the rat spinal cord.

(d) L- and D-Serine (Table 1, 19 & 20). In the present experiments on the frog cord the L form had only a very weak depolarizing action while the D form was more potent, being about one quarter as strong as L-glutamate. This finding is in agreement with previous studies on the unblocked amphibian spinal cord (Curtis *et al.*, 1961). When passed as a cation from solutions of pH 3 in electrophoretic experiments on the rat, D-serine caused delayed high-frequency discharges which could not be clearly differentiated from H^+ ion effects. L-Serine was not tested on rat spinal neurones, but has previously been found to have a strychnine-sensitive depressant action in the cat spinal cord (Curtis, Hösli & Johnston, 1968).

(e) L- and D-Malic acid (Table 1, 21 & 22). On the frog spinal cord, the D form had a weak depolarizing action, but no activity was demonstrated with the L form (5 mM). Neither compound was tested on rat spinal neurones. These compounds have previously been found to be weakly excitatory in the unblocked amphibian spinal cord, the D form again being the more potent (Curtis *et al.*, 1961), but neither compound had any detectable action when administered electrophoretically around cat spinal neurones (Curtis & Watkins, 1960).

Discussion

A close parallelism has been observed between the relative potencies of a range of amino acid excitants tested on frog and rat spinal neurones. The only major difference observed in the two series of experiments was in the ineffectiveness of 6-OH-DOPA on microelectrophoretic administration around rat spinal neurones. However, this anomaly was readily explained by the instability of the compound, as discussed in the Results section. It can thus be concluded that the receptors for excitatory amino acids on spinal neurones of the two species are similar.

From the structure-activity relations summarized in Table 1, it appears that a variety of terminal groups can replace the ω-carboxylic terminal of glutamate or aspartate with retention, or indeed in many cases pronounced enhancement of excitatory activity. The

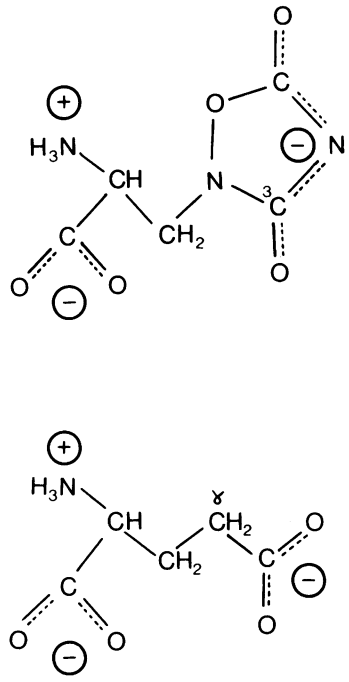


Figure 3 Proposed anionic structure of quisqualate (above) in comparison with glutamate (below).

allowable variation embraces carboxylic, sulphonic, thiosulphonic, phenolic, hydroxypyridine (keto-form) and the unusual heterocyclic (oxadiazolidine) dione system of quisqualic acid. Other examples have been quoted in our preliminary communication (Biscoe *et al.*, 1975).

Quisqualate, domoate and kainate were by far the most potent excitants. Experiments with high-voltage paper electrophoresis showed that all three substances are anionic at physiological pH. In the case of quisqualic acid (Table 1; 1) this observation presumably indicates that the ring system loses the sole proton and becomes negatively charged, the ionized form probably being stabilized by a resonance hybrid structure involving the carbonyl groups. Thus the charge may be distributed between the nitrogen atom and the two oxygen atoms (Figure 3). Of these three atoms, the C_3-oxygen atom seems particularly favourably situated with respect to the amino and carboxylate groups, since in certain conformations of the molecule, this oxygen atom can be isosteric with one of the γ-carboxylate oxygen atoms of glutamate (Figure 3).

The high activity of domoate and kainate (Table 1; 2 and 3 respectively) taken in conjunction with the low activity of α-allo-kainate (Table 1; 13) and also of dihydrokainate (Johnston *et al.*, 1974), suggests that the potency of the former two substances is associated with unsaturation in the side chain and a *cis*-relation between the C_3 and C_4 substituents. The *cis* relationship of these substituents results in a mutual steric hindrance which greatly restricts the possible conformations of both substituents. It is therefore possible that in the relatively fixed conformation of the C_3 side chain, the carboxyl group is particularly well oriented with respect to a corresponding electropositive region of the receptor. Alternatively, or in addition, the unsaturated groups may themselves bind to lipophilic areas close to the electropositive (and presumably hydrophilic) regions of the receptor, so stabilizing the complex.

Among the other compounds tested, most interest attaches to the activity of certain pyridol-related compounds (Table 1; 8 and 23) and particularly of the phenolic substances, 6-OH-DOPA and L-DOPA (Table 1; 7 and 18, respectively). Our findings in regard to the latter compound confirm and extend the observations of Krnjević & Phillis (1963) on neurones in the cat cerebral cortex. Whether this direct excitatory effect of L-DOPA on central neurones is relevant to the therapeutic action of the substance in the treatment of Parkinsonism is a question warranting further investigation.

An important aspect of the activity of the phenolic and pyridol-related compounds is that, on the basis of paper electrophoresis experiments, only mimosine (Table 1; 23), which is a very weak excitant, carries a net negative (anionic) charge at physiological pH, and the ionization of the ring-attached oxygen atoms is only partial even in this case. It has previously been considered (Curtis & Watkins, 1960) that glutamate-like activity requires one cationic and two anionic groups in the active form of the molecule. These groups were envisaged to interact with oppositely charged groups in the receptor molecule. It seems possible, therefore, that if these aromatic compounds do indeed interact with L-glutamate (or L-aspartate) receptors, they do so *via* a metal ion component of the receptor (e.g. Ca^{2+}) which could conceivably form coordinate bonds with undissociated hydroxyl groups (with displacement of a proton) as well as with ionized oxygen functions. The actions of asparagine and serine (mainly the D form in both cases) might be explained on a similar basis; in this case the coordinate link with the receptor metal ion would involve the ω-carboxamide and alcoholic OH groups, respectively. The suggestion that bound Ca^{2+} may be an integral part of excitatory amino acid receptors has previously been made (Curtis & Watkins, 1960; Curtis *et al.*, 1961; Watkins, 1965).

The duration of action of these compounds on the frog spinal cord (Table 1) appeared to be independent of depolarizing potency. In both the frog and rat experiments, slower recoveries were generally observed in the case of the more lipophilic compounds. Similarly, it was previously noted that *N*-

alkyl derivatives of D-aspartic acid had durations of action which were directly related to the length of the alkyl chain (Curtis & Watkins, 1963).

In spite of the similarity of the chemical structure of the compounds and of the nature of the observed responses, it should be emphasized that the present experiments do not provide any evidence as to whether the substances all act on the same receptors, or whether they induce the same ionic permeability changes in the neuronal membrane. These considerations must await the development of specific antagonists of amino acid actions, and/or a detailed study of the ionic basis of the potential changes produced in each case.

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