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MIXED-AGONIST ACTION OF EXCITATORY AMINO ACIDS ON MOUSE SPINAL CORD NEURONES UNDER VOLTAGE CLAMP

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SUMMARY

1. Neurones from the ventral half of mouse embryo spinal cord were grown in tissue culture and voltage clamped with two micro-electrodes. The current–voltage relation of responses evoked by brief pressure applications of excitatory amino acids was examined over a membrane potential range of −100 to +70 mV.

2. Three types of current–voltage relation were observed. Responses to kainic and quisqualic acids were relatively linear within ±20 mV of the resting potential. *N*-methyl-D-aspartate (NMDA) and L-aspartic acid responses had a negative slope conductance at membrane potentials more negative than −30 mV. In contrast, over the same potential range the slope conductance of responses evoked by L-glutamic and L-homocysteic acids was close to zero.

3. The membrane potential–chord conductance relation of the ionic mechanism activated by excitatory amino acids, derived using the driving force for ionic current, showed two types of behaviour. The conductance linked to NMDA receptors was highly voltage sensitive and increased on depolarization; a much weaker voltage sensitivity was observed for responses evoked by kainic and quisqualic acids. L-glutamic and L-homocysteic acid responses behaved as though due to simultaneous activation of both NMDA and either kainate or quisqualate receptors.

4. In the presence of the NMDA receptor antagonist (±)-2-aminophosphonovaleric acid (2-APV) the response to L-glutamate became less voltage sensitive and resembled responses evoked by kainate or quisqualate. Simultaneous activation of both conductance mechanisms by mixtures of kainate and NMDA produced current–voltage and membrane potential–chord conductance relations similar to those of L-glutamate.

5 The voltage sensitivity of the L-glutamate response was inversely related to the dose; for low doses of L-glutamate the slope conductance of responses recorded near the resting potential was close to zero. However, larger doses of L-glutamate evoked responses with a voltage sensitivity similar to that of kainate.

6. We suggest that L-glutamate acts as a mixed agonist at both NMDA and

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non-NMDA receptors. This can explain the results of previous experiments that failed to demonstrate a membrane resistance change during L-glutamate-induced depolarizations.

INTRODUCTION

On the basis of experiments with conformationally restricted agonists and selective antagonists it has been suggested that, in the spinal cord, there exist three pharmacologically distinct excitatory amino acid receptors (McLennan, 1981*a*, *b*; Watkins, 1981*a*, *b*). These have been classified as *N*-methyl-D-aspartate (NMDA), kainate and quisqualate receptors on the basis of rank order of potency of these agonists, selective antagonism of NMDA responses by magnesium ions (Davies & Watkins, 1977; Ault, Evans, Francis, Oakes & Watkins, 1980) and by receptor antagonists such as (±)-2-amino-5-phosphonovaleric acid (2-APV) (Davies, Francis, Jones & Watkins, 1981), and antagonism of NMDA and kainate but not quisqualate responses by γ-D-glutamylglycine (Davies & Watkins, 1981). Experiments with these antagonists have examined the possibility that the excitatory neurotransmitter candidates, L-aspartic and L-glutamic acids, act as mixed agonists at more than one amino acid receptor (Davies & Watkins, 1979; Watkins, 1981*b*). The interpretation of results from such experiments becomes complicated if the membrane conductance change produced by excitatory amino acids shows voltage dependence.

Recent electrophysiological experiments, performed using intracellular recording of membrane potential, suggest that two distinct conductance mechanisms are separately linked to excitatory amino acid receptors. NMDA responses appear to be generated by a voltage-dependent conductance (Engberg, Flatman & Lambert, 1978; MacDonald & Wojtowicz, 1982; MacDonald & Porietis, 1982), while the conductance mechanism linked to kainate and quisqualate receptors is less sensitive to the membrane potential (Engberg *et al.* 1978; MacDonald & Porietis, 1982). Voltage-clamp recording has recently shown that L-aspartic acid and NMDA produce a region of negative slope conductance in the membrane of mammalian neurones (MacDonald, Porietis & Wojtowicz, 1982; Flatman, Schwindt, Crill & Stafstrom, 1983), thus confirming that NMDA receptors are linked to a voltage-regulated mechanism. Such voltage-sensitive responses greatly complicate the interpretation of those experiments in which membrane potential recording is used to study the action of excitatory amino acids. For instance several reports have appeared suggesting that the depolarizing action of L-glutamate is not accompanied by any membrane conductance change (Bernadi, Zieglgänsberger, Herz & Puil, 1972; Altmann, Ten Bruggencate, Pickelmann & Steinberg, 1976; Segal, 1981; Hablitz & Langmoen, 1982). It is possible that in the mammalian nervous system the action of L-glutamate is unconventional and that electrogenic uptake of L-glutamate accounts for its depolarizing action (cf. Kehoe, 1975). However, in invertebrate muscle excitatory amino acid-activated ion channels have been directly identified using the patch-clamp technique (Cull-Candy, Miledi & Parker, 1981; Gration, Lambert, Ramsey & Usherwood, 1981).

We have used voltage-clamp recording to study the membrane current produced by several conformationally restricted amino acids, and in the same spinal cord neurones examined the action of the endogenous excitatory amino acid transmitter candidates, L-glutamate and L-aspartate. Our results suggest that an action of

L-glutamate as a mixed agonist at both NMDA and non-NMDA receptors can account for the apparent absence of a membrane conductance change during the response evoked by L-glutamate.

METHODS

Tissue culture

The spinal cord and dorsal root ganglia of 13–14-day-old mouse embryos (C57BL/6) were dissected and grown in antibiotic-free media as monolayer cultures according to methods previously developed by Ransom, Neale, Henkart, Bullock & Nelson (1977), with the modification that the growth medium was changed on day 1 to Eagle's MEM supplemented with the combination of a chemically defined nutrient supplement (Romijn, Habets, Mud & Wolters, 1982) and 5% (v/v) horse serum. In the majority of experiments the ventral half of the spinal cord was dissected from surrounding tissue (Guthrie & Brenneman, 1982) giving a higher yield of well-dispersed large neurones. After 18–28 days in culture, spinal cord neurones were impaled with two independent micro-electrodes for voltage clamp using techniques similar to those described previously (Mayer & Westbrook, 1983).

Electrophysiological recording

Experiments were performed at room temperature (stable at 27–28 °C) on the stage of a Zeiss inverted phase-contrast microscope. Micro-electrodes (60–100 MΩ) were filled with 1 M-caesium chloride or in a few control experiments 1 M-caesium sulphate or 3 M-potassium chloride. The voltage recording electrode was guarded using a driven shield extending to the bath surface. Typically, voltage control to 25 mV step changes in membrane potential was achieved within 0·5 ms. The clamp was operated at a d.c. gain of 12500 and the frequency response extended using the mixed system described by Smith, Barker, Smith & Colburn (1981). In the majority of experiments, membrane current was recorded using a virtual ground circuit with a 3 dB point of 1·6 kHz.

The membrane potential and current to ground were recorded at several gains on a Gould Brush recorder and on a storage oscilloscope. An LS1-11 based microcomputer was used to digitize membrane potential and current on line, and to generate step voltage commands for the voltage clamp. The records shown were photographed directly from the chart traces or prints of the computer generated data files.

Caesium, a potassium channel blocker, reduced but did eliminate time-dependent outward rectification activated by depolarizing voltage jumps even after periods of voltage clamp at holding potentials depolarized to 0 mV, suggesting that high intracellular caesium concentrations were not obtained; this may reflect low leakage of electrolyte from the fine tip micro-electrodes, and possible efflux of caesium across the cell membrane.

Composition of media

The normal recording medium contained (mM): 143, NaCl; 4·8, KCl; 1, $MgCl_2$; 5, $CaCl_2$; 10, glucose; 10, HEPES; 0·01 mg phenol red/ml and was titrated to pH 7·3. Sucrose was added to match the osmolarity of the growth medium (325 mosmol). Tetrodotoxin (0·6 μM) was added to block spontaneous activity and action potential generation; spontaneous inward synaptic currents were often recorded in the presence of tetrodotoxin suggesting quantal release of transmitter (Fatt & Katz, 1952). In some experiments 2-APV (0·25 mM) was added to the recording medium. Experiments on a single plate were performed for periods of up to 3–5 h.

Extracellular perfusion technique

Excitatory amino acids were dissolved in the bathing medium and applied to individual neurones by pressure ejection from extracellular micropipettes. These were fashioned on a two-stage puller and were similar to those used for patch recording. Such pipettes had tip diameters of 1–2 μm, and were very reliable in their release characteristics. Drug solutions were ejected using pressure pulses of 10–1000 ms duration, 1–4 lbf/in^2, applied to pipettes positioned 10–50 μm from individual neurones and aimed at the soma. This method of drug application allowed us to obtain brief agonist responses (see Fig. 1), but is limited in that the actual concentration of excitatory amino acid at the membrane surface is unknown. Considerable dilution certainly occurs but we have no

quantitative information concerning this. The following drugs, purchased from Sigma and Cambridge Research Biochemicals, were used in the present study: L-aspartic acid (1 mM); *N*-methyl-D-aspartic acid (1 mM); *N*-methyl-DL-aspartic acid (1 mM); L-glutamic acid (1 mM); L-homocysteic acid (1 mM); D-homocysteic acid (1 mM); kainic acid (0·2 mM); quisqualic acid (0·05 mM); a mixture of L-glutamic acid (0·2 mM) and (±)-2-amino-5-phosphonovaleric acid (2 mM).

Experimental protocol

To examine the voltage sensitivity of responses to excitatory amino acids the following protocol was used: neurones were initially voltage clamped at −50 or −60 mV and a pair of drug pipettes positioned to obtain responses which reached peak amplitudes of 1–1·5 nA, usually within 500 ms; clamp control was such that voltage escape was negligible (see Fig. 1). Drug applications were

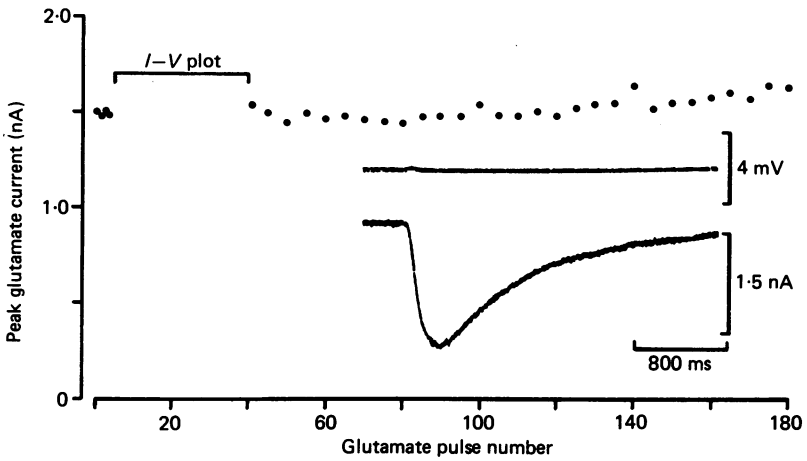

Fig. 1. Stability of L-glutamate responses under voltage clamp. The inset shows an oscillographic record of an inward current response produced by pressure application of L-glutamate (18 ms, 1 lbf/in²) from a micropipette positioned close to the soma of a spinal cord neurone. At the peak of the inward current trace the voltage is well controlled (holding potential −50 mV). Such responses were evoked every 7·6 s. The graph shows a plot of the membrane current response amplitude in nA (ordinate) plotted against the cumulative L-glutamate pressure pulse number. During the period marked '*I–V* plot' the membrane potential was varied between −80 and +5 mV. On return to −50 mV the amplitude of the L-glutamate response was similar to initial control values and remained stable for a total of 23 min.

repeated using a regular time cycle, until reproducible responses were obtained at the initial holding potential. The membrane potential was then altered in 10 mV increments, initially in the hyperpolarizing direction and subsequently in the depolarizing direction over the potential range −70 to +20 mV. Usually, two or three responses were obtained at each value of membrane potential. If the current electrode was of low enough resistance, the voltage range was extended between −100 and +70 mV.

This experimental protocol was used in preference to the alternative approach of recording currents evoked by voltage jumps in the presence and absence of a steady drug current. Slow depolarization of the membrane potential inactivated a portion of the outward rectifier current (see Aldrich, Getting & Thompson, 1979; Adams, Brown & Constanti, 1982), enabling small drug currents to be accurately recorded close to the reversal potential. Our method rests on the assumption that each pulse of amino acid activates a constant number of amino acid receptors, and that depolarizing the membrane potential does not seriously alter transmembrane ion gradients and thus change the reversal potential of the amino acid response. Fig. 1 shows a plot of the peak amplitude of L-glutamate currents recorded over a period of 23 min. Initially four responses were evoked at

−50 mV, before commencing a current–voltage run. The membrane potential was then returned to −50 mV for a further 17 min. The consistency of the responses suggests that the release of L-glutamate is constant, and that the reversal potential does not greatly change during the experiment. This d.c. polarization protocol provides no kinetic information, but avoids the activation of large outward currents during depolarizing jumps which hinder measurement of amino acid responses close to the reversal potential. In some neurones, a hyperpolarizing voltage-jump protocol was used to measure membrane conductance during a constant application of amino acid.

RESULTS

All spinal cord neurones ($n = 73$) responded to the application of excitatory amino acids with membrane potential depolarization or, when voltage clamped at their resting potential, an inward current.

Voltage sensitivity of amino acid responses

Excitatory amino acid responses showed three characteristic types of voltage sensitivity. Individual agonists gave highly consistent responses in different neurones, such that it was possible to guess the type of agonist from its voltage sensitivity. At holding potentials of −50 to −60 mV, the dose of amino acid was usually adjusted to give peak inward currents of 1·0–1·5 nA, except for NMDA and L-aspartic acid for which it was usually difficult to obtain currents larger than 0·5–1·0 nA. Plots of the peak amplitude of the amino acid-evoked current response against membrane potential reflected the three classes of voltage sensitivity.

One end of the spectrum of voltage sensitivity was characterized by kainic acid ($n = 5$) and quisqualic acid ($n = 5$), weakly voltage-sensitive agonists. Current–voltage plots of responses evoked by kainate and quisqualate were relatively linear, showing slight outward rectification as the membrane was depolarized (Fig. 2). Slope conductance measurements, obtained from tangents to these current–voltage plots at −60 and +10 mV increased by a factor of 1·5–2·6 as the membrane potential was depolarized (see Table 1).

NMDA and its racemic mixture NMA ($n = 11$) and L-aspartic acid ($n = 6$) typified the other end of the spectrum of voltage sensitivity. Responses evoked by these agonists were strongly dependent on the membrane potential, resulting in J-shaped current–voltage plots with a peak inward current occurring between −25 and −35 mV and a negative slope conductance at more hyperpolarized membrane potentials (Fig. 3). The slope conductance of the positive limb of the NMDA and L-aspartate current–voltage plot increased markedly with depolarization beyond −30 mV (see Table 1). The reversal potential of responses evoked by kainic acid (−5 to −1 mV, mean −2·8) and quisqualic acid (−6 to +2 mV, mean −1·3) was similar to that recorded for NMDA and NMA (−6 to +3 mV, mean −0·7) and L-aspartic acid (−4 to +2 mV, mean −0·8).

The other amino acids examined gave responses that were intermediate in their voltage sensitivity between kainate and quisqualate on the one hand and NMDA and L-aspartate on the other. The current–voltage relation of responses evoked by L-homocysteate ($n = 8$) was of nearly zero slope conductance over the membrane potential range −40 to −70 mV (see Fig. 4 and Table 1). Depolarized beyond −30 mV the slope conductance increased sharply to large positive values. Responses

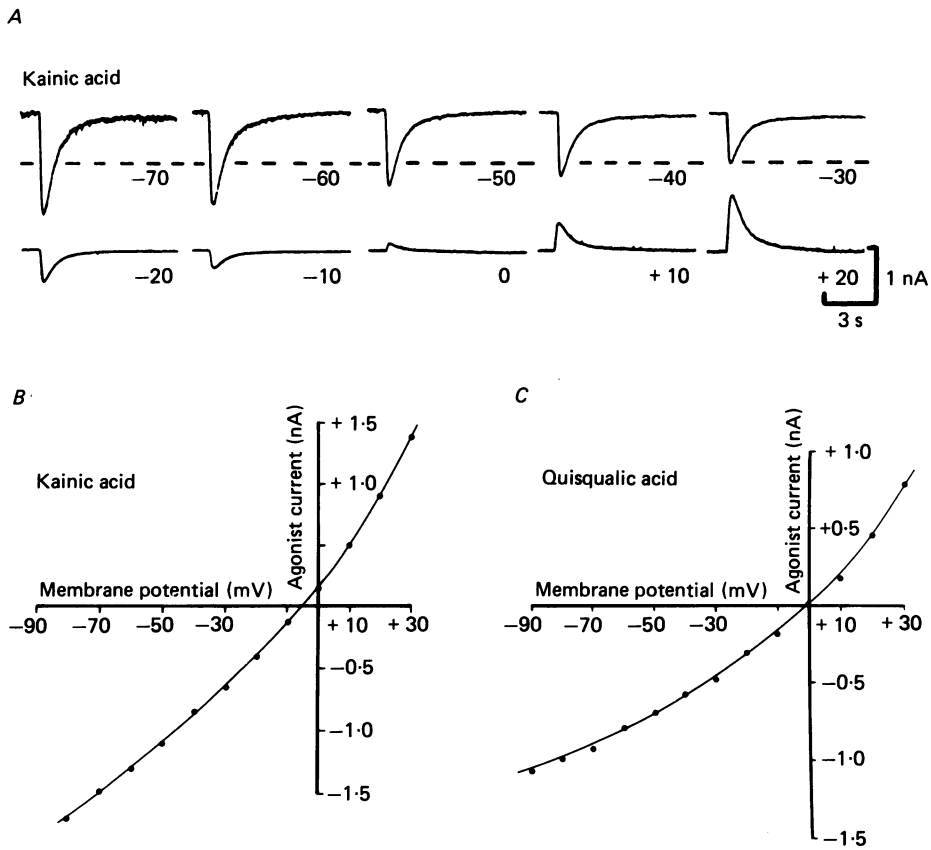

Fig. 2. *A*, voltage sensitivity of responses evoked by kainic acid. The current responses evoked by brief pressure applications of kainic acid were recorded at several values of membrane potential, and are presented with the holding currents offset to the same base line at each membrane potential. The dashed lines indicate the amino acid current response amplitude at −30 mV. The responses evoked by kainic acid (and quisqualate, not shown) change in amplitude in an approximately linear fashion between −70 and +20 mV. *B*, *C*, current–voltage relations of responses evoked by kainic acid (*B*) and quisqualic acid (*C*). Each current–voltage plot (Figs. 2, 3, 4 and 5) presents data from one spinal cord neurone. Membrane potential (abscissa) is plotted against the amplitude of amino acid current responses (ordinate), and each data point is the mean of two observations.

evoked by D-homocysteate ($n = 6$) were less voltage sensitive than those evoked by L-homocysteate (Fig. 4). L-glutamate responses also showed intermediate voltage sensitivity (Fig. 5), although at membrane potentials more negative than −30 mV, the slope conductance usually had small positive values (see Table 1).

Since pharmacological evidence suggests that there are distinct NMDA and non-NMDA (kainate and quisqualate) receptors (Watkins, 1981*a*, *b*), the intermediate voltage-sensitive behaviour of L-glutamate and L-homocysteate is consistent with a simultaneous action at both NMDA and non-NMDA receptors. To examine this possibility, we recorded the response of spinal cord neurones to mixtures of kainic acid and NMDA applied from the same micropipette. The current–voltage relation of responses evoked by such a mixed-agonist application ($n = 6$) strongly resembled

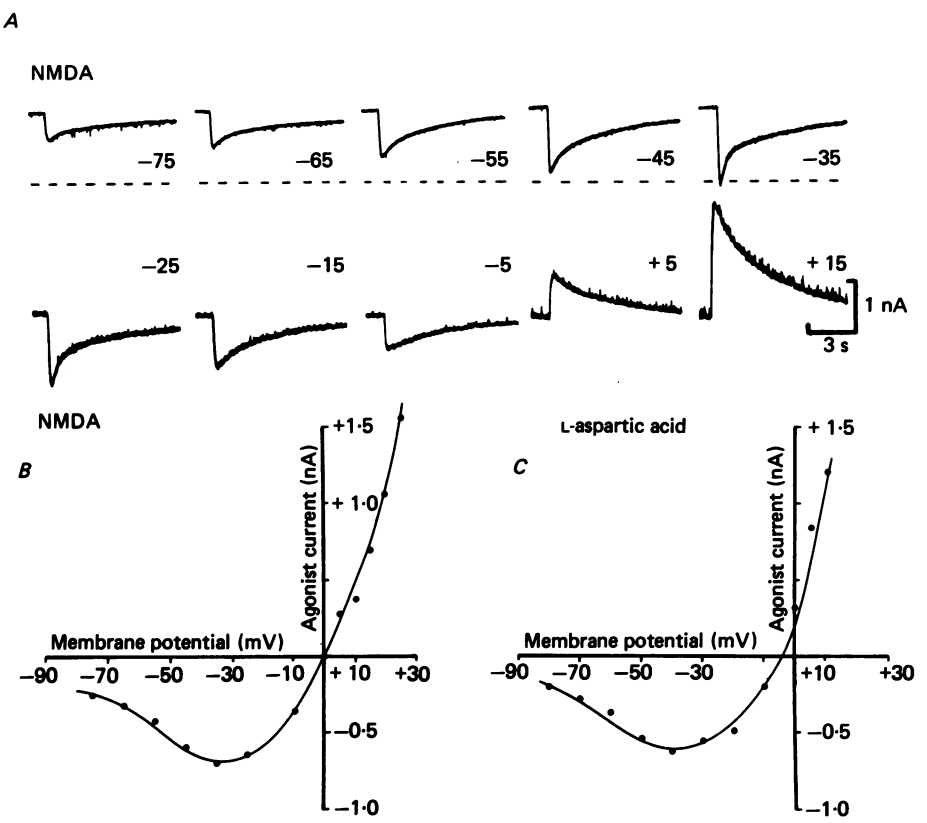

Fig. 3. *A*, voltage sensitivity of responses evoked by NMDA. Holding currents are offset to the same base line at each membrane potential. The dashed line is the NMDA current amplitude at −30 mV. In contrast to kainic or quisqualic acids (see Fig. 2), responses evoked by NMDA increase in amplitude as the membrane potential is hyperpolarized from −5 to −25 mV, but then decrease in amplitude on further hyperpolarization from −35 to −75 mV; depolarized beyond its reversal potential (0 mV) NMDA-evoked outward current responses are of much larger amplitude than inward current responses recorded at more hyperpolarized membrane potentials (cf. responses recorded at +15 and −15 mV). *B* and *C*, current–voltage relations for NMDA (*B*) and L-aspartic acid (*C*). Both plots are J-shaped with a negative slope at membrane potentials more hyperpolarized than −30 mV and a positive slope at potentials more depolarized than −30 mV.

that produced by L-glutamic and L-homocysteic acids (Fig. 5, see Table 1 for slope conductance measurements).

The reversal potential of responses evoked by L-glutamate (−4 to +3 mV, mean −0·6) and L-homocysteate (−5 to +2 mV, mean −1·2) was similar to that of the other acidic amino acids. The use of caesium chloride electrodes did not appear substantially to alter the voltage sensitivity or reversal potential of the responses evoked by L-glutamate since similar responses were recorded using potassium chloride ($n = 2$) or caesium sulphate ($n = 3$) in the recording electrodes. Such results suggest that caesium may be highly permeable through the ion channels activated by L-glutamate. At the frog end-plate there is a similar lack of discrimination in the permeability to small cations (Adams, Dwyer & Hille, 1980).

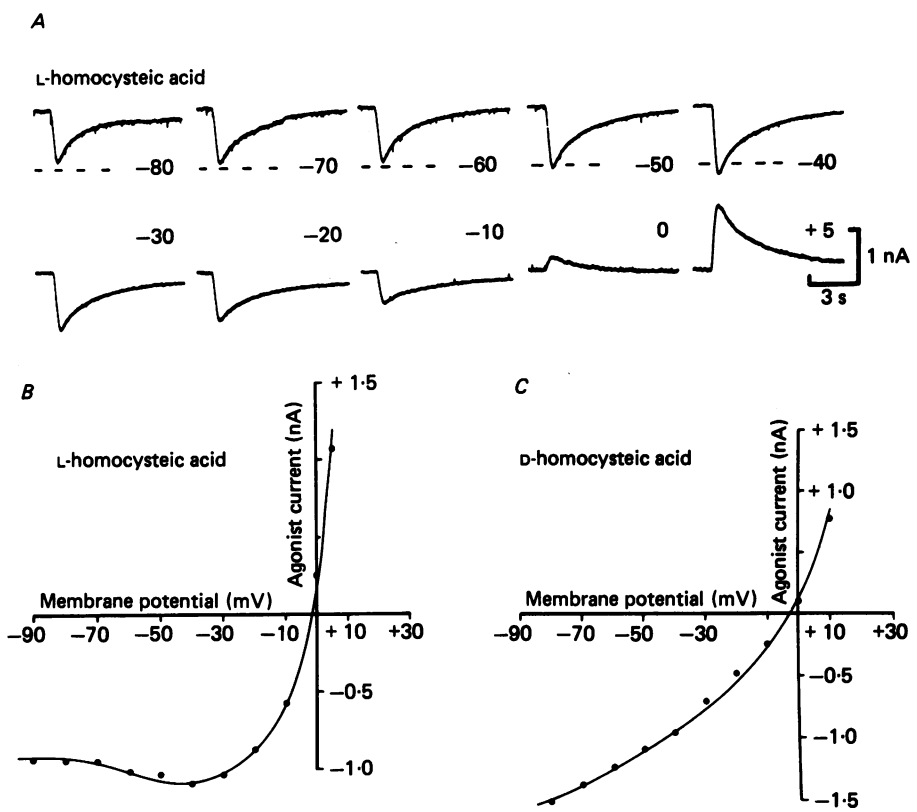

Fig. 4. *A*, voltage sensitivity of responses evoked by L-homocysteic acid. Holding currents are offset to the same base-line value at each membrane potential. The dashed line is the L-homocysteate current amplitude at −30 mV. In contrast to responses evoked by kainic acid (Fig. 2) or NMDA (Fig. 3), responses evoked by L-homocysteate remain relatively constant in amplitude as the membrane potential is hyperpolarized from −30 to −80 mV. *B* and *C*, current–voltage relations of responses evoked by L-homocysteic acid (*B*) and D-homocysteic acid (*C*). While responses evoked by L-homocysteic acid show only a small change in amplitude as the membrane potential is depolarized from −80 to −30 mV (cf. responses shown in Fig. 3), further depolarization evokes large increases in the slope conductance (*B*). In contrast, D-homocysteic acid responses result in a more linear current–voltage relation.

Membrane potential–conductance relation of amino acid responses

Changes in the slope of amino acid current–voltage relations highlight the voltage sensitivity of the response, but reveal little about the underlying conductance mechanism. For a parallel conductance model (Ginsborg, 1967) the agonist-evoked current I_x is related to the agonist-activated ionic (chord) conductance G_x by the equation:

$$I_x = G_x(E_m - E_x),$$

where E_m is the membrane potential and E_x the reversal potential of the agonist-activated current. We were always able to record amino acid-evoked currents on both sides of the reversal potential and could therefore accurately estimate the driving force $(E_m - E_x)$ either by direct measurement or by interpolation of the reversal

potential for each neurone. The amino acid-activated ionic conductance was then calculated and plotted *versus* the membrane potential (Fig. 6).

The membrane potential–conductance relation of responses evoked by kainic acid and quisqualic acid was relatively flat. Outward rectification ratios were obtained by dividing the chord conductance at +20 mV by the chord conductance at −70 mV. The mean ratio was 1·38 for kainic acid and 1·78 for quisqualic acid (Table 2). Thus, the conductance mechanism linked to these receptors is only slightly sensitive to the membrane potential. In contrast, the conductance linked to NMDA receptors is lowest at hyperpolarized membrane potentials and increases continuously with depolarization over the entire range of potentials investigated. The large rectification ratios for NMDA (28·7) and L-aspartic acid (22·7) reflect this voltage sensitivity (Table 2).

TABLE 1. Slope conductance measurements obtained from current–voltage plots of the response to various excitatory amino acids under voltage clamp. Measurements were made at membrane potentials of −60 mV ($G_{S_{-60}}$) and +10 mV ($G_{S_{+10}}$). For NMDA, L-aspartate, and in some neurones the response to L-homocysteic acid and the mixture of kainic and NMDA, the slope conductance at −60 mV was negative; for these agonists, and for L-glutamate and D-homocysteate the slope conductance at +10 mV was large and positive. In contrast, the slope conductance of responses evoked by kainic and quisqualic acids showed less change on depolarizing from −60 to +10 mV

		$G_{S_{-60}}$ (nS)			$G_{S_{+10}}$ (nS)		
Agonist	(*n*)	Mean	Range	S.E. of the mean	Mean	Range	S.E. of the mean
NMDA + NMA	(10)	−15·3	−9 to −29	2·26	+69·4	+35 to +115	7·1
L-aspartate	(5)	−14·8	−10 to −22	2·07	+91·8	+75 to +107	6·8
L-homocysteate	(8)	−2·3	−10 to +4	1·74	+167	+75 to +433	39·4
Mix (kainate + NMDA)	(6)	+3·8	−2 to +8	1·59	+88·9	+23 to +190	24·4
D-homocysteate	(6)	+16·5	+9 to +31	3·37	+89·8	+42 to +123	11·7
L-glutamate	(16)	+5·6	+1 to +9	0·93	+74·7	+17 to +139	9·7
L-glutamate + 2-APV	(9)	+5·2	+6 to +21	1·74	+38·1	+17 to +67	5·5
Quisqualate	(5)	+8·6	+6 to +13	1·19	+22·5	+10 to +36	4·3
Kainate	(4)	+30·4	+12 to +44	7·68	+46·0	+12 to +85	16·3

L-homocysteic acid, D-homocysteic acid and L-glutamic acid gave intermediate responses. At hyperpolarized membrane potentials the conductance activated by L-glutamate and D- and L-homocysteate was of the order of 10–20 nS, similar to values recorded for quisqualate and kainate but considerably higher than that recorded for NMDA and L-aspartate. As the membrane was depolarized, the conductance activated by D- and L-homocysteate and L-glutamate increased steeply, in a similar fashion to that activated by NMDA and L-aspartic acid (Table 2). Thus, chord conductance measurements suggest a mixed-agonist action of L-glutamate, L-homocysteate and D-homocysteate on two separate receptor linked mechanisms.

Agonist ionophoresis by membrane current does not explain voltage dependence of L-glutamate responses

An action of L-glutamate as a mixed agonist at two receptors, one the NMDA receptor linked to a highly voltage-sensitive mechanism, the other a quisqualate or kainate receptor linked to a more conventional agonist-activated conductance, is

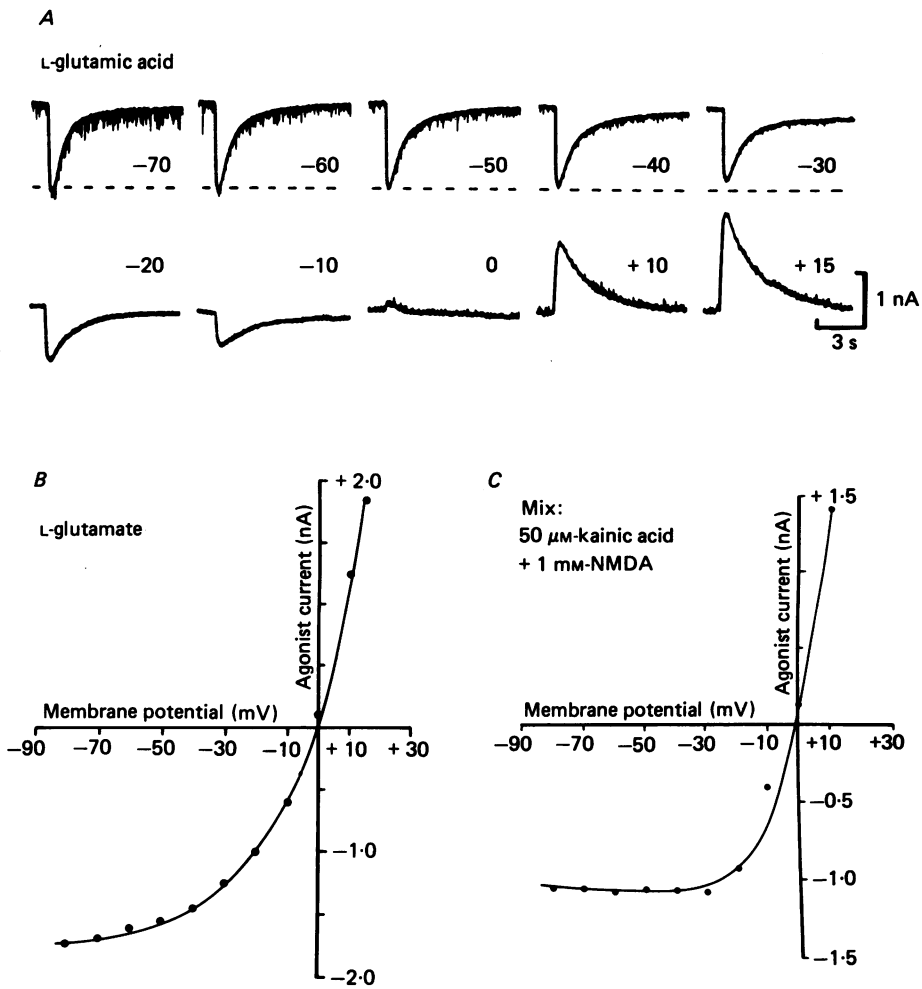

Fig. 5. *A*, voltage sensitivity of responses evoked by pressure application of L-glutamic acid. Holding currents were offset to the same base-line value at each membrane potential. The dashed lines indicate the current response amplitude recorded at −50 mV; note that responses evoked by L-glutamate show only a small increase in amplitude as the membrane potential is hyperpolarized from −30 to −70 mV. Small fluctuations in the current traces are spontaneous synaptic currents which reverse near −20 mV. Note that it is easy to distinguish between the fast synaptic and slower drug-evoked responses, and that the synaptic responses have a more linear voltage dependence than the responses evoked by L-glutamate. No attempt was made to ascertain if the synaptic responses were due to packets of inhibitory transmitter (the intracellular micro-electrodes contained caesium chloride) or if they were excitatory synaptic responses, however, since the reversal potential of stimulus-evoked e.p.s.c.s occurs close to 0 mV it is probable that many of these spontaneous events are i.p.s.c.s mediated by inward chloride currents. *B* and *C*, current–voltage relations of responses evoked by L-glutamic acid (*B*) and a mixture of 50 μM-kainic acid and 1 mM-NMDA acid (*C*). Responses evoked by L-glutamic acid (*B*) and the mixture (*C*), both yield current–voltage plots that are characteristic in shape: in each case there is only a small increase in slope conductance with depolarization over the range −80 mV to −30 mV; however, further depolarization, beyond −30 mV, produces much larger increase in slope conductance.

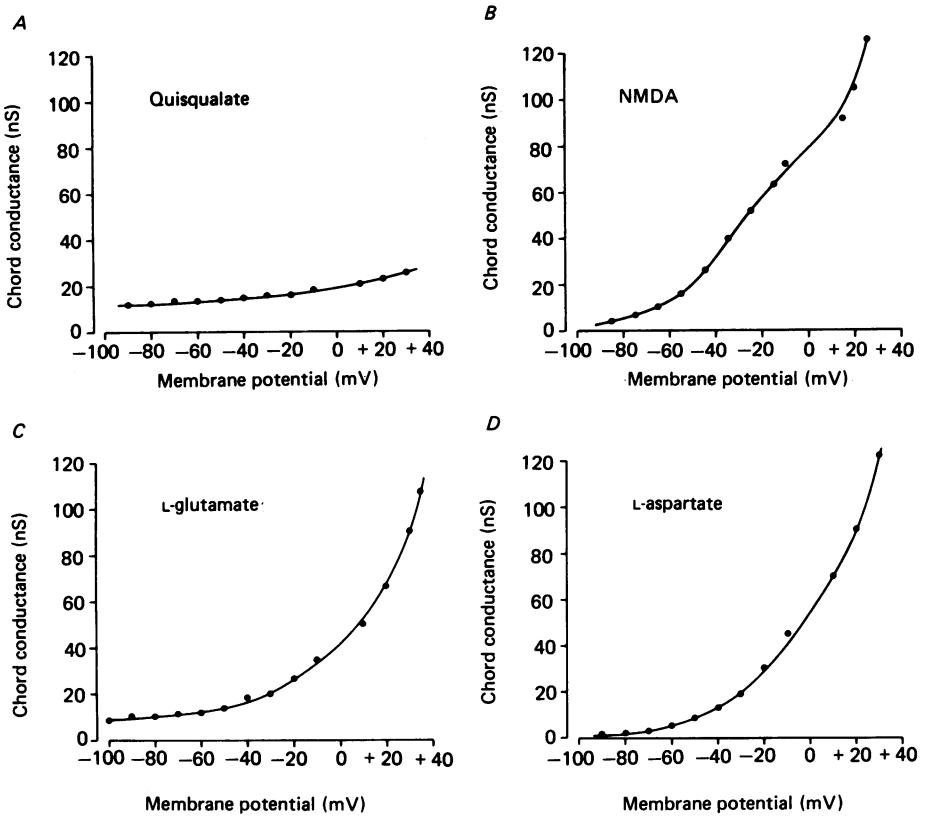

Fig. 6. Chord conductance–membrane potential relations for *A*, quisqualic acid, *B*, NMDA, *C*, L-glutamic acid and *D*, L-aspartic acid. Chord conductance values were calculated by dividing the current response amplitude at a given membrane potential by the driving force for amino acid-activated ionic current. The conductance mechanism linked to quisqualate receptors (*A*) and kainate receptors (not shown) was relatively insensitive to the membrane potential. In contrast, the conductance mechanisms activated by NMDA (*B*), L-glutamate (*C*) and L-aspartate (*D*) behave as outward rectifiers, and increase in amplitude as the membrane is depolarized. The inflexion in the conductance plot for NMDA over the potential range −50 to +20 mV was not observed in all neurones. Although similar in over-all shape, conductance plots for L-aspartate and L-glutamate responses differ in that the conductance activated by L-glutamate does not fall below 8–10 nS at −100 mV; in contrast at −90 mV the L-aspartate response is less than 2 nS.

sufficient to explain the characteristic shape of the L-glutamate current–voltage relation. To exclude the possibility that the voltage dependence of L-glutamate action is due to ionophoresis of agonist into the synaptic membrane, the response produced by two doses of L-glutamate was recorded over the potential range −70 to +15 mV (Fig. 7). The result suggests that ionophoresis of agonist does not occur since this would result in a non-linear relation (see Dionne & Stevens, 1975, for a discussion of similar experiments on the response to acetylcholine at the frog end-plate). A linear relation suggests that even with the low dose of L-glutamate there is activation of both NMDA and non-NMDA receptors.

Action of NMDA receptor antagonists on L-glutamate responses

The recent development of selective antagonists acting at excitatory amino acid receptors provides a useful experimental tool to examine the mixed-agonist action of L-glutamic acid. We used 2-APV, a potent NMDA receptor antagonist (Davies *et al.* 1981). To confirm the specificity of action of 2-APV in cultures of mouse spinal neurones we tested its effect on depolarization evoked by NMDA and kainic acid. 2-APV clearly blocked the depolarization evoked by NMDA with little action on the response to kainic acid (Fig. 8).

TABLE 2. Chord (ionic) conductance measurements calculated from amino acid-evoked responses using the driving force for ionic current (see text on p. 36). Measurements were made at membrane potentials of −70 mV ($G_{c_{-70}}$) and +20 mV ($G_{c_{+20}}$). An outward rectification ratio ($G_{c_{20/70}}$) was obtained by dividing the chord conductance at +20 mV by the chord conductance at −70 mV. The amino acid responses fell into three categories. NMDA and L-aspartic acid, agonists showing the greatest voltage sensitivity, have outward rectification ratios exceeding 20. L-homocysteic acid, D-homocysteic acid, L-glutamic acid and the mixture of kainic acid + NMDA have rectification ratios of 4·5–12·5, consistent with their action as mixed agonists. Quisqualic acid, kainic acid, and L-glutamic acid in the presence of 2-APV, form the third group of agonists showing only weak voltage sensitivity, with rectification ratios of 1·4–1·9

		$G_{c_{-70}}$ (nS)			$G_{c_{+20}}$ (nS)			
Agonist	(*n*)	Mean	Range	S.E. of the mean	Mean	Range	S.E. of the mean	$G_{c_{20/70}}$
NMDA + NMA	(10)	3·9	0·8–7·5	0·7	79·3	27·3–122	9·1	28·7
L-aspartate	(5)	4·3	2·8–5·7	0·6	91·8	78·2–110	5·2	22·7
L-homocysteate	(8)	12·6	5·4–17·2	1·8	131	85–140	10·6	12·5
Mix (kainate + NMDA)	(6)	12·9	6·6–15·6	1·3	71·3	27·5–117	12·6	5·3
D-homocysteate	(6)	21·1	6·7–28	3·2	91·8	45–155	16·2	4·7
L-glutamate	(14)	18·8	7·2–27·4	2·0	84·8	28–138	9·3	4·5
L-glutamate + 2-APV	(8)	20·3	11·8–28·9	2·2	38·1	22·7–65·8	4·9	1·9
Quisqualate	(5)	11·8	8·3–16·7	1·6	21·6	11·1–34·3	4·2	1·8
Kainate	(4)	32·8	10·8–52·2	9·6	47·5	11·2–86·4	15·9	1·4

If L-glutamate activates NMDA receptors, then 2-APV should convert the response evoked by L-glutamate to one resembling that produced by kainate and quisqualate. The current–voltage relation of L-glutamate responses recorded in the presence of 2-APV ($n = 9$) was considerably more linear than obtained in control experiments (see Fig. 9 and Table 1). Chord conductance–membrane potential plots for L-glutamate showed less outward rectification in the presence of 2-APV (Fig. 9); as a consequence, the rectification ratio was also considerably less and approached those obtained for kainate and quisqualate (Table 2).

In control experiments, the slope of the L-glutamate current–voltage relation in the region of resting potential is close to zero (Figs. 5 and 9). In the presence of 2-APV, for equivalent inward current responses, the slope of the L-glutamate current–voltage relation has more positive values (Fig. 9 and Table 1). Our results explain previous reports suggesting lack of a membrane conductance change during the action of L-glutamate (Puil, 1982). In the absence of 2-APV, the increase in membrane

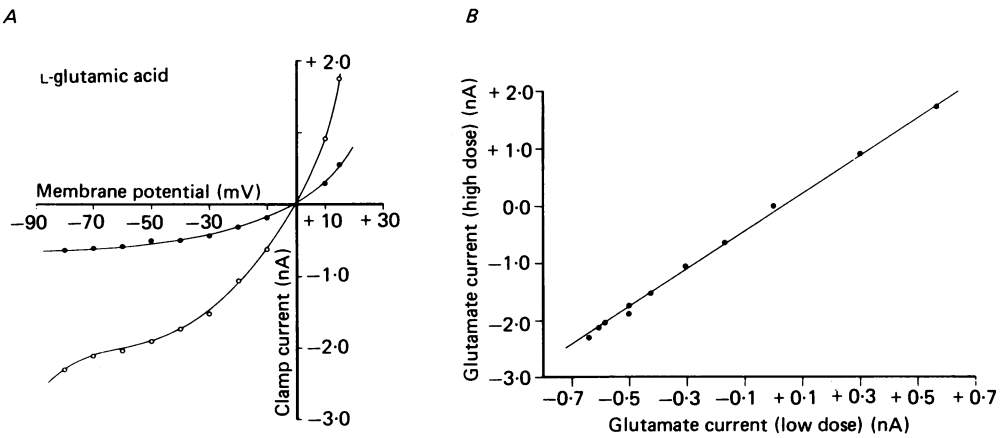

Fig. 7. Voltage dependence of L-glutamate action is independent of agonist concentration at low doses of L-glutamate. *A* shows the current–voltage relation of responses evoked by two doses of L-glutamate adjusted to give inward currents of 2·0 and 0·5 nA at −50 mV. The response to both doses is non-linear with respect to membrane potential. *B* shows a plot of the amplitude of the response evoked by the higher dose of L-glutamate *versus* the amplitude of the response evoked by the lower dose recorded at the same holding potential, over the range −80 to +15 mV. The two responses are highly correlated suggesting that over this membrane potential and agonist concentration range the voltage dependence of L-glutamate action is not a function of the agonist concentration *per se*.

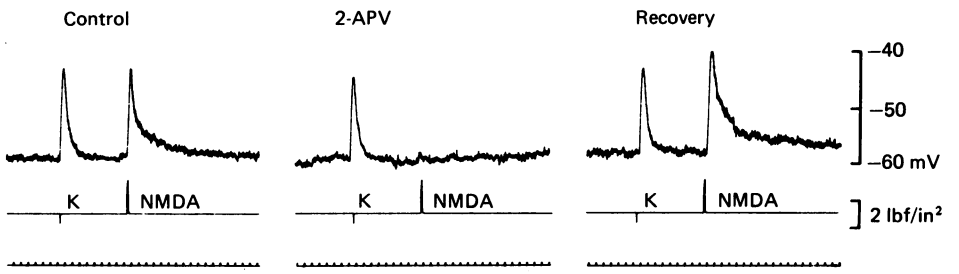

Fig. 8. Selective antagonism of NMDA but not kainic acid (K) depolarizing responses by 2-APV. Each trace shows a chart record of the membrane potential of a spinal cord neurone (upper trace) during pressure application of pulses of kainate and NMDA. The middle record shows the output from a pressure transducer monitoring the period of drug application. Leakage of 2-APV from a broken pipette containing 2 mM-antagonist selectively and reversibly antagonized the membrane potential depolarization evoked by NMDA. The lower record shows 1 s time marks.

conductance recorded using a hyperpolarizing voltage jump during a steady inward current evoked by L-glutamate is much less than expected for a classical excitatory synaptic mechanism. After block of NMDA receptors by 2-APV, L-glutamate produces easily measurable increases in membrane conductance (Fig. 9).

Amino acid dose–membrane conductance relation

To examine the relation between membrane conductance and different doses of excitatory amino acids, we performed voltage-jump experiments during steady inward currents evoked by kainic acid and L-glutamic acid. However, the performance

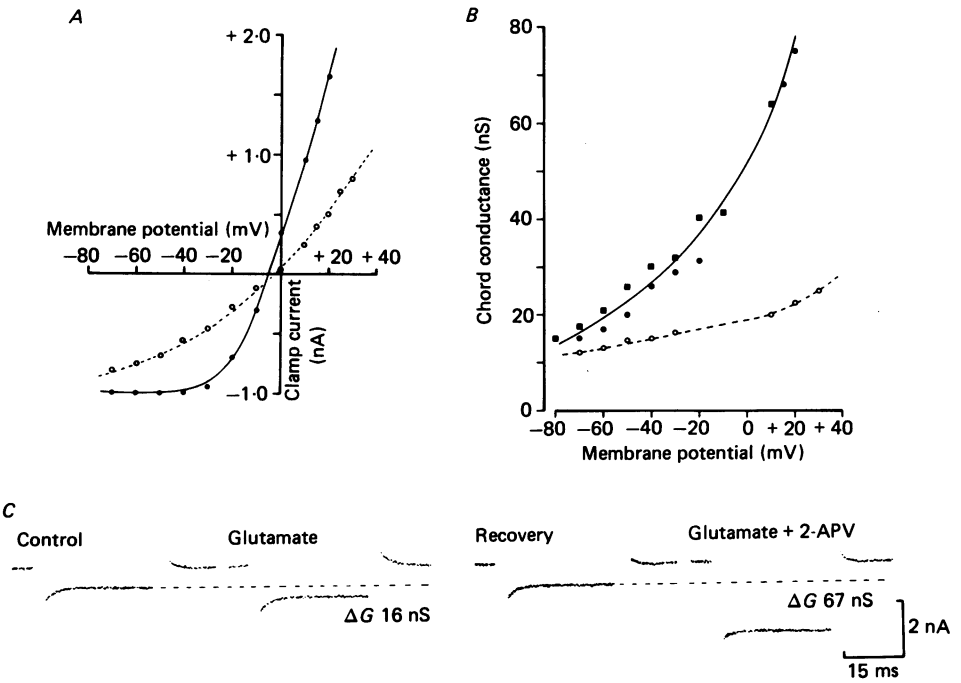

Fig. 9. Action of the NMDA receptor antagonist (±)-2-APV on the response to L-glutamic acid. *A* shows current–voltage plots of the response evoked by application of L-glutamate during diffusion of (±)-2-APV from a micropipette containing 250 μM-antagonist (○) and following recovery from the effect of 2-APV (●). *B* shows chord conductance–membrane potential plots derived from the L-glutamate responses shown in *A*. Control responses (■) and recovery (●) show strong outward rectification; during application of 2-APV (○) the L-glutamate response shows considerably less rectification. *C* shows current relaxations produced by 25 mV step hyperpolarizing voltage jumps from a holding potential of −50 mV, and recorded from one spinal cord neurone. The resting membrane conductance was 28 nS; during the application of L-glutamate sufficient to evoke 2·1 nA of inward current at −50 mV, the membrane conductance increased to 44 nS (dashed lines indicate the conductance recorded immediately before application of L-glutamate or L-glutamate+2-APV). On recovery from the response to L-glutamate, the resting conductance was 31 nS. During the simultaneous application of L-glutamate and (±)-2-APV, sufficient to evoke 2·3 nA of inward current at −50 mV, the membrane conductance increased to 98 nS. Each record is the average of ten responses.

of our voltage clamp was not sufficiently fast (settling time 0·5 ms) to accurately measure the instantaneous membrane chord conductance in the presence of rapid time- and voltage-dependent conductance changes. Since with L-glutamic acid the membrane conductance changes so rapidly with time over the potential range −50 to −100 mV (Fig. 10), that it is effectively constant for even 10–20 ms voltage jumps, we were unable to estimate the instantaneous current–voltage relation of the membrane conductance mechanism activated by L-glutamate. In contrast, the conductance mechanism linked to kainic acid receptors is not very sensitive to the membrane potential and the membrane current response to this amino acid does not show fast relaxations following voltage jumps. Thus, brief (30 ms) voltage jumps were used to estimate the steady-state membrane current–voltage relation in the presence of several doses of kainic acid and L-glutamic acid (Fig. 10).

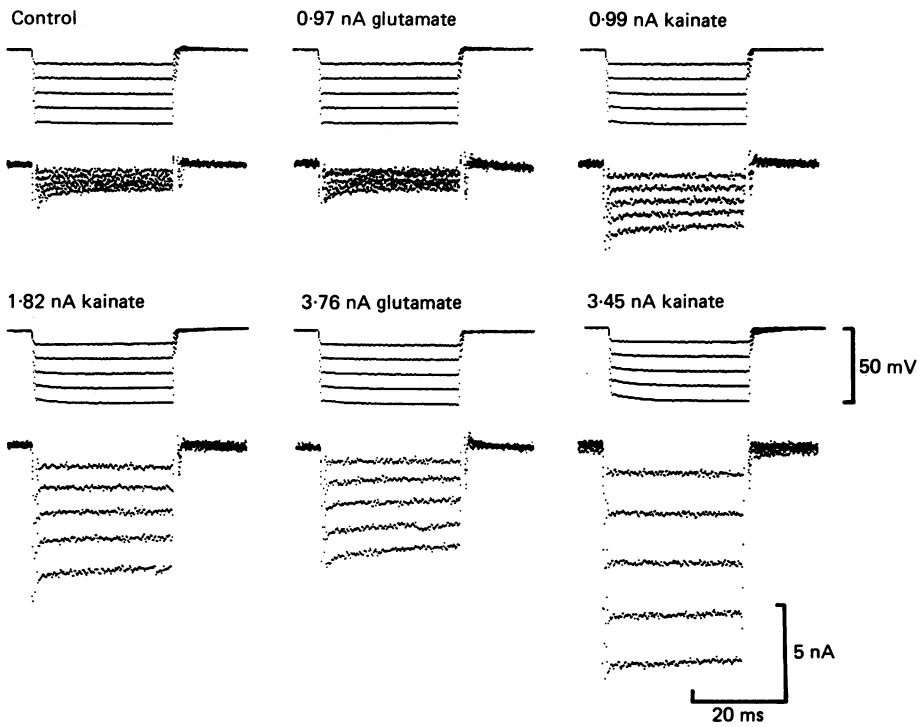

Fig. 10. Membrane conductance changes evoked by several doses of kainic acid and L-glutamic acid. Each set of records, obtained from the same spinal cord neurone, shows membrane potential (upper record) and membrane current (lower record) during 30 ms step hyperpolarizing voltage jumps incrementing by −10 mV from a holding potential of −50 mV. The resting current–voltage relation was linear (upper left) and the membrane conductance at the end of the 30 ms voltage jump was 20 nS (control). Steady inward currents were then evoked by application of L-glutamate and kainate. During a steady inward current of 0·97 nA evoked by application of L-glutamate (upper middle) the membrane conductance was unchanged. Kainic acid was then applied to evoke a steady inward current of 0·99 nA (upper right), and this was accompanied by an obvious membrane conductance increase. Larger doses of kainic acid (1·82 nA, 3·45 nA lower left and lower right) evoked further increases of membrane conductance. In contrast, the membrane conductance increase during a large current evoked by L-glutamate (3·76 nA, lower middle) was much less than that recorded during the 3·45 nA response evoked by kainic acid. Between each application of amino acid a control current–voltage plot was obtained to ensure that the resting membrane conductance remained stable.

For kainic acid, subtraction of the resting membrane chord conductance from that recorded during a steady inward current evoked by the application of amino acid gives a true estimate of the increase in chord conductance (ΔG) accompanying the amino acid response. In contrast, for L-glutamate the subtraction procedure measures only the change in membrane slope conductance produced by L-glutamate.

For responses evoked by several doses of kainic acid there was an approximately linear relation between the amplitude of the steady drug current recorded at the holding potential of −50 mV, and the increase in membrane chord conductance (ΔG) recorded during a series of hyperpolarizing voltage jumps to −100 mV (Fig. 10). Low doses of L-glutamate, producing about 1 nA of inward current at −50 mV, evoke small increases, small decreases, or no apparent change in membrane slope con-

ductance; larger doses of L-glutamate evoke membrane conductance increases, but these are of much smaller amplitude than those evoked by doses of kainic acid producing equivalent inward current responses at a holding potential of −50 mV (Fig. 10).

The resting membrane current–voltage relation was usually linear between −50 and −100 mV for 30 ms voltage jumps, and remained linear in the presence of either kainate or L-glutamate (Fig. 11). For a voltage-insensitive agonist, extrapolation of the membrane current–voltage relations recorded in the presence and absence of agonist will intercept at the reversal potential of the agonist-activated conductance; kainic acid produced increases in the slope of the current–voltage relation which confirmed this (Fig. 11). However, during large sustained currents evoked by kainic acid the extrapolated reversal potential was often shifted in the hyperpolarizing direction suggesting changes in ionic gradients.

In contrast, since small doses of L-glutamate produce little change in the slope of the membrane current–voltage relation, regression lines plotted through data points recorded in the presence and absence of L-glutamate were nearly parallel. Higher doses of L-glutamate did produce increases in membrane chord conductance (ΔG); however, extrapolaation often gave estimates of the reversal potential more positive than those recorded experimentally. When several doses of L-glutamate were applied to a single neurone, a plot of ΔG *versus* the L-glutamate activated current at −50 mV was non-linear, consistent with a negative slope conductance for small doses of L-glutamate, although this was not confirmed experimentally. The highest doses of L-glutamate used in our experiments produced membrane chord conductance increases comparable to those evoked by kainate. Such a dose-dependent action of L-glutamate as a mixed agonist has not been clearly demonstrated before. Although dose-dependent membrane resistance changes for the action of L-glutamate have been reported for current-clamp experiments (MacDonald & Wojtowicz, 1982), the depolarization associated with larger doses of L-glutamate is sufficient to enter a region where voltage-sensitive potassium currents are activated and the slope of the L-glutamate current–voltage relation has large positive values (see Fig. 5).

Interaction between amino acid responses

The negative slope conductance associated with the activation of NMDA receptors should potentiate any depolarizing stimulus, such as an excitatory post-synaptic potential (e.p.s.p.), if the membrane potential is within the negative slope region of the NMDA current–voltage relation. To model this situation, we evoked depolarizing responses with a voltage-insensitive amino acid, kainic acid, and applied doses of NMDA that in isolation produced only small depolarizations. Responses evoked by kainic acid were clearly potentiated by NMDA (Fig. 12). NMDA receptor agonists and mixed agonists may produce a similar modulation of excitatory synaptic potentials *in vivo*; Engberg, Flatman & Lambert (1979) have shown potentiation by DL-homocysteic acid of Ia e.p.s.p.s recorded in motoneurones. This is consistent with the activation of NMDA receptors by the mixed agonists, D- and L-homocysteic acids (Fig. 4).

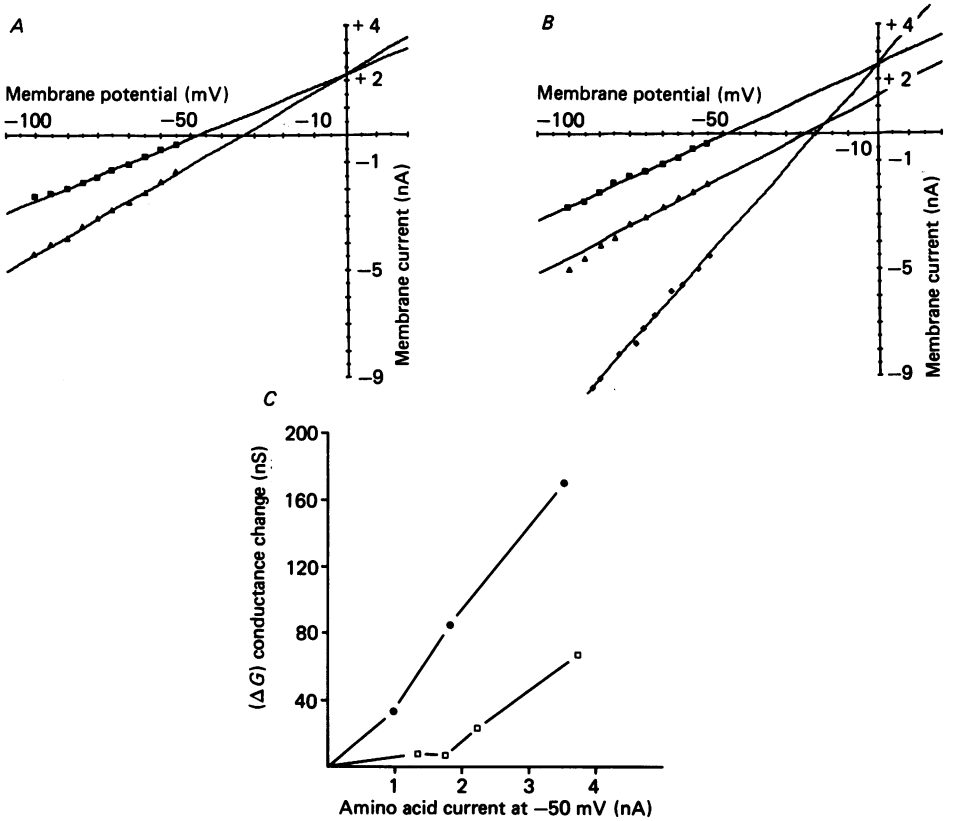

Fig. 11. *A* and *B* show membrane current–voltage relations recorded from spinal cord neurones voltage clamped at a holding potential of −50 mV. Step hyperpolarizing voltage jumps (30 ms, incrementing by −5 mV) were used to measure the membrane conductance at rest and during steady inward currents evoked by amino acids; membrane potential and current were sampled at 5 kHz and linear regression analysis used to estimate membrane chord conductance. A linear portion of the current–voltage relation was used for the regression analysis (−50 to −100 mV in *A*, and −50 to −80 mV in *B*). Application of kainic acid sufficient to evoke 1·0 nA of inward current at −50 mV (*A*) increased the membrane conductance by 20·3 nS. Control (■) and kainate (▲) regression lines intersect at 0 mV, the extrapolated reversal potential of the kainic acid current. *B* shows responses of a second neurone to two doses of L-glutamate. Application of L-glutamate sufficient to evoke 1·0 nA of inward current at −50 mV increased the membrane conductance by 0·2 nS; thus control (■) and L-glutamate (▲) regression lines are virtually parallel and there is no apparent reversal potential for the L-glutamate current. Since the input resistance of this neurone was 27 MΩ, this dose of L-glutamate would have depolarized the membrane potential by 27 mV if the neurone had not been voltage clamped. A much larger dose of L-glutamate, sufficient to evoke 3·7 nA of inward current at −50 mV (◆), was associated with a membrane conductance increase of 93·7 nS. Regression analysis showed an extrapolated reversal potential for this L-glutamate response of 0 mV. *C* shows membrane conductance changes, measured as in *A* and *B*, associated with several doses of kainate (●, one neurone) and L-glutamate (□, a second neurone) and plotted against the agonist-activated current recorded at −50 mV. Kainic acid responses show a relatively constant increase in membrane conductance with dose of amino acid; conductance changes associated with L-glutamate responses show a non-linear dose dependence with only larger doses of L-glutamate evoking appreciable membrane chord conductance increases. Higher doses of kainic acid gave a larger conductance change than expected for a driving force of 50 mV, presumably due to shifts in ionic gradients induced by large kainic acid currents.

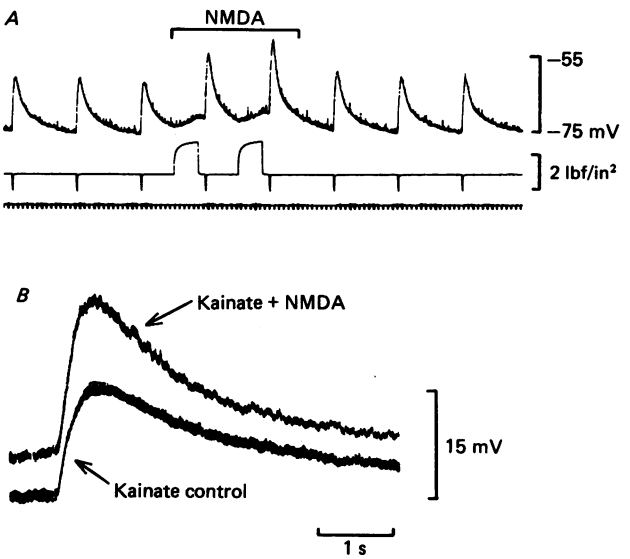

Fig. 12. Potentiation of kainic acid responses by NMDA. *A* shows a chart record of the membrane potential of a spinal cord neurone (upper trace) and the output of a pressure transducer used to monitor the period of application of amino acids (middle trace). The lower record shows 1 s time marks. The kainic acid pipette was positioned close to the spinal cord neurone to evoke brisk depolarizing responses; the pipette containing NMDA was deliberately placed several hundred micrometres away to evoke small prolonged depolarizing responses. Depolarizing responses evoked by kainic acid were clearly potentiated during application of NMDA. *B* shows oscillographic records of the depolarizing response to kainate before and during the application of sufficient NMDA to depolarize the membrane by 5 mV. The control response to kainate produced a depolarization of 14 mV; during the simultaneous application of NMDA the amplitude of the kainic acid response increased to 20 mV.

DISCUSSION

The depolarization of mouse spinal neurones in culture by acidic amino acids is consistent with the well-documented excitatory action of these amino acids *in vivo*. Our results, obtained under voltage clamp, provide further insight into the current–voltage relation and membrane conductance change produced by a number of different acidic amino acids in mammalian neurones. Earlier studies on spinal cord neurones *in vivo* and in culture clearly showed that excitatory amino acid receptors are linked to at least two distinct conductance mechanisms (Engberg *et al.* 1978; MacDonald & Porietis, 1982); however, the limitations imposed by intracellular recording of membrane potential during current injection with a single micro-electrode hinders measurement of the current–voltage relation of excitatory amino acids responses and associated membrane conductance changes (see MacDonald & Porietis, 1982, fig. 3). In addition, voltage sensitivity of the conductance linked to NMDA receptors (MacDonald *et al.* 1982; Flatman *et al.* 1983) greatly complicates the interpretation of such data. Thus voltage-clamp recording is a prerequisite for more detailed studies of excitatory amino acid action in mammalian neurones.

Two membrane conductance mechanisms

The reversal potential of responses evoked by agonists acting at NMDA and non-NMDA receptors is similar and close to 0 mV. This suggests that in mouse spinal neurones activation of excitatory amino acid receptors does not act to increase the membrane permeability selectively to either sodium or calcium ions (cf. Dingledine, 1983), nor to decrease the resting membrane permeability to potassium ions (cf. Hablitz, 1982). In addition, a dual conductance mechanism of the type in which excitatory amino acids decrease the membrane permeability to potassium ions and increase the membrane permeability to sodium or calcium ions (Puil, 1982) is also excluded since such a system would show a reversal potential outside the range of Nernstian equilibrium potentials of these ions, i.e. hyperpolarized to E_K or depolarized to E_{Na} or E_{Ca} (Brown, Muller & Murray, 1971). At the vertebrate neuromuscular junction the reversal potential of the end-plate current varies between -15 and 0 mV (Fatt & Katz, 1951; Takeuchi & Takeuchi, 1960) and ion substitution experiments suggest an increase in membrane permeability to both sodium and potassium ions. It seems probable that a similar mechanism, possibly including a sizeable calcium ion component, underlies the action of excitatory amino acids on spinal neurones; ion substitution experiments to test this are in progress. In our experiments, multiple reversal potentials to L-glutamic acid were observed only occasionally (see Wojtowicz, Gysen & MacDonald, 1981) in that small biphasic currents (early outward, late inward) were sometimes recorded within a few millivolts of zero membrane potential (i.e. the reversal potential of the amino acid current), but we are uncertain if this reflects a small degree of space-clamp error or the activation of two conductance mechanisms with nearly identical reversal potentials.

Despite the similarity of the reversal potential of currents evoked by agonists selective for NMDA and non-NMDA receptors, the current–voltage relation of the two responses is markedly different. The conductance linked to NMDA receptors increases as the membrane potential is depolarized such that the conductance available at $+20$ mV is approximately 28 times larger than at -70 mV. The conductance linked to non-NMDA receptors shows a much weaker dependence on the membrane potential and the conductance available at $+20$ mV is only 1·5 times larger than that available at -70 mV. This suggests, but does not prove, that NMDA and non-NMDA receptors are linked to two distinct conductance mechanisms. The response evoked by L-glutamate appears to be produced by activation of both mechanisms, rather than one of intermediate voltage sensitivity.

The results presented in this paper reveal little about the mechanism of the voltage sensitivity of acidic amino acid responses, but it is useful to consider some of the following possible causes.

(1) Activation of NMDA receptors increases the maximum conductance available for a voltage-sensitive mechanism active in the absence of NMDA (see Dingledine, 1983); it is unlikely that a calcium-specific channel is the underlying charge carrier, despite the precedent for modulation of calcium conductance by β-adrenergic agonists in cardiac muscle membranes (Reuter, 1983), since the reversal potential of the NMDA current, around 0 mV, is considerably more negative than that expected for a calcium current. It is plausible that NMDA could act to modulate voltage-sensitive

conductances of the type carrying persistent inward current in motoneurones (Schwindt & Crill, 1980) and neocortical pyramidal cells (Stafstrom, Schwindt & Crill, 1982); until the reversal potential of these currents is measured further comparison with responses evoked by NMDA is premature.

(2) NMDA receptors are linked to ion channels not serving any other function, in a similar fashion to the coupling between nicotinic receptors and end-plate channels, and the kinetics of the NMDA receptor activated channels depend on the membrane potential.

(3) The binding of NMDA to its receptor may depend on the membrane electric field if the receptor molecule has a dipole in or near the agonist binding site (cf. Adams & Sakmann, 1978).

(4) Divalent cations in the extracellular fluid may interfere with current flow through NMDA activated ion channels (see Adams *et al.* 1980), the binding of divalent cations to the ion channel being a function of membrane potential. Preliminary results (Mayer & Westbrook, 1984) suggest that physiological concentrations of magnesium have a voltage-dependent regulatory action on the conductance linked to NMDA receptors. Single channel recording experiments have recently been used to show that magnesium ions block ion channels activated by L-glutamate (Nowak, Bregestovski, Ascher, Herbet & Prochiantz, 1984).

Mixed-agonist action

The excitation of Renshaw cells and other interneurones in the cat spinal cord by acidic amino acids has been used to examine the nature of the receptors activated by ionophoretic application of L-glutamic and L-aspartic acids *in vivo*. The results obtained from such extracellular recording experiments reveal depression of L-glutamate and L-aspartate responses, with varying degrees of efficacy and discrimination, by NMDA receptor antagonists (for a recent review see Davies, Evans, Jones, Smith & Watkins, 1982). Responses evoked by L-aspartate are more susceptible to antagonism than responses evoked by L-glutamate. Thus, Watkins (1981*b*) suggests that L-aspartate acts mainly on NMDA receptors, and L-glutamate mainly on quisqualate receptors. Other studies on neurones in the rat cerebral cortex, caudate nucleus and cuneate nucleus confirm that in these areas NMDA receptor antagonists reduce the response to L-aspartate to a greater degree than responses evoked by L-glutamate (Stone, 1979).

Our results suggest that close to the resting potential, and at low doses of agonist, approximately 50% of the inward current response to L-glutamate may be due to activation of NMDA receptors. Thus, the small amplitude of the slope conductance of the response to L-glutamate and L-homocysteate recorded near resting potential is simply explained by two simultaneous responses: an increase in inward current during membrane hyperpolarization due to an increase in driving force for current flow through quisqualate receptor activated channels counterbalanced by a decrease in inward current during membrane hyperpolarization due to inactivation of current flow through NMDA receptor activated channels. The similarity of the response to mixtures of NMDA and kainic acid compared to that evoked by L-glutamate and L-homocysteate provides support for this hypothesis. Thus, measurement of the voltage sensitivity of the membrane response to excitatory amino acids provides

evidence of a mixed-agonist action of L-glutamate independent of any classification based on the use of antagonists. Of the agonists studied, we suggest a rank order of selectivity at NMDA receptors (based on the voltage sensitivity of the responses) of NMDA $\geqslant$ L-aspartate > L-homocysteate > L-glutamate $\geqslant$ D-homocysteate $\gg$ quisqualate = kainate. On the basis of studies with various antagonists, Davies *et al.* (1982) suggest the following rank order, NMDA > L-homocysteate > D-homocysteate > L-aspartate > L-glutamate $\gg$ kainate = quisqualate. The major differences between our results and those of Davies *et al.* (1982) are that we find L-aspartate to be highly selective for NMDA receptors. In our experiments L-glutamate and D-homocysteate appear to have quite similar behaviour as mixed agonists at NMDA and non-NMDA receptors. We suggest that since the degree of mixed-agonist action varies with the dose of agonist (see below), differences in experimental protocols may in part explain these results; thus, with L-aspartate and L-glutamate our results might be expected if we evoked responses with lower concentrations of agonist than Davies *et al.* (1982).

A second line of evidence supporting a mixed-agonist action of L-glutamate is provided by block of the voltage-sensitive component of the response to L-glutamate by the NMDA receptor antagonist 2-APV. Recent data from receptor binding experiments suggest that L-glutamate has a high affinity for the NMDA receptor (Jones, Olverman & Watkins, 1983). In the presence of 2-APV, voltage sensitivity of the response to L-glutamate is similar to that evoked by quisqualate. In slices of cerebral cortex, incomplete antagonism by 2-APV of the depolarization evoked by L-glutamate also suggests an action of L-glutamate at both NMDA and non-NMDA receptors (N. L. Harrison, personal communication). In this tissue, the sensitivity of the response to L-aspartate to antagonism by 2-APV is intermediate between that of NMDA and L-glutamate, suggesting that L-aspartate may also have an agonist action at non-NMDA receptors (N. L. Harrison, unpublished data). In our experiments voltage dependence of the conductance activated by L-aspartic acid closely resembled the response to NMDA. It is probable that the proportion of NMDA to non-NMDA receptors activated by L-aspartic acid varies with the dose of agonist (cf. the response to L-glutamate) and that high doses of L-aspartic acid may activate quisqualate receptors. A higher affinity of L-glutamate and L-aspartate for NMDA receptors compared to non-NMDA receptors could explain the observed dose-dependent effect (cf. Jones *et al.* 1983), i.e. low doses of these agonists activate NMDA receptors whereas higher doses activate an increasing proportion of non-NMDA receptors.

The mixed-agonist action of a number of amino acids at more than one receptor greatly complicates quantitative study of excitatory amino acid pharmacology. Measurement of the depolarization, or inward current evoked by various agonists gives no indication at which receptor type the agonist is acting. For example, in the presence of a competitive NMDA receptor antagonist, increasing the concentration of L-glutamate to overcome competitive block of NMDA receptors may shift the action of L-glutamate from a relatively specific one at NMDA receptors at low doses of L-glutamate, to a mixed-agonist action at higher doses of agonist. Further receptor binding studies and quantitative dose conductance measurements are required to clarify this issue.

Apparent membrane resistance changes

NMDA receptor agonists, including NMDA, L-aspartic acid and DL-homocysteic acid produce membrane potential depolarizations accompanied by an *apparent* membrane resistance increase (Engberg *et al.* 1978, 1979; MacDonald & Wojtowicz, 1982). The current–voltage relation of responses evoked by NMDA and L-aspartic acid has a negative slope hyperpolarized beyond −30 mV, thus hyperpolarizing electrotonic potentials used to measure membrane resistance result in a progressive decrease of the inward *depolarizing* current activated by NMDA receptors, resulting in an *apparent* membrane resistance increase during the depolarizing response to NMDA (see Jack, Noble & Tsien, 1983).

At membrane potentials depolarized beyond −30 mV, the slope of the NMDA current–voltage relation becomes positive, thus in current-clamp experiments NMDA responses recorded at such membrane potentials will be accompanied by a membrane resistance decrease (see Dingledine, 1983, Fig. 5; Hablitz, 1982). The membrane potential at which the inward peak of the current–voltage relation of the NMDA response occurs will influence the membrane potential value at which a change in sign of the membrane resistance change evoked by NMDA is recorded.

The depolarizing action of L-glutamate often occurs with little change in *apparent* membrane resistance (Puil, 1981). This is not surprising in view of the fact that the current–voltage relation of L-glutamate responses has a slope close to zero in the vicinity of the resting potential. Non-linearities in the resting membrane current–voltage relation tend to obscure the small change in slope conductance produced by L-glutamate. This in part helps to explain the variable actions of L-glutamate on membrane resistance changes recorded in current-clamp experiments (Puil, 1982). For example, in neurones in which depolarization activates a persistent inward current (Schwindt & Crill, 1980; Stafstrom *et al.* 1982) the response to L-glutamate may be accompanied by an *apparent* membrane resistance increase. In those neurones where membrane depolarization activates predominantly outward rectifiers, the depolarizing response to L-glutamate may be accompanied by a membrane resistance decrease. In addition, since the slope of the L-glutamate current–voltage relation becomes positive depolarized beyond approximately −30 mV, the membrane potential value at which the response to L-glutamate is measured also influences the sign of the *apparent* resistance change recorded in current-clamp experiments (see MacDonald & Wojtowicz, 1982).

Micromolar concentrations of L-glutamate may selectively activate NMDA receptors (Jones *et al.* 1983). In our experiments, low doses of L-glutamate produced voltage-dependent responses. At high doses, the voltage-sensitive behaviour of L-glutamate is much less evident due to the activation of large numbers of kainate or quisqualate receptors. Such dose-dependent effects could result in biphasic changes in membrane resistance during L-glutamate-induced depolarizations (see Engberg *et al.* 1979; MacDonald & Wojtowicz, 1982). The mixed-agonist action of L-glutamate could also influence the sign and magnitude of the *apparent* membrane resistance change evoked by this amino acid if the ratio of NMDA to non-NMDA receptors is not constant on different neurones.

The current–voltage relation of responses evoked by quisqualic and kainic acids

has a relatively constant positive slope between −70 and +20 mV. Thus, the depolarization evoked by these substances is accompanied by a readily measured resistance decrease, which is independent of the membrane potential (see MacDonald & Wojtowicz, 1982). Since the NMDA receptor antagonist 2-APV removes the voltage-sensitive component of the response to L-glutamate, the membrane potential depolarization evoked by L-glutamate after block of NMDA receptors is also accompanied by an easily measured decrease in membrane resistance. The result of this experiment renders unnecessary the suggestion that the dendritic location of L-glutamate receptors, electrotonically remote from the intrasomatic recording site, obscures measurement of membrane resistance changes accompanying responses to L-glutamate (Bernadi *et al.* 1972; Altmann *et al.* 1976; Segal, 1982), but suggests instead that the voltage-sensitive action of L-glutamate as a mixed agonist is responsible for the lack of an *apparent* membrane resistance change.

Synaptic receptors

In our experiments, and those performed with ionophoresis into the extracellular environment of spinal neurones *in vivo*, excitatory amino acid responses undoubtedly result from activation of both synaptic and extrasynaptic receptors. If there are discrete hot spots of either quisqualate or NMDA receptors at the subsynaptic membrane, then it is likely that with L-glutamate, the membrane physiology of the synaptic response may be determined by the nature of the subsynaptic receptor rather than the synaptic transmitter *per se*. Experiments with receptor antagonists (Davies & Watkins, 1979) suggest activation of NMDA receptors during polysynaptic transmission in feline spinal cord. However, monosynaptic excitatory post-synaptic currents (e.p.s.c.s) evoked in motoneurones by I a afferents show much weaker voltage dependence (Finkel & Redman, 1983) than NMDA responses This raises the possibility that in the spinal cord NMDA receptors are widely distributed and act as modulators of neuronal excitability rather than in classical excitatory synaptic mechanisms.

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