Br. J. Pharmac. (1985), **86**, 19–25

Pharmacological characterization of D-aminophosphonovaleric acid antagonism of amino acid and synaptically evoked excitations on frog motoneurones *in vitro*: an intracellular study

R. Corradetti[1], Anne E. King, A. Nistri[2], Catherine Rovira[3] & Lucia Sivilotti

Department of Pharmacology, St Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ

1 The effect of D-aminophosphonovaleric acid (D-APV) on the depolarizations induced by N-methyl-D-aspartate (NMDA), glutamate, aspartate or quisqualate was studied with intracellular recordings from frog motoneurones *in vitro*.

2 D-APV (0.5–10 μM) produced a slight hyperpolarization of the motoneuronal membrane without significant changes in input conductance.

3 In control and tetrodotoxin-containing solutions the depolarizations induced by NMDA were strongly reduced by D-APV while quisqualate depolarizations were unaffected. Responses to glutamate and aspartate were antagonized to an intermediate level. The relatively small conductance increases evoked by excitatory amino acids were unaltered in solutions containing D-APV.

4 The amplitude of monosynaptic excitatory postsynaptic potentials (e.p.s.ps) was strongly depressed by D-APV. The amplitude of polysynaptic e.p.s.ps was little changed but their decay time was reduced.

5 It is suggested that D-APV is a powerful and selective NMDA receptor antagonist and that an endogenous amino acid acting via NMDA receptors may be the transmitter of monosynaptic e.p.s.ps on frog motoneurones.

Introduction

The pharmacological classification of excitatory amino acid receptors has been greatly aided by using selective antagonists to distinguish various receptor classes (Watkins & Evans, 1981). Among several phosphonic derivatives of amino acids apparently displaying specific antagonism to the excitant N-methyl-D-aspartate (NMDA), the D isomer of 2-aminophosphonovaleric acid (D-APV) seems to be very potent on spinal cord preparations (Evans *et al.*, 1982). Other studies (reviewed by McLennan, 1983) have found a similar pharmacology at a variety of central nervous system sites, although most authors have used the racemic mixture of 2-aminophosphonovaleric acid rather than its D form (the L form is described as virtually inactive; Evans *et al.*, 1982; McLennan, 1982). In the original work by Evans *et al.* (1982) extracellular recordings from the *in vitro* spinal cord of the neonatal rat and the adult frog were employed so that detailed information on the neuronal membrane mechanisms underlying the observed effects was not available. Many other investigators have likewise based their experiments on extracellular recording techniques (McLennan, 1983).

We therefore attempted to study the pharmacological actions of D-APV, using intracellular recordings from frog spinal motoneurones, as an approach to a direct demonstration of its pharmacological selectivity towards exogenous excitants and excitatory postsynaptic potentials (e.p.s.ps). We used the adult frog as the experimental animal rather than the neonatal rat since we wished to record at low temperature to reduce amino acid uptake (Davidoff &

[1] Present address: Department of Pharmacology, University of Florence, Viale Morgagni 65, 50134 Florence, Italy.
[2] Author for correspondence.
[3] Present address: Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC 27514, U.S.A.

Adair, 1975), and also to avoid the possibility of immaturity of the amino acid receptor systems (Saito *et al.*, 1982; Seno *et al.*, 1984).

Methods

Experiments were carried out on lumbar motoneurones of the frog (*R. temporaria*) spinal cord slice preparation as previously described (Nistri & Arenson, 1983). Animals were kept in an aquarium at 6–7°C before use. After decerebration and removal of the spinal cord, a parasagittal spinal slice was prepared and placed in a 0.2 ml bath continuously superfused with oxygenated Ringer solution at about 10 ml min^{-1} for 1–3 days. The composition of the Ringer solution was as follows (mM): NaCl 111, KCl 2.5, $NaHCO_3$ 17, NaH_2PO_4 .$2H_2O$ 0.1, $CaCl_2$ 2, glucose 4, and the solution was gassed with 95% O_2 and 5% CO_2. The bath temperature was maintained at 7°C and monitored with an electronic thermometer via a miniature probe located near the slice.

Motoneurones were impaled with microelectrodes filled with 3 M KCl (50–80 MΩ d.c. resistance) and functionally identified on the basis of their characteristic short latency, all-or-none antidromic spike elicited by ventral root stimulation. Two dorsal and two ventral lumbar roots contained in sealed bath side chambers were connected to suction electrodes (filled with Ringer solution) which provided rectangular electrical pulses (0.20–0.25 Hz; 0.1 ms; variable intensity) from isolated stimulators. A Ag/AgCl pellet was used as ground electrode and placed downstream from the slice. Intracellularly recorded responses were amplified through a WPI M-707 electrometer with facilities for bridge balancing, current injection and capacity neutralization. Responses were displayed on a storage oscilloscope, recorded on line on a two channel pen recorder and also stored on FM magnetic tape (frequency response d.c. – 2.5 kHz) for further analysis. Retrieval and playback analysis were aided by a Tektronix 5D10 waveform digitizer. Resting membrane potential was measured, taking as zero the potential recorded after electrode withdrawal from the cell. Input conductance was calculated from electrotonic hyperpolarizing potentials regularly elicited by intracellularly-applied current pulses (600 ms) and from the slope of current/voltage plots usually constructed within ± 20 mV from resting membrane potential. All drugs were applied to the bath through pre-cooled individual flowlines.

The following compounds were used: sodium L-glutamate (BDH), sodium L-aspartate (Sigma), NMDA (Cambridge Research Biochemicals), quisqualic acid (kindly donated by Dr H. Shinozaki or purchased from Cambridge Research Biochemicals), D-APV (Cambridge Research Biochemicals), tetrodotoxin (Sigma). Results are expressed as mean ± s.e.mean.

Results

Control responses to excitatory amino acids

Control data for this study were obtained from 53 motoneurones with stable resting potential (− 63 ± 1 mV) and input conductance (96 ± 7 nS) and with antidromic action potentials overshooting the zero reference line by 15–20 mV. Control applications of glutamate (2 mM; 26 cells), NMDA (30 μM; 20 cells), aspartate (2 mM; 6 cells) or quisqualate (30 μM; 16 cells) produced matched amplitude depolarizations of 10 ± 1, 10 ± 2, 10 ± 1 and 13 ± 2 mV respectively. These responses were accompanied by an increased voltage noise, as shown by the thicker baseline during the glutamate amino acid application (see Figure 3 top left). In about 30% of these cells the depolarizations were preceded by a small hyperpolarizing component acociated with a conductance increase (cf. Nistri *et al.*, 1985). The percentage apparent input conductance increases at the peak of the depolarizing responses to these amino acids were 21 ± 8, 22 ± 6, 39 ± 17 and 27 ± 20, respectively. For each cell the experimental protocol consisted of initially testing dorsal root-evoked excitatory synaptic transmission as well as responses to at least two amino acids during superfusion with normal Ringer solution and subsequently retesting synaptic potentials and amino acids effects in the presence of D-APV (minimum exposure 15 min): successful runs were completed with 31 cells.

Finally, five motoneurones were first tested for their responsiveness to glutamate and later superfused with tetrodotoxin (0.6–1.2 μM) which, after about 30 min, fully blocked regenerative electrical activity including that due to interneurones. The resting potential and conductance values for these cells in tetrodotoxin solution were − 79 ± 9 mV and 46 ± 9 nS respectively. Peak depolarizations to glutamate, NMDA and quisqualate were 10 ± 2, 7 ± 1 and 9 ± 3 mV respectively (cf. top tracings of Figure 3). Their corresponding percentage actual conductance increases were 22 ± 8, 3 ± 1 and 39 ± 24, respectively. These cells, too, were subsequently bathed in a solution containing D-APV and tetrodotoxin.

Effects of D-APV on motoneurones and their responsiveness to amino acids

On 31 motoneurones D-APV was applied in concentrations ranging from 0.5 to 10 μM. In the presence of D-APV motoneurones displayed a slight hyperpolarizations which reached an average maximum of 3.1 ± 1.4 mV after approximately 15 min. Applica-

tions of D-APV produced no change in the slope of the current/voltage line, as shown in Figure 1 (only in one cell was there a modest increase in the slope), or in the amplitude of electrotonic hyperpolarizing potentials

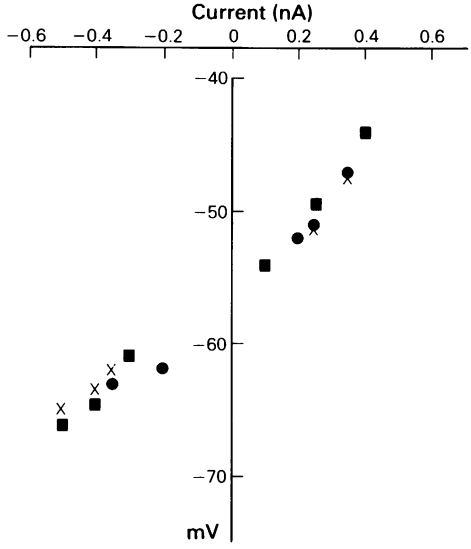

Figure 1 Current/voltage relation for a single frog motoneurone in control Ringer (●), in the presence of 10 μM D-aminophosphonovaleric acid (D-APV) (■) and 30 min after D-APV washout (x). The relation, apparently linear within the values of membrane potentials monitored, is typical of that found for the majority of motoneurones (Schwindt, 1976). Resting potentials in control and D-APV Ringer were −57 mV and −58 mV respectively.

elicited by a constant current pulse; both tests thus indicated a lack of significant input conductance changes following D-APV application. No alteration in the configuration of the motoneuronal action potential was observed in the presence of D-APV.

Changes in the amplitude of excitatory amino acid-induced depolarizations (expressed as % of controls in normal Ringer) following 15–20 min exposure to various concentrations of D-APV are depicted in Figure 2. D-APV 1 μM was found to ensure 85% block of NMDA responses while glutamate and aspartate responses were reduced by 37% and 39% respectively (quisqualate responses were unchanged). Longer exposures to D-APV (> 1 h) produced no further reduction in the amino-acid responses.

Attempts to measure D-APV antagonism of amino acid-induced conductance changes were made difficult by the variability of modest control conductance increases. Hence in the presence of D-APV (0.5–10 μM) the apparently larger average increase in conductance evoked by glutamate (49 ± 17%) and the smaller one produced by NMDA (7 ± 6%) were not

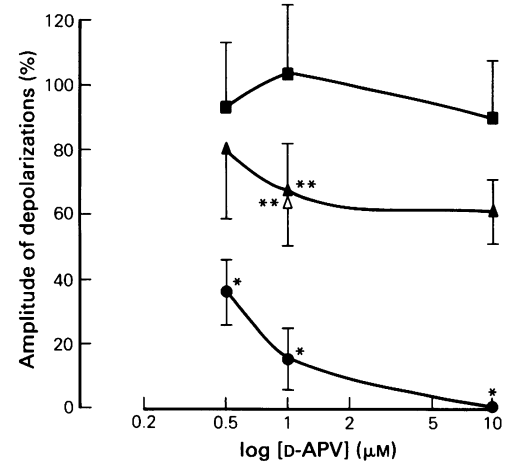

Figure 2 Plot of increasing concentrations of D-aminophosphonovaleric acid (D-APV) versus amplitude of depolarizations (expressed as % of controls) produced by glutamate (▲), N-methyl-D-aspartate (●) quisqualate (■) and aspartate (△). Each point is the mean and vertical lines s.e.mean of 4–16 responses. * $P < 0.01$ and ** $P < 0.05$, significantly different from control; Wilcoxon signed ranks test (see Colquhoun, 1971).

significantly different from their control values ($P > 0.05$; Wilcoxon signed-ranks test; see Colquhoun, 1971). Since indirect effects via activation of interneurones may be a complicating factor, a solution containing tetrodotoxin (0.6–1.2 μM) was used for 5 neurones. D-APV (10 μM) hyperpolarized these cells by −4 ± 1 mV (conductance values were 85 ± 15% of tetrodotoxin data). In the presence of D-APV and tetrodotoxin the NMDA-induced depolarization was suppressed while that to glutamate was reduced and the response to quisqualate unaffected (Figure 3; bottom tracings). Regarding the conductance values, the average data (Table 1) confirm the lack of a consistent effect of D-APV on this parameter.

Table 1 Effect of 10 μM D-aminophosphonovaleric acid (D-APV) on amino-acid-evoked conductance changes of frog motoneurones in Ringer solution solution containing tetrodotoxin (TTX)

	TTX ΔG	*TTX + D-APV* ΔG
Glutamate (2 mM)	8 ± 2	8 ± 5
NMDA (30 μM)	1 ± 1	−3 ± 2
Quisqualate (30 μM)	13 ± 7	7 ± 4

Data are expressed as changes (Δ G measured in nS) in input conductance, during the application of the amino acid, with respect to resting conductance values. $n = 5$. NMDA = N-methyl-D-aspartate.

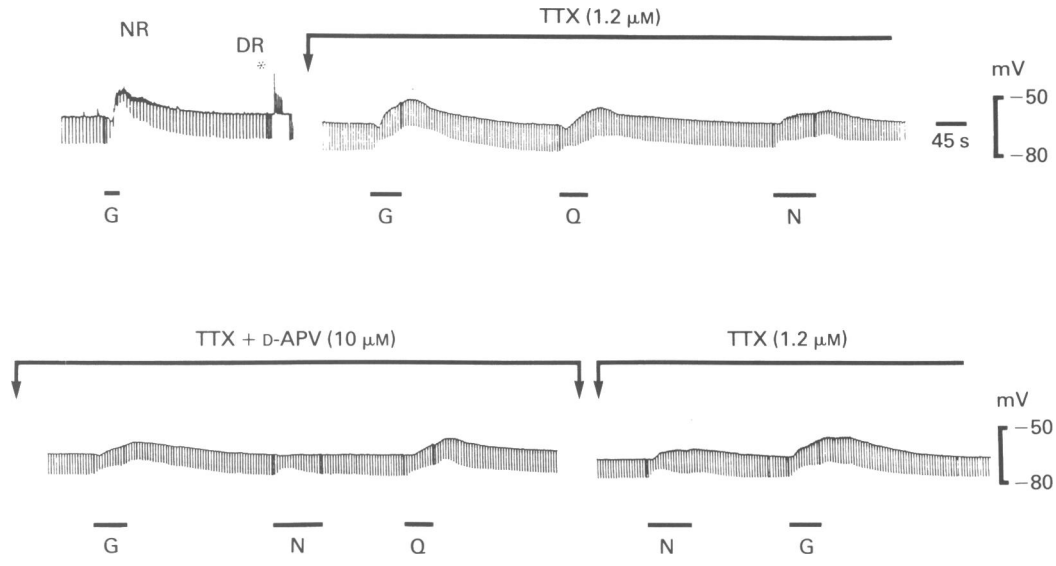

Figure 3 Membrane potential records obtained from a single motoneurone showing the effect of D-aminophosphonovaleric acid (D-APV) on responses to glutamate (G; 2 mM), quisqualate (Q; 30 μM) and N-methyl-D-aspartate (N; 30 μM); the durations of application are represented by horizontal bars. Upper traces: NR indicates a glutamate response in control Ringer and is followed by amino acid responses after 35 min exposure to 1.2 μM tetrodotoxin (TTX), note dorsal root-evoked excitatory potentials (* DR) in NR. Lower traces: responses after superfusion with a solution containing TTX and 10 μM D-APV and subsequent recovery after the removal of D-APV. The downward deflections are hyperpolarizing electrotonic potentials evoked by intracellular current injection (−0.87 nA and −0.75 nA for top and bottom tracings respectively).

Effects of D-APV on synaptic transmission

The same motoneurones used for tests on the excitatory amino acid pharmacology in the absence of tetrodotoxin were also investigated for the effect of D-APV on excitatory synaptic transmission. D-APV 80.5–10 μM) usually reduced the cell spontaneous voltage noise arising from asynchronous discharges from interneurones. Although it was not practical to quantitate this reduction it was nevertheless clear that there was no complete suppression of spontaneous synaptic activity. It was possible to study quantitatively the effects of D-APV on e.p.s.ps elicited by electrical stimulation of dorsal root fibres. Monosynaptic e.p.s.ps were distinguished from polysynaptic e.p.s.ps on the basis of their shorter latency, low activation threshold (i.e. produced by stimuli < 1.5 times those producing threshold response; cf. Eccles, 1946; Eccles *et al.*, 1957), rather small amplitude (2–3 mV; cf. Shapovalov & Shiriaev, 1980) and inability to evoke orthodromic action potentials (Eccles, 1946; Fadiga & Brookhart, 1960). Some of these features are illustrated in Figure 4 in which (a) shows several superimposed polysynaptic e.p.s.ps near threshold for action

Table 2 Effect of D-aminophosphonovaleric acid (D-APV) on the amplitude of e.p.s.ps

	Control (mV)	*D-APV (mV)*	P	*% change*
Monosynaptic e.p.s.ps	2.3 ± 0.2	0.9 ± 0.4	<0.05	− 61
Polysynaptic e.p.s.ps	15.0 ± 4.5	13.6 ± 4.5	>0.05	− 10

Results are means (± s.e.mean) from 7 motoneurones; in each cell the amplitudes of mono- or polysynaptic e.p.s.ps were averaged from a minimum of 5 tests in control Ringer and 5 in D-APV (1–10 μM) solution (at least 15 min exposure). As data with 1 or 10 μM D-APV were similar, they were pooled. Statistical analysis was done with the Wilcoxon signed ranks test (Colquhoun, 1971). Values of $P < 0.05$ were taken as indicative of a statistically significant difference.

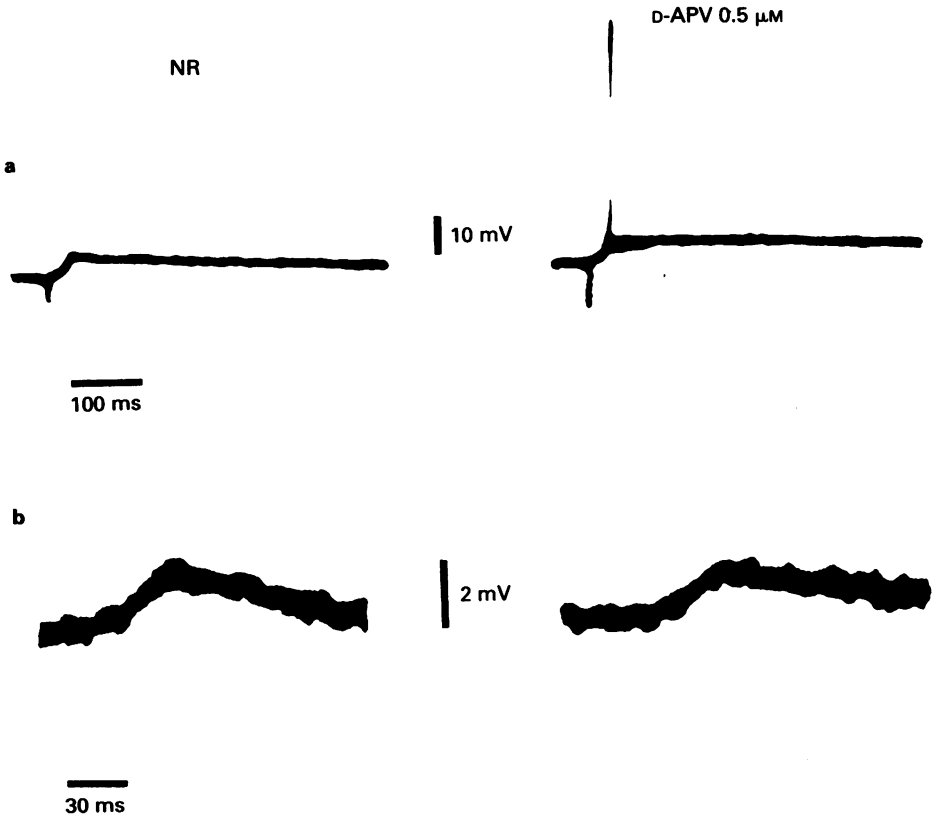

Figure 4 Polysynaptic (a) and monosynaptic (b) e.p.s.ps of frog motoneurones in normal Ringer solution (NR) or after 26 min exposure to 0.5 μM D-aminophosphonovaleric acid (D-APV). Each panel shows several superimposed oscilloscope tracings. Note depression of monosynaptic e.p.s.p. without reduction of polysynaptic one. In 0.5 μM D-APV the polysynaptic e.p.s.p. reaches threshold to initiate action potential. Initial resting membrane potential of −76 mV was not changed by D-APV.

potential generation whereas (b) displays monosynaptic e.p.s.ps. The latter were reduced by 30% after 26 min in the presence of a concentration of D-APV as low as 0.5 μM (lower right hand panel of Figure 4) while polysynaptic transmission was not depressed (these e.p.s.ps actually produced action potentials; upper right hand panel of Figure 4a). On this cell the D-APV concentration decreased the NMDA-induced depolarization by 40% without blocking the quisqualate-induced response. Table 2 shows that the mean reduction in monosynaptic e.p.s.p. amplitude in the presence of higher concentrations of D-APV (1 or 10 μM) was 61% (in 3 of these cells the e.p.s.ps disappeared within the baseline noise which was approximately 0.3 mV).

Any apparent depression of polysynaptic e.p.s.p. amplitude was not statistically significant (Table 2). However, during exposure to 1–10 μM D-APV the decay of polysynaptic e.p.s.ps (from peak to baseline crossing) was significantly ($P = 0.05$) reduced from 1.9 ± 0.2 to 1.1 ± 0.2 s (38% decrease). Conversely, there was no significant difference in the decay of the monosynaptic e.p.s.ps.

Discussion

The principal finding of our study is the direct demonstration of the high potency and selectivity of D-APV as an antagonist of NMDA-induced depolarizations and of monosynaptic e.p.s.ps on frog spinal motoneurones. These actions of D-APV were associated with a small membrane hyperpolarization probably in part caused by reduction in the tonic activity of interneurones impinging upon recorded motoneurones. This notion is supported by the absence of significant changes in neuronal conductance (which make a direct inhibitory action of the antagonist unlikely) and by the observed depression of spontaneous synaptic activity (cf. also extracellular

data of Evans *et al.*, 1982). A slight direct hyperpolarizing action of D-APV was observed by Evans *et al.* (1982) and confirmed in the present experiments performed in tetrodotoxin solution. While the precise mechanism of this hyperpolarization remains unclear, it is unlikely to result from activation of an electrogenic pump since the present experiments, as well as those of Evans *et al.*, were conducted at low temperatures which would largely depress the activity of energy-dependent membrane carrier processes. In our study D-APV was a far more potent antagonist of NMDA than of glutamate and aspartate and is to date the most powerful antagonist of NMDA-induced depolarizations on frog motoneurones.

Whereas antagonism of depolarizing responses to NMDA (and, to a lesser extent, to glutamate and aspartate) was clearly observed in solutions containing D-APV, it was not possible to detect significant alterations in amino-acid-induced conductance responses even when interneuronal activity was blocked by tetrodotoxin. The present data thus support the notion that glutamate and related analogues produce only small alterations in membrane conductance of spinal motoneurones (Engberg *et al.*, 1979; Nistri *et al.*, 1985). On cultured spinal neurones a glutamate-induced conductance increase was revealed by D-APV (Mayer & Westbrook, 1984). This finding was not obtained in the present study. The discrepancy may arise for a variety of reasons; for example, cultured vs *in vitro* neurones, immature vs mature cells. In particular the composition of the bathing solutions should be considered since, unlike in this study, Mayer & Westbrook (1984) used solutions with high concentrations of Ca^{2+} and Mg^{2+} which are known to affect amino acid responses (Ault *et al.*, 1980).

The much stronger antagonism by D-APV for NMDA depolarizations than for similar responses to glutamate and aspartate (quisqualate responses were not blocked) supports previous receptor classification based on extracellular recordings from the spinal cord (Watkins & Evans, 1981) and accords with previous work on mammalian cortical neurones (Perkins *et al.*, 1981). Interestingly, there was an apparent saturation in the D-APV antagonism of glutamate responses at about 60% of control ones; these results thus indicate that glutamate is probably a mixed agonist acting partly on NMDA receptors and partly on APV-insensitive quisqualate receptors (Watkins & Evans, 1981; Mayer & Westbrook, 1984). In analogy to a recent intracellular study on rat hippocampal neurones *in vitro* (Crunelli *et al.*, 1983), the present investigation did not find evidence for preferential antagonism by D-APV of responses to glutamate or aspartate. In fact as aspartate responses were only partially blocked by D-APV, it is likely that aspartate is also a mixed agonist on frog motoneurones.

Unlike the e.p.s.ps on hippocampal granule cells (Crunelli *et al.*, 1983) the monosynaptic e.p.s.ps of frog motoneurones were antagonized by D-APV. Motoneuronal polysynaptic e.p.s.ps were not blocked as they could still elicit action potentials, although the e.p.s.p. decay time was reduced possibly because D-APV was bound to the excitatory amino-acid receptors in the interneuronal network (Ryan *et al.*, 1984) responsible for the late components of the synaptic response (cf. also depression of spontaneous synaptic discharges). In an extracellular study by Evans *et al.* (1982) it was suggested that APV antagonized polysynaptic rather than monosynaptic e.p.s.ps. Nevertheless, extracellular recordings are by necessity not very suitable for distinguishing between these synaptic potentials since monosynaptic e.p.s.ps in the frog are typically very small (Shapovalov & Shiriaev, 1980) and even unable to generate a motoneuronal action potential (Eccles, 1946; Fadiga & Brookhart, 1960). The differential blockade by D-APV of mono- and polysynaptic potentials cannot be attributed to the fact that the monosynaptic e.p.s.p. is simply a small response mediated by submaximal receptor activation and hence more susceptible to antagonism. In fact, the motoneuronal monosynaptic e.p.s.p. is believed to be generated in an all-or-none manner by receptor saturation at each subsynaptic site (Redman & Walmsley, 1983; Shapovalov & Shiriaev, 1980) and any apparently graded nature of the composite monosynaptic e.p.s.p. is probably due to summation of all-or-none transmission processes at individual synaptic boutons. Consequently, the preferential block by D-APV of monosynaptic e.p.s.ps highlights a pharmacological difference in the receptor mechanisms mediating mono- and polysynaptic transmission rather than a mere difference in synaptic receptor numbers. In the rat cerebral cortex a low threshold e.p.s.p. is also apparently mediated by NMDA receptors (Thomson *et al.*, 1985).

From the present results the possibility exists that the natural transmitter of the monosynaptic e.p.s.p. in the frog spinal cord is an excitatory amino acid acting via D-APV-sensitive receptors. However, it is not possible at this stage to say whether glutamate or aspartate (or a mixture of both) is such a transmitter. The answer to this question will probably be aided by neurochemical experiments on the release of these endogenous amino acids. Even though the brief time scale of the synaptic events must make it difficult to obtain accurate correlations between released compounds and synaptic potentials, some powerful analytical techniques which have recently been used for this work (Takeuchi *et al.*, 1983) might clarify this issue.

This work was supported by a project grant from the Joint Research Board of St. Bartholomew's Hospital. R.C. was a

Fellow of the European Molecular Biology Organization (EMBO). A.E.K. is a Postdoctoral Fellow of the Medical Research Council; C.R. was a Fellow of the European Science Foundation. L.S. held a Fellowship from the Royal Society under their exchange programme with the Accademia dei Lincei, Rome. We wish to thank Miss Carol Brown for typing this manuscript and Mr G. Davis for photography.

References

AULT, B., EVANS, R.H., FRANCIS, A.A., OAKES, D.J. & WATKINS, J.C. (1980). Selective depression of excitatory amino acid-induced depolarizations by magnesium ions in isolated spinal cord preparations. *J. Physiol.*, **307**, 413–428.

COLQUHOUN, D. (1971). *Lectures on Biostatistics*, p. 425 Oxford: Oxford University Press.

CRUNELLI, V., FORDA, S. & KELLY, J.S. (1983). Blockade of amino acid-induced depolarizations and inhibitions of excitatory post-synaptic potentials in rat dentate gyrus. *J. Physiol.*, **341**, 627–640.

DAVIDOFF, R.A. & ADAIR, R. (1975). High affinity amino acid transport by frog spinal cord slices. *J. Neurochem.*, **24**, 545–552.

ECCLES, J.C. (1946). Synaptic potentials of motoneurones. *J. Neurophysiol.*, **9**, 87–120.

ECCLES, J.C., ECCLES, R.M. & LUNDBERG, A. (1957). Synaptic actions on motoneurones in relation to the two components of the group I muscle afferent volley. *J. Physiol.*, **136**, 527–546.

ENGBERG, I., FLATMAN, J.A. & LAMBERT, J.D.C. (1979). The actions of excitatory amino acids on motoneurones in the feline spinal cord. *J. Physiol.*, **288**, 227–262.

EVANS, R.H., FRANCIS, A.A., JONES, A.W., SMITH, D.A.S. & WATKINS, J.C. (1982). The effects of a series of ω-phosphonic α-carboxylic amino acids on electrically evoked and excitant amino acid-induced responses in isolated spinal cord preparations. *Br. J. Pharmac.*, **75**, 65–75.

FADIGA, E. & BROOKHART, J.M. (1960). Mono-synaptic activation of different portions of the motor neuron membrane. *Am. J. Physiol.*, **198**, 693–703.

MAYER, M. & WESTBROOK, G.L. (1984). Mixed-agonist action of excitatory amino acids on mouse spinal cord neurones under voltage clamp. *J. Physiol.*, **354**, 29–53.

McLENNAN, H. (1982). The isomers of 2-amino-5-phosphonovalerate as excitatory amino acid antagonists – a reappraisal. *Eur. J. Pharmac.*, **79**, 135–137.

McLENNAN, H. (1983). Receptors for the excitatory amino acids in the mammalian central nervous system. *Prog. Neurobiol.*, **20**, 251–271.

NISTRI, A. & ARENSON, M.S. (1983). Differential sensitivity of spinal neurones to amino acids: an intracellular study on the frog spinal cord. *Neuroscience*, **8**, 115–122.

NISTRI, A., ARENSON, M.S. & KING, A. (1985). Excitatory amino acid-induced responses of frog motoneurones bathed in low Na^+ media: intracellular study. *Neuroscience*, **14**, 921–927.

PERKINS, M.N., STONE, T.W., COLLINS, J.F. & CURRY, K. (1981). Phosphonate analogues of carboxylic acids as amino acid antagonists on rat cortical neurones. *Neurosci. Lett.*, **23**, 333–336.

REDMAN, S. & WALMSLEY, B. (1983). Amplitude fluctuations in synaptic potentials evoked in cat spinal motoneurones at identified group Ia synapses. *J. Physiol.*, **343**, 135–145.

RYAN, G.P., HACKMAN, J.C., WOHLBERG, C.J. & DAVIDOFF, R.A. (1984). Spontaneous dorsal root potentials arise from interneuronal activity in the isolated frog spinal cord. *Brain Res.*, **301**, 331–341.

SAITO, K., GOTO, M. & FUKUDA, H. (1982). Postnatal development of the GABA system in the rat spinal cord. *Jap. J. Pharmac.*, **32**, 1–7.

SCHWINDT, P.C. (1976). Electrical properties of motoneurons. In *Frog Neurobiology*, ed. Llinás, R. & Precht, W. pp. 750–764. Berlin: Springer.

SENO, N., ITO, S. & OHGA, A. (1984). The development of responsiveness to substance P and glutamate in the spinal motoneurons of rat fetuses. *Brain Res.*, **298**, 366–369.

SHAPOVALOV, A.I. & SHIRIAEV, B.I. (1980). Dual mode of junctional transmission at synapses between single primary afferent fibres and motoneurones in the amphibian. *J. Physiol.*, **306**, 1–15.

TAKEUCHI, A., ONODERA, K. & KAWAGOE, R. (1983). The effects of dorsal root stimulation on the release of endogenous glutamate from the frog spinal cord. *Proc. Jap. Acad. B.*, **59**, 88–92.

THOMSON, A.M., WEST, D.C. & LODGE, D. (1985). An N-methylaspartate receptor-mediated synapse in rat cerebral cortex: a site of action of ketamine? *Nature*, **313**, 479–481.

WATKINS, J.C. & EVANS, R.H. (1981). Excitatory amino acid transmitters. *A. Rev. Pharmac. Tox.*, **21**, 165–204.

(Received October 11, 1984.
Revised April 24, 1985.
Accepted May 24, 1985.)