J. Physiol. (1986), **376**, *pp.* 13–29
With 5 text-figures
Printed in Great Britain

AMINO ACID RECEPTOR MEDIATED EXCITATORY SYNAPTIC TRANSMISSION IN THE CAT RED NUCLEUS

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(*Received 7 August 1985*)

SUMMARY

1. A study has been made of the effects of the selective *N*-methyl-D-aspartate receptor antagonist, 2-amino-5-phosphonovalerate (APV), and the broad spectrum excitatory amino acid antagonists, γ-D-glutamylglycine (γ-DGG), γ-D-glutamyl-aminomethylsulphonate (GAMS), 4(*p*-chlorobenzoyl)-*cis*-piperazine-2, 3-dicarboxylate (*p*CB-PzDA) and kynurenate, have been examined on excitation evoked on neurones in the magnocellular red nucleus (m.r.n.) of the anaesthetized cat by stimulation of the interpositus nucleus (i.p.n.) and sensorimotor cortex, and by ionophoresed excitant amino acid agonists.

2. The profile of activity of the excitatory amino acid antagonists on m.r.n. neurones was similar to that described on neurones in other areas of the central nervous system. APV selectively depressed responses to *N*-methyl-D-aspartate (NMDA), whereas the broader spectrum antagonists reduced responses to kainate and quisqualate as well as to NMDA. Neuronal responses to L-glutamate and L-aspartate were depressed by all the antagonists tested.

3. I.p.n.-evoked monosynaptic responses of m.r.n. neurones were reversibly reduced by the broad spectrum antagonists, but were unaffected by APV.

4. Cortically evoked mono- and polysynaptic excitatory responses were reversibly depressed by APV and the broad spectrum antagonist, *p*CB-PzDA. The action of APV corresponded with its ability to antagonize responses to NMDA. However, the cortically evoked responses appeared to be more sensitive to the actions of *p*CB-PzDA than to those of APV, although the former is a less effective antagonist of NMDA-induced excitation compared with APV.

5. APV depressed excitation induced by cortical stimuli and L-glutamate and L-aspartate. However, there was no obvious correlation between the actions of the broad spectrum amino acid antagonists on synaptically evoked responses and those induced by L-glutamate or L-aspartate on the few neurones tested.

6. These results are consistent with an amino acid being the transmitter in the interposito-rubral and cortico-rubral excitatory pathways which interacts with non-NMDA and both NMDA and non-NMDA receptors respectively. However, the identity of the transmitter acting at these receptors remains to be determined.

INTRODUCTION

Neurones of the magnocellular region of the cat red nucleus receive two distinct monosynaptic excitatory inputs, one from the contralateral interpositus nucleus (i.p.n.) and one from the ipsilateral sensorimotor cortex (s.m.c.) (Massion, 1967). Results from several different laboratories suggest that excitatory amino acids, such as L-glutamate and L-aspartate, may function as neurotransmitters in these two pathways. Both L-glutamate and L-aspartate are present in the red nucleus (Perry, Berry, Hansen, Diamond & Mok, 1971), and *in vivo* and *in vitro* studies demonstrate that both substances depolarize magnocellular red nucleus (m.r.n.) neurones and that this is accompanied by an increase in conductance (Altman, Ten Bruggencate, Pickleman & Steinberg, 1976; Huffman & Davis, 1977; Sakaguchi, Kubata, Nakamura & Tsukahara, 1984). Furthermore, lesions of either the sensorimotor cortex or cerebellar nuclei result in significant reductions in the high-affinity uptake of L-glutamate into the caudal part of the red nucleus (Kerkerian, Nieoullon & Dusticier, 1983; Nieoullon, Kerkerian & Dusticier, 1984). However, more direct evidence supporting a neurotransmitter role for excitatory amino acids in these pathways is lacking.

Recent evidence suggests that several different receptors exist for excitatory amino acids in the mammalian central nervous system (Watkins & Evans, 1981; McLennan, 1983). These receptors can be broadly classified as NMDA and non-NMDA receptors. NMDA receptors are most readily classified. They are activated by the agonist *N*-methyl-D-aspartate (NMDA) and blocked by a variety of antagonists, of which one of the most potent is 2-amino-5-phosphonovalerate (APV) (Davies & Watkins, 1982). Non-NMDA receptors are less readily classified and probably do not constitute a homogenous group. They are activated by kainate and quisqualate, are unaffected by selective NMDA receptor antagonists but are differentially sensitive to several less selective excitatory amino acid antagonists (broad spectrum antagonists) such as γ-D-glutamylglycine (γ-DGG) and γ-D-glutamylaminomethylsulphonate (GAMS) (Davies and Watkins, 1981, 1985). In the present study we have examined the effects of some of the more recent selective and broad spectrum excitatory amino acid antagonists on excitatory synaptic responses evoked in m.r.n. neurones in the anaesthetized cat. The aims were to try and obtain more direct evidence of a transmitter role for excitatory amino acids in the cortico-rubral and interposito-rubral pathways, and to determine the receptor types mediating the ensuing synaptic events.

Some of these results have appeared in a preliminary form (Davies, Miller & Sheardown, 1985).

METHODS

Experiments were performed on cats of either sex anaesthetized with α-chloralose (50 mg/kg I.V.) following induction with halothane. Supplementary doses of either sodium pentobarbitone (3·5 mg/kg) or α-chloralose (10 mg/kg) were administered via a cannula positioned in the femoral vein if required. The animal's core temperature was maintained at 36–38 °C by means of a thermostatically controlled heating blanket. Femoral arterial blood pressure was continually monitored, experiments being terminated if the systolic pressure fell below 80 mmHg. The head of the animal was placed in a stereotaxic frame and the skull overlying the left sensorimotor cortex

and red nucleus, and right cerebellar hemisphere was removed. The posterior fossa was opened and the first cervical segment of the spinal cord was exposed.

Stimulation. Following excision of the dura, bipolar silver ball electrodes were positioned on the surface of the left sensorimotor cortex (s.m.c.) and right dorsolateral funiculus (d.l.f.). Concentric bipolar steel stimulating electrodes (Rhodes Medical Instruments) were introduced stereotaxically into the right i.p.n. and, in some experiments, the left cerebral peduncle. To avoid the bony tentorium the i.p.n. electrode was introduced at an angle of 40 deg with the tip angled rostrally, 4·5 mm lateral to the mid line. The peduncle electrode was inserted 5·0–6·0 mm lateral to the mid line with the tip angled 20 deg caudally. In three cats the left pyramid was approached ventrally and bipolar silver electrodes were placed longitudinally on the surface at the level of the trapezoid body. In two of these cats bipolar electrodes were inserted through the cerebellum and brain stem until the electrode tip was positioned (under visual control from the ventral surface) within the left pyramidal tract in close proximity to the surface electrode. All stimuli consisted of single pulses (0·1 ms duration) and intensities up to 100 μA (i.p.n. and peduncle electrodes) or 1 mA (pyramidal tract electrode). At the termination of each experiment, electrolytic lesions were made with the i.p.n., peduncle and pyramidal tract electrodes and their tip locations in the appropriate structure were confirmed in subsequent 40 μM transverse frozen sections with reference to the atlas of Berman (1968).

Recordings. Extracellular recording of single unit activity in the red nucleus was obtained via the centre barrel (4 M-NaCl) of a seven-barrelled micro-electrode. This electrode was positioned vertically in the red nucleus (coordinates: A 4·0, L 2·0, D −2 to −3, Berman 1968). Neuronal action potentials were amplified, displayed on an oscilloscope, electronically counted, the counted pulses being recorded on a chart recorder or fed into a small computer (Neurolog) to construct peristimulus time histograms (p.s.t.h.s) of synaptic events. Each peripheral barrel of the micro-electrode contained one of the following compounds which were ejected from the electrode using standard ionophoretic techniques: Na kainate (0·02 M in 0·18 M-NaCl, pH 7·0), sodium *N*-methyl-D-aspartate (NMDA) (0·05 M in 0·15 M-NaCl, pH 7·0), sodium Na quisqualate (0·02 M in 0·18 M-NaCl, pH 7·0), sodium L-glutamate, sodium L-aspartate, sodium γ-D-glutamylglycine (γ-DGG), sodium γ-D-glutamyl-aminomethylsulphonate (GAMS), sodium 4-(*p*-chlorobenzoyl)-*cis*-piperazine-2,3-dicarboxylate (*p*CB-PzDA) and sodium kynurenate (all 0·2 M pH 7·0–7·2), sodium DL-2-amino-5-phosphonovalerate (APV) (0·05 M in 0·1 M-NaCl, pH 7·0) and acetylcholine chloride (0·5 M, pH 5·5). One barrel in each electrode contained 0·165 M-NaCl and was used for current neutralization. In initial experiments Pontamine Sky Blue (2% w/v in 0·5 M-sodium acetate solution) was used in place of NaCl in order to deposit dye and verify the location of the electrode tip within the red nucleus in subsequent histological sections. This proved to be unnecessary in later experiments (see Results).

RESULTS

Recordings were obtained from eighty cells located in the m.r.n.; approximately 5% of these neurones were spontaneously active.

Identification of m.r.n. neurones

Neurones in the m.r.n. were identified in initial experiments on the basis of both their monosynaptic excitatory response evoked by electrical stimulation of the contralateral i.p.n. and by their antidromic response to stimulation of the contralateral d.l.f. (see Eccles, Scheid & Taborikova, 1975 for references). Stimulation of the i.p.n. gives rise to two field potentials in the red nucleus (Eccles *et al.* 1975). The first is the presynaptic spike potential which is evident while the recording electrode is some distance dorsal to the nucleus. The second is the synaptically evoked field potential recorded when the electrode is just superficial to the nucleus. Both fields became maximal in size within the m.r.n., and spike potentials of individual m.r.n. neurones encountered are superimposed upon the second potential (Fig. 1*B*). The appearance of these fields and superimposed spikes were regarded as sufficient evidence that the

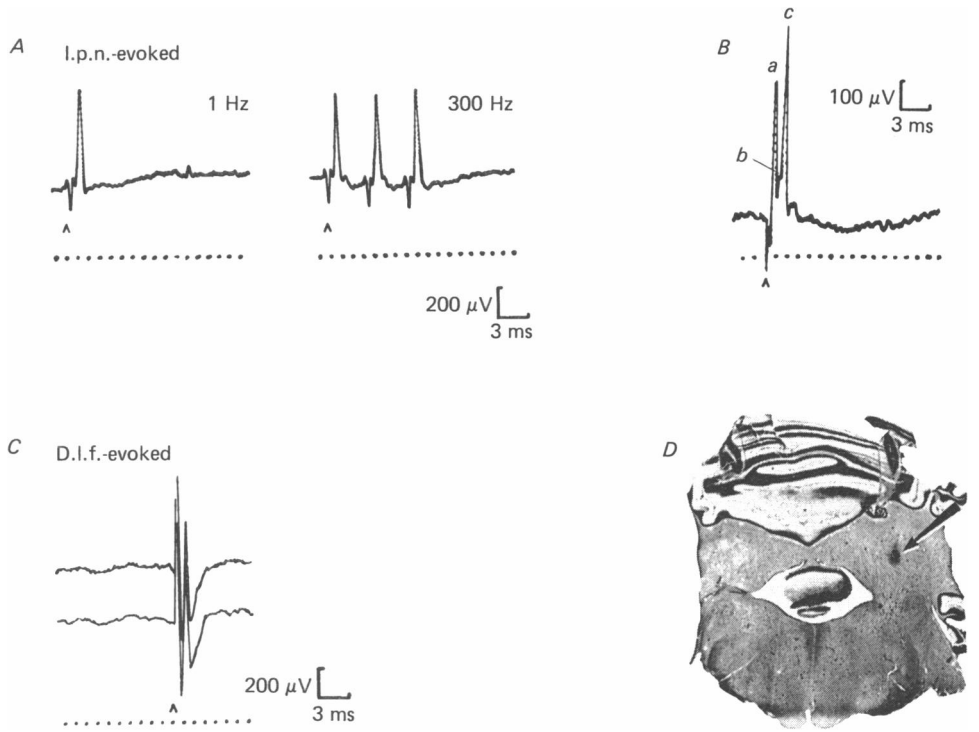

Fig. 1. Orthodromic and antidromic excitation of a red nucleus neurone. *A*, monosynaptic excitation evoked by stimulation of the interpositus nucleus (i.p.n.) at threshold intensity (40 μA, 0·1 ms pulse) at 1 and 300 Hz. *B*, as in *A*, but at higher gain and i.p.n. stimulus intensity 60 μA; note presynaptic field (*a*) followed by post-synaptic field (*b*) with superimposed monosynaptic action potential (*c*). *C*, antidromic response evoked in same neurone by stimulation of the contralateral d.l.f. at C1. *D*, transverse section through the hind brain, illustrating lesion (arrow) made in the right i.p.n. with the stimulating electrode. The position of the stimulus artifact in this and subsequent Figures is indicated by the arrowhead below each record.

recording electrode was located in the m.r.n. in many subsequent experiments. This was verified histologically. Spikes were evoked using stimulus intensities of 20–100 μA (60 ± 18 A, mean $\pm$ S.D.). They faithfully followed high frequencies of stimulation (100 Hz or more) (Fig. 1*A*), had small latency variations (0·2 ms) and onset latencies 1·2–2·0 ms ($1{\cdot}6 \pm 0{\cdot}2$, mean $\pm$ S.D., $n = 60$). These latencies correspond to conduction velocities in the range of 15–20 m/s, which are consistent with those expected for monosynaptic excitation via the i.p.n.–rubral pathway (Eccles *et al.* 1975). Neurones responding in this characteristic manner to stimulation of the i.p.n. were also invaded antidromically on stimulation of the d.l.f. at the C1 level (Fig. 1*C*), confirming that they were rubrospinal neurones and that the recording site was in the magnocellular region of the red nucleus.

Responses to cortical stimulation

In fifty m.r.n. neurones, identified as described above, single stimuli applied to the surface of the ipsilateral pericruciate cerebral cortex evoked single or multiple action

potentials. The earliest cortically evoked spike in 44% (twenty-two cells) of these neurones occurred with latency variations of less than 0·2 ms and followed stimulus frequencies of 100 Hz or more, and is therefore likely to be evoked monosynaptically. The latency to onset of this excitation varied between 2·6 and 6·5 ms (4·3 ± 1·2 ms, $n = 16$). The onset latency of the first spikes evoked by cortical stimuli in the remaining twenty-eight neurones varied by at least 1 ms, to sometimes more than 5 ms, in individual neurones. Over-all, minimum onset latencies in these neurones were between 2·7 and 15 ms, and none was capable of following high-frequency cortical stimuli. These responses were classified as being polysynaptically mediated.

Stimulation of the cerebral peduncle at A 5·0 evoked single, presumably monosynaptic, spikes in six m.r.n. neurones that occurred at short constant latencies of 1·5–1·9 ms (1·17 ± 0·16 ms) and followed frequencies of at least 100 Hz. Five of these neurones were excited polysynaptically from the cerebral cortex (Fig. 5).

Response to pyramidal stimulation. The pyramidal (corticospinal) tract was stimulated in an attempt to differentiate between m.r.n. neurones activated from the cortex via collaterals of this tract and those excited via the cortico-rubral pathway (Tsukahara, Fuller & Brooks, 1968). However, none of twenty-five neurones activated by cortical stimuli (eleven of which were monosynaptically activated) was affected by pyramidal tract stimulation although neurones within the sensorimotor cortex, studied subsequently in the same experiments, were antidromically or othodromically excited by low-intensity pyramidal stimuli (e.g. 50 μA), establishing that these fibres were indeed activated.

Excitatory amino acid antagonists

Excitant amino acids. Prior to examining the effects of excitant amino acid antagonists on synaptic responses evoked in m.r.n. neurones, their effects were determined on the responses of these cells to the excitant amino acids kainate, quisqualate and NMDA. Kainate and quisqualate readily excited neurones when ejected with currents in the range of 20–100 nA (63 ± 14 nA, mean ± S.D.) and 15–100 nA (43 ± 21 nA) respectively. These currents are similar to those found to excite neurones in other areas of the central nervous system (C.N.S.) (e.g. see Davies & Watkins, 1983, 1985). However, to obtain comparable excitatory responses to NMDA, ejecting currents of 70–300 nA (160 ± 31 nA) were required. These currents are considerably higher than those generally necessary to excite other C.N.S. neurones (Davies & Watkins, 1983, 1985). To ascertain whether the apparent low sensitivity of m.r.n. neurones to NMDA was due to inadequate ionophoretic release of this excitant the sensitivity of neurones in other C.N.S. regions to the excitants, released from the micro-electrodes previously tested in the red nucleus, was determined. Little difference was found between the range of currents required for a given amino acid to excite neurones in the thalamus, cuneate nucleus and cerebellar cortex. The over-all range of effective currents for the excitants in these areas was: 30–90 nA (53 ± 21 nA, $n = 16$) for kainate, 20–80 nA (50 ± 17 nA, $n = 17$) for quisqualate and 40–115 nA (68 ± 22 nA, $n = 19$) for NMDA. This indicates that m.r.n. neurones are less sensitive to the actions of NMDA compared with neurones tested in the other C.N.S. areas.

The effects of the excitatory amino acid antagonists on responses of m.r.n. neurones to the agonists are summarized in Table 1. The selective NMDA-receptor antagonist

TABLE 1. Effects of antagonists on amino acid induced excitation of red nucleus neurones

Antagonist	Ejection current† (nA)	Number of cells tested	Effect on response* induced by: NMDA No. dep.	NMDA % dep.	Kainate No. dep.	Kainate % dep.	Quisqualate No. dep.	Quisqualate % dep.	L-Glutamate No. dep.	L-Glutamate % dep.	L-Aspartate No. dep.	L-Aspartate % dep.
APV	83±23	17	17	93±12	0	0	0	0	—	—	—	—
	40±23	4	4	85±15	—	—	—	—	2	33	4	58±14
	62±21	6	6	94±10	—	—	—	—	6	68±20	6	78±12
γ-DGG	62±26	6	5	77±23	6	70±20	6	57±21	—	—	—	—
	80	2	—	—	2	68	1	60	2	23	1	25
GAMS	40±18	6	3	53±12	6	85±16	5	92±14	—	—	—	—
*p*CB-PzDA	47±20	12	11	70±28	12	83±23	12	81±24	—	—	—	—
	60±21	7	—	—	—	—	7	79±30	4	63±26	4	61±24
Kynurenate	31±20	4	4	95±10	4	75±30	4	75±30	—	—	—	—
	40±10	3	3	96±5	—	—	3	78±18	3	77±21	3	89±9

* Data show the number of cells in which responses to the excitants were depressed (dep.) by the antagonists and the mean±S.D. percentage depression.

† The ejection currents shown are the mean±S.D. current (nA) from tests against various combinations of agonists on the same cells.

APV (20–100 nA) reduced, and often abolished, responses to NMDA while having little or no effect on responses of the same neurones to kainate or quisqualate (Figs. 2 and 4, and Table 1). Currents of APV reducing responses to NMDA by about 90% also markedly reduced responses to L-glutamate and L-aspartate in tests on six m.r.n. neurones. However, relatively lower currents of APV depressed responses to L-aspartate more than those to L-glutamate in tests on four of these cells (Table 1).

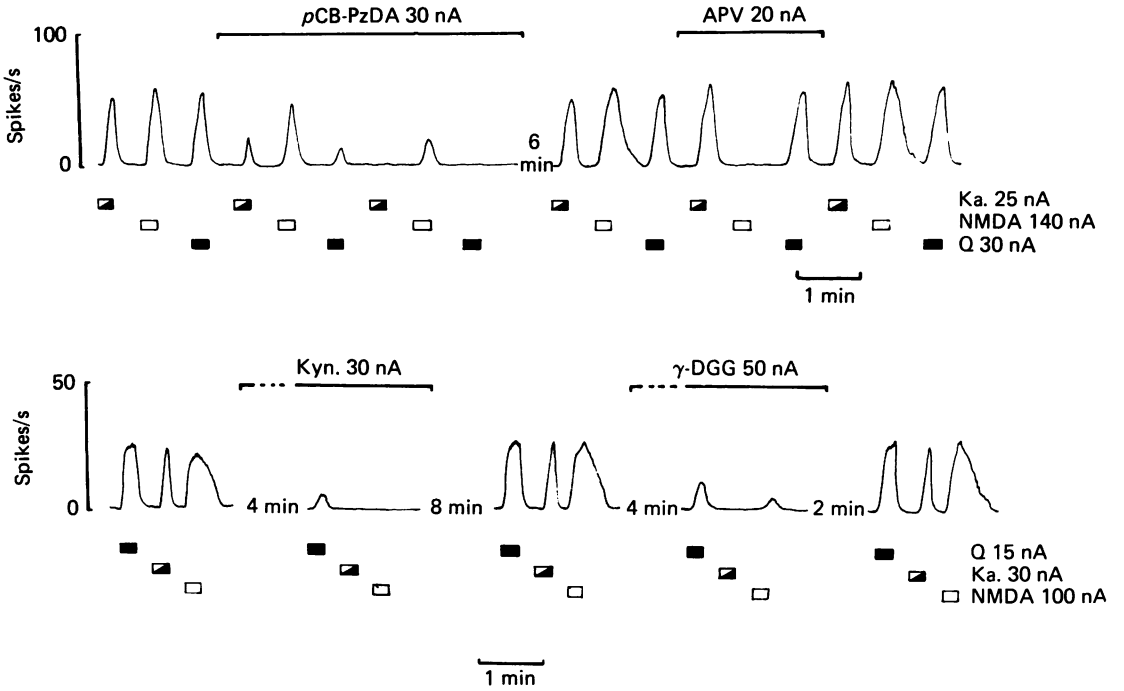

Fig. 2. Rate-meter records from two different red nucleus neurones (upper and lower traces) illustrating the effects of amino acid antagonists on excitatory responses induced by kainate (Ka.), *N*-methyl-D-aspartate (NMDA) and quisqualate (Q). 4-(*p*-chlorobenzoyl)-*cis*-piperazine-2,3-dicarboxylate (*p*CB-PzDA), kynurenate (Kyn.) and γ-D-glutamylglycine (γ-DGG) depressed neuronal responses to all three excitants whereas 2-amino-5-phosphonovalerate (APV) only depressed responses to NMDA. Note: much larger ejection currents of NMDA are required to excite both neurones compared with those for Ka. and Q. The bars below the record indicate the excitant ejected and the length of the bar corresponds to the duration of ejection.

The non-selective excitatory amino acid antagonists, γ-DGG, *p*CB-PzDA and kynurenate, reversibly reduced responses induced by kainate, quisqualate and NMDA, showing no clear differential antagonism of any particular amino acid induced responses (Fig. 2). In agreement with our earlier findings (Davies & Watkins, 1985), GAMS reduced responses to quisqualate and kainate more than those to NMDA. Indeed GAMS failed to antagonize the actions of NMDA in three of six neurones (Table 1). On the few neurones tested, the broad spectrum antagonists also depressed responses of neurones to L-glutamate and L-aspartate. γ-DGG and *p*CB-PzDA had less marked depressant effects on responses to L-glutamate and L-aspartate

relative to their effects on the other excitants tested, whereas kynurenate reduced responses to all the excitants more or less in parallel (Table 1).

Acetylcholine was ionophoresed into the vicinity of ten m.r.n. neurones in two cats with the intention of verifying the selectivity of the amino acid antagonists. However, no observable effect was observed with this agent (up to 200 nA).

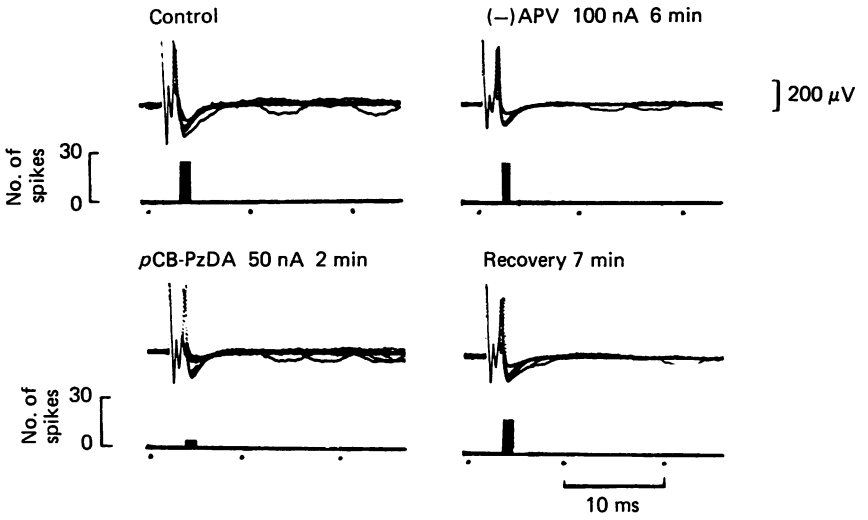

Fig. 3. Effects of APV and *p*CB-PzDA on i.p.n.-evoked monosynaptic responses of a red nucleus neurone. The upper trace in each record is the superimposed (thirty-two) oscilloscope sweep of the neuronal response to i.p.n. stimulation (2 Hz, 60 μA, 0·1 ms), and the lower trace, the p.s.t.h.s computed from this response and analysed in 1 ms intervals. During the control period (top left) the response faithfully followed each stimulus and was unaffected by a 6 min ejection of 100 nA of APV (top right). However, 3 min later a 2 min ejection of 50 nA *p*CB-PzDA depressed the monosynaptic response to 16 % of the control level (bottom left). The evoked response was fully reversible 7 min after terminating the *p*CB-PzDA ejection (bottom right).

I.p.n.-evoked excitations. APV, ejected with currents of 60–120 nA for 40–10 min, had no effect on the monosynaptic excitation of thirty-four m.r.n. neurones evoked by stimulation of the i.p.n. at intensities near threshold for activation (20–60 A) and frequencies of 0·5–10 Hz (e.g. Figs. 3 and 4, and Table 2). In tests on some of these neurones (at least two with each micro-electrode used) these currents of APV were sufficient to selectively abolish responses to NMDA (Fig. 4) and to depress responses to L-glutamate and L-aspartate by 70 % or more. In contrast to the lack of effect of APV, the broad spectrum antagonists γ-DGG, GAMS, *p*CB-PzDA and kynurenate reduced or abolished these synaptic responses in all m.r.n. neurones tested (Table 2). This depression was usually apparent within 1–3 min of commencing the antagonist ejection and was fully reversible 1–10 min after terminating it. Examples of the effects of some of these antagonists on this synaptic response are illustrated in Fig. 3. No attempt was made to compare the relative potencies of the different antagonists, as each electrode used rarely contained more than one of them (in combination with APV and a series of agonists). Responses to quisqualate, kainate and NMDA were

also depressed by *p*CB-PzDA, γ-DGG and kynurenate with currents that clearly reduced the synaptically evoked excitation in the same neurones. However, currents of GAMS that reduced responses to i.p.n. stimulation by 45–100% failed to affect responses to NMDA in three cells, and reduced the synaptic response more than the

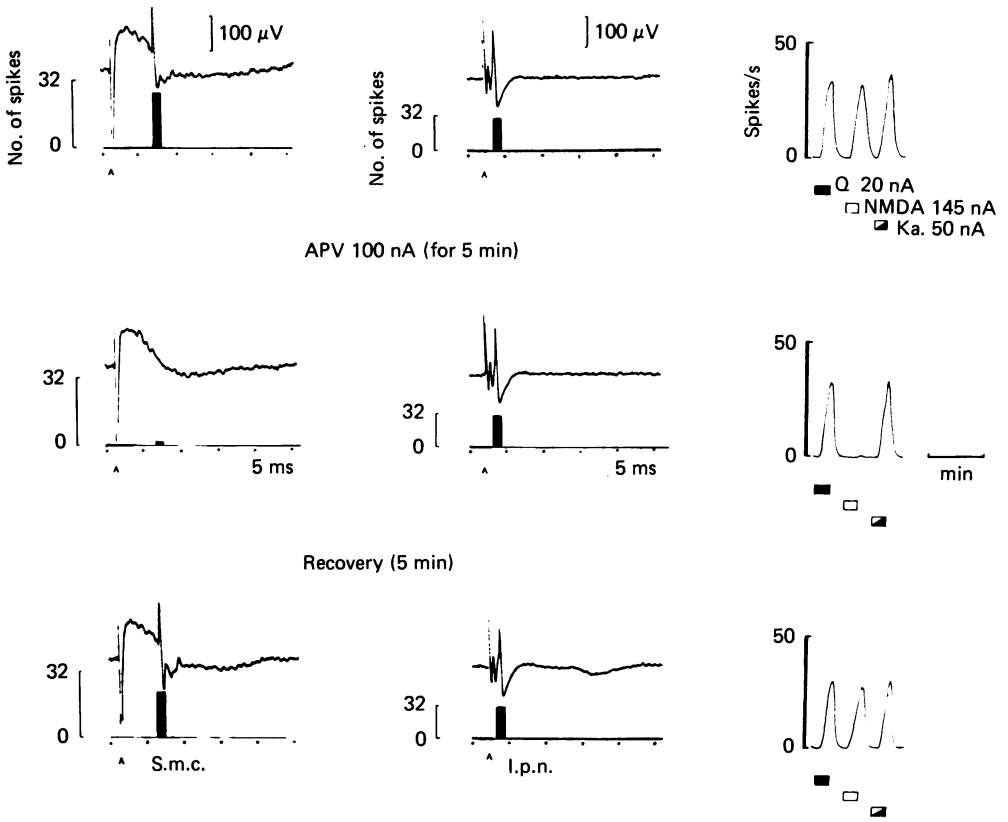

Fig. 4. Effects of APV on cortically evoked, i.p.n.-evoked and amino acid induced excitation of the same red nucleus neurone. The traces from left to right were recorded sequentially over a period of 2 min. Stimulation of the sensorimotor cortex (s.m.c.) and i.p.n. monosynaptically excited this neurone (left and centre records respectively). Each trace shows a single oscilloscope sweep of the synaptic response (top) and the p.s.t.h. (bottom) computed from thirty-two such sweeps and analysed in 1 ms intervals (stimulation rate 2 Hz). The rate-meter records to the right of the Figure illustrate the responses of the same neurone to quisqualate (Q, 20 nA), NMDA (145 nA) and kainate (Ka., 50 nA). The upper records are control responses, the middle records were obtained during the final 2 min of a 5 min ejection of 100 nA APV, and the lower records, 5 min after terminating the APV ejection. Note: only the s.m.c.-evoked monosynaptic excitation and response to NMDA were depressed by APV.

response to NMDA in three other neurones. In contrast kainate- and quisqualate-induced excitations were at least as sensitive as the i.p.n.-evoked response to the depressant action of GAMS. Relative to its depressant action on i.p.n.-evoked excitation, γ-DGG had little effect on excitations induced by L-glutamate and L-aspartate (two cells). *p*CB-PzDA had no effect on responses to L-glutamate and

L-aspartate in three cells but reduced responses to these in four other neurones, in parallel with the reduction in the responses to i.p.n. stimulation.

Cortically evoked excitations. The effects of APV on the constant-latency, presumed monosynaptic, excitation of a m.r.n. neurone evoked by stimulation of the cortical surface is illustrated in Fig. 4. Here an ejection of 100 nA APV, lasting 5 min, completely and reversibly abolished the cortically evoked monosynaptic response. This ejection of APV also selectively depressed the response of the neurone to NMDA but had no effect on the i.p.n.-evoked monosynaptic excitation of the cell. Similar depressant actions of APV were observed on cortically evoked monosynaptic responses of ten other m.r.n. neurones (Table 2), and in tests on four of these excitation induced by L-glutamate and L-aspartate was also markedly reduced. However, monosynaptic responses in five neurones were insensitive to APV (up to 150 nA for 10 min). Currents of APV below 70 nA had no significant depressant action on cortically evoked excitation in any of the sixteen cells studied. Polysynaptic excitatory responses, induced by cortical stimuli in seventeen m.r.n. neurones, were depressed by ejections of APV (40–100 nA, but more usually, 80–100 nA) that also selectively antagonized responses to NMDA. However, polysynaptic responses evoked in eleven other m.r.n. neurones were unaffected by prolonged (10 min) ejections of 150 nA APV.

Single, short-latency, monosynaptic spikes could be evoked by stimulation of the cerebral peduncle in five m.r.n. neurones that responded polysynaptically to cortical stimuli. APV reversibly reduced the cortically and peduncle-evoked responses in four (by 27–100%) (Table 2, Fig. 5). The excitation evoked only by stimulation of the peduncle in another m.r.n. neurone was unaffected by APV (100 nA).

The non-selective excitatory amino acid antagonist, *p*CB-PzDA, depressed cortically evoked mono- and polysynaptic excitation of m.r.n. neurones irrespective of the sensitivity of these responses to APV (Table 2). Excitatory responses to kainate, quisqualate and NMDA were also reduced by currents of *p*CB-PzDA that depressed these synaptic responses. Cortically evoked excitation seemed to be more sensitive to the depressant actions of *p*CB-PzDA compared to those of APV. Thus, in comparisons on seven neurones the cortically evoked response was depressed by 33–100% (67 ± 22%, mean ± S.D.) with 80–150 nA (104 ± 21 nA, mean ± S.D.) of APV, and by 50–100% (89 ± 19%) using currents of 40–100 nA (69 ± 20 nA) of *p*CB-PzDA. However, cortically evoked mono- and polysynaptic responses were more resistant to the effects of *p*CB-PzDA than were i.p.n.-evoked monosynaptic responses in the same neurones. This is illustrated in Fig. 5 where *p*CB-PzDA depressed the response to i.p.n. stimulation more than that to cortical stimulation. Comparable differential sensitivities of the synaptic responses to *p*CB-PzDA were observed in four other neurones.

DISCUSSION

Synaptic responses

Stimulation of the i.p.n. may evoke monosynaptic or antidromic responses in m.r.n. neurones (Eccles *et al.* 1975). The i.p.n.-evoked excitations in the present study are considered to be monosynaptic for two reasons. First, their latency to onset exceed those reported for antidromic activation but is entirely consistent with published

TABLE 2. Effects of antagonists on synaptically evoked excitation of red nucleus neurones

	Synaptic activation by stimulation of											
	Interpositus nucleus				Sensorimotor cortex				Cerebral peduncle			
Antagonist	Ejection current (nA)	Type	No. dep./ no. tested	% dep.	Ejection current (nA)	Type	No. dep./ no. tested	% dep.	Ejection current (nA)	Type	No. dep./ no. tested	% dep.
APV	91 ± 18	m.s.	0/34	—	98 ± 17	m.s.	11/16	67 ± 18	97 ± 8	m.s.	5/6	82 ± 15
					90 ± 20	p.s.	17/28	64 ± 24				
*p*CB-PzDA	58 ± 20	m.s.	17/17	84 ± 20	61 ± 19	m.s.	7/7	95 ± 7				
					67 ± 24	p.s.	8/8	91 ± 12				
γ-DGG	72 ± 24	m.s.	11/11	84 ± 17								
GAMS	46 ± 23	m.s.	10/10	79 ± 20								
Kynurenate	37 ± 19	m.s.	5/5	96 ± 22								

Data show the mean ± S.D. ejection current of antagonist (nA) tested on cells monosynaptically (m.s.) or polysynaptically (p.s.) activated, the number of cells in which synaptic responses were depressed by the antagonists over the total number tested and the mean ± S.D. percentage depression.

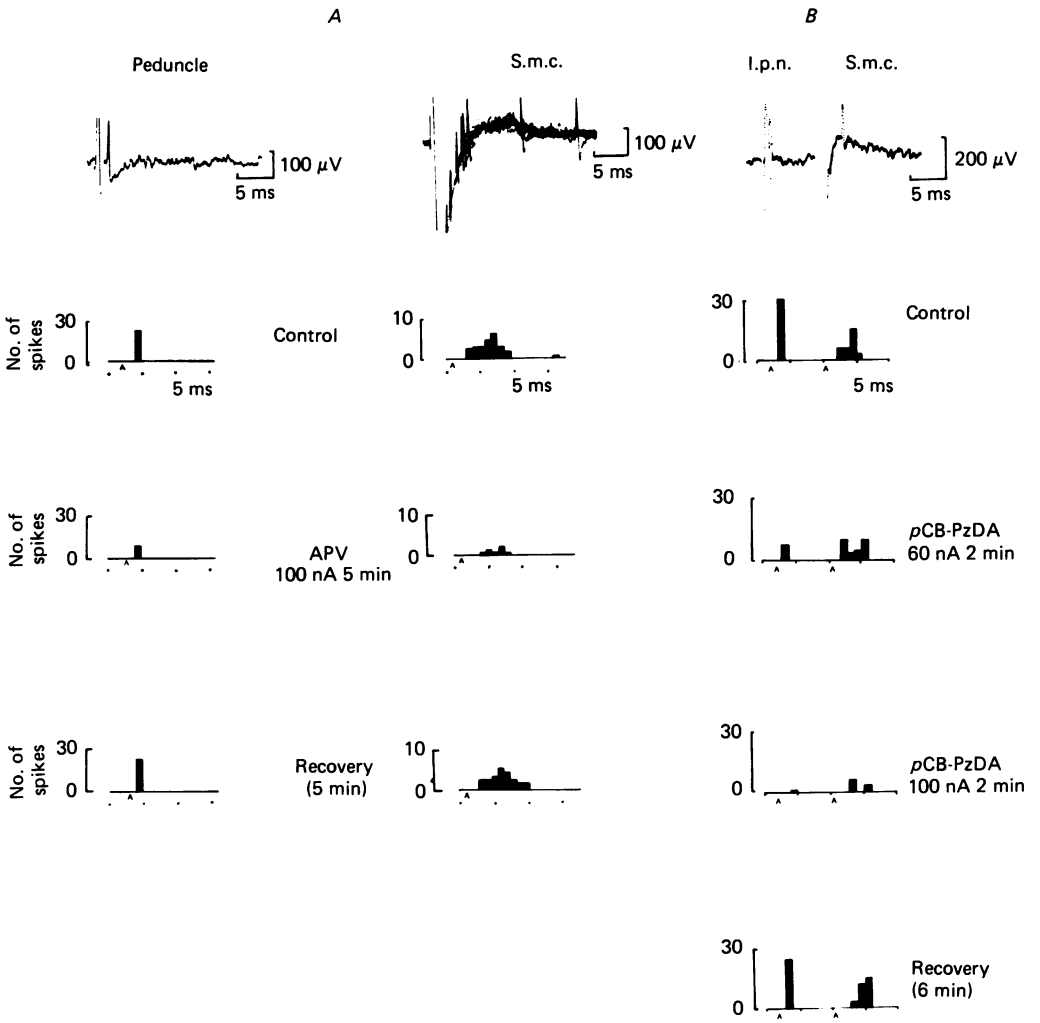

Fig. 5. The records in *A* and *B* are from two different red nucleus neurones. The upper trace in each record illustrates single or superimposed oscilloscope sweeps of the evoked synaptic responses; in sequence below these are p.s.t.h.s (computed from thirty-two sweeps and analysed in 1 ms intervals) obtained before (control), during and after (recovery) the ionophoresis of an amino acid antagonist. In *A*, stimulation of the peduncle at A 5·0 evoked a short, constant-latency, monosynaptic spike, whereas sensorimotor cortex (s.m.c.) stimulation excited the same neurone polysynaptically. APV 100 nA ejected for 5 min depressed both the mono- and polysynaptic response, recovery occurring 5 min after terminating the APV ejection. In *B*, stimulation of the interpositus nucleus (i.p.n.) and 8 ms later the s.m.c., evoked monosynaptic and polysynaptic spikes respectively (stimulus intensities were at threshold for evoking these synaptic responses). An ejection of 60 nA of the broad spectrum amino acid antagonist *p*CB-PzDA lasting 2 min only depressed the i.p.n.-evoked response. Increasing the ejection of *p*CB-PzDA to 100 nA for 2 min caused a further depression of the i.p.n.-evoked response and also reduced the s.m.c.-evoked excitation. The depressant effect of *p*CB-PzDA on both synaptic responses was fully reversible 6 min later.

monosynaptic latencies (Toyama, Tsukahara, Kosaka & Matsunami, 1970; Eccles *et al.* 1975). Secondly, antidromic activation is only observed in about 5% of m.r.n. neurones and requires stimulus intensities above the threshold for orthodromic activation (Eccles *et al.* 1975).

Neurones in the red nucleus can be excited monosynaptically from the sensorimotor cortex via activation of either cortico-rubral afferents (Tsukahara & Kosaka, 1968) or collaterals of slow corticospinal afferents (Tsukahara *et al.* 1968). In this study 44% of the cortically evoked responses were considered to be monosynaptically induced from cortico-rubral afferents because the evoked excitation (a) occurred at constant latencies compatible with previously reported monosynaptic latencies (Tsukahara & Kosaka, 1968; Anderson, 1971; Jeneskog & Padel, 1983), (b) faithfully followed high-frequency cortical stimulation rates, and (c) could not be evoked by stimulation of the corticospinal tract.

56% of cortically evoked excitations of m.r.n. neurones occurred at variable onset latencies indicating they were evoked polysynaptically. The pathway relaying these responses may have been the cortico-ponto–interposito-rubral pathway (Tsukahara, Korn & Stone, 1968), or the direct cortico-rubral pathway subsequent to its transynaptic activation from intracortical neuronal elements. The latter pathway is implicated by the observation that m.r.n. neurones excited polysynaptically from the cortex could also be excited by stimulation of the cerebral peduncle at latencies consistent with monosynaptic activation (Tsukahara & Kosaka, 1968).

Excitatory amino acid antagonists

In this study APV selectively depressed responses to NMDA, whereas γ-DGG, *p*CB-PzDA and kynurenate reduced responses to NMDA, kainate and quisqualate. The sulphonate analogue of γ-DGG, GAMS, depressed responses to kainate and quisqualate more than those to NMDA. This profile of activity of the excitatory amino acid antagonists is similar to that reported for neurones in other areas of the mammalian C.N.S. (Perkins & Stone, 1982, 1984; Davies & Watkins, 1983, 1985; Davies, Jones, Sheardown, Smith & Watkins, 1984; Herrling, 1984) and is consistent with the existence of at least two types of receptors for excitatory amino acids, viz. NMDA and non-NMDA receptors (Watkins & Evans, 1981).

L-Glutamate and L-aspartate are thought to be mixed agonists, producing their effects at NMDA and non-NMDA receptors (Watkins & Evans, 1981). In keeping with this notion, responses of m.r.n. neurones to both amino acids were depressed by the selective NMDA-receptor antagonist, APV, and by the broad spectrum antagonists in this study. Interestingly, ejections of APV that markedly reduced responses to NMDA also markedly depressed excitations to L-glutamate as well as to L-aspartate. In contrast, γ-DGG and *p*CB-PzDA had relatively weaker effects on responses to L-glutamate and L-aspartate compared to their effect on responses to kainate and quisqualate. One interpretation of these observations is that responses to L-glutamate, as well as those to L-aspartate, are mainly mediated at NMDA receptors, contrary to an earlier suggestion (Watkins & Evans, 1981). This notion must be viewed with caution however, as these excitants were only tested on a small number of m.r.n. neurones. Nevertheless, it is interesting to note, first, that γ-DGG and *p*CB-PzDA are relatively weak antagonists of responses to NMDA compared to APV (Davies &

Watkins, 1981; Davies *et al.* 1984) and, secondly, that recent *in vitro* electrophysiological and binding studies indicate that L-glutamate is a potent agonist at NMDA-type receptors (Mayer & Westbrook, 1984; Nowak, Bregestovski, Ascher, Herbet & Prochiantz, 1984; Olverman, Jones & Watkins, 1984).

An unexpected finding was the high ejecting currents (usually > 100 nA) of NMDA needed to excite m.r.n. neurones relative to those required for quisqualate and kainate. In studies on spinal neurones we have rarely needed more than 100 nA NMDA to excite cells adequately (e.g. see Figs. 1, 2 and 3 & Davies & Watkins, 1983). On the basis that neurones in the thalamus, cerebellum and cuneate nucleus were consistently excited by much lower ejection currents of NMDA compared with m.r.n. neurones tested with the same micro-electrode, it would seem that the latter cells are genuinely less sensitive to the excitant actions of NMDA. This could arise if m.r.n. neurones have fewer NMDA receptors than cells in other brain regions, or if these receptors are located some distance from the micro-electrode tip, which is assumed to be situated near the cell soma.

The available evidence indicates that the excitatory amino acid antagonists employed in this study depress amino acid induced excitation in the absence of either effects on that not induced by amino acids or direct effects on post-synaptic membranes (Perkins & Stone, 1982; Davies & Watkins, 1983, 1985; Ganong, Lanthorn & Cotman, 1983; Davies *et al.* 1984; Mayer & Westbrook, 1984; Sawada & Yamamoto, 1984; Herrling, 1985). Thus, the present observation that these agents reversibly antagonized cortico-rubral and interposito-rubral evoked excitation of m.r.n. neurones suggests that neurotransmission in these pathways is mediated via an amino acid receptor. The failure of the selective NMDA antagonist APV to influence i.p.n.-evoked monosynaptic excitation, but the reversible antagonism of this response by γ-DGG, GAMS, *p*CB-PzDA and kynurenate (that additionally act on non-NMDA receptors) suggests that the transmitter mediating this synaptic event interacts with non-NMDA amino acid receptors. This notion is further supported by the finding that GAMS depressed excitation evoked by i.p.n. stimulation, kainate and quisqualate but not by NMDA on some m.r.n. neurones. However, whether the non-NMDA receptors involved in this synaptic response are of the kainate or quisqualate type is not known.

APV reversibly antagonized monosynaptic and polysynaptic excitation evoked in many m.r.n. neurones by stimulation of the sensorimotor cortex, using ejecting currents that also selectively depressed responses to NMDA. This indicates that transmitter interaction with NMDA receptors mediates these synaptic events. The APV-sensitive response is probably due to activation of cortico-rubral afferents, but the neuronal pathway transmitting the APV-sensitive polysynaptic response is not definitely known (see earlier discussion). Activation of the cortico-ponto–interposito-rubral polysynaptic pathway is unlikely to be involved as i.p.n.-evoked excitation was insensitive to APV. APV-sensitive polysynaptic responses may have resulted from indirect activation of cortico-rubral afferents since stimulation of the cerebral peduncle evoked monosynaptic responses in the same m.r.n. neurones that were also antagonized by APV.

Cortically evoked mono- and polysynaptic excitation of some m.r.n. neurones was unaffected by APV, but was depressed by the broad spectrum antagonist *p*CB-PzDA.

This reflects an interaction of the synaptically released transmitter with non-NMDA receptors on these neurones. Alternatively, an insufficient concentration of APV may have reached the active synapses in these instances. Indeed, cortico-rubral afferents terminate on distal dendrites of m.r.n. neurones (Pizzini, Tredici & Miani, 1975) and thus may be some distance from the recording or drug delivery site which is assumed to be near the cell soma. The remote location of these synapses, as opposed to the termination of i.p.n. afferents on proximal dendrites or somas of m.r.n. neurones (Nakamura & Mizuno, 1971), could also explain why *p*CB-PzDA depressed cortically evoked responses less effectively than i.p.n.-evoked responses on the same neurones. However, while it is possible that only non-NMDA receptors mediate cortically evoked excitation at APV-insensitive sites, both NMDA and non-NMDA receptors appear to be implicated in APV-sensitive cortically evoked events. This notion arises from the observation that cortically evoked responses were more readily antagonized by *p*CB-PzDA than by APV, yet the former is less effective than APV as an antagonist of NMDA-induced excitation (Davies *et al.* 1984). Recent patch-clamp and voltage-clamp studies on cultured spinal neurones reveal that membrane conductance linked to NMDA receptors is highly voltage dependent, increasing with membrane depolarization (Mayer & Westbrook, 1984; Novak *et al.* 1984), whereas that linked to non-NMDA receptors is much less voltage dependent (Mayer & Westbrook, 1984). Thus, if NMDA and non-NMDA receptors are involved in mediating cortico-rubral excitation, transmitter activation of voltage-sensitive ion channels linked to NMDA receptors may serve to regulate neuronal responses resulting from concomitant transmitter interaction with non-NMDA receptors. However, further clarification of the role of NMDA and non-NMDA receptors in these cortically evoked responses of m.r.n. neurones must await the development of potent and selective non-NMDA-receptor antagonists.

While the present results are consistent with an amino acid being a transmitter in the interposito-rubral and cortico-rubral excitatory pathways they do not establish the identity of the synaptically released transmitter. Ejecting currents of APV that depressed synaptic excitation significantly reduced responses to both L-aspartate and L-glutamate on the limited number of neurones tested in this study. Hence, either of these amino acids may possibly be the transmitter acting at NMDA receptors. However, transmission in m.r.n. neurones involving non-NMDA receptors was more sensitive to the broad spectrum excitatory amino acid antagonists than neuronal responses to either L-glutamate or L-aspartate in some neurones. While this may reflect a greater action of exogenously administered L-glutamate and L-aspartate on NMDA receptors relative to non-NMDA receptors, the possibility must be considered that some other endogenous amino acid is the transmitter interacting with these amino acid receptors.

This work was supported by the M.R.C.

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