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Quantitative studies on some antagonists of N-methyl D-aspartate in slices of rat cerebral cortex

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1 Coronal sections of rat brain (500 μm thick) were trimmed to form 'wedges' of tissue consisting of cerebral cortex and corpus callosum.

2 When these slices were placed in a two-compartment bath, the cortical tissue could be depolarized, relative to the corpus callosum, by superfusions of high K^+, or by amino acids such as L-glutamate, L-aspartate, quisqualate, kainate and N-methyl D-aspartate (NMDA).

3 Responses to NMDA were reduced by magnesium ions, by the organic antagonists (−)-2-amino 5-phosphonovalerate (APV) and 2-amino 7-phosphonoheptanoate (APH), and by the dissociative anaesthetic ketamine.

4 In this preparation, all these antagonists shifted the NMDA dose-response curve to the right in a parallel manner. A Schild plot for Mg^{2+} had a slope significantly less than unity, indicative of a non-competitive action, whilst Schild plots for (−)-APV, APH and ketamine appeared linear and had slopes of approximately 1.

5 Analysis of the results of combination experiments suggested that the presumed competitive antagonists, (−)-APV and APH, share a common site of action as NMDA antagonists, and that this site is distinct from that at which ketamine exerts its action. The action of Mg^{2+} is clearly different from that of either (−)-APV or ketamine. It is concluded that ketamine is a non-competitive antagonist of NMDA and may act at an allosteric site on the NMDA receptor complex to influence its function.

Introduction

The endogenous amino acids L-glutamate and L-aspartate excite neurones throughout the mammalian central nervous system and may function as neurotransmitters in certain areas (see Watkins & Evans, 1981). The development of antagonists of excitatory amino acids has led to the proposal that L-glutamate and L-aspartate do not act on a single receptor population, and to the identification of excitants which appear to interact preferentially with discrete receptor populations, designated quisqualate-, N-methyl D-aspartate (NMDA)-, and kainate-preferring. These receptor types, whose existence was first suggested on the basis of electrophysiological evidence, have now been visualised autoradiographically in the hippocampus (Monaghan *et al.*, 1983).

It is well established that the excitation of central neurones by NMDA is sensitive to a variety of antagonists. Mg^{2+} ions have been shown to attenuate NMDA responses in both the cat and frog spinal cord (Davies & Watkins, 1977; Ault *et al.*, 1980). Several organic antagonists have also been shown to reduce responses to NMDA, the most potent and selective of these being (−)-2-amino 5-phosphonovalerate, which is reported to antagonize NMDA in the cat spinal cord (Davies & Watkins, 1982) and rat hippocampus (Collingridge *et al.*, 1983; Crunelli *et al.*, 1983). In addition the 'dissociative' anaesthetic ketamine has been reported to prevent selectively the excitation of spinal neurones by NMDA (Anis *et al.*, 1983).

Much of the substantial progress in this field has been achieved through the use of the iontophoretic technique, but drug concentrations at the receptor sites are unknown and this limits the extent to which quantitative analysis of drug action can be pursued. An alternative approach is to employ bath application of drugs to isolated neuronal preparations, for example, the isolated hemisected frog spinal cord (Ault *et al.*, 1980). Such an approach has proved useful in the

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study of receptors for γ-aminobutyric acid (GABA) in the cuneate nucleus of the rat (Simmonds, 1978).

For our present purposes we decided to use a preparation of cerebral cortex, since it has long been known that virtually all cortical neurones are excited by L-glutamate and other amino acids (Krnjević & Phillis, 1963; Crawford & Curtis, 1964). We set out to prepare a slice of brain tissue containing cortical efferents which could be placed in a two-compartment bath so that the depolarization of individual neurones by L-glutamate could be recorded as a population response. Having achieved this aim, we have used the preparation to study the actions of some NMDA antagonists in the rat cerebral cortex.

Some of these results have appeared previously in preliminary form (Harrison & Simmonds, 1984).

Methods

Preparation of slices

'Wedges' of rat cerebral cortex were prepared from the brains of male Wistar rats (150–350 g). Whole brains were rapidly removed and placed, ventral surface uppermost, in an agar brain mould (Brown & Halliwell, 1981). The ventral surface of the tissue was removed to a depth of 2–3 mm, using a hand-held blade, and discarded. The remaining block of brain tissue was then removed to a tissue chopper (Halliwell *et al.*, 1984), and fixed to an agar pad with cyanoacrylate glue at the caudal end of its dorsal surface, so that the cut surface of the brain was uppermost. Vertical sections were made by a falling razor strip, the first section being made at the level of the anterior commissure. The rostral portion was discarded; subsequent coronal sections yielded a series of slices of thickness 500–600 μm, consisting of cerebral cortex, corpus callosum and striatal tissue. The slices were rapidly placed in oxygenated Krebs medium, and divided at the mid-line by hand. Further cuts were made on either side of the mid-line to produce wedge-shaped pieces of tissue consisting of cerebral cortex and corpus callosum, which were ≃1.5 mm wide at the pial surface and ≃ 1 mm wide at the corpus callosum.

Experimental procedure

After 2–3 h of incubation in oxygenated Krebs medium at room temperature, each wedge was placed in a two-compartment bath (Figure 1). The arrangement was such that the cortical tissue was contained almost entirely in one compartment and the ventral margin of the cortex passed through a greased slot

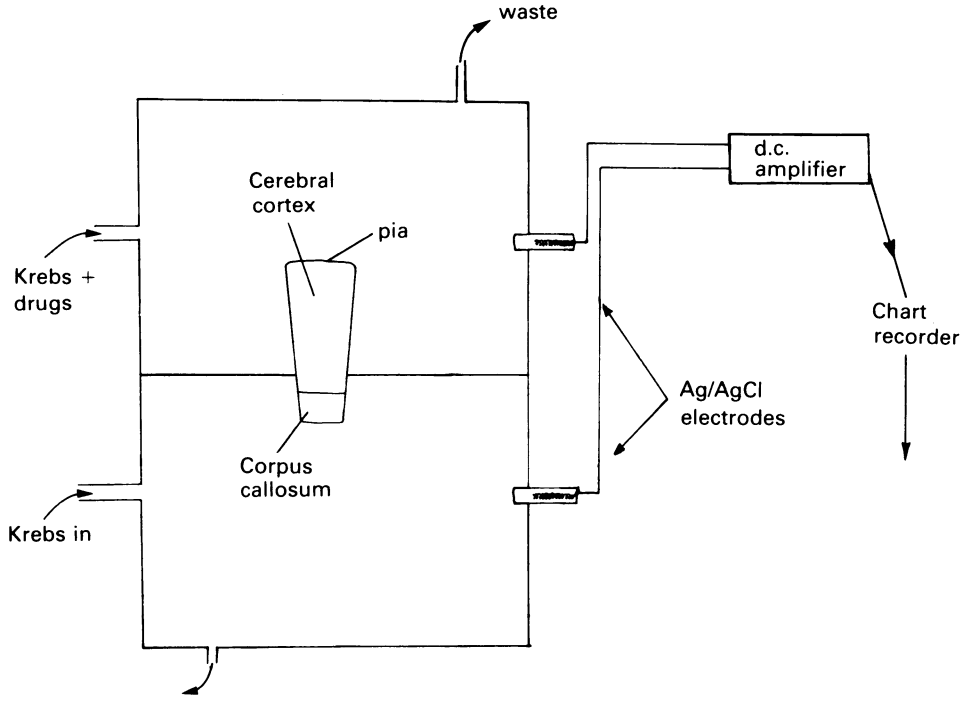

Figure 1 Diagram (not drawn to scale) illustrating the form of the 'wedges' of tissue and their position in the two-compartment bath.

(high vacuum silicone grease, BDH) so that the corpus callosum was entirely contained in the other compartment. The two compartments were perfused independently with Krebs medium at 2 ml min^{-1}, drugs generally being applied only to the cortical end of the preparation. The d.c. potential between the two compartments was continuously monitored via Ag/AgCl electrodes embedded in 3% agar in saline and a high input impedance amplifier, and displayed on a chart recorder. No consistent change in this d.c. potential was measured after insertion of the slices; drug-induced deviations from this baseline d.c. potential were measured at peak amplitude.

Approximately 30 min after the tissue was placed in

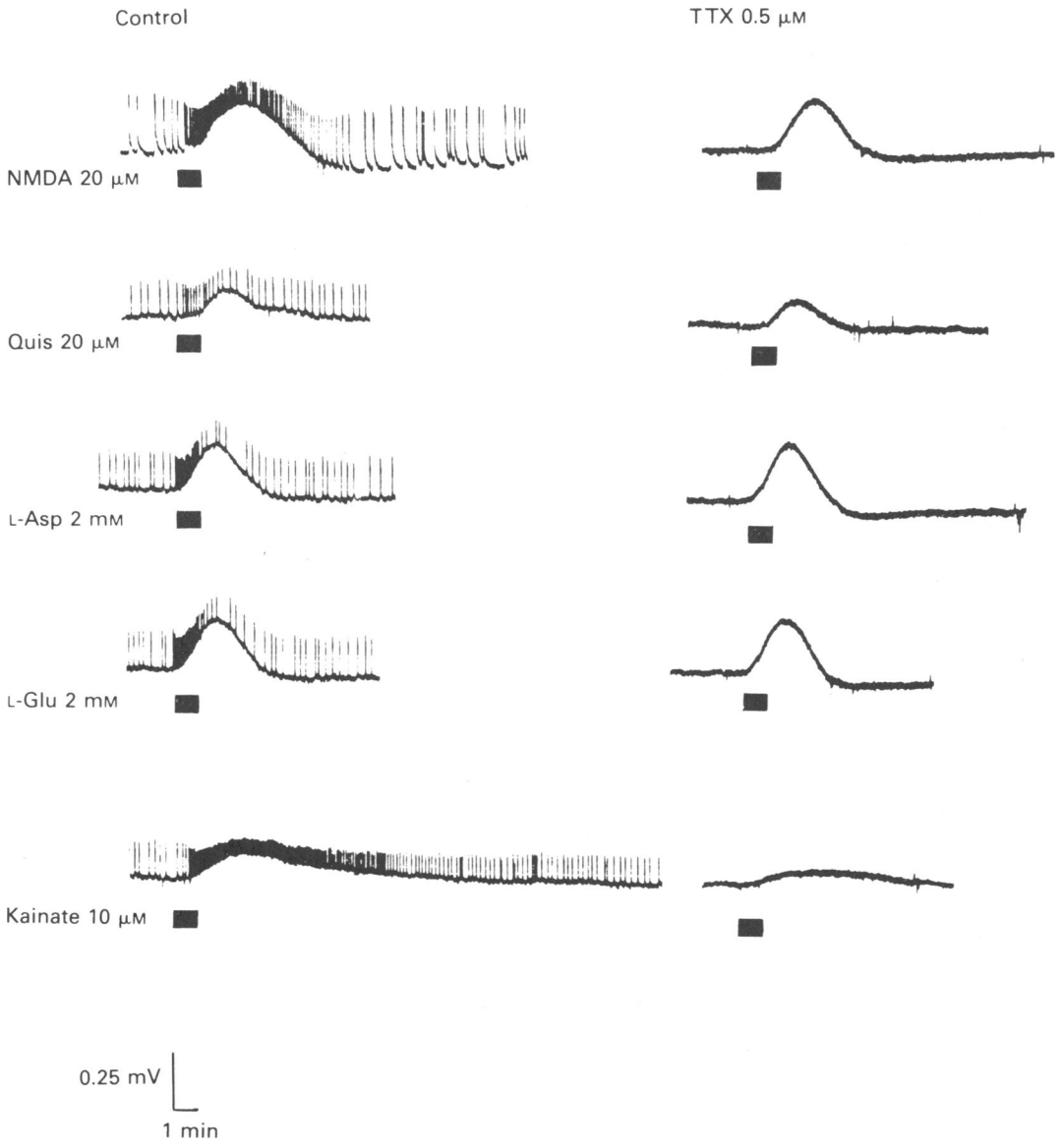

Figure 2 Increase in frequency of spontaneous activity in one preparation produced by 1 min applications of excitatory amino acids to the cortical tissue. The cortical tissue is depolarized relative to the corpus callosum by superfusions of excitatory amino acids, the amplitude of these depolarizing responses being unaltered after the suppression of spontaneous activity by tetrodotoxin (TTX) 0.5 μM. In this experiment the depolarizing response to kainate was somewhat depressed in TTX, but this was not a consistent finding.

the bath, the bathing solution was replaced with a Mg^{2+}-free Krebs solution, pH 7.4, containing (mM): NaCl 118, KCl 2.1, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25 and glucose 11. The solution was continuously bubbled with 95%O_2/5%CO_2. Mg^{2+} was omitted from the experimental medium, since Mg^{2+} ions antagonize certain excitatory amino acids (Davies & Watkins, 1977; Ault *et al.*, 1980), but the slices were initially incubated in a Krebs medium containing Mg^{2+} 2mM in order to assist recovery. Except where otherwise stated, amino acids were applied by 2 min superfusion, and other drugs were perfused for at least 30 min before retesting agonist responses. About one in three of slices prepared and set up in this way gave adequate responses to the amino acids, and these slices remained viable for many hours. On several occasions, consistent responses to quisqualate or NMDA were elicited over a period of 18 h, illustrating the stability of both the tissue response and of the 'grease-gap' seal; responses to high K^+ application also remained stable for very long periods.

Sources of drugs and chemicals

Bulk chemicals were obtained from BDH; other substances as follows: sodium L-glutamate, L-aspartic acid, acetylcholine chloride, nicotine hydrogen tartrate, kainic acid, γ-aminobutyric acid and tetrodotoxin (Sigma); quisqualic acid, N-methyl D-aspartic acid, 2-amino 7-phosphonoheptanoic acid and D(−)-2-amino 5-phosphonovalerate (Cambridge Research Biochemicals). A separate sample of D(−)2-APV was obtained from Tocris Chemicals. Ketamine HCl was a gift from Warner-Lambert UK Ltd.

Analysis of data

Where antagonist drugs produced a parallel shift of the NMDA dose-response curve, NMDA doses were increased so as to bridge the same response level as the control dose-response curves, and thus enable dose ratios to be calculated. Schild plots were constructed and, where appropriate, 'best-fit' equations were obtained by least-squares linear regression analysis to yeild estimates of Schild plot slopes and pA_2 values.

In combination experiments, antagonists were combined and the dose-ratios obtained for an antagonist B in the presence of antagonist A compared with those obtained for antagonist B acting alone. If antagonists A and B act independently of each other, these values should not differ significantly from one another (see Table 2). Statistical comparisons were made using Student's *t* test.

Results

Spontaneous activity

During the initial 30 min superfusion of slices with Krebs medium containing Mg^{2+} 2mM, a stable d.c. potential between the two compartments was attained with no evidence of spontaneous activity. When the solution was changed to Mg^{2+}-free Krebs medium, spontaneous activity appeared within 30 min in about a quarter of the slices. Superfusion with any of the excitatory amino acids increased the frequency of this activity (Figure 2) and with large depolarizations reduced its amplitude. Tetrodotoxin (TTX), 0.5 μM, abolished the spontaneous activity within about 25 min.

In three experiments, the depolarization responses to the amino acids L-glutamate, L-aspartate, NMDA and quisqualate were determined during the occurrence of spontaneous activity and after its abolition by TTX. There was little difference between the depolarization responses recorded under the two conditions (Figure 2). Therefore, TTX was not routinely included in the Krebs medium.

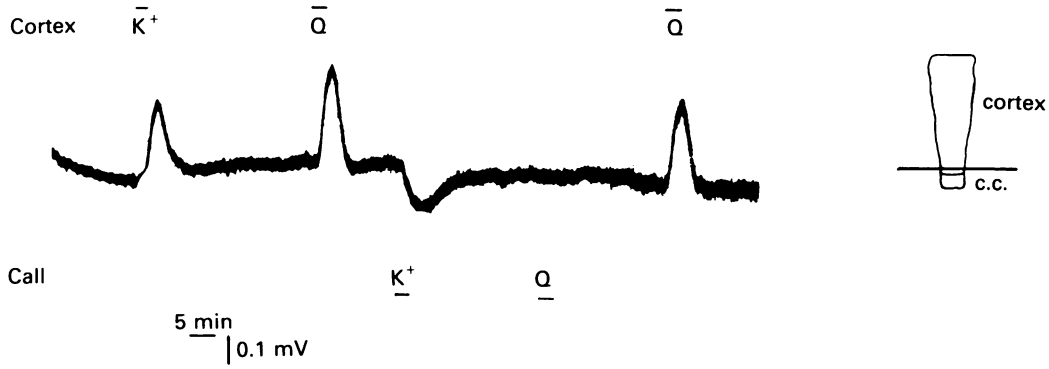

Figure 3 Depolarization (upward deflection) of cortical tissue relative to the corpus callosum by 2 min applications of 9 mM K^+ and 10 μM quisqualate (Q), followed by depolarization (downwards) of the corpus callosum by 9 mM K^+. Quisqualate 10 μM failed to depolarize the white matter.

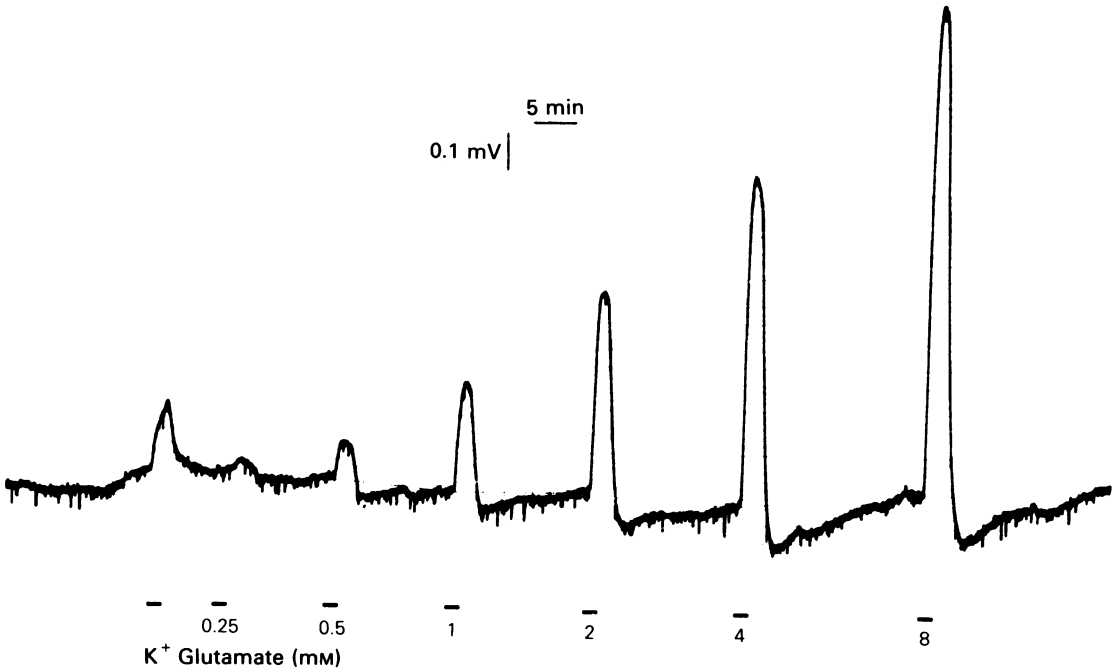

Figure 4 Dose-dependent depolarizations of the cortical tissue produced by 2 min superfusions of L-glutamate. Note the after-hyperpolarizations following large depolarizations.

Depolarization responses

K^+

Superfusion with Krebs containing increased K^+ produced a rapid and concentration-related depolarization of the cortical tissue which reversed rapidly on washout. In two experiments 9 mM K^+ was superfused over the corpus callosum. The depolarization of the white matter was much smaller than that of the cortical tissue and was slower in onset and decline (Figure 3).

L-Glutamate

L-Glutamate produced a rapid and reversible depolarization of the cortical tissue which was concentration-dependent (Figure 4), the threshold concentration being 100–500 μM. No obvious maximum to the dose-response curve was observed with concentrations of L-glutamate up to 8 mM. Responses to the higher doses of L-glutamate were often biphasic, large depolarizations being followed by a pronounced after-hyperpolarization (Figure 4); this type of after-hyperpolarization has also been observed in sucrose-gap recordings from the hemisected frog spinal cord, and ascribed to activation of the electrogenic Na^+ pump (Padjen & Smith, 1983). Very high doses (8 mM) of L-glutamate were not used routinely since they resulted in depression of subsequent responses to amino acids of all types, possibly due to neurotoxic actions of high doses of L-glutamate.

L-Aspartate

L-Aspartate also produced depolarizing responses which were rapid in onset and decline. Threshold concentrations were similar to those for L-glutamate and in most experiments the two amino acids appeared almost equipotent. After-hyperpolarizations followed large depolarizing responses to L-aspartate and high doses depressed subsequent responses. As with L-glutamate it was impossible to estimate a maximum response.

Quisqualate

Quisqualate produced depolarizing responses which were as rapid in onset and decline as those produced by L-glutamate and L-aspartate. Threshold concentrations were between 1 and 10 μM. The dose-response curve had a readily observable maximum, reached at 50–100 μM quisqualate. After-hyperpolarization was not so marked as with L-aspartate and L-glutamate

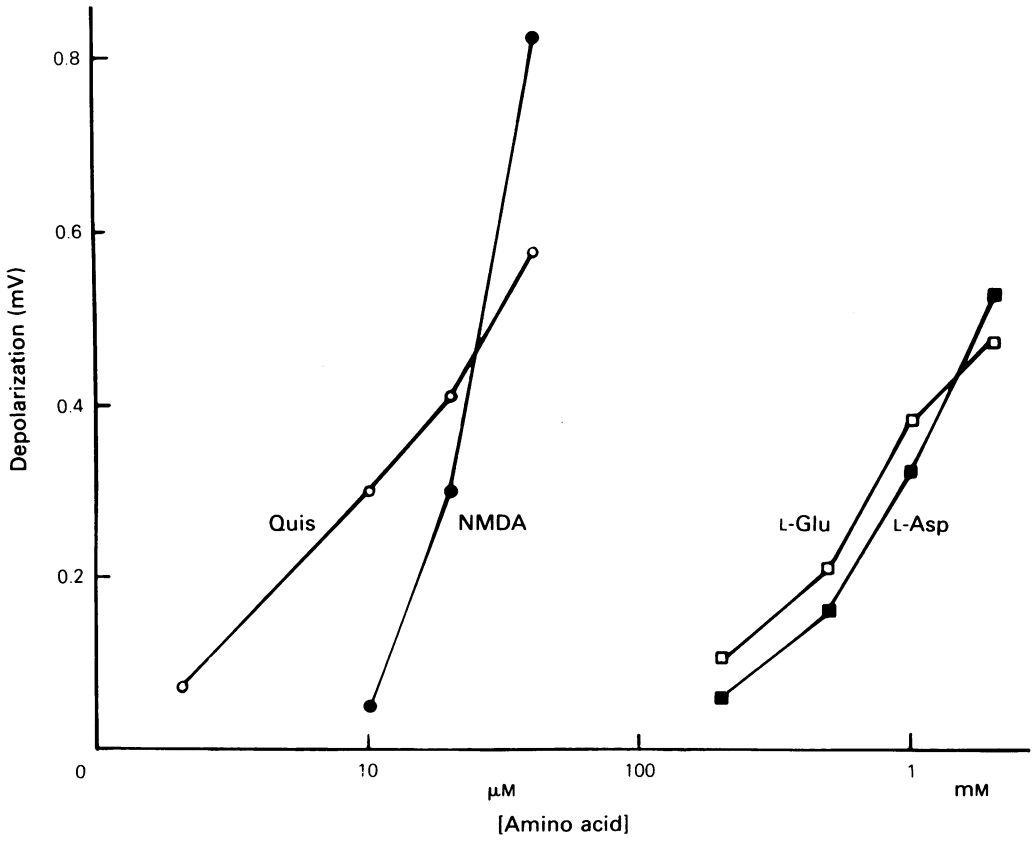

Figure 5 Dose-response relationships obtained in one slice preparation for N-methyl D-aspartate (NMDA), quisqualate (Quis), L-glutamate (L-Glu) and L-aspartate (L-Asp). Each point represents a single measurement made after stable responses had initially been obtained to test doses of the amino acids. In this example, no maximum was determined for quisqualate; this was usually reached between 50–100 μM.

and even applications of 100 μM quisqualate did not depress subsequent responses. When the corpus callosum was superfused with quisqualate (10 μM), no response was observed (Figure 3). All the other amino acids tested produced no depolarization of the corpus callosum, except for a low amplitude response to kainate.

N-methyl-D-aspartate

NMDA produced depolarizing responses which were slower in onset and decline than responses of equal amplitude to L-glutamate, L-aspartate or quisqualate, probably due to the lack of an efficient uptake process for NMDA within the slice. Threshold concentrations were 2–10 μM and the dose-response curve was notably steeper than those for the other three amino acids (Figure 5). After concentrations of NMDA of 100 μM or greater, the cortical tissue failed to repolarize completely and subsequent amino acid responses were depressed. For this reason, it was often not possible to obtain a maximum for the NMDA dose-response curve. Even modest responses to NMDA were followed by pronounced after-hyperpolarization.

Kainate

Kainate produced depolarizing responses which were extremely slow in onset and offset, and of low amplitude. The dose-response curve for kainate was generally shallow in comparison with those for the other amino acids. Measurable responses were obtained with 5–10 μM kainate; relatively fewer experiments were performed with kainate due to its known neurotoxic properties.

γ-Aminobutyric acid and muscimol

GABA (1 mM) or muscimol (20–50 μM) produced small depolarizing responses comparable in amplitude to those obtained with 0.5 mM L-glutamate.

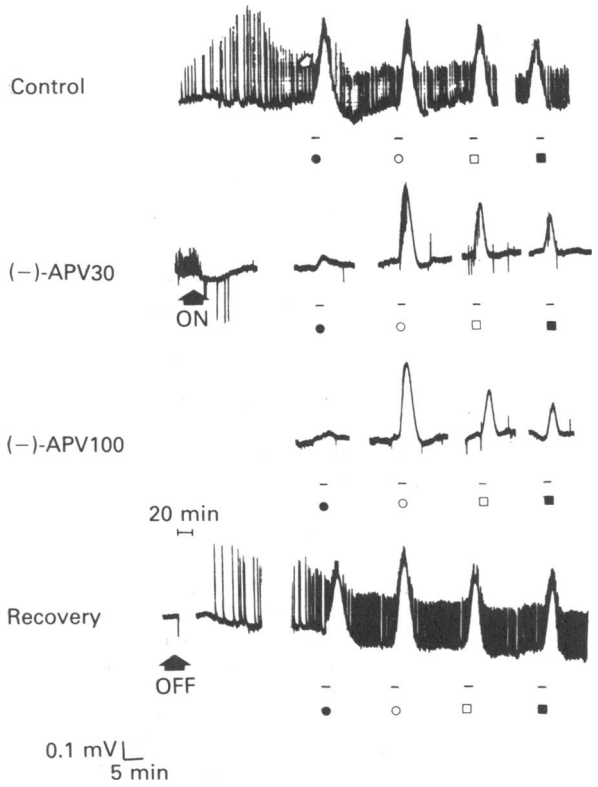

Figure 6 Depolarizing responses to N-methyl D-aspartate 20 μM (●), quisqualate 20 μM (○), L-glutamate 1 mM (□) and L-aspartate 1 mM (■), before, during and after superfusion of (−)-2-amino-5-phosphonovalerate ((−)-APV), 30 and 100 μM. Note that increasing the concentration of (−)-APV from 30–100 μM produced only a small further decrease in the amplitude of responses to L-glutamate and L-aspartate. Spontaneous activity was increased in frequency by amino acid application (open arrow), and suppressed by (−)-APV, this effect being reversible within 20 min of washout.

Acetylcholine and nicotine

No responses to these substances were observed at 1 mM.

Actions of some antagonists of N-methyl-D-aspartate

Four antagonists of NMDA have been studied in the present experiments: Mg^{2+}, (−)-2-amino 5-phosphonovalerate (APV), 2-amino 7-phosphonoheptanoate (APH) and ketamine. All except APH were evaluated against quisqualate, L-glutamate and L-aspartate as well as NMDA.

In Figure 6 are shown results of one experiment with (−)-APV 100 μM, which failed to influence the quisqualate response although the NMDA response was almost entirely abolished; similar results in terms of selectivity were obtained with Mg^{2+}. NMDA antagonism by either Mg^{2+} or (−)-APV was readily reversed on washout, full recovery being obtained within 1 h. Ketamine also reduced responses to NMDA without affecting those to quisqualate, but it was not possible to demonstrate reversal of this action of ketamine during a washout period of 3 h (Figure 7). A similar lack of reversibility has been observed for ketamine as an antagonist of ACh at electric organ nicotinic receptors (Volle *et al.*, 1982); this may reflect extensive penetration of the drug into lipophilic compartments from which it is slowly released.

All three of these NMDA antagonists also reduced responses to L-glutamate and L-aspartate, the percentage reduction of L-aspartate responses being rather greater than for L-glutamate responses. This reduction by NMDA antagonists was incomplete (Figure 6). In separate experiments, (−)-APV proved to have no effect on the responses to kainate. This pattern of selectivity, with NMDA being most susceptible, then L-aspartate and L-glutamate, with quisqualate and kainate unaffected, was common to all of the NMDA antagonists studied here.

In order to determine the potency of the NMDA antagonists, dose-response relationships were established for NMDA before and during superfusions of antagonist drugs. All of the antagonists caused approximately parallel shifts of the NMDA dose-

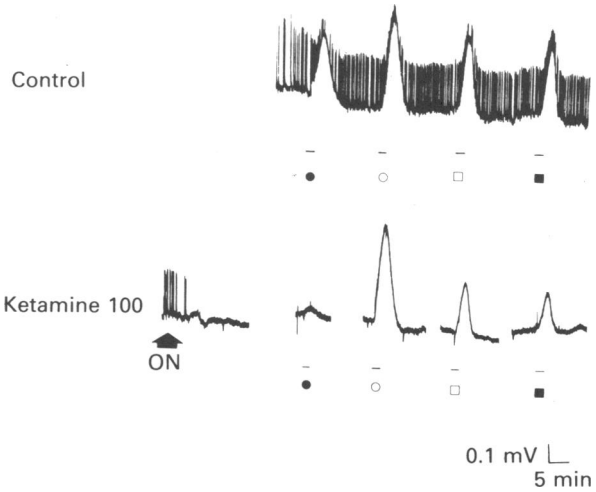

Figure 7 Depolarizing responses to the same amino acids as in Figure 6, before and during superfusion of 100 μM ketamine. This experiment was performed on the same preparation as in Figure 6, following full recovery from (−)-APV. Ketamine also abolished the spontaneous activity in this experiment. N-methyl D-aspartate 20 μM (●); quisqualate 20 μM (○); L-glutamate 1 mM (□); L-aspartate 1 mM (■).

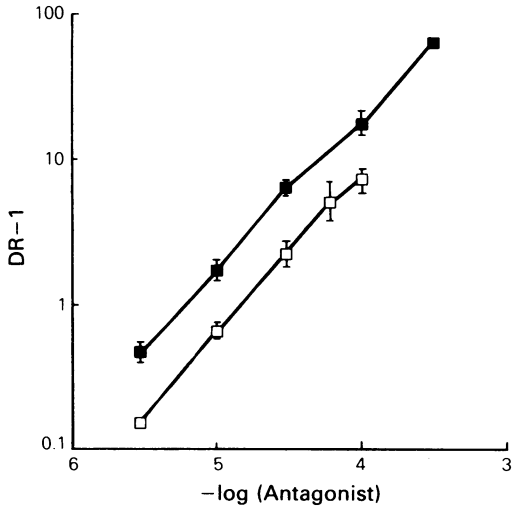

Figure 8 Schild plots for (−)-2-amino 5-phosphonovalerate (■) and 2-amino-7-phosphonoheptanoate (□) as antagonists of N-methyl D-aspartate (NMDA). Each point represents the mean of 3–16 individual experiments; error bars represent s.e. mean.

response curves, allowing Schild plots of log [NMDA dose-ratio − 1] against −log [antagonist concentration] to be constructed (Figures 8 and 9).

The Schild plots for (−)-APV, APH and ketamine were all linear with slopes not significantly different from unity. The slopes and pA_2 values estimated from the data are shown in Table 1. At ketamine concentrations above 100 μM, there was some flattening of the NMDA dose-response curves which prevented the extension of the Schild plot for ketamine.

The Schild plot for Mg^{2+} had a slope significantly ($P < 0.01$) less than unity (Table 1); nevertheless,

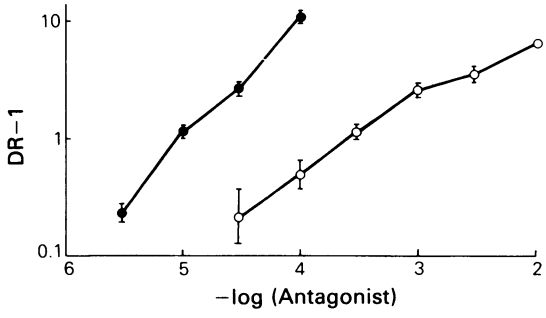

Figure 9 Schild plots for Mg^{2+} (○) and ketamine (●) as antagonists of N-methyl D-aspartate (NMDA). Each point is the mean of 3–14 experiments; s.e. mean shown by vertical lines.

Table 1 Slopes of Schild plots and pA_2 values, derived from the data illustrated in Figures 8 and 9

Antagonist	*Slope of Schild plot*	*pA_2*
(−)-APV	1.12 ± 0.07	5.2
APH	1.11 ± 0.09	4.8
Ketamine	1.05 ± 0.06	5.0
Mg^{2+}	0.58 ± 0.04	3.6

(−)-APV = (−)-2-amino 5-phosphonovalerate; APH = 2-amino 7-phosphonoheptanoate.

NMDA dose-response curves were always shifted in a parallel manner by Mg^{2+}.

To ascertain whether (−)-APV, APH and ketamine all act at a common site to antagonize NMDA, combinations of (−)-APV with either ketamine or APH were tested. Combinations of Mg^{2+} with either (−)-APV or ketamine were also tested.

The data for APH in the presence of (−)-APV (Table 2) show clearly that APH produces a significantly smaller dose-ratio in the presence of (−)-APV than is produced when APH is added alone, indicating that these drugs share a common site in antagonizing NMDA. In addition, the results for the two drugs in combination accord closely with the theoretically predicted value for the two drugs acting at a common site. In contrast, ketamine appeared to *increase* the potency of (−)-APV, when (−)-APV was added after ketamine. Such a result indicates that although (−)-APV and ketamine do not share a common site, these sites may interact. The result for the drugs in combination here is significantly different from the common site prediction.

The combination experiments with Mg^{2+} show that Mg^{2+} and (−)-APV also appear to act at separate sites, although there is some indication from the data (Table 3) that these antagonists may increase each other's potency. Combination experiments with Mg^{2+} and ketamine show no significant differences in the dose-ratios produced by these drugs acting alone or in the presence of the other. It thus seems likely that these drugs act at separate sites to antagonize NMDA.

Discussion

The depolarizing responses recorded here using the 'barrier' technique depend upon the number of neurones that project their axons in the plane of the slice, and on the density of the appropriate receptors on those neurones. The rather low proportion of slices giving adequate responses may reflect the lack of the clear guidelines for cutting the slices in the plane of the pyramidal cell projections via the corpus callosum.

Table 2 Antagonism of N-methyl D-aspartate (NMDA) by (−)-2-amino 5-phosphonovalerate ((−)-APV), individually or in combination with ketamine or 2-amino 7-phosphonoheptanoate (APH)

	Antagonist (B)		*(−)-APV* (10 μM) (A)		*Combinations*[a]			*Predicted values for combinations*[b]	
Antagonist (B)	*Alone*	*Added to A*	*Alone*	*Added to B*	*A + B*	*B + A*	*Total* (8)	*Common site*	*Independent sites*
		$P < 0.01$[c]		$P < 0.01$					
APH 60 μM	8.3±0.8	2.7±0.2	3.0±0.2	1.5±0.1	8.2±1.0	12.3±0.6	10.3±0.9	11.1	24.5
Ketamine 30 μM	3.6±0.7	4.2±1.2	2.9±0.2	5.4±0.7	12.2±3.4	19.4±3.5	15.8±2.6	6.2	10.4
	NS			$P < 0.01$					

Values shown are mean NMDA dose ratios ± s.e. mean (four experiments).
[a]In individual experiments either antagonist A was added first and then B subsequently (A + B), or B first followed by A (B + A).
[b]Predicted values for antagonist combinations were determined as follows: for independent sites, multiply the value for A alone by the value for B alone (Barlow, 1980); for a common site, determine from Figure 8 the (−)-APV concentration equipotent with B alone then add 10 μM and read off the NMDA dose-atio from Figure 8.
[c]Values are significantly different (Student's *t* test); NS = not significant.

Table 3 Antagonism of N-methyl D-aspartate (NMDA) by Mg^{2+}, individually or in combination with ketamine or (−)-2-amino 5-phosphanovalerate ((−-APV.)

	Antagonist (B)		Mg^{2+} (1 mM) (A)		*Combinations*[a]			*Predicted values for combinations*[b]	
Antagonist (B)	*Alone*	*Added to A*	*Alone*	*Added to B*	*A + B*	*B + A*	*Total* (8)	*Common site*	*Independent sites*
	NS[c]		NS						
(−)-APV 10 μM	2.8±0.7	4.9±0.7	3.0±0.5	4.5±0.5	14.2±1.5	13.2±4.1	13.7±2.0	3.9	8.5
Ketamine 30 μM	3.8±0.6	3.1±0.4	4.1±0.4	3.1±0.6	12.7±2.0	11.1±1.4	11.9±1.1	4.3	15.4
		NS		NS					

Values shown are mean NMDA dose-ratios ± s.e. mean (four experiments).
[a]In individual experiments either antagonist A was added first and then B subsequently (A + B), or B first followed by A (B + A).
[b]Predicted values for antagonist combinations were determined as follows: for independent sites, multiply the value for A alone by the value for B alone (Barlow, 1980); for a common site, determine from Figure 9 the Mg^{2+} concentration equipotent with B alone, then add 1 mM and read off the NMDA dose-ratio from Figure 9.
[c]NS = difference not significant.

It appears that only receptors for kainate may be present on axons in the corpus callosum. The signals recorded from the cortex, therefore, probably arise as a consequence of the activation of receptors on the cell bodies and dendrites of pyramidal cells. Such an interpretation is compatible with observations made during intracellular recordings from pyramidal cells in slices prepared as described here, showing that NMDA and quisqualate receptors are indeed present on these neurones (J.V. Halliwell, unpublished observations).

The spontaneous activity seen in Mg^{2+}-free medium did not affect the depolarizing responses since their amplitude was unaltered after suppression of spontaneous activity with TTX. The results obtained with antagonist drugs were the same in slices showing spontaneous activity as in the many preparations which did not show spontaneous activity. It is interesting to note that all of the exictatory amino acids increased the frequency of the activity, while this was markedly decreased by Mg^{2+} and other NMDA antagonists (Figures 6 and 7) (cf.Evans *et al.*, 1982).

(−)-APV and ketamine have been reported to be selective antagonists of NMDA (Davies & Watkins, 1982; Collingridge *et al.*, 1983; Crunelli *et al.*, 1983; Anis *et al.*, 1983), and the present results confirm these observations. Quisqualate and kainate were not antagonized but both L-glutamate and L-aspartate were, to some extent. Again these findings confirm the view that L-glutamate and L-aspartate are 'mixed agonists', acting on both NMDA and non-NMDA receptors (Watkins & Evans, 1981). The present data do not, however, allow a quantitative evaluation of the relative contribution of these receptor types to the responses elicited by L-glutamate or L-aspartate, nor do they identify the receptor types involved in the generation of that fraction of the responses which is insensitive to NMDA antagonists. This will be the subject of a further study.

The observation that the higher homologue of APV, APH, shares a common site with (−)-APV in antagonizing NMDA is in line with the accepted idea of these compounds as competitive antagonists of NMDA. The Schild plots for (−)-APV and for (±)-APH reveal that (±)-APH is 2.5 times less potent than the (−)-isomer of APV. This potency ratio is consistent with reports that (±)-APV is slightly more potent than (±)-APH as an NMDA antagonist in the frog spinal cord (Evans *et al.*, 1982), and with the relative potencies of these agents in displacing [^{3}H]-(−)-APV binding (Olverman *et al.*, 1984).

The estimated K_d of 6.4 μM for (−)-APV at the NMDA receptor obtained here is a little higher than the estimate for (±)-APV in experiments in the isolated hemisected spinal cord of frog (1.4 μM; Evans *et al.*, 1982), but this difference in itself is insufficient reason to suppose that the NMDA receptor studied here differs materially from that in the frog spinal cord.

In view of the Schild plot slope of 1 for ketamine in these experiments, it might have been expected that ketamine also was a competitive antagonist of NMDA and shared a common site with (−)-APV. However, the results obtained when these drugs were combined strongly suggest that ketamine acts at a site distinct from the NMDA binding site. The existence of a very large receptor reserve may account for the observation that ketamine has a Schild plot slope of 1, although it is not a competitive antagonist (Pennefather & Quastel, 1983). The findings from binding studies that NMDA is a potent displacer of [^{3}H]-(−)-APV binding (Olverman *et al.*, 1984), whilst ketamine is inactive (J.C. Watkins, personal communication) also support the idea of a non-competitive action of ketamine. It is apparent, therefore, that a Schild plot slope of 1 is not always reliable evidence of competitive antagonism.

The action of ketamine at the NMDA receptor-ionophore complex may be analogous with the action of the non-competitive GABA antagonist picrotoxinin, in decreasing the rate of flow of ions through the channels without directly decreasing individual channel conductance (Barker *et al.*, 1983) or agonist binding. The mechanism of action of ketamine appears distinct from the action of Mg^{2+} in these experiments by virtue of the different slopes of the Schild plots and the results of the combination experiments. Present evidence indicates that Mg^{2+} ions produce a highly voltage-dependent block of the channels operated by NMDA (Mayer *et al.*, 1984; Nowak *et al.*, 1984).

In conclusion, the NMDA receptor complex on cortical neurones of the rat appears to be qualitatively similar to that described in the spinal cord in terms of the susceptibility of NMDA responses to antagonism by Mg^{2+}, APV and ketamine. The present experiments have enabled us to discern different loci of action for each of these agents, and to determine their potencies. The slice preparation described here should prove useful in the identification of antagonists of excitatory amino acids, and in estimating their potency. This is likely to be a growing field of interest in view of reports of the anticonvulsant activities of NMDA antagonists (Croucher *et al.*, 1982).

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