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EXCITATORY AMINO ACIDS IN SYNAPTIC TRANSMISSION IN THE SCHAFFER COLLATERAL–COMMISSURAL PATHWAY OF THE RAT HIPPOCAMPUS

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SUMMARY

1. The effects of excitatory amino acids and some antagonists applied by iontophoresis to stratum radiatum in the CA1 region of rat hippocampal slices were examined on the locally recorded field e.p.s.p. evoked by stimulation of the Schaffer collateral–commissural projection.

2. L-glutamate, L-aspartate and the more potent and selective excitatory amino acids quisqualate, kainate and *N*-methyl-DL-aspartate (NMA) depressed the e.p.s.p., presumably through depolarization and/or a change in membrane conductance.

3. The depression induced by kainate considerably outlasted the period of ejection whereas NMA depressions were rapidly reversible and were often followed by a potentiation of the e.p.s.p. In higher doses NMA also depressed the presynaptic fibre volley. The possible involvement of these effects in neurotoxicity and synaptic plasticity is raised.

4. The selective NMA antagonist, DL-2-amino-5-phosphonovalerate (APV) applied in doses which abolished responses to NMA, had no effect on the e.p.s.p. but prevented long term potentiation (l.t.p.) of synaptic transmission evoked by high frequency stimulation of the Schaffer collateral–commissural pathway. Other antagonists which had little or no effect on normal synaptic transmission included D-α-aminoadipate (DAA), the optical isomers of 2-amino-4-phosphonobutyrate (APB) and L-glutamate diethylester (GDEE).

5. In contrast, γ-D-glutamylglycine (DGG), applied in amounts which affected quisqualate and kainate actions as well as those of NMA, was an effective synaptic antagonist whilst having no effect on the presynaptic fibre volley.

6. These results indicate that the synaptic receptor in the Schaffer collateral–commissural pathway may be of the kainate or quisqualate type. Although NMA receptors do not appear to be involved in normal synaptic transmission in this pathway they may play a role in synaptic plasticity. The interaction of L-glutamate and L-aspartate with these receptors is discussed.

INTRODUCTION

The major excitatory input to CA1 hippocampal pyramidal neurones originates from CA3 pyramidal cells located in both the ipsilateral and contralateral hippocampus. The ipsilateral projection, the Schaffer collateral pathway, terminates mainly on the apical dendrites in stratum radiatum and to a lesser extent on the basal dendrites in stratum oriens, whereas the smaller commissural pathway shows the opposite pattern of termination on CA1 cells (Hjorth-Simonsen, 1973; Swanson, Wyss & Cowan, 1978; Laurberg, 1979). There is considerable neurochemical evidence that the neurotransmitter secreted by these fibres is an excitatory amino acid, probably L-glutamate and/or L-asparate. Thus the levels (Nitsch, 1981), uptake (Storm-Mathisen, 1981) and K^+-evoked release (Nadler, Vaca, White, Lynch & Cotman, 1976) of these amino acids are reduced by lesioning of these fibres. Furthermore, electrical stimulation of the Schaffer collateral–commissural pathway increases uptake (Wieraszko, 1981) and elicits a Ca^{2+}-dependent release of acidic amino acids (Malthe-Sørenssen, Skrede & Fonnum, 1979; Wieraszko & Lynch, 1979; Spencer, Tominez & Halpern, 1981). Due to the lack of potent and selective antagonists however, there has been little electrophysiological evidence to support a role of excitatory amino acids as neurotransmitters in this system. The compounds that have been reported to affect the commissural or Schaffer collateral pathways such as L-glutamate diethylester (GDEE), DL-2-amino-4-phosphonobutyrate (APB) and 1-hydroxy-3-aminopyrrolidone-2 (HA-966) (Segal, 1976; 1978; White, Nadler & Cotman, 1979) are not sufficiently selective as antagonists in the high doses required to achieve effects (Baudry & Lynch, 1981; Watkins & Evans, 1981). Consequently we have examined the sensitivity of the Schaffer collateral–commissural system to a range of substances that have recently been described as potent and selective excitatory amino acid antagonists in the spinal cord (Watkins & Evans, 1981; McLennan & Liu, 1982). The selectivity of these compounds administered to stratum radiatum of the CA1 region upon the responses of single CA1 neurones has been determined and is described in the preceding paper (Collingridge, Kehl & McLennan, 1983).

The Schaffer collateral–commissural projection displays considerable neuronal plasticity. Most striking is the long term potentiation (l.t.p.) produced by brief, high frequency stimulation of the pathway (Schwartzkroin & Wester, 1975; Alger & Teyler, 1976; Dunwiddle & Lynch, 1978; Andersen, Sundberg, Sveen & Wigstrom, 1980). This potentiation, which *in vivo* may last for days (Buzsaki, 1980), has been considered as a useful model for certain aspects of memory (see Cotman, Foster & Lanthorn, 1981). The phenomenon has been localized to the synapse, however whether it is pre- or post-synaptically produced is not known. Furthermore it is not understood what triggers l.t.p., although the observation that it could be prevented by DL-2-amino-4-phosphonobutyrate (Dunwiddie, Madison & Lynch, 1978; Cotman *et al.* 1981), a glutamate antagonist in invertebrates (Cull-Candy, Donnellan, James & Lunt, 1976) raises the possibility that l.t.p. may be produced by a post-synaptic action of an excitatory amino acid. Accordingly we have examined the long term effects of excitatory amino acids applied to the synaptic region on the field e.p.s.p. and also the ability of some antagonists to affect l.t.p.

Some of these results have appeared in preliminary form (Collingridge, Kehl & McLennan, 1982*b*).

METHODS

Experiments were performed on transverse rat hippocampal slices prepared and maintained as described in the previous paper (Collingridge *et al.* 1983). Field potentials were evoked using single monophasic square wave pulses (0·1 msec duration, 3–95 μA intensity) delivered at 0·1 Hz through a glass micro-electrode (tip diameter 0·5–3·0 μm) filled with 4M-NaCl. This electrode was positioned

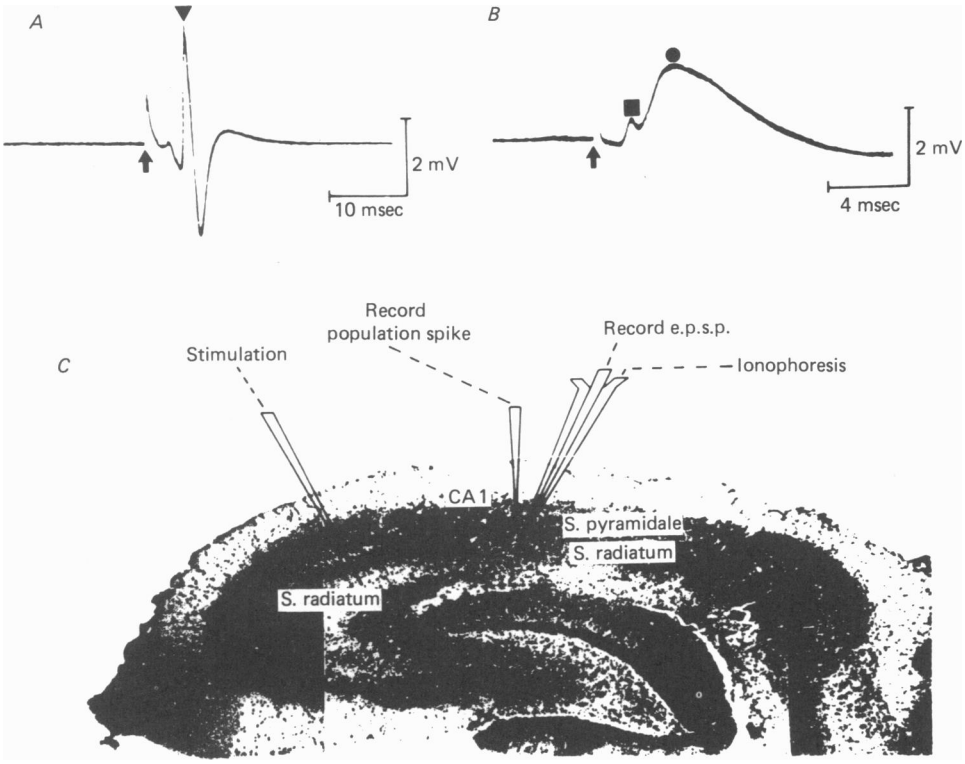

Fig. 1. Electrode placements and field potentials in the hippocampal slice. *C*, a Cresyl Violet-stained section (10 μm thick) prepared from a hippocampal slice at the end of an experiment illustrating the electrode placements. *A*, three superimposed traces illustrating the response to stimulation in stratum radiatum recorded by the single-barrelled electrode in stratum pyramidale. The amplitude of the population spike (▼) was measured from the pre-stimulus base line or from the peak of the initial positive deflexion. *B*, five superimposed traces of the response recorded in stratum radiatum through the central barrel of the multibarrelled electrode in response to stimulation in stratum radiatum. The amplitude of the presynaptic fibre volley (■) and e.p.s.p. (●) were measured from the pre-stimulus base line response. In both records the time of stimulation is shown by an arrow under the record and is followed by a small stimulus artifact. Negativity is upwards in both records.

under visual control in stratum radiation of the CA1, CA2 or CA3 region of the slice (Fig. 1) and its depth was adjusted to produce the maximal response at the particular stimulus intensity used. High frequency stimulation (100 Hz, 1 sec) was employed to generate l.t.p.

A multibarrelled electrode was positioned in stratum radiatum in the CA1 region approximately the same distance from stratum pyramidale as the stimulating electrode so as to be in the terminal region of the activated fibres (Andersen, Silfvenius, Sundberg & Sveen, 1980). The central barrel (4M-NaCl) was used to record the field potential which was comprised by two predominantly negative waves (Fig. 1). The initial component resulted from the depolarization of presynaptic

elements (presynaptic fibre volley: Andersen, Silfvenius, Sundberg, Sveen & Wigstrom, 1978) whereas the second component was generated by the extracellular current flow resulting from depolarization of the apical dendrites (field excitatory post-synaptic potential, e.p.s.p.: Andersen, 1960). The positive deflexion which followed the e.p.s.p. was largely a result of the a.c. coupling in the recording system. The stimulus intensity was selected to evoke a submaximal e.p.s.p. of 0·5–5·0 mV peak amplitude which was usually not contaminated by a population spike. This response remained fairly constant from about 1·5 to 10 hr after preparing the slices. The amplitudes at a fixed latency (usually the peak amplitudes) of the e.p.s.p., and sometimes of the fibre volley, were measured in response to successive stimulus presentations, either visually from an oscilloscope or electronically using a small computer.

In some experiments a single-barrelled glass electrode (4 M-NaCl) was positioned in stratum pyramidale 100–300 μm away from the multibarrelled electrode in stratum radiatum (Fig. 1). The field potential recorded by this electrode consisted of the positive reflexion of the e.p.s.p. superimposed on which was a negative wave generated by the synchronous discharge of CA1 neurones (population spike: Andersen, Bliss & Skrede, 1971). A stimulus intensity was chosen which evoked a stable submaximal population spike of 0·5–4·0 mV and the peak negative deflexion from the initial base line or preceding peak positivity was measured in response to successive stimuli.

Drugs were applied to stratum radiatum from the peripheral barrels of the seven-barrelled micro-electrodes using standard ionophoretic techniques. The solutions filling the barrels were the same as those described in the companion paper (Collingridge *et al.* 1983), with the following additions: quisqualate (50 mM in 100 mM-NaCl, pH 8), kainate (up to 50 mM in 100 mM-NaCl, pH 8) and *N*-methyl-DL-aspartate (NMA; 100 mM, pH 8). Excitants were applied for periods of 10–120 sec whereas antagonists were usually administered until a constant effect was achieved. The action of an antagonist was considered real only if the size of the e.p.s.p. measured over a period of 1 min (six successive measurements) during the administration of the antagonist was significantly different ($P < 0·05$) from the amplitude before and after recovery from the drug application (two-tailed unpaired t tests). Effects were highly reproducible when tests were repeated using the same slice and were never mimicked by appropriate current controls. Results presented are means ± S.E. of the mean.

TABLE 1. Effect of excitants on the Schaffer collateral–commissural–evoked e.p.s.p.

Excitant	E.p.s.p.s depressed/ e.p.s.p.s tested	Percent reduction (mean ± S.E.M. all slices tested)
NMA	24/24	77·4 ± 3·6*
Kainate	22/22	65·8 ± 4·8*
Quisqualate	15/16	45·4 ± 5·8†
L-aspartate	5/6	31·7 ± 8·7
L-glutamate	2/4	10·0 ± 5·8

* The percentage depressions of the e.p.s.p. induced by NMA and kainate were not significantly different from one another but were greater than the depression induced by the other excitants ($P < 0·01$).

† Quisqualate was more potent than glutamate ($P < 0·01$) but there was no significant difference between glutamate- and aspartate-induced effects.

RESULTS

Effects of excitants on e.p.s.p.s

The excitants, applied ionophoretically at the recording site in stratum radiatum, depressed extracellularly recorded e.p.s.p.s (Table 1). L-Glutamate and L-aspartate were the least potent as judged by the intensities of the ejecting currents needed and the magnitude of the effects, and reductions in e.p.s.p.s were fully reversed 1–2 min after terminating the ionophoretic application. Quisqualate was more potent but elicited qualitatively similar depressions which usually fully recovered within 1–3 min (Fig. 2*A*). More potent still was kainate and its effects were usually long lasting

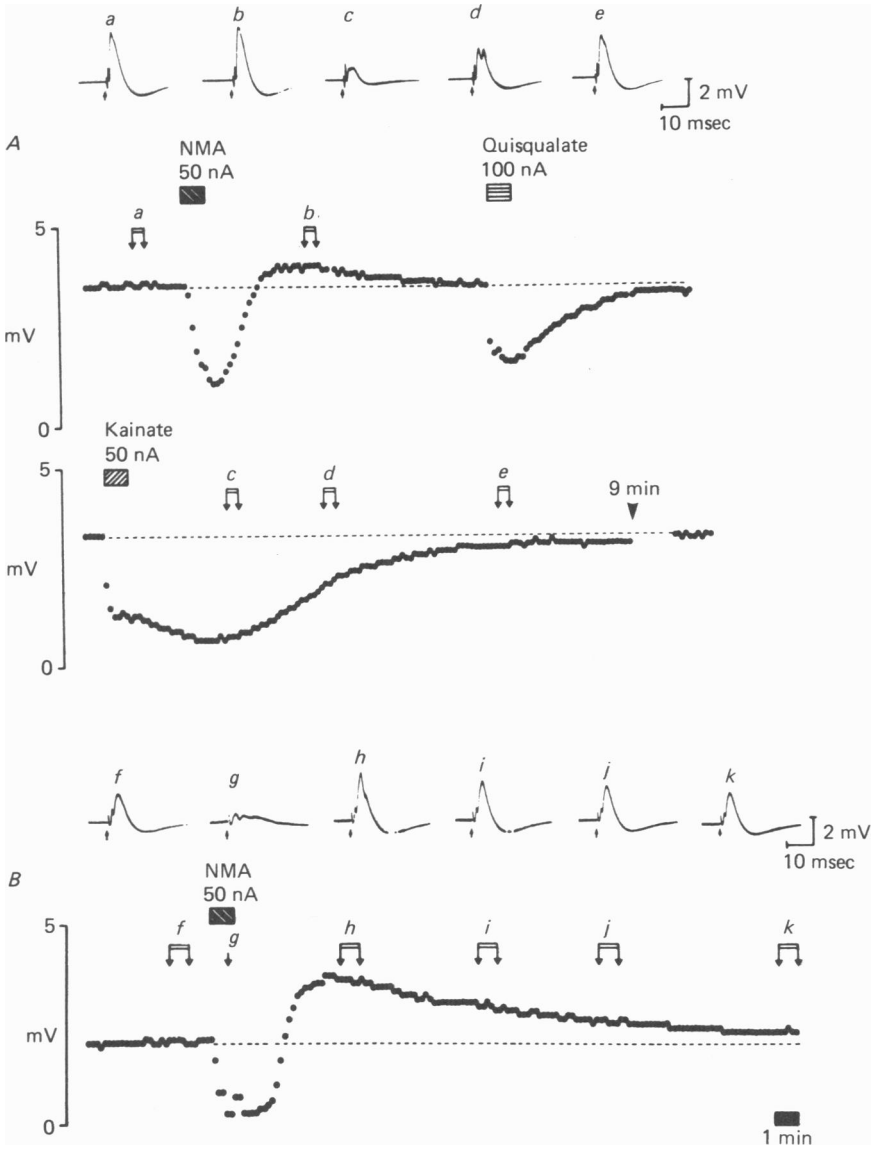

Fig. 2. Effect of excitants on the Schaffer collateral–commissural evoked field e.p.s.p. *A*, in this record the effects of NMA, quisqualate and kainate were compared on the same slice. The peak amplitude of each e.p.s.p. was measured in response to successive stimuli delivered at 0·1 Hz, and the values were plotted. Drugs were applied ionophoretically at the recording site for the periods indicated by the bars above the graph. Representative responses comprising five superimposed oscilloscope records were taken at the times indicated by the small letters (*a–e*). All three excitants depressed the e.p.s.p. Note that recovery from the NMA-induced depression was followed by potentiation (*b*) and that the slow recovery from the kainate-induced depression was associated with the generation of a population spike (*d*). *B*, in this slice NMA produced a much greater potentiation of the e.p.s.p. following recovery from the depression. Note also the reduced size but broadened presynaptic fibre volley, and the virtual abolition of the e.p.s.p. during the administration of NMA (*g*).

(Fig. 2*A*). Full recovery was observed with nine slices within 3–25 min but in the other thirteen recovery was incomplete throughout the period of observation (4–20 min). Although NMA was of similar potency to kainate in causing depression of the e.p.s.p., recovery was much more rapid. In eighteen slices recovery was complete in 1–3 min and was followed by an increase (6–165 %) in the amplitude of the e.p.s.p. (Fig. 2*A*, *B*). This potentiation lasted 9 min in one slice (Fig. 2*A*) but in the remaining slices the e.p.s.p. did not return to control levels for periods up to 24 min (Fig. 2*B*). In six slices where potentiation of the e.p.s.p. was not observed, full recovery from the NMA-injected depression took 1·5–7 min.

Depression of the e.p.s.p. was often observed without any detectable change in the size of the presynaptic fibre volley; however, with higher doses of the excitants reductions in the size of the fibre volley were sometimes observed. With kainate and quisqualate these presynaptic effects were always small, whereas NMA completely abolished the fibre volley on several occasions. Recovery of the fibre volley always preceded that of the e.p.s.p. (Figs. 2*B* and 3).

These effects of the excitants on the field potentials could be antagonized and the selectivity resembled that observed when the antagonists were examined against the excitation of single units (Collingridge *et al.* 1983). Thus APV completely prevented the NMA-induced reduction in the e.p.s.p. and fibre volley, as well as the ensuing potentiation of the e.p.s.p. in all eleven slices examined (Fig. 3). In the same slices, quisqualate responses were either unaffected (3/6 slices) or increased in duration (3/6 slices; Fig. 3) by APV, and kainate responses similarly were either unaffected (1/3 slices) or slightly potentiated (2/3 slices). DGG also abolished NMA responses (3/3 slices), reduced or abolished kainate responses (2/3 slices) and slightly reduced quisqualate responses (3/4 slices; Fig. 4*B*).

TABLE 2. Effects of antagonists on the Schaffer collateral–commissural-evoked e.p.s.p.

Antagonists	E.p.s.p.s depressed/ e.p.s.p.s tested	Percent reduction
DGG	21/23	21·3 ± 2·5*
L-APB	4/9	8·2 ± 2·4
D-APB	3/6	6·4 ± 3·4
DAA	2/4	4·2 ± 2·2
APV	10/26	3·3 ± 1·3
GDEE	0/4	0·1 ± 1·9

Column 2 gives the no. of slices in which the e.p.s.p. was significantly ($P < 0·05$) depressed compared to both pre- and post-drug administration, and the total no. of slices tested. The third column presents the mean ± S.E. of the mean % depression calculated for all slices tested.

* DGG was significantly ($P < 0·05$) more potent than the other compounds. There were no significant differences between the potencies of the other five substances.

Effects of antagonists on e.p.s.p.s

The excitatory amino acid antagonists, also administered at the recording site in stratum radiatum, had actions on evoked e.p.s.p.s which are summarized in Table 2. APV, although expelled in doses in excess of those needed to abolish the NMA-induced depresions, had little or no effect on the e.p.s.p amplitude (Fig. 3). DAA, GDEE and the optical isomers of APB were all similarly ineffective as antagonists of the Schaffer collateral–commissural pathway.

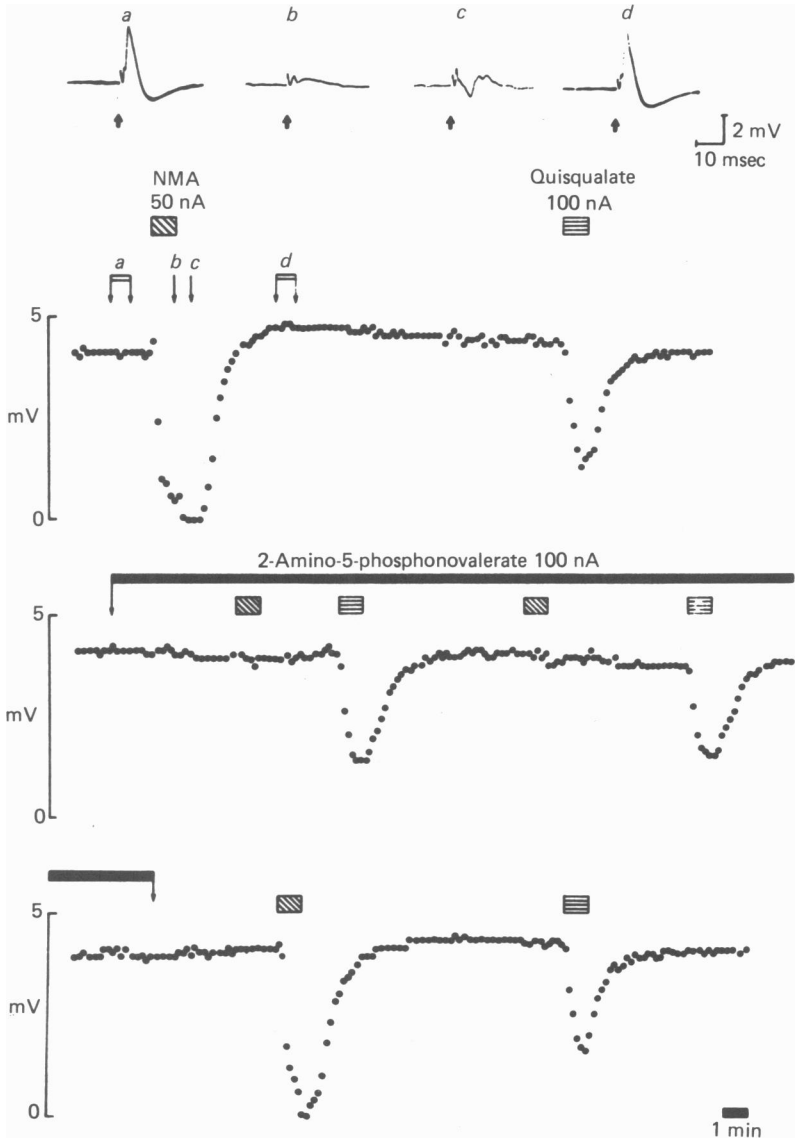

Fig. 3. Effect of 2-amino-5-phosphonovalerate on the Schaffer collateral–commissural-evoked e.p.s.p. and on the depressions of this response induced by NMA and quisqualate. The graph is continuous and was constructed as for Fig. 2. APV completely abolished the depression induced by NMA and slightly potentiated the quisqualate-induced depression. The antagonist had no effect on the amplitude of the e.p.s.p. Note also that the depression of the fibre volley evident during the application of NMA (*b*) recovered more quickly than the reduction in the e.p.s.p. (*c*).

By contrast DGG was an effective synaptic antagonist of this pathway when applied with doses which would have been expected to reduce kainate and quisqualate responses as well as those induced by NMA (Collingridge *et al.* 1983 and see Fig. 4*B*). In fourteen tests using nine separate slices, where this dipeptide caused depressions of the e.p.s.p. of 13·0–30·7 %, the amplitude of the presynaptic fibre volley was

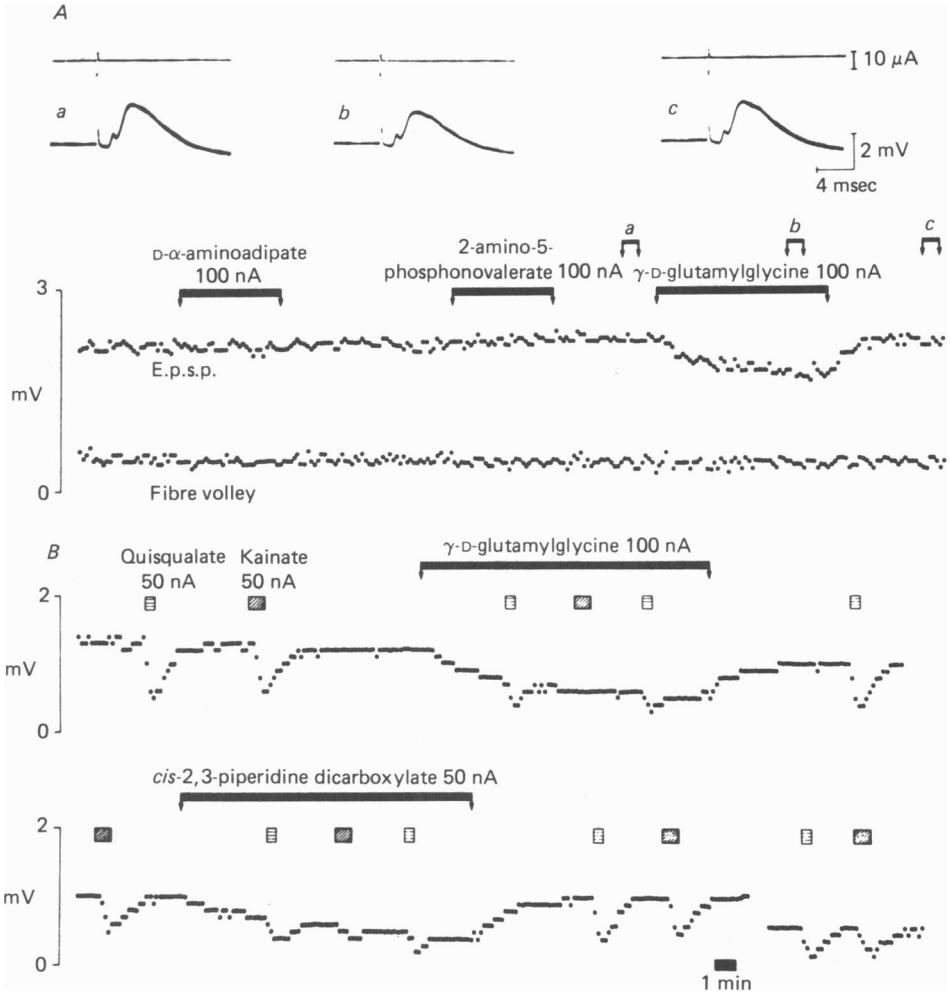

Fig. 4. Effects of antagonists on the Schaffer collateral–commissural-evoked field potential and on the depression of this response by excitants. *A*, the upper record of the graph is a plot of e.p.s.p. amplitude, the lower that of presynaptic fibre volley amplitude in response to successive stimuli. Of the three antagonists tested only DGG reduced the e.p.s.p. but was without effect on the fibre volley. Oscilloscope records each of five consecutive sweeps taken at the times indicated by (*a–c*) are shown above. The uppermost part of these records indicates the time and strength of the stimulus. *B*, in another slice both DGG and PDA reduced the e.p.s.p. and reduced or abolished the depression of the e.p.s.p. caused by quisqualate and kainate. The final applications of quisqualate and kainate were made with the stimulus intensity reduced so that the amplitude of the control e.p.s.p.s matched those which were obtained in the presence of the antagonists. The reduced effectiveness of the two excitants in the presence of the antagonists thus is not attributable simply to a smaller e.p.s.p.

simultaneously measured and on all but one occassion was not significantly affected (Fig. 4*A*). Generally the DGG-induced depression of the e.p.s.p. was detected 10–30 sec after the start of the application, reached a maximum within 3–6 min and recovered to control levels 2–4 min after terminating the administration of antagonist.

Two other substances were tested and proved to be effective at reducing the

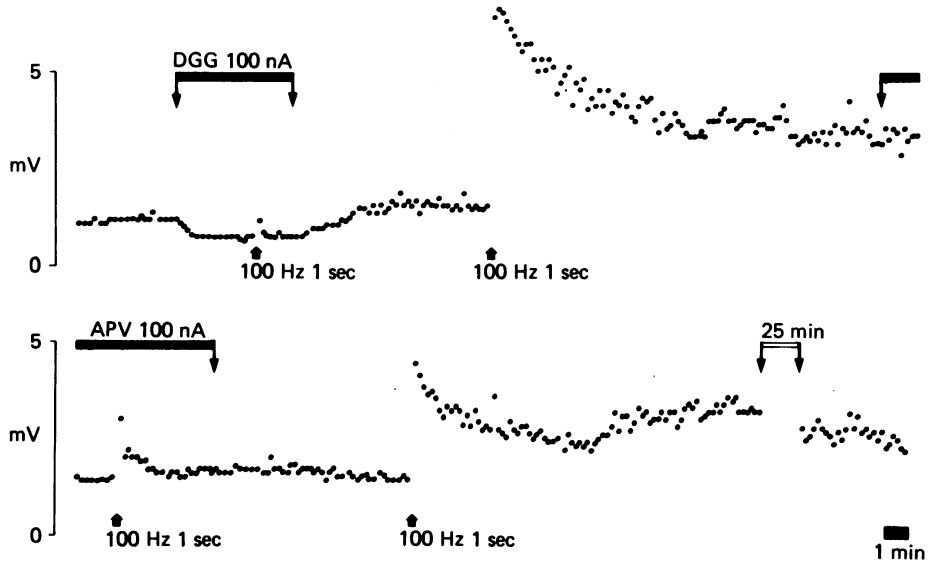

Fig. 5. Effects of antagonists on the population spike and on the generation of l.t.p. The amplitude of the population spike was recorded in stratum pyramidale in response to 0·1 Hz stimulation of the Schaffer collateral–commissural projection and antagonists were administered ionophoretically in stratum radiatum for the durations indicated by the bars. The pathway was stimulated at 100 Hz at the times shown by the arrows below the trace. DGG reduced the amplitude of the population spike and prevented l.t.p. in response to high frequency stimulation. Following recovery from the effects of DGG, l.t.p. was produced using a period of identical high frequency stimulation. After 17 min, APV was applied and had no effect on the population spike. 2 min later the stimulus intensity was reduced (lower record) and 3 min later the effects of high frequency stimulation were again tested. Although some short lasting post-tetanic potentiation resulted, l.t.p. was prevented. After the APV injection had been terminated for 8 min, 100 Hz stimulation for 1 sec was again able to produce l.t.p. which did not recover completely over the time that responses were measured (45 min).

synaptic response. These were DL-α-aminoadipate (7/8 slices, $21{\cdot}4 \pm 5{\cdot}1$ % depression) and ($\pm$) cis-2, 3-piperidine dicarboxylate (PDA) (12/12 slices, $46{\cdot}0 \pm 6{\cdot}5$ %, Fig. 4*B*. However, both this PDA (Collingridge *et al.* 1983) and L-α-aminoadipate (Hall, Hicks, McLennan, Richardson & Wheal, 1979) are weakly excitatory, which complicates the interpretation of these results.

Effects of antagonists on population spikes and synaptic plasticity

The effects of APV and DGG applied to stratum radiatum were examined on the population spike recorded in stratum pyramidale. Consistent with its lack of effect on the e.p.s.p., APV had no effect on the population spike (Fig. 5). In contrast DGG reversibly reduced or abolished the response, and this action occurred in parallel with the reduction in the e.p.s.p. on the three occasions when they were simultaneously recorded.

Brief tetanic stimulation (100 Hz, 1 sec) of the Schaffer collateral–commissural pathway often produced a large and long lasting potentiation of the population spike. This phenomenon was reversibly reduced or abolished by the ionophoretic application of DGG (4/4 slices) and APV (8/12 slices; Fig. 5).

DISCUSSION

The discrete electrical stimulation used in the present study activated predominantly Schaffer collateral and probably to a considerably smaller extent commissural fibres (Voneida, Vardaris, Fish & Reiheld, 1981). Both projections appear to originate from the same CA3 neurones (Swanson, Sawchenko & Cowan, 1980; Laurberg & Sorensen, 1981) and so pharmacologically can probably be assumed to be homogeneous. Effects produced by other fibre systems which course through stratum radiatum and remain intact in transversely cut slices would be at most of minor consequence since a large proportion of the terminals on the apical dendrites of CA1 cells originate from CA3 neurones (Goldowitz, Scheff & Cotman, 1979).

The ionophoretic application of excitants reduced the field e.p.s.p. presumably by depolarizing and/or increasing the conductance of CA1 neurones. The sensitivity of this effect to antagonism is essentially identical to that of the excitation of single neurones described in the companion paper (Collingridge *et al.* 1983). The antagonism of the depression of e.p.s.p.s induced by various excitants may therefore be used to check the specificity of an antagonist as it is tested against a synaptic response (Figs. 3 and 4 *B*). If high currents are used to eject potent agonists such as kainate or NMA, e.p.s.p.s can be abolished and hence the excitant can be assumed to be affecting the entire region that is generating the recorded synaptic response. In turn then, antagonists such as APV which can prevent these large depressions similarly can be assumed to be reaching the whole area from which the field potential is produced. Conversely under conditions where synaptic responses are unaffected it can be assumed that a given type of receptor is not involved in mediating the responses, rather than that the antagonist is not reaching the synaptic receptor (e.g. Fig. 3).

The depressions induced by L-glutamate, L-aspartate and quisqualate were relatively weak and short lasting probably due to uptake of these compounds. In contrast, kainate produced a massive reduction in the e.p.s.p that was at best only slowly reversible. The effect was associated with the generation or increase in size of the population spike either recorded locally (Fig. 2*A*) or at the cell body region (authors' unpublished observations). These data suggest that kainate produces a long lasting depolarization and/or conductance increase which facilitates synaptic excitation; an hypothesis which is consistent with intracellular (Robinson & Deadwyler, 1981; Segal, 1981) and extracellular DC recording (Lambert, Flatman & Jahnsen, 1981) in this part of the brain. The possibility that the resulting intensification of synaptic excitation is the basis of kainate's neurotoxicity has already been suggested (Collingridge & McLennan, 1981).

Although NMA produced a similarly large depression of the e.p.s.p. as did kainate there were two very different aspects to its action. Firstly, with high doses NMA produced a pronounced APV-sensitive reduction in the fibre volley suggesting that it may additionally have presynaptic actions in the hippocampus. Secondly, this compound often potentiated the amplitude of the e.p.s.p. for long periods following recovery from the depression. This potentiation may have been due to a long lasting alteration in membrane potential or conductance. Studies on motoneurones (Lambert, Flatman & Engberg, 1981) have suggested that NMA depolarizes neurones and increases their input resistance. Possibly a residual increase in resistance could

explain the observed increase in the e.p.s.p. Alternatively the potentiation of the e.p.s.p. may be related to the phenomenon of l.t.p., and consistent with this idea were the observations that both types of potentiation were susceptible to antagonism by APV. It is interesting that NMA produces a larger Ca^{2+} influx into spinal neurones than other acidic amino acids (Padjen & Smith, 1981) since post-synaptic Ca^{2+} entry has been implicated in l.t.p. (Baudry & Lynch, 1981).

The firmest conclusion that can be drawn from the antagonist studies is that the NMA receptor is not involved in mediating synaptic excitation in the Schaffer collateral–commissural pathway. Thus applications of APV that were greater than those required to abolish NMA induced depressions of the e.p.s.p. or increases in CA1 firing rate (Collingridge *et al.* 1982*c*) had little or no effect on this synaptic mechanism. It is highly unlikely that the absence of antagonism with APV was due to inadequate accessibility to synaptic receptors since firstly, the antagonists were administered at the dendritic region of the slice where the stimulated fibres terminated, secondly, using the same electrodes APV effectively antagonized the lateral perforant path input into the dentate gyrus even with much lower ejection currents (authors' unpublished observations) and thirdly, other substances applied from the same electrode depressed the Schaffer collateral–commissural evoked response. That DAA did not affect this pathway while being a selective NMA antagonist in the hippocampus (Collingridge *et al.* 1983) also indicates that an endogenous activator of the NMA receptor is not involved.

Unlike the selective NMA antagonists DAA and APV, DGG consistently depressed the synaptic response and with the high ejection currents required to do this it also antagonized kainate- and quisqualate-induced depressions of the e.p.s.p. and the excitation of single CA1 neurones (Collingridge *et al.* 1983). It is therefore possible that either the kainate or quisqualate receptor mediates synaptic excitation in the Schaffer collateral–commissural pathway. The observation that DGG had no appreciable effect on the fibre volley indicates that the synaptic antagonism is unlikely to be caused by a separate action on the excitability of presynaptic terminals. Furthermore, post-synaptic effects unrelated to synaptic antagonism are unlikely to account for the action since the DGG-induced reduction of the e.p.s.p. evoked by stimulation of the medial perforant path in the dentate gyrus was not associated with any change in membrane potential, input resistance or neuronal excitability (Collingridge, Crunelli, Forda & Kelly, 1982*a*). Similarly Segal (1981) has briefly mentioned that topical application of DGG to the hippocampal slice reduced the Schaffer collateral–commissural e.p.s.p. without affecting membrane potential or resistance. Confirmation that either the kainate or quisqualate receptor is the synaptic receptor in this pathway must await the development of more potent and selective antagonists for these compounds.

The observation that GDEE, an effective quisqualate antagonist in the spinal cord (Davies & Watkins, 1979; McLennan & Lodge, 1979) did not affect the synaptic response does not preclude a role of quisqualate receptors in this pathway since satisfactory antagonisms of responses of single CA1 neurones to this agonist by GDEE were never obtained (Collingridge *et al.* 1983).

The optical isomers of APB were similarly weak at depressing the e.p.s.p. but probably acted by different mechanisms since D-APB had a pharmacological profile

similar to but weaker than DGG whereas L-APB was weakly excitatory and devoid of antagonist activity (Collingridge *et al.* 1983). Thus although the depression of the Schaffer collateral–commissural e.p.s.p. observed following superfusion of hippocampal slices with high doses of the racaemic mixture of APB (Dunwiddie *et al.* 1978; White *et al.* 1979) theoretically could be due to an action of either or both of the stereoisomers, an effect of D-APB on the NMA receptor is presumably not involved. On the other hand such an action might explain why the racaemate was able to abolish l.t.p. (Dunwiddie *et al.* 1978; Cotman *et al.* 1981), since we have shown here that l.t.p. can be prevented by APV, an NMA antagonist.

To attribute a role to either L-glutamate or L-aspartate as the natural transmitter in the Schaffer colateral–commissural projection is complicated by the fact that both amino acids may be capable of interaction to varying extents with the three excitatory amino acid receptors so far identified, i.e. those selectively activated by NMA, kainate and quisqualate (Watkins & Evans, 1981; McLennan, 1981). It has, however, been suggested from the relative sensitivities of L-glutamate and L-aspartate to blockade by a range of antagonists that these acidic amino acids preferentially interact with the quisqualate and NMA receptors respectively (Watkins & Evans, 1981). Since a similar pattern of antagonism is seen in the hippocampus (Collingridge *et al.* 1983) the present data would, therefore, favour L-glutamate as the transmitter in the Schaffer collateral-commissural pathway. The majority of neurochemical evidence also favours L-glutamate rather than L-aspartate in this regard (Nitsch, 1981; Wieraszko, 1981; Wieraszko & Lynch, 1979); and recently depolarization by glutamate was shown to have the same reversal potential as the e.p.s.p. evoked by stimulation in stratum radiatum (Hablitz & Langmoen, 1982). By the same reasoning L-aspartate could be more involved in synaptic plasticity by a predominant action on the NMA receptor. The possibility, however, that other acidic amino acids or related compounds may be the natural ligands for these receptors cannot be excluded (Stone & Perkins, 1981; Watkins & Evans, 1981).

In conclusion, the present study has shown that the NMA receptor plays no role in the mediation of synaptic excitation but may be involved in the generation of l.t.p. in the Schaffer collateral–commissural projection to the CA1 region of the rat hippocampus. However, the demonstration that DGG affects this pathway when applied in sufficient amounts to antagonize the kainate and quisqualate receptors while having no effect on presynaptic or post-synaptic excitability provides strong evidence that an excitatory amino acid is utilized as the neurotransmitter in this pathway.

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