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BLOCKADE OF AMINO ACID-INDUCED DEPOLARIZATIONS AND INHIBITION OF EXCITATORY POST-SYNAPTIC POTENTIALS IN RAT DENTATE GYRUS

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SUMMARY

1. Excitatory post-synaptic potentials (e.p.s.p.s) evoked by stimulation of the medial perforant path and depolarizations induced by excitatory amino acids were recorded from granule cells in the preparation of the hippocampal slice from the rat. The effects of (±)-2-amino-5-phosphonovalerate (APV), γ-D-glutamylglycine (γDGG) and *cis*-2,3-piperidinedicarboxylate (PDA), antagonists of excitatory amino acids on these phenomena were compared.

2. γDGG was the most effective antagonist of the e.p.s.p. Its action was reversible and not associated with any change in the passive membrane properties of the granule cells or in the apparent reversal potential of the e.p.s.p. Quantal analysis showed that the reduction in the e.p.s.p. paralleled the decrease in quantal size rather than quantal content, confirming a post-synaptic site of the action of γDGG.

3. The potency of γDGG against the exogenous agonists was *N*-methyl-D-aspartate > kainate ≥ quisqualate.

4. APV had very little effect on the e.p.s.p. but was a selective antagonist of *N*-methyl-D-aspartate-induced depolarizations.

5. PDA depolarized granule cells and increased their membrane input resistance.

6. Although γDGG was a potent antagonist of both glutamate- and aspartate-induced depolarizations, no clear pattern of specificity could be found. The action of glutamate was unaffected by APV.

7. These results indicate that the receptor for the transmitter at the synapses formed by the fibres of the perforant path with the granule cells is of the quisqualate and/or kainate type. The present data are consistent with the biochemical evidence that glutamate may be the endogenous transmitter at this synapse.

INTRODUCTION

Perforant path fibres, originating in the entorhinal cortex, constitute the major excitatory input to the hippocampal formation (Lorente de No, 1934; Hjorth-Simonsen & Jeune, 1972; Lomo, 1971; Stewart, 1976). They synapse extensively onto the dendrites of granule cells in the dentate gyrus and there is evidence favouring an excitatory amino acid as the endogenous transmitter released at these synapses

(Nadler, White, Vaca, Redburn & Cotman, 1977; Storm-Mathisen & Iversen, 1979; White, Nadler, Hamberger, Cotman & Cummins, 1977). However lack of potent and selective antagonists of excitatory amino acids (Watkins & Evans, 1981) as well as difficulties in obtaining long-lasting and stable impalement of the small granule cell bodies (Andersen, Holmqvist & Voorhoeve, 1966; Lomo, 1970) have precluded a detailed electrophysiological investigation of the receptor mediating the excitatory post-synaptic potential (e.p.s.p.) in these cells evoked by stimulation of the perforant path.

Recently the existence in the spinal cord of three types of pharmacologically distinct receptors for excitatory amino acids has been proposed (Watkins & Evans, 1981; McLennan, 1981). They have been classified as *N*-methyl-D-aspartate, kainate or quisqualate receptors on the basis of rank orders of potencies of these agonists, or by the selectivity of available antagonists. Thus (±)-2-amino-5-phosphonovalerate (APV) has been shown to be the most potent and selective *N*-methyl-D-aspartate receptor antagonist (Davies & Watkins, 1982; Evans, Francis, Jones, Smith & Watkins, 1982; Davies, Francis, Jones & Watkins, 1981*b*); γ-D-glutamylglycine (γDGG) in addition to blocking *N*-methyl-D-aspartate receptors also antagonizes responses to kainate (Davies & Watkins, 1981), whereas *cis*-2,3-piperidinedicarboxylate (PDA) antagonizes all three receptor types (Davies, Evans, Francis, Jones & Watkins, 1981*a*).

In the present paper we have used the hippocampal slice preparation (Dingledine, Dodd & Kelly, 1980) to investigate the effect of APV, γDGG and PDA on the e.p.s.p. evoked by stimulation of the medial perforant path and on the depolarization of granule cells induced by application of excitatory amino acids. Preliminary reports of some of these results have been published (Collingridge, Crunelli, Forda & Kelly, 1982; Crunelli, Forda, Collingridge & Kelly, 1982).

METHODS

Slice preparation and recordings

Hippocampal slices (400 μm thick) were prepared from decapitated rats (200 g) using a McIlwain tissue chopper. The slices were maintained and subsequently used for electrophysiological recording as previously described (Assaf, Crunelli & Kelly, 1981). Briefly, they were initially kept in an incubation chamber (Nicoll & Alger, 1981) where they remained viable for more than 10 hr. 1 hr before the beginning of each experiment, slices were transferred to the recording chamber and perfused with a warmed (36·0±0·5 °C) continuously oxygenated (95% O_2, 5% CO_2) medium consisting of 134 mM-NaCl, 5 mM-KCl, 1·25 mM-KH_2PO_4, 2 mM-Mg_2SO_4, 2 mM-$CaCl_2$, 16 mM-$NaHCO_3$ and 10 mM-glucose.

Intracellular electrodes were made from glass tubing ('Kwick-fil', GC 120F-10, Clark Electromedical Instruments) and filled with 1 M-potassium acetate (resistances 80–120 MΩ). Potentials were recorded with a precision electrometer (M-707, WP Instruments) which was also used to inject current through the recording micro-electrode. The resting membrane potential of the cell was measured as the change in potential seen on withdrawal of the electrode from the cell. The time constant was measured from voltage transients produced by small-amplitude, hyperpolarizing pulses of current which lay within the linear portion of the voltage–current plot. The initial 60 msec of each voltage transient was normalized with respect to the plateau voltage and the logarithm plotted *versus* time. The time constant was calculated from the line fitted by the least-squares method. Glass micropipettes (3–4 μm tip diameter) filled with 1 M-NaCl were used as stimulating electrodes (15–50 μsec, 1–60 V, 0·5–1 Hz) and positioned along the afferent fibres of the medial perforant path in the middle third of the molecular layer (McNaughton & Barnes, 1977; McNaughton, 1980), 1–2 mm away from the recorded cell.

Drugs were ejected ionophoretically from an independently mounted six-barrelled micropipette (6–9 μm tip diameter) positioned along the dendritic tree of the impaled neurone in the molecular layer of the dentate gyrus at the same level of the stimulating electrode. After the cell had been impaled, the tip of the ionophoretic electrode was slowly advanced into the slice in 5–10 μm steps until a fast-rising depolarization of at least 5 mV could be observed in response to a brief (500–700 msec) application of glutamate. Ionophoretic barrels contained various combinations of the following drugs: L-glutamate (1 M; pH 8), L-aspartate (1 M; pH 8), *N*-methyl-D-aspartate (20 mM in 150 mM-NaCl; pH 8), quisqualate (20 mM in 150 mM-NaCl; pH 8), kainate (20 mM in 150 mM-NaCl; pH 8), APV (50 mM in 100 mM-NaCl; pH 8) γDGG (200 mM; pH 8), PDA (200 mM; pH 8) and NaCl (1 M; pH 8). Retaining currents of 1–5 nA were applied to the individual barrels when necessary. At the end of each impalement, the effect of ionophoretic application of the drugs was re-tested to evaluate any electrical coupling between the ionophoretic pipette and the recording electrode. Results were stored on a Racal FM 4D tape recorder and later analysed with a PDP 11/23 computer (Cambridge Electronic Design Ltd.).

Quantal analysis

During the collection of trains of e.p.s.p. for the quantal analysis, stimulus intensity in the control situation was carefully adjusted to obtain an e.p.s.p. not bigger than 10–14 mV. Such an e.p.s.p. would generally be 50–60% below threshold for firing (Crunelli, Assaf & Kelly, 1983; cf. McNaughton, Barnes & Andersen, 1981), thus minimizing problems associated with membrane rectification and non-linear summation. Since the contribution of noise to the fluctuation in the amplitude of a small e.p.s.p. is substantial, the ionophoretic current used to eject γDGG was adjusted to obtain an e.p.s.p. not smaller than 2·5–3 mV. The mean quantal size (q) and quantal content (m) were determined using the variance method (Hubbard, Llinas & Quastel, 1969):

$$m = \frac{\bar{v}'^2}{var\ v' - var\ noise},$$

and

$$q = \frac{var\ v' - var\ noise}{\bar{v}'},$$

where

var noise is the variance of the noise calculated as the root mean square of the noise level in each experiment;

$\bar{v}'$ and *var* v' are the mean and variance respectively of the e.p.s.p.s amplitude corrected for non-linear summation. This correction was made according to the following equation:

$$v' = v \Big/ \left[1 - \frac{v}{V - V_0}\right],$$

where

v is the e.p.s.p. amplitude;

V is the resting membrane potential of the cell;

V_0 is the equilibrium potential of the e.p.s.p. determined by extrapolation.

In each cell 150–300 e.p.s.p.s were collected in the absence and presence of γDGG at a rate of 1 Hz. Data were rejected if there was a lack of stability in the mean e.p.s.p. amplitude with time (see Fig. 2), or because no recovery from antagonism occurred.

RESULTS

Impaled neurones were characterized as granule cells by their position in the granular layer of the dentate gyrus, by orthodromic activation from the medial perforant path and by antidromic invasion from the mossy fibres region of CA_3. Experiments were performed only on those granule cells ($n = 54$) whose resting membrane potential (mean $\pm$ S.E. of the mean: $-66 \pm 1{\cdot}8$ mV; range 59–75) and membrane input resistance (mean $\pm$ S.E. of the mean: $48{\cdot}4 \pm 2{\cdot}7$ MΩ; range: 19–80)

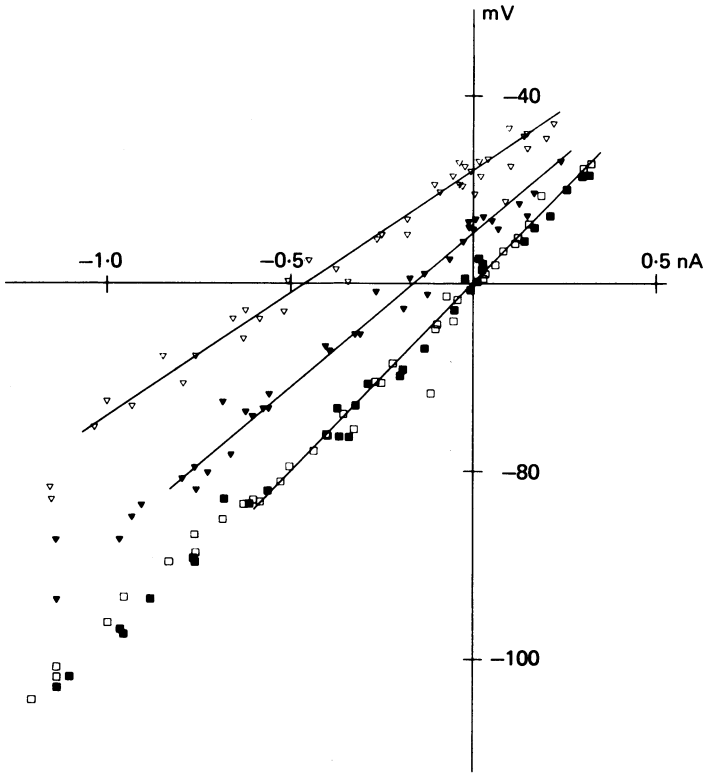

Fig. 1. Voltage–current plots show the reduction of the medial perforant path evoked e.p.s.p. by γDGG to be associated with an increase in the membrane resistance during the peak of the e.p.s.p. The membrane potential and resting membrane input resistance were unchanged. The reversal potential computed by extrapolation of all the three possible pairs of lines differed by less than 4 mV. Families of hyperpolarizing and depolarizing pulses (120–200 msec) of current were passed through the recording micro-electrode and the corresponding voltage displacements used to measure membrane resistance. Depolarizing pulses of greater amplitude than shown evoked action potentials and could no longer be used to calculate the passive properties of the membrane. The application of 50 nA of γDGG (■) did not change the resting membrane input resistance from the control value (□) (41 MΩ) and, statistically, the regression lines ($r = 0{\cdot}99$) through both sets of points were identical. The amplitude of the e.p.s.p. was reduced by γDGG from 13·1 mV to 5·2 mV. In the control situation the input resistance measured near the peak of the e.p.s.p. was 24 MΩ (▽) and it increased significantly to 31 MΩ (▼) during the application of γDGG ($t = 6{\cdot}19$; d.f. = 61; $P < 0{\cdot}001$). (Table 1, cell 1).

remained stable throughout the course of the experiment. The time constant of these cells ranged between 7·5 to 14·3 msec (mean ± S.E. of the mean: 10·2 ± 0·5) and discrete electrical stimulation of the medial perforant path evoked an e.p.s.p. of 8–20 mV in amplitude, yet below threshold for initiating action potentials.

Synaptic antagonism

In thirty-three out of thirty-five granule cells tested, the ionophoretic application of γDGG (25–400 nA) markedly reduced the e.p.s.p. evoked by stimulation of the

medial perforant path (Figs. 1 and 2). The reduction ranged from 20 to 85% (mean 62%) and in twenty-nine neurones it was rapidly reversible upon termination of the ionophoretic current (four cells were lost before a complete recovery could be observed). With the ionophoretic electrode in an 'optimum' position (see Methods) the latency of onset of the γDGG-induced block was as short as 5–8 sec. The latency for maximum antagonism by γDGG was observed between 0·5 and 3 min (mean ± S.E. of the mean: 2·0 ± 0·5). The action of γDGG was not associated with any change in the resting membrane potential of the cell (Fig. 1). (see Segal, 1981). Moreover, voltage–current plots showed the resting input resistance to be unaltered (Fig. 1 and Table 1) as was the excitability of the cell, tested both by determining the threshold voltage required for antidromic invasion and by the direct injection of depolarizing current. Furthermore, the amplitude and duration of the single and averaged mass presynaptic fibre volley was unaltered by γDGG (see Crunelli *et al.* 1982).

However these data do not exclude the possibility that γDGG exerts its effect by a presynaptic mechanism and more direct evidence was obtained from a detailed analysis of the e.p.s.p. Voltage–current plots were determined from changes in the peak e.p.s.p. amplitude produced by the intracellular injection of long pulses (120–200 msec) of depolarizing and hyperpolarizing current. They showed no significant deviation from linearity. Thus the reduction in the e.p.s.p. amplitude during the application of the antagonist was not caused by changes in the time constant of the cell soma or principal dendrites or by the intracellular injection of current (Fig. 1). As expected, the peak of the e.p.s.p. was associated with a marked decrease in membrane input resistance (47 ± 4%, $n = 8$) (Fig. 1 and Table 1). In addition the apparent reversal potential of the e.p.s.p. (−12·6 ± 3·1 mV, $n = 6$, Table 1) was not affected by the ionophoretic application of γDGG i.e. no systematic alteration occurred in the point of intersection of the extrapolated voltage–current plots derived on and off the peak of the e.p.s.p. in the presence and absence of γDGG. In effect, in seven out of eight granule cells, the fall in input resistance associated with the e.p.s.p. was reduced during exposure to γDGG (Fig. 1) and this change in input resistance was statistically significant (Table 1). Finally, the 50% decay time of the e.p.s.p. was also unchanged by γDGG (Table 1).

In four cells the fluctuation of the e.p.s.p. amplitude in the presence and absence of γDGG conformed to the Poisson distribution (i.e. the mean and variance were proportional) and quantal analysis was carried out using the variance method (Hubbard *et al.* 1969; Kuno, 1971). Each e.p.s.p. was corrected for non-linear summation (see Methods) using the apparent reversal level of the e.p.s.p. for that cell computed by extrapolation.

Typical data from two cells are shown in Fig. 2 and a summary of the results from the four cells is given in Table 2. It is evident that the mean quantal content of all four cells is practically unaffected (control, 47; γDGG, 49) while the mean quantal size is decreased to a level which would account for the depression of the mean e.p.s.p. amplitude by 48% (control 230 μV; γDGG 113 μV). Since the quantal model of transmitter release attributes the determinants of quantal content to the presynaptic elements and those of quantal size to the post-synaptic cells (Katz, 1966; Kuno, 1971; Martin, 1977; Takeuchi, 1977), the findings strongly support the view that γDGG acts post-synaptically.

TABLE 1. Passive properties of granule cells and effect of γDGG on the medial perforant path evoked e.p.s.p.

	Resting membrane potential (mV)		Membrane input resistance at rest (a) (MΩ)		Membrane input resistance during e.p.s.p. (a) (MΩ)		Amplitude of the e.p.s.p. (b) (mV)		Apparent reversal level of the e.p.s.p. (c) (mV)		50% decay time of the e.p.s.p. (d) (msec)	
Cell	Control	γDGG	Control	γDGG	Control	γDGG	Control	γDGG	Control	γDGG	Control	γDGG
1	60	61	41	41	24	31**††	13·1	5·2	22·1	24·6	7·5	7·6
2	70	68	52	53	35	42**††	8·5	3·2	8·7	8·2	8·8	8·1
3	65	67	44	43	21	24††	10·2	7·8	—	—	11·4	11·6
4	75	76	45	45	19	24*††	12·5	6·7	9·6	8·1	9·6	9·4
5	67	68	31	33	15	22**†	11·5	4·0	—	—	6·5	6·3
6	62	64	57	58	35	43**††	7·8	2·8	12·3	13·8	9·4	9·3
7	61	61	48	45	32	48**	7·9	6·3	8·9	8·1	9·8	9·6
8	63	65	50	51	21	39**††	10·8	5·8	14·2	13·5	8·5	8·9

(a) Measured as the slope of regression lines in voltage–current plots (see Fig. 1, legend).

(b) Each value represents the computer average of fifty successive e.p.s.p. (uncorrected for non-linear summation).

(c) Each value is negative in respect to zero potential and has been calculated by extrapolation from regression lines in voltage–current plots (see Fig. 1, legend).

(d) Each value is the mean of fifty samples.

* $P < 0.01$, ** $P < 0.001$: compared to control membrane input resistance during e.p.s.p.

† $P < 0.01$, †† $P < 0.001$: compared to membrane input resistance at rest.

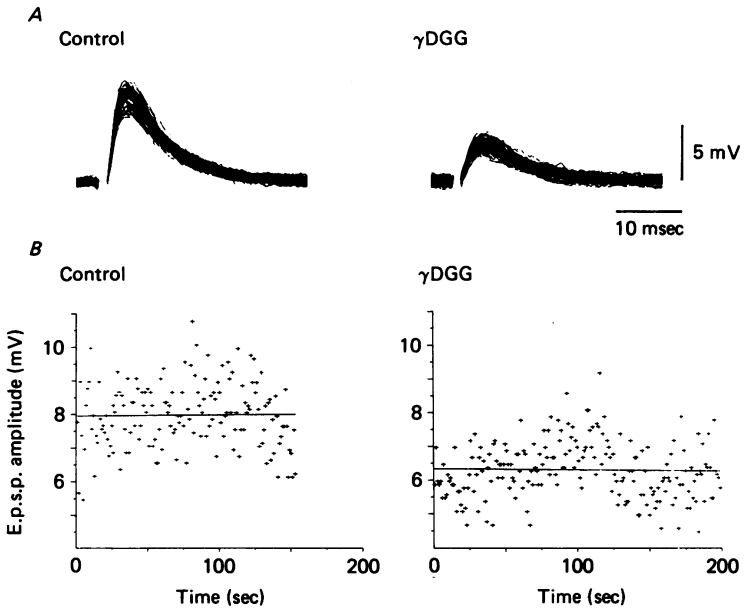

Fig. 2. Quantal analysis of the medial perforant path evoked e.p.s.p. to show the post-synaptic nature of γDGG antagonism. *A*, superimposed ($n = 150$) computer drawn e.p.s.p.s recorded before (control) and during (γDGG) the ionophoretic application of 200 nA of γDGG. A clear reduction in fluctuation accompanies the decline of the e.p.s.p. amplitude produced by γDGG. This is reflected in a decrease in quantal size (q) with no change in quantal content (m) (Table 1 and 2, cell 2), indicating the post-synaptic nature of γDGG effect. Note that during the application of γDGG the fluctuation in the e.p.s.p. amplitude is still bigger than the noise level. *B*, plots of e.p.s.p. amplitude *versus* time to show that no clear sign of synaptic habituation occurs both in control condition and during the ionophoretic application of γDGG, when reasonable steady-state conditions have been reached. Note that the decrease in the e.p.s.p. amplitude is associated with a reduction in the fluctuation (decrease in the variance of the mean e.p.s.p. amplitude) indicating a post-synaptic action of γDGG (Tables 1 and 2, cell 7) (data uncorrected for non-linear summation).

TABLE 2. The effect of γDGG on quantal parameters for the medial perforant path evoked e.p.s.p.

	Quantal content (m)		Quantal size (q) (μV)			Reduction of e.p.s.p. amplitude*
Cell	Control	γDGG	Control	γDGG	Reduction (%)	(%)
2	42	47	275	96	65	62
6	53	44	154	71	54	64
7	53	49	172	135	22	20
8	38	57	322	151	53	46
Mean	47	49	230	113	49	48

* See Table 1.

APV, the most potent and selective *N*-methyl-D-aspartate receptor antagonist in the spinal cord (Watkins & Evans, 1981), had practically no effect on the e.p.s.p. evoked by stimulation of the medial perforant path. The ionophoretic application of APV (50–500 nA) was in fact ineffective on eight out of nine neurones on which γDGG reversibly reduced the e.p.s.p. In the only granule cell on which it appeared to be

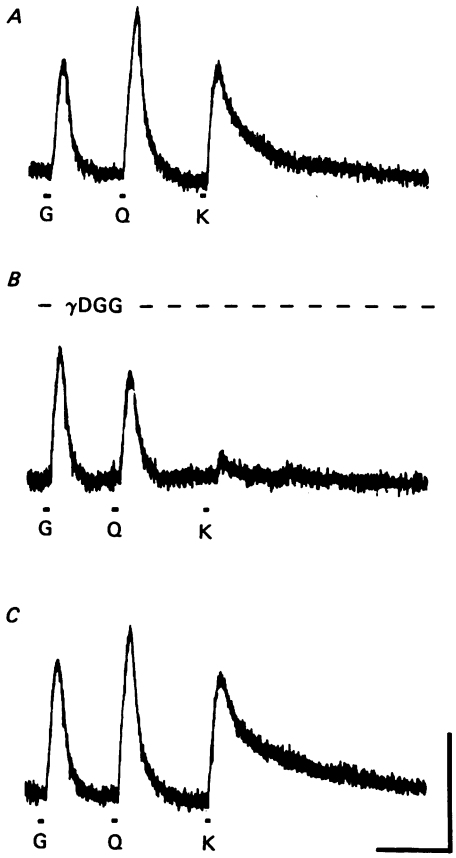

Fig. 3. Intracellular recordings from a granule cell showing the effect of γDGG on the kainate (K)-, quisqualate (Q)- and glutamate (G)-induced depolarization. The ionophoretic application of γDGG (400 nA) abolished the kainate response, reduced quisqualate to 35 % of control response and left the glutamate-induced depolarization unaffected. The gap between *A* and *B* is 7 sec while *B* and *C* are consecutive traces. In this and the following Figure, the action potentials evoked by the drug-induced depolarizations have been attenuated by the computer plotting sub-routine. Duration of drug pulses is indicated by the bar below records. Drug ejection currents were: kainate, 300 nA; quisqualate, 400 nA and glutamate 400 nA. Calibration bars represent 10 mV and 20 sec. Resting membrane potential: −64 mV; membrane input resistance: 49 MΩ.

active, the decrease (25 %) in the e.p.s.p. amplitude occurred in the absence of any change in membrane potential or resting membrane input resistance.

The other antagonist, PDA, was tested on eleven neurones. On seven occasions it excited the cells by depolarizing them and increasing their membrane input resistance (see Segal, 1981). This depolarization was seen with ionophoretic currents as low as

10 nA with a latency to onset as short as 3 sec. The excitation always consisted of burst-like activity and from our experience with *N*-methyl-D-aspartate (unpublished observations), this would be in keeping with an action on the proposed *N*-methyl-D-aspartate receptor (Davies *et al.* 1981*a*; Collingridge, Kehl & McLennan, 1983; Salt & Hill, 1982). In the remaining four neurones, which were not excited, no effect of PDA on the e.p.s.p. amplitude was observed.

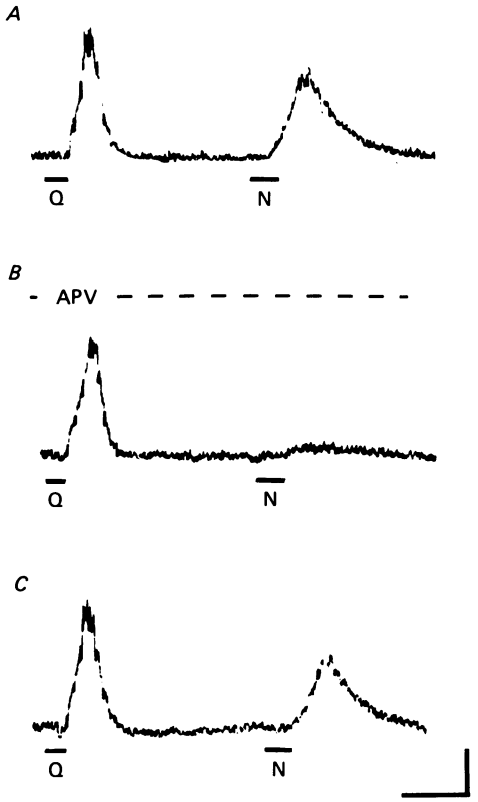

Fig. 4. Intracellular recordings from a granule cell showing the selective antagonism by APV of the *N*-methyl-D-aspartate (N)-*versus* quisqualate (*Q*)-induced depolarization. The ionophoretic application of 100 nA of APV almost abolished the *N*-methyl-D-aspartate response leaving the quisqualate-induced depolarization unaffected. *A*, *B* and *C* are consecutive traces. Drug ejection currents were: *N*-methyl-D-aspartate 150 nA and quisqualate, 120 nA. Calibration bars represent 10 mV and 10 sec. Resting membrane potential: −61 mV; membrane input resistance: 38 MΩ. Other details as for Fig. 3.

Antagonism of excitation induced by amino acids

The ionophoretic application of *N*-methyl-D-aspartate, quisqualate and kainate to the dendrites of the granule cells readily depolarized the cells and produced a complex pattern of changes in input resistance (unpublished observations). In a recent paper (Crunelli *et al.* 1982) we compared the action of γDGG on the e.p.s.p. evoked by stimulation of the perforant path and on the depolarization produced by these potent exogenous agonists. The response produced by *N*-methyl-D-aspartate was readily blocked by γDGG (25–150 nA; 7–15 sec). However, the reduction in the e.p.s.p.

amplitude only occurred when the application of γDGG was increased sufficiently (100–400 nA; 8–34 sec) to reduce the depolarization evoked by quisqualate and/or kainate (Fig. 3). Unfortunately separation between these two types of receptors was difficult since in seven experiments γDGG did not appear to select between quisqualate-and kainate-induced depolarization of granule cells.

The selectivity of APV was also tested, and on a further four granule cells it was found to be a more potent antagonist of *N*-methyl-D-aspartate responses than was γDGG. In each cell, brief (6–8 sec) ionophoretic applications (25 nA) were sufficient to abolish *N*-methyl-D-aspartate-induced depolarization, leaving quisqualate and kainate responses unaffected (Fig. 4). Moreover on the same cell γDGG but not APV was shown on five occasions to antagonize glutamate-induced depolarization and the e.p.s.p. evoked by stimulation of the perforant path (unpublished observations).

The direct excitatory action of PDA on granule cells made it impossible to test the effect of this compound against *N*-methyl-D-aspartate-, quisqualate- and kainate-induced depolarizations. However on the cells where PDA did not produce an excitation it was found to be an effective antagonist of both *N*-methyl-D-aspartate- and quisqualate-induced depolarizations.

In the spinal cord, γDGG has been shown to antagonize *N*-methyl-D-aspartate- and aspartate-induced excitation and to leave quisqualate and glutamate responses relatively unaffected (McLennan & Liu, 1982; Davies & Watkins, 1982; Evans, Francis, Jones, Smith & Watkins, 1982). In the six granule cells tested, γDGG showed no clear pattern of selectivity against the two endogenous amino acids glutamate and aspartate. In four of these cells the reduction in the responses to glutamate and aspartate were identical, in one cell the aspartate-induced depolarization was more readily blocked and in the remaining cell the glutamate responses appeared more sensitive.

DISCUSSION

The primary conclusion of this investigation is that quisqualate/kainate receptors mediate excitation at the synapses formed by the perforant path fibres with the granule cells. This is based on (1) the higher potency of γDGG compared with APV in antagonizing the e.p.s.p. evoked by stimulation of the medial perforant path; (2) the true post-synaptic site of action of γDGG and (3) the selectivity of γDGG and APV against *N*-methyl-D-aspartate-, quisqualate- and kainate-induced depolarization of granule cells.

Synaptic antagonism

γDGG was found to be more potent than APV in antagonizing the e.p.s.p. evoked in granule cells by stimulation of the medial perforant path. It is unlikely that the lack of effect of APV was due to inadequate accessibility, since both APV and γDGG were administered from adjacent barrels of the ionophoretic electrode at the same position along the dendritic tree of the impaled neurone and applications of APV smaller than those shown to have no effect on the e.p.s.p. blocked the *N*-methyl-D-aspartate response. Thus, *N*-methyl-D-aspartate receptors do not appear to be involved in the excitation at the synapses formed by the fibres of the perforant path

with the granule cells. Instead an involvement of quisqualate and/or kainate receptors is very likely since synaptic antagonism by γDGG only occurred when the application was sufficient to block quisqualate and/or kainate responses. At the moment differentiation between these two receptor types seems difficult. Although other workers (Davies & Watkins, 1981; see Watkins & Evans, 1981) found that γDGG preferentially antagonized responses to kainate rather than quisqualate, we were unable to distinguish between the two types of receptors (in agreement with the findings of Salt & Hill, 1982 and Collingridge *et al.* 1983). Thus, the separation of quisqualate from kainate receptors must await the development of more specific antagonists.

In a recent paper focussed on the lateral perforant path, Koerner & Cotman (1981) have reported *l*-2-amino-4-phosphonobutyrate as the most potent antagonist against the extracellularly recorded synaptic field potential in the middle molecular layer of the dentate gyrus and suggested the existence of 'a novel L-glutamate receptor not previously described'. However, any attempt to compare their results with the present data is hindered by methodological differences and the fact that these authors concentrated only on the action of the ω-phosphate series of excitatory amino acid antagonists. Furthermore the potency and selectivity of *l*-2-amino-4-phosphonobutyrate as an excitatory amino acid antagonist has been strongly questioned at the spinal cord level (Evans *et al.* 1982) and more recently also on hippocampal CA_1 pyramidal cells (Collingridge *et al.* 1983), where it not only enhanced the action of *N*-methyl-D-aspartate and quisqualate but also showed some excitatory action of its own. By the same reasoning, PDA was rejected as a useful antagonist in this study because of its excitatory action.

Mechanism of action of γDGG

The current view of e.p.s.p. generation at chemical synapses suggests that the e.p.s.p. amplitude is determined by the summation of a large number of ionic currents of similar amplitude generated by the opening of transmitter specific channels (Eccles, 1964; Ginsborg, 1967). One prediction of such a theory is that a continuous linear relationship exists between the amplitude of the synaptic potential and the associated change in conductance and that antagonists simply combine with specific receptors to reduce the number of channels. The synaptic antagonism by γDGG reported in this study closely followed these predictions. Thus, γDGG reduced the amplitude of the e.p.s.p. without any alteration in its apparent reversal level by limiting the change in membrane input resistance associated with the e.p.s.p. i.e. reducing the number of channels opened by the endogenous transmitter. The term 'apparent reversal level' has been used in this study since the value was determined by extrapolation. However, no marked deviation from linearity in the voltage–current relationship of granule cells was observed either in the hyperpolarizing direction (0 to −30 mV) (see Brown, Fricke & Perkel, 1981) or in the depolarizing direction (0 to +15 mV). This linear relationship is in agreement with the results of Barnes & McNaughton (1980), who also reported an extrapolated reversal potential of the e.p.s.p.s of −18 mV.

According to the quantal hypothesis of transmitter release the size of the miniature e.p.s.p. is determined by the sensitivity of the post-synaptic membrane to the endogenous transmitter (Katz, 1966; Martin, 1977; Takeuchi, 1977; Kuno, 1971).

Thus the results of the quantal analysis performed in the presence of γDGG (i.e. a decrease in q with no change in m) provide further and independent evidence that γDGG acts directly on the granule cell membrane, decreasing its sensitivity to the endogenous transmitter. The validity of this analysis is supported by the similarity between the values of m and q reported in this study and those recently published by McNaughton *et al.* (1981, see Fig. 5, p. 960), who have calculated the quantal parameters of the e.p.s.p. evoked by stimulation of the medial perforant path using both the variance method and the method of failures and a different approach to correct the e.p.s.p. for non-linear summation.

The transmitter of the medial perforant path

Glutamate and aspartate have long been suggested as the most likely candidates for a transmitter role at the synapses formed by the fibres of the perforant path with the granule cells (Nadler *et al.* 1977; Storm-Mathisen & Iversen, 1979; Wheal & Miller, 1980), with strong biochemical evidence favouring glutamate (White *et al.* 1977). In the spinal cord and higher centres it has recently been proposed that glutamate and aspartate function as mixed agonists since they are able to interact with all three types of excitatory amino acid receptor (cf. Watkins, 1980). Moreover, it has been shown that compounds which antagonize the *N*-methyl-D-aspartate receptor tend also to antagonize aspartate-induced excitations leaving glutamate relatively unaffected (Hicks, Hall & McLennan, 1978; Watkins & Evans, 1981). This observation has led to the suggestion that aspartate preferentially interacts with *N*-methyl-D-aspartate receptors while glutamate preferentially interacts with the quisqualate ones. In our system a reduction in the e.p.s.p. could occur in the absence of glutamate blockade (see Fig. 3) and γDGG reduced the responses to glutamate and aspartate equally (in agreement with Salt & Hill, 1982), so it is not possible from these data to give preference to either glutamate or aspartate as being the endogenous transmitter of the perforant path. However, over-all the e.p.s.p. evoked by stimulation of the medial perforant path was always reduced by γDGG during blockade of quisqualate and/or kainate receptors and in those cells where γDGG was concomitantly tested against the e.p.s.p., aspartate and glutamate, the e.p.s.p. amplitude decreased by the same degree as the glutamate response. Thus, despite the fact that on the granule cells γDGG appears not to be as selective an inhibitor on aspartate-induced responses as in the spinal cord, we favour the view that the endogenous transmitter of the perforant path, and glutamate, both interact with the same receptor, and that the effect of both are blocked by γDGG. However, other amino acids or related acidic compounds cannot be excluded as the natural ligand for the synaptic receptors.

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