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GLUTAMATE ANTAGONISTS IN RAT HIPPOCAMPUS

M. SEGAL
Isotope Department, Weizmann Institute of Science, Rehovot, Israel

1 Hippocampal cellular responses to acidic amino acids and some of their antagonists were measured in the rat anaesthetized with urethane. The effects of these antagonists on the field responses of the rat hippocampus to afferent stimulation were measured in acute as well as chronically prepared rats.

2 Hippocampal pyramidal cells were excited by microiontophoretic application of glutamate and aspartate. These responses were antagonized by glutamic acid diethyl ester (GDEE), glutamic acid dimethyl ester (GDME) and by proline. Partial specificity could be seen as excitatory responses to acetylcholine were less susceptible to the antagonists.

3 Field responses of the hippocampus to commissural stimulation were reduced significantly in both an acutely prepared or the conscious rat following parenteral administration of GDEE and GDME but not proline. Responses to perforant path stimulation were not affected by these drugs.

4 It is suggested that an acidic amino acid may serve as a neurotransmitter in the commissural path to-area CA1 of the dorsal hippocampus.

Introduction

The acidic amino acids, glutamate and aspartate, have long been suggested as putative neurotransmitters in the nervous system (Curtis, Duggan, Felix, Johnston, Tebécis & Watkins, 1972). They exert potent excitatory action towards neuronal action potentials when applied iontophoretically (Curtis *et al.*, 1972; Johnson, 1972) in nearly every part of the brain tested. Several antagonists to the action of glutamate have been suggested; glutamic acid diethyl ester (GDEE) among them has been reported to reduce the excitatory effects of glutamate without affecting responses to acetylcholine (ACh) in spinal cord, cuneate nucleus, ventrobasal thalamus, lateral geniculate and cerebral cortex (Curtis *et al.*, 1972; Haldeman & McLennan, 1972). Furthermore, GDEE blocks excitatory action of input pathways to some of these structures when applied iontophoretically (Haldeman & McLennan, 1972) or parenterally (Stone, 1973). Glutamic acid dimethyl ester (GDME) is a glutamate uptake blocker (Haldeman & McLennan, 1973) and has been shown in various preparations to potentiate cellular responses to glutamate. Its iontophoretic application in the hippocampus might be expected to produce effects opposite to those produced by GDEE. Recently, Van Harreveld & Fifkova (1973) have suggested that proline may antagonize glutamate responses in chicken isolated retina and elsewhere, whereas Felix & Künzle (1974) claimed that proline is a putative inhibitory transmitter in the cerebellum. A test of the efficacy of this compound in antagonizing acidic amino acid excitation was also attempted.

The hippocampus receives three main extrinsic excitatory inputs, two of which utilize neurotransmitters of unknown species. In this study the responses of hippocampal cells to acidic amino acids were tested. An attempt was made to antagonize these responses with GDEE, GDME and proline and the suceptibility of afferent responses to these antagonists was tested.

Methods

Adult (200–300 g) male Wistar rats of a local breeding colony were used. Two types of experiments were performed using the acutely anaesthetized and the freely moving conscious rat.

Rats prepared for the acute experiments were anaesthetized with urethane (1 g/kg) and placed in a stereotaxic frame. The skin overlying the skull was reflected and a hole 2 mm in diameter drilled 3.5 mm posterior to bregma and 1.5 mm lateral to the midline suture. The dura was removed and the cortex covered with warm 3% agar. A 5-barrel micropipette 4–6 μm in diameter was inserted to a depth corresponding to that of the pyramidal cell layer of area CAl of the dorsal hippocampus. A bipolar concentric electrode

was inserted at a similar point in the contralateral hippocampus. Another electrode was placed in the entorhinal cortex, the source of the perforant path to the hippocampus. The localization of the electrodes was verified histologically.

Three of the four outer pipettes of the 5-barrel micropipette were filled with the following compounds: acetylcholine chloride (2.5 M, Calbiochem), L-aspartic acid (Merck, 0.1 M, pH 8.0), DL-glutamic acid (K & K Laboratories, pH 8, 0.1 M), L-glutamic acid diethyl ester hydrochloride (GDEE, 0.1 M, Sigma), L-glutamic acid dimethyl ester hydrochloride (GDME, 0.1 M, Sigma) and L-proline (0.1 M, Fluka AG). The fourth barrel was filled with 5 M NaCl and served for testing current effects and to neutralize tip currents (Geller & Woodward, 1972).

The neuronal signals were recorded with the centre barrel which was filled with 5 M NaCl. The signals were fed via a preamplifier into an amplifier-filter which, in one channel, amplified the high frequency band (1–10 kHz) and recorded spike potentials. This amplifier was connected to a spike-height window discriminator and a ratemeter which displayed on a chart recorder integrated unitary firing rates.

In the other channel a low frequency band (1–1 kHz) for measuring evoked field potentials was amplified and connected to an Ortec signal averager which was set to average 16–32 traces. The hippocampal afferents were stimulated with 0.2 ms 40–100 μA monophasic pulses delivered through a battery operated simulator which was triggered by a Devices Digitimer which also triggered the signal averager.

The rats prepared for the chronic experiments were anaesthetized with sodium pentobarbitone (Nembutal, 50 mg/kg) and implanted with twisted 100 μm bipolar electrodes aimed at the entorhinal cortex and the hippocampus contralateral to the recording site where 62 μm microwire was implanted for recording. All wires were cemented to the skull and assembled in a plastic cap. After 3–4 days for recovery the rat was introduced into the test chamber, connected to a ten wire cable which delivered the stimuli to the rat and the responses through a preamplifier to the recording system described above. After termination of the experiments the rats were injected with an overdose of Nembutal perfused with 10% buffered formaldehyde and, at a later stage, sectioned on a freezing stage for determination of electrode placements. Further details of the methods are given elsewhere (Segal, 1973).

Results

As already reported (Biscoe & Straughan, 1966), glutamate caused an increase in firing rates of hippocampal cells when ejected with very low currents (0–10 nA). All 57 cells tested were excited with a short latency (less than 2 s) compared to the latency of the excitatory action of acetylcholine (ACh, 3–5 seconds).

The effects of glutamate were also of shorter duration than those of ACh and often an apparent inhibition of spontaneous firing rates which follows an initial excitatory response to glutamate was observed. This inhibition was often accompanied by a reduction of spike size. Responses similar to those produced by glutamate in both the current needed to generate an excitation and the time course of the effects resulted from iontophoretically applied aspartate.

The effects of GDEE on the excitatory action of glutamate were tested in 27 cells. On most cells tested (Table 1) GDEE exhibited a potent direct inhibitory action accompanied by a reduction of spike size when applied with a medium to high current (40–80 nA). However, when applied with low to moderate currents (20–40 nA) it had no noticeable effect on spontaneous activity or spike configuration and with such a dose it antagonized the responses to glutamate in 20 of 27 cells tested (Figure 1). GDEE action was considered antagonistic when it caused at least a 50% reduction in response to glutamate in three successive test sequences. Its effects were relatively specific in the current range used; although some antagonistic action towards the excitatory responses of 6 cells to ACh was noticed, a complete blockade of cellular responses to ACh was seen in only one cell.

GDME had a direct excitatory action towards most of the cells tested without affecting spike size (Table 1), as might be expected from its presumed glutamate uptake blocking action. When tested against the effects of glutamate, it did antagonize the action of glutamate in 7 of 13 cells tested. This

Table 1 Effects of glutamic acid diethylester (GDEE), glutamic acid dimethyl ester (GDME) and proline on the responses of hippocampal cells to glutamate (Glu) and acetylcholine (ACh) and on their spontaneous activity

	Glu		*ACh*		*Direct action*		
	Block	*No effect*	*Block*	*No effect*	*Increase*	*No effect*	*Decrease*
GDEE	20	7	1	5	0	12	15
GDME	7	6	2	2	6	6	1
Proline	14	2	3	2	1	11	4

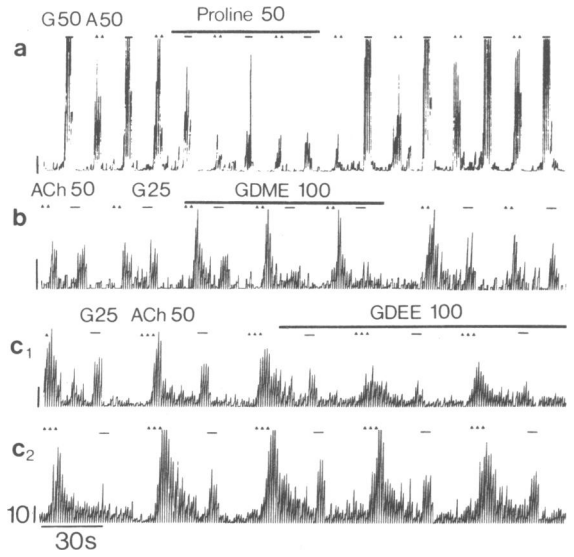

Figure 1 Effects of (a) proline, (b) glutamic acid dimethyl ester (GDME) and ($c_I c_{II}$) glutamic acid diethyl ester (GDEE) on excitatory cellular responses to putative neutrotransmitters. (a) Cumulative spike records comparing the effects of glutamate (G) and aspartate (A) on cellular firing rates and the effects of proline on these responses. Drugs were applied at regular intervals for a duration indicated by a bar with an ejection current in nA indicated by the numbers above the bars (50 nA for glutamate). Note the slightly longer recovery time needed for aspartate responses compared with responses to glutamate. (b) Comparison of the effects of glutamate with those of acetylcholine (ACh) and the action of GDME towards these excitatory actions. Note the apparent increase of ACh responses during GDME administration along with the complete blockade of glutamate responses and the relative absence of effects on spontaneous firing rates of GDME. ($c_I c_{II}$) Two successive records of the effects of GDEE towards glutamate and ACh. An apparent antagonistic action of GDEE towards both ACh and glutamate can be seen. No direct action on the spontaneous firing rates was seen. Note the slower recovery of responses to glutamate as compared with those of ACh. In all traces the abscissa scale denotes time (30 s bar) and the ordinate scale. spikes per s (10 spikes per s bar).

proportion is far lower than that produced by GDEE towards glutamate excitation. In 2 of 4 cells tested the action of ACh was at least partially antagonized by GDME.

Proline seemed to have the least direct action on spontaneous cell activity and spike configuration among the drugs tested. Most of the cells (11 of 16 cells) were not directly affected by proline. Proline exerted a potent antagonistic action towards the effect of glutamate; in 14 of 16 cells tested the responses to glutamate were at least partially antagonized by proline. The effects of proline were also tested against aspartate in three cells. Proline antagonized responses to aspartate in all of these cells. When tested simultaneously, proline appeared to have a slightly better antagonistic action towards aspartate than towards glutamate (Figure 1a).

Hippocampal CA1 responses to commissural stimulation consisted of a large negative potential with a latency of 16–24 ms, recorded in stratum radiation, below the pyramidal cell layer. This potential is generated in the terminal area of the commissural path and its magnitude and polarity varies within the hippocampus (Andersen, 1960). When applied iontophoretically neither of the three glutamate antagonists could block the responses to stimulation of the commissural pathway without a primary potent direct action towards the spontaneous activity of the recorded cells. Therefore no further attempts to antagonize the responses to stimulation of the input pathways by iontophoretic administration of glutamate antagonists were made and instead the antagonists were administered parenterally. Intraperitoneal administration of GDEE, GDME and proline was attempted in four of the above rats. In three of them the evoked field responses to commissural stimulation were reduced to 30–50% of control (Figure 2) with little effect on the responses to perforant path stimulation. GDME had a similar potency in these three rats in that it reduced responses to commissural stimulation to 40–70% of control values without affecting perforant path responses. Proline has no effect at the dose level used (200 mg/kg i.p.).

The conscious rat preparation has the advantages of allowing the effects of drugs to be tested without interference of anaesthetics and enabling comparative tests of various drugs to be made since the original response to afferent stimulation can be maintained over several days (Segal, unpublished observations). The effects of GDEE, GDME and proline were tested in 6 rats. Both GDEE and GDME reduced selectively the commissural responses within 2–5 min after injection (Figure 3), while proline, as in the acute experiments, was without effects. The action of GDEE was pronounced (60–80% decrease in the commissural response) in one rat, moderate (20–25% decrease in the response magnitude) in 4 more rats and ineffective in one rat. GDME action was pronounced in two rats, moderate in one rat and ineffective in the other two rats tested. There were no conspicuous effects of these drugs on the behaviour of the tested rats.

Discussion

The present results indicate that hippocampal neurones are excited by glutamic acid and that GDEE

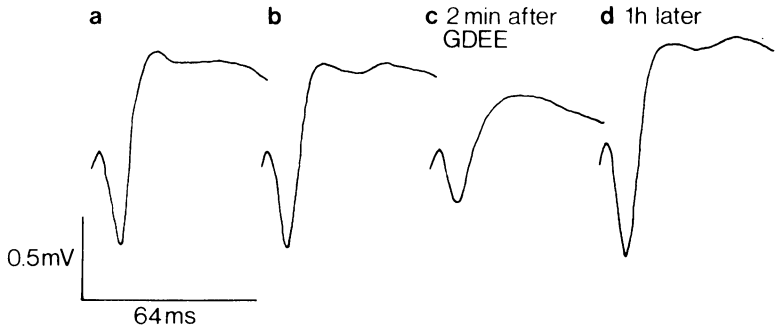

Figure 2 Mean evoked commissural responses in an anaesthetized rat. Each trace is the mean response to 16 stimuli (4 V, 0.2 ms duration, 1 Hz frequency). The recording electrode was placed just below the pyramidal cell layer. A stable response (a & b) was reduced to (c) less than 50% of the control value 2 min after intraperitoneal injection of 200 mg/kg glutamic acid diethyl ester (GDEE). (d) Complete recovery was seen 1 h later. In this figure positivity is upwards.

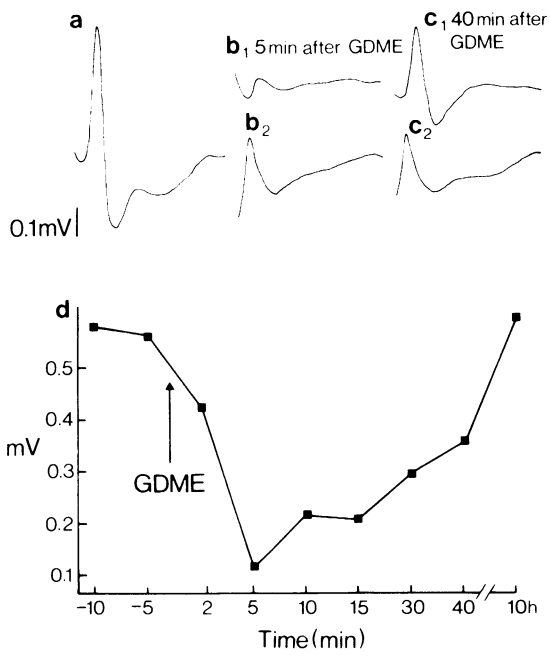

Figure 3 Effects of glutamic acid dimethyl ester (GDME) on commissural responses in the conscious rat. (a) Mean response to commissural stimulation before drug administration; (b) 5 min after GDME injection: (b_1) response to commissural stimulation; (b_2) response to entorhinal (perforant path) stimulation; (c_1 and c_2) responses to commissural and entorhinal stimulation respectively, 40 min after drug injection. In all these records negativity is upwards. (d) Time course of the changes in the magnitude of the commissural response after GDME injection.

antagonizes these excitatory responses. Another drug known to interact with glutamate, GDME, previously reported to block glutamate uptake and potentiate glutamate responses (Haldeman & McLennan, 1973) also had an antagonistic action towards glutamate in the present experiments. Similarly, proline, an amino acid reported to have some glutamate antagonistic action (Van Harreveld & Fifkova, 1973) exhibited this effect in the present study.

When the effects of these drugs on responses to stimulation of pathways afferent to the hippocampus were tested, it was found that two of them, GDEE and GDME, had a selective antagonistic action, in at least some cases, towards the responses to the commissural input. This effect could not be demonstrated when the antagonists were administered iontophoretically. The separation between the recording site, at or near the pyramidal cell layer of field CA1 of dorsal hippocampus, and the terminal field of the commissural path is some 100–500 μm. It is therefore unlikely that sufficient amounts of the antagonist could have arrived at the terminal field and have a selective antagonistic action towards glutamate terminals. Parenteral drug application, on the other hand, presents other problems of interpretation, since the drug may also change the excitability of the presynaptic fibres or the stimulated area or even the recorded cells in a way that may not affect the responses to the other input pathway tested, the perforant path. Tests for these possibilities are certainly pertinent.

It is interesting to note, in this context, that although proline was a potent antagonist of glutamate responses, it had little effect on the response to the stimulation of the pathway. It is possible that proline, being a natural amino acid, was taken up mainly by other mechanisms and only a little of it arrived at the

site where antagonistic action towards the pathway could be manifested.

The evidence for a transmitter role for glutamate or aspartate in the hippocampus is as yet incomplete. It has been reported thus far that aspartate uptake is reduced in decommisurized brain slices indicating that aspartate may be involved in commissural transmission (Nadler, Vaca, Cotman & Lynch, 1975). It was also suggested that glutamate is released upon stimulation of an intrinsic hippocampal pathway (Crawford & Connor, 1973). Recently, Spencer, Gribkoff, Cotman & Lynch (1976) have also reported that GDEE antagonizes hippocampal CA1 cellular responses to glutamate and aspartate. They, too, were unable to differentiate between glutamate and aspartate.

Finally, Iversen & Storm-Mathisen (1976) found a selective uptake of [^{3}H]-glutamate into excitatory terminals which belong, in part, to the commissural path. Obviously with the small selection of putative excitatory neurotransmitters available for study it is almost inevitable that at least one of the two extrinsic excitatory and two intrinsic excitatory synapses in the hippocampus would utilize an acidic amino acid as a neurotransmitter. Our data in agreement with Nadler *et al.* (1975) supports the suggestion that glutamate or aspartate might be the transmitter of the commissural path. With the present lack of specific antagonists to glutamate or aspartate a more definite suggestion cannot be made on the basis of the present data. Further investigation is needed to obtain evidence which will allow a comparison of responses to both putative neurotransmitter application and stimulation of the pathway at the membrane level.

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