Br. J. Pharmac. (1972), **44**, 435–441.

Effects of a focal penicillin lesion on responses of rabbit cortical neurones to putative neurotransmitters

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Summary

1. Epileptogenic foci were generated in rabbit cerebral cortex by the topical application of penicillin.

2. Responses to iontophoretically applied putative neurotransmitters were compared in cortical neurones firing spontaneously with those driven by applied excitant substances, both before and after establishing the penicillin focus.

3. In the presence of the spiking focus, currents of γ-aminobutyric acid, which normally produced 100% depression of neuronal firing, were ineffective.

4. In the same situation, currents of (±)-homocysteic acid, glutamate and acetylcholine produced predominantly depolarization block responses, and hence depression of firing.

5. It is concluded that an epileptogenic focus can alter the responses of rabbit cortical neurones to microiontophoretically applied neurotransmitter substances. Possible mechanisms for the spread of seizure activity are discussed.

Introduction

Action potentials recorded from single neurones in the mammalian cerebral cortex normally have little correlation with the gross electrical activity of the brain, as exemplified by the conventional electroencephalogram (Buchwald, Halas & Schramm, 1966). In the epileptic animal, however, surface paroxysmal activity can be shown to be synchronous with burst firing in single units (Ward, 1960 ; Sypert & Ward, 1967 ; see also Fig. 1).

It seemed worthwhile therefore to study the pharmacological responses on single neurones in an animal with an epileptogenic seizure induced with penicillin (Ajmone-Marsan, 1969), and to look for changes in response to putative neurotransmitters applied microiontophoretically. Any such changes in response might help to explain the mechanism underlying the synchronous discharge of neuronal aggregates, resulting in the surface 'spiking' activity seen in the epileptiform electroencephalogram (EEG).

Methods

Eight New Zealand white rabbits, of either sex, weighing 2–3 kg, were anaesthetized with 50% nitrous oxide in oxygen with 1·5% halothane. The trachea was cannulated and the head fixed in a stereotaxic apparatus. The posterior sensorimotor cortex (Bures, Petran & Zachar, 1967) was exposed by removal of the overlying skull and dura mater, and the surface of the brain was kept in place by a

celluloid pressor plate. In all animals the electroencephalogram was monitored from stainless steel skull electrodes close to the craniotomy. In some experiments, the electroencephalogram was additionally recorded from a monopolar silver ball electrode on the exposed cortex or from the micropipette itself.

Five to seven barrelled micropipettes (Roberts & Straughan, 1967) were used with tip diameters of 5–7 microns. Two barrels were filled with 3 M NaCl solution and the rest filled with solutions of (±)-homocysteic acid (DLH), (−)-sodium glutamate, acetylcholine chloride (ACh) and γ-aminobutyric acid (GABA). These drug solutions were made up to 0·2 M and adjusted to a suitable pH for iontophoresis. Of the two sodium chloride barrels, one was used to apply a ' balancing ' current so as to minimize the effects of current flow upon neuronal firing. The other barrel was used for recording extracellular action potentials relative to an indifferent electrode (silver/silver chloride plate), placed under the skin of the neck. The spikes were amplified and displayed on a cathode ray oscilloscope, counted over consecutive 10 s epochs, and registered on one DC channel of a polygraph as successive peaks. The height of each peak was proportional to the number of spikes counted in the preceding 10 s period.

Penicillin foci were produced by the application of approximately 10 μl of a 600 mg/ml (1 mega-unit per ml) solution of sodium benzyl penicillin to the exposed cortical surface, immediately adjacent to the micropipette penetration.

Each animal was used as its own control in that recordings were made from units at depths ranging down to 3 mm from the surface of the cortex, before the penicillin lesion was made. This helped to confirm that both the micropipette and the animal were giving reproducible and typical responses, the same pipette being used before and after making the lesion in each case.

Each drug used was expelled until a maximal effect was observed, that is, depression of firing rate by γ-aminobutyric acid, or increase in firing rate by the other substances. In the case of the excitant substances, supramaximal currents sometimes progressively stopped cell firing, by producing depolarization block. This was easily distinguished from γ-aminobutyric acid type depression by its accompanying degradation of spike height, and by delayed recovery when the drug was turned off (Krnjević & Phillis, 1963a). In several cases an individual neurone was held long enough to observe its sensitivity both before and after making the lesion, and the information gained in this way suggests that we were not selecting a different population of neurones after the focus had become established.

Drugs

Acetylcholine chloride (B.D.H.); (±)-homocysteic acid (Calbiochem); gamma-aminobutyric acid (Sigma); (−)-sodium glutamate (B.D.H.); sodium benzylpenicillin (Crystapen) (Glaxo).

Results

Electroencephalogram

Before making the lesion the electroencephalogram exhibited primarily high voltage slow activity, characteristic of an animal lightly anaesthetized with halothane-nitrous oxide (Fig. 2).

After applying the penicillin, the characteristic progression from isolated paroxysmal surface 'spikes' to repeated ictal events was invariably seen (Matsumoto & Ajmone-Marsan, 1964a; also Fig. 3). Unit activity was studied both during the interictal 'spiking' stage, and when regular ictal events had become established.

In general, good synchronization was observed between surface paroxysmal activity and neuronal action potentials (Fig. 1), although during an ictal event the unit activity could be altered by application of drugs from the micropipette, without affecting the gross seizure pattern (Fig. 3).

Unit activity

γ-Aminobutyric acid

Before the application of penicillin, microiontophoretically applied γ-aminobutyric acid caused depression of firing in spontaneously active neurones and also in those neurones being driven by excitant substances applied iontophoretically. Currents of 10–40 nA routinely completely abolished the firing of these cells (Fig. 2).

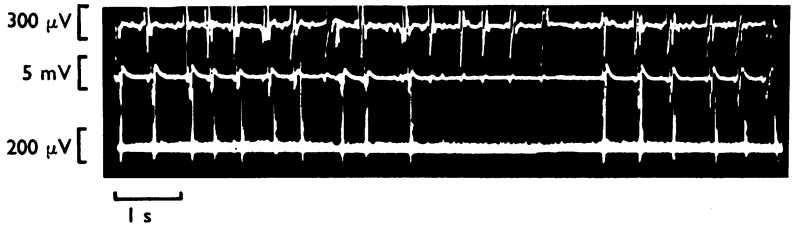

FIG. 1. Photographic record showing the synchronization between EEG 'spikes' and neuronal firing in an epileptic rabbit cortical neurone (2·57 mm deep in the cortex). Thirty min previously 6 mg/10 μl of sodium benzyl penicillin was applied to the brain surface adjacent to the micropipette. Time mark, 1 s. Top trace, EEG via skull electrodes; middle trace, record from micropipette without band pass filters to show EEG; lower trace, spikes from the same micropipette, band pass filters in circuit, higher gain. Animal anaesthetized with N_2O/halothane and respiring spontaneously.

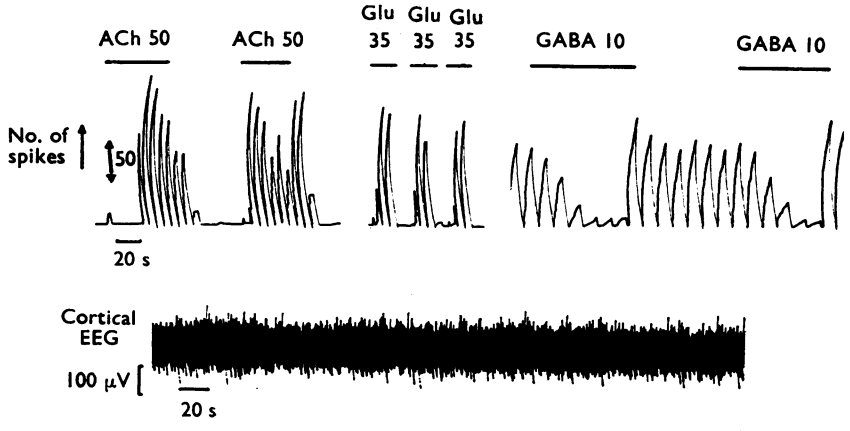

FIG. 2. Control responses of anaesthetized rabbit cortical neurones and surface EEG. Upper trace, action potential counter record of excitatory responses to acetylcholine chloride (50 nA) and sodium glutamate (35 nA) (each peak represents the number of neuronal spikes in a 5 s epoch). A depressant response to GABA (10 nA) is also shown (each peak represents a 10 s epoch). Lower trace, normal cortical EEG. Vertical scale fifty spikes (upper), 100 μV (lower).

After establishing the focus, there was a marked reduction in the sensitivity of all the cells studied to the depressant action of γ-aminobutyric acid. In some experiments, this was apparent before any signs of paroxysmal activity were evident in the electroencephalogram. When the focus was well established, and units were only firing in synchrony with the overall seizure activity, it was virtually impossible to inhibit the firing of a cell with γ-aminobutyric acid, even though currents 4 or 5 times as large as those normally producing 100% inhibition were used (Fig. 3).

Excitant agonists

Glutamate and (±)-homocysteic acid, when applied iontophoretically, increase the firing rate of most cortical neurones. The effect is rapid in onset and the response decays equally quickly on turning off the drug. Before applying the penicillin, currents of glutamate in the order of 25–50 nA caused marked increases in firing rate. The more potent depolarizing agent (±)-homocysteic acid gave similar increases with currents of 5–25 nA.

Under control conditions acetylcholine gave its characteristic excitatory response (Krnjević & Phillis, 1963b). That is, applications of the drug with currents of 25–75 nA caused a prolonged excitation after an initial delay of 5–15 seconds.

After producing the lesion, both glutamate and (±)-homocysteic acid had little effect when used in doses which produced excitation under control conditions. When the applying current was increased by about 50%, two effects were noted. In the

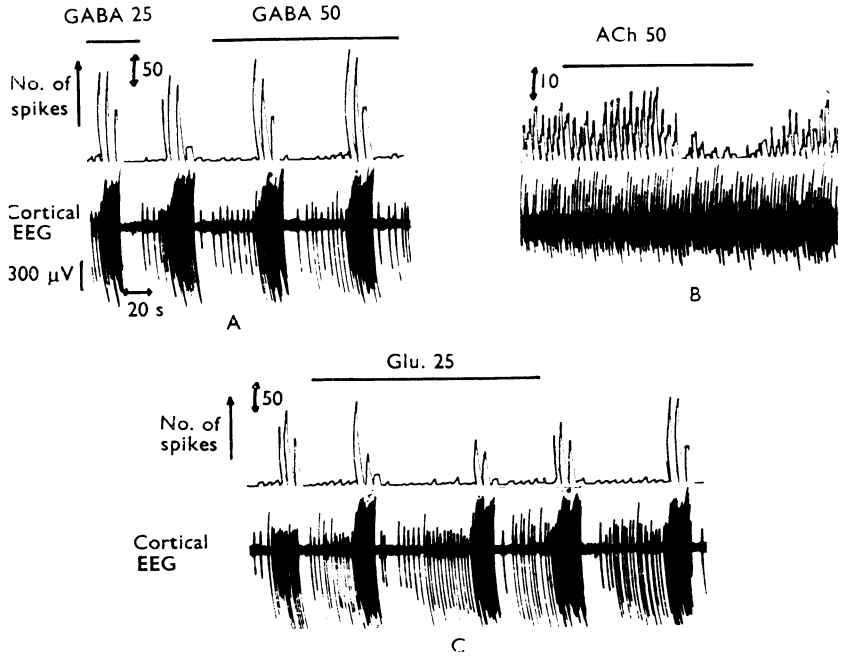

FIG. 3. Action potential counter and surface EEG records from two deep cortical cells of an anaesthetized rabbit after surface application of penicillin. The effect of GABA (A) and glutamate (C) are shown on the same cell in an animal with regular ictal episodes. GABA (25–50 nA) caused no reduction in firing rate whereas glutamate (25 nA) caused a depression of firing due to depolarization block (cf. Fig. 2). Acetylcholine (B) applied to a different cortical neurone in an animal showing interictal 'spiking' but before seizures had occurred. (Note the initial small increase followed by a rapid reduction in firing rate.)

interictal 'spiking' situation it was sometimes possible to drive a cell faster than the rate at which it was firing under the influence of the focus, but depression of the firing of the unit by intense depolarization was commonly seen. When the cell was tested during an ictal event, it appeared that a maximal firing rate had already been achieved, as cells could not be made to fire faster with glutamate or (±)-homocysteic acid and depolarization block was the first effect seen (Fig. 3).

In control conditions cells were more readily excited by (±)-homocysteic acid than by glutamate, and in the presence of the penicillin focus it was easier to depress cells with (±)-homocysteic acid, indicating that the depression seen is in fact related to their relative potency as depolarizing agents.

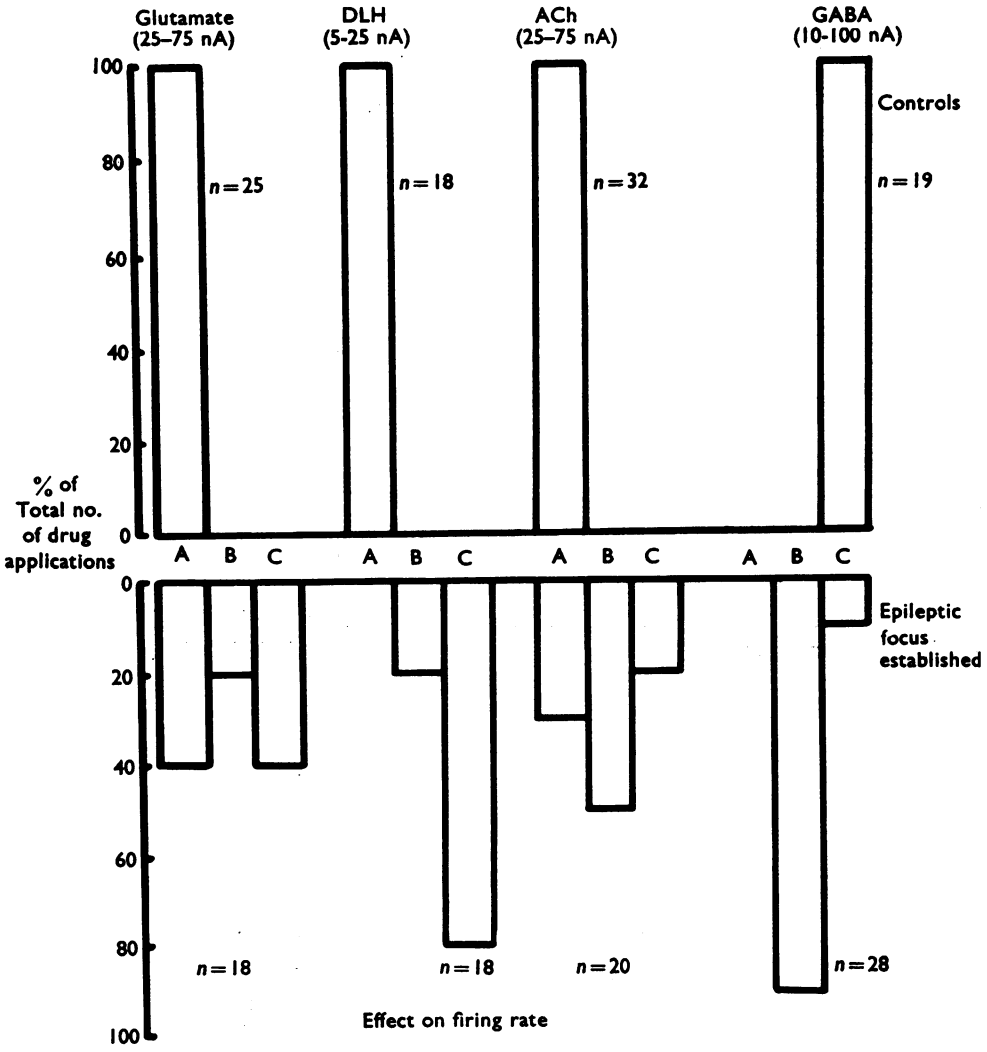

FIG. 4. A double histogram showing the neuronal responses to four drugs. Data obtained from sixty-four cortical neurones, upper section, thirty before establishing the epileptic focus and lower section, thirty-four after making the focus (note the reversal of the scale in the lower section). In the normal state, glutamate, DLH and ACh caused an increase in firing after every application (Column A). GABA caused a decrease in firing in every case (Column C). After establishing a focus GABA was almost always ineffective (Column B), DLH invariably caused depression of firing and glutamate and ACh gave varied responses (see text).

The responses to acetylcholine in the lesioned state were indistinguishable from those to glutamate and they seemed to be roughly equipotent in producing depolarization block.

These results are summarized in Fig. 4.

Discussion

An explanation of the above results must take into account the fact that the units studied probably fell into two groups. The first group would comprise those neurones nearer the surface of the brain which came into direct contact with penicillin, as a result of diffusion through the brain tissue and permeation down the micropipette track. The second group would be those neurones which had no contact with the lesioning agent, but owed their epileptic character to neural connexion with the directly affected area.

When considering those neurones directly affected by penicillin, it is possible to draw an analogy with observations made on isolated nervous tissue. Intracellular records made in the crayfish stretch receptor (Ayala, Lin & Vasconetto, 1970) and the stellate ganglion: giant axon system of the squid (Ayala, Spencer & Gumnit, 1971), indicate that penicillin produces a slow depolarization of neurones exposed to its action. A similar effect on neurones in the central nervous system may help to explain our results, as a lowered resting membrane potential would make depolarizing substances, such as glutamate, appear more effective and make hyperpolarizing agents, such as γ-aminobutyric acid, appear less effective.

In our experiments, it was not possible to distinguish between cells directly affected by penicillin, and those being neuronally driven, on the basis of their sensitivity to drugs applied iontophoretically. It is therefore necessary to produce a mechanism to account for the changed responses of the second group of neurones. Intracellular records, taken from a cortical cell during an ictal event (Matsumoto & Ajmone-Marsan, 1964b) show that there is a steady decrease in membrane potential during the seizure, and that this can reach a level so low that any small extra depolarization will abolish neuronal action potentials. In addition to this it has been found that prolonged stimulation of a neurone can cause retention of sodium and hence depolarization (Sherman & Atwood, 1971), and facilitation of excitatory synaptic action. Retention of sodium will also tend to increase the extracellular potassium concentration, and this can also exert a powerful depolarizing action on adjacent cells (Zuckermann & Glaser, 1970).

If one accepts the considerable weight of evidence (Krnjević, 1970) that suggests that γ-aminobutyric acid and glutamate may be the main inhibitory and excitatory transmitters, respectively, in the mammalian cerebral cortex, and that acetylcholine is almost certainly a central excitatory transmitter, then the above discussion may help to explain how the initial paroxysmal discharge originates and is maintained. Stated simply, glutamate or acetylcholine mediated excitation would be enhanced, whilst on the same neurone γ-aminobutyric acid mediated inhibition would be rendered less effective by penicillin-induced depolarization and ion movement.

Given a sufficiently large population of neurones directly affected by penicillin, the disturbance produced in the normal balance of excitation and inhibition could result in the setting up of a positive feedback loop resulting in a synchronous paroxysmal discharge from the lesioned population. A powerful enough discharge

could drive neighbouring neurones not directly affected by penicillin, and thereby lead to spread of the seizure activity.

The mechanisms discussed above can therefore be seen to offer an explanation for the changed responses of neurones directly affected by penicillin and of neurones relatively distant from the site of application of the lesioning substance. Our suggestion that a neuronal aggregate must be affected by penicillin before the seizure will spread is supported by recent work in which penicillin was applied iontophoretically to single neurones in cat cortex (Walsh, 1970). These observations demonstrated that no paroxysmal discharge resulted unless a more gross application of penicillin to the cortex was made, although penicillin was quite clearly reaching the neurone when iontophoresed as an increase in the size of the EPSP was detectable.

It can therefore be stated that topical application of penicillin to the cortex of the rabbit can result in a pronounced change in the sensitivity of single neurones to putative neurotransmitter substances. Additionally, it is possible that many convulsant substances could, *per se*, make the endogenous inhibitory transmitter less effective without postulating blockade of inhibitory receptors on the postsynaptic membrane (Curtis, Duggan, Felix & Johnston, 1970).

We are grateful to Professor D. W. Straughan and to Dr. M. A. Simmonds for much help and discussion during the preparation of this paper. The help of the Wellcome Research Laboratories through a Studentship to R.G.H. and the Medical Research Council via a Training Award to G.C. is gratefully acknowledged. This work was supported in part by a grant from the M.R.C. to D.W.S.

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(*Received July* 20, 1971)