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THE ACTIONS OF DIAZEPAM AND PHENYTOIN ON A LOW DOSE PENICILLIN EPILEPTIFORM FOCUS IN THE ANAESTHETISED RAT

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1 A technique is described for the induction of an acute short-lived epileptiform focus by the local injection of benzyl penicillin 25 units into the cerebral cortex of rats anaesthetized with urethane. The effects of anticonvulsants on this microfocus have been studied.

2 Phenytoin 20 mg/kg and diazepam 1 mg/kg intravenously significantly reduced the frequency of the normally occurring surface positive electrocorticogram (ECoG) plateaux and caused a greater decrease in the frequency of the epileptiform spikes normally associated with these plateaux.

3 A novel effect was seen in that both anticonvulsant drugs significantly reduced the amplitude of the epileptiform spike within the focus, as well as causing an even larger decrease in the amplitude of the propagated spike. The contribution of the anticonvulsant drug effect on the focal spike to its effect on propagated activity is discussed.

Introduction

The epileptiform focus produced by application of penicillin to the cerebral cortex of various animals is a commonly used model for experimental studies of epilepsy (Prince, 1972). However, penicillin-induced epileptiform foci in the rat appear to be resistant to various anticonvulsant drugs (Edmonds, Stark & Hollinger, 1974; Forrester & Gartside, 1975). As the rat is a convenient and economical laboratory animal and as penicillin foci are easy to produce, it would be useful to be able to produce a penicillin focus in the rat which is susceptible to the actions of reasonable doses of anticonvulsants. Edmonds *et al.*, 1974, showed that the rat was more sensitive to penicillin as an epileptogenic agent than other commonly used laboratory animals. They quoted the minimum reliable doses of penicillin to produce generalized convulsions following intracortical injection to be as follows: cat, 10,000 units, monkey, 1000 units, dog, 500 units, and rat 200 units. It seemed worthwhile to reduce this dose still further in the anaesthetized rat and this has resulted in a minimal focus which has been found to be sensitive to the anticonvulsant actions of both diazepam and phenytoin.

Methods

Twelve rats weighing 200 to 300 g were anaesthetized with ethyl carbamate 1.6 to 1.8 g/kg and the dorsal surface of the skull exposed. Three burr holes were made above the forepaw area of both sensorimotor cortices and also above the parietal cortex. Intracortical injections of 5 μl of a solution of 5 ku/ml (a total dose of 25 u) of penicillin G (Crystapen, Glaxo) in 0.9% w/v NaCl solution (saline) were made into one sensorimotor cortex at a depth of 0.5 to 1 mm and the syringe needle was used as an electrode to record focal activity. The indifferent electrode was a silver wire placed in the conjunctival sac. Stainless steel screws (8BA) were placed in the remaining burr holes to record propagated epileptiform activity from the contralateral sensorimotor cortex and the ipsilateral parietal cortex. The anaesthetic depth was adjusted so that no muscle twitching accompanied electrocorticogram (ECoG) spikes, and thus these spikes were uncontaminated with electromyographic activity. From these ECoG recordings, the frequency of occurrence of ECoG 'plateau' and epileptiform spiking were derived automatically using a Schmidt trigger, fed into a cumulative analogue counter (counting over a period of 30 s) and displayed, along with femoral arterial pressure and heart rate.

Phenytoin sodium was dissolved in saline (20 mg/ml). Diazepam in the commercial vehicle was diluted with saline to a concentration of 1 mg/ml, as this removed the depressant effects on heart rate and blood pressure seen with the undiluted vehicle alone. All injections were made through a cannula in the femoral vein. Drugs and control injections were given in a randomized order, with at least a 30 min interval between injections.

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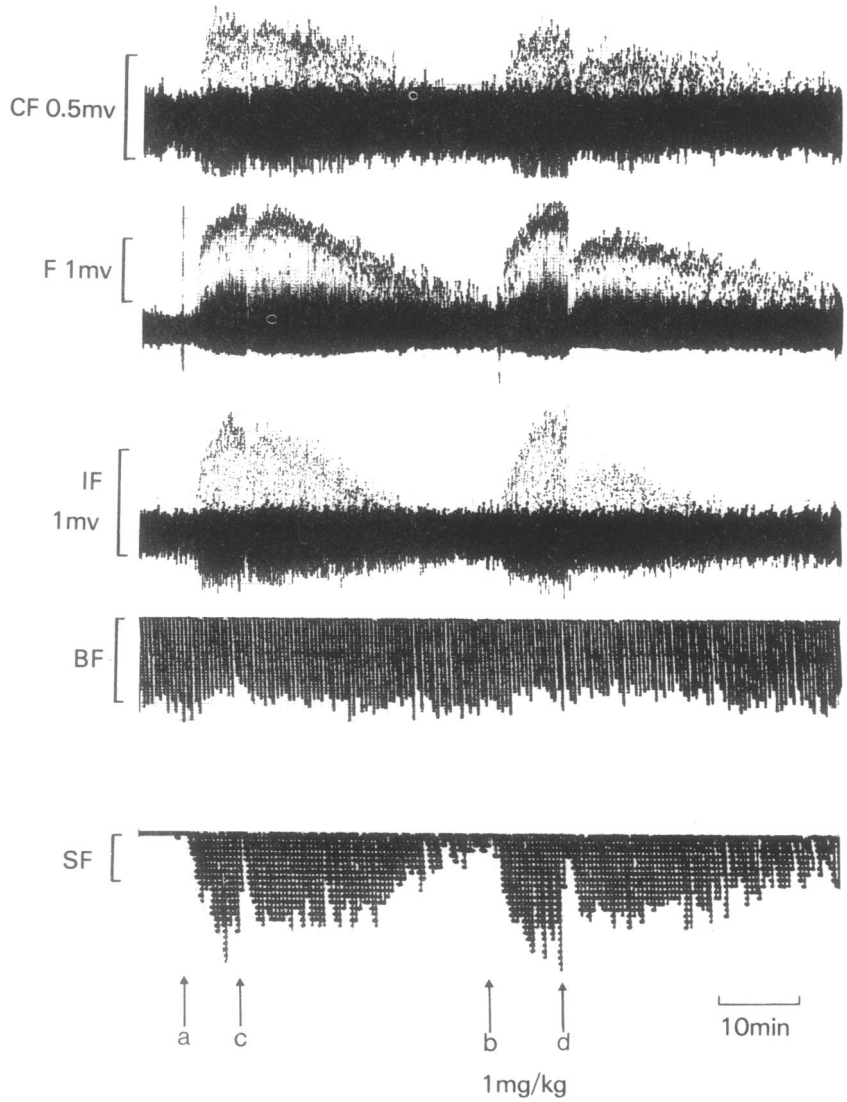

Figure 1 The effects of intravenous injections of 0.2 ml of saline-diluted diazepam vehicle (at c) and 0.2 ml of a similar solution containing 1 mg/ml of diazepam, at (d) on two separate penicillin epileptiform foci in the anaesthetized rat. Penicillin (25 units) was injected at both (a) and (b). Records, from top to bottom are: CF—ECoG recorded contralateral to the focus; F—ECoG recorded from the focus itself; IF—ECoG recorded from parietal cortex ipsilateral to the focus; BF—cumulative record of counts of spike activity over 30 s (record inverted). Calibration bar, 60 plateaux/minute. SF—cumulative record of counts of epileptiform spike activity over 30 s (record inverted). Calibration bar, 20 spikes/minute. Time bar = 10 minutes. Note that diazepam has a greater depressant effect on all parameters than the control injection, except for plateau frequency, which was hardly affected in this experiment.

Results

The normal ECoG in the rat consists of a series of surface positive flat topped deflections (plateaux). These plateaux have a duration of 20 to 200 ms and a frequency of 0.1 to 4 hertz.

The epileptiform activity produced by the intracortical injection of 25 u penicillin is qualitatively no different from that produced by much larger (1 ku) doses in this preparation. Following injection of 25 u penicillin into the sensorimotor cortex, the first epileptiform spike occurs within 1 to 3 min of the injec-

Table 1 The effects of injections of saline, diazepam (1 mg/kg) or diphenylhydantoin (20 mg/kg) on various measures of epileptiform activity

	Saline control	*Diazepam (1 mg/kg)*	*Diphenylhydantoin (20 mg/kg)*
n	9	11	11
ECoG plateau frequency	1.003 ± 0.014	0.858 ± 0.093	0.801 ± 0.040**
Epileptiform spike frequency	0.987 ± 0.035	0.629 ± 0.081**	0.762 ± 0.047**
Focal spike amplitude	0.949 ± 0.026	0.711 ± 0.071*	0.824 ± 0.048*
Contralateral driven spike amplitude	0.944 ± 0.038	0.503 ± 0.080**	0.624 ± 0.046**
Ipsilateral driven spike amplitude	0.954 ± 0.059	0.328 ± 0.040**	0.563 ± 0.054**

Results are expressed as the ratios ± s.e. mean of the various measures immediately before and 5 min after the injection. Significant differences from control are indicated as * $P < 0.05$; ** $P < 0.01$.

tion. The spikes steadily increase in size and reach a peak amplitude in 5 to 10 minutes. The spike amplitude then remains constant for a further 5 to 20 min before beginning a slow decay over a period of 20 to 40 minutes. Thus, the total lifetime of such a focus is 30 to 60 minutes. With a 1 ku focus the time to onset of the first spike and to maximal spike amplitudes are the same but the maximal spike amplitude is maintained for 1 to 2 h and the decay time is much greater giving a total lifetime of 3 to 6 h for the focus. Due to the relatively short duration of these small penicillin foci, the drug or control injections were given after the focal spikes reached peak amplitude. Measures of focal and propagated spike amplitude and ECoG plateau and spike frequencies were made immediately before and 5 min after the administration of the drug. Each measure was expressed as the ratio before and after drug. Table 1 shows these ratios for each treatment. It can be seen that each measure of epileptiform activity is depressed slightly by the control injection and consequently all drug effects are compared with control.

Plateau frequency appears to be inversely related to the extent of central depression (Gartside, unpublished observations), and is only minimally decreased by diazepam and phenytoin. This 'sedative' effect was only significant with phenytoin. However, both drugs produced a significant depression of spike frequency. This may not represent an anticonvulsant action *per se* as in this preparation epileptiform spikes always occur on and may be triggered by ECoG plateau (Forrester & Gartside, 1975) and the decrease in spike frequency may merely reflect the drop in plateau frequency. Using a related *t* test, the drop in spike frequency following diazepam was found to be significantly greater than that expected from the reduction of that in plateau frequency alone ($P < 0.05$), but the drop in frequency following diphenylhydantoin was not significantly different. Both drugs significantly depressed the amplitude of both focal and driven spikes relative to the controls, the depression of driven spikes being the greater.

Discussion

This study shows that it is possible to induce, in the rat, an epileptiform focus which is susceptible to the actions of anticonvulsants by the use of penicillin, provided that the dose of penicillin is kept very small.

In this study the effects of the anticonvulsants on the propagated epileptiform activity are greater than those on the focus itself. This is consistent with the conclusions of Morrell, Bradley & Ptashne (1959). They suggested that, 'possibly the greater part of the anticonvulsant mechanism is the reinforcement of those intrinsic systems within the brain which serve to limit propagation, rather than a direct action on epileptogenic tissue'. Alternatively, if the relationship between the amplitude of focal activity and propagated activity is a steep one, then a small decrease in focal spike amplitude (as seen in Table 1) might cause the decrease in drive spike amplitude. Hence the apparently greater effects of anticonvulsants on propagated spike activity may be due primarily to their effects on focal activity rather than in the mechanism of spread as was suggested by Morrell *et al.* (1959).

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