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Comparative convulsant activity of various penicillins after intracerebral injection in mice

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The convulsant effects of penicillin derivatives (penicillin G, phenoxymethylpenicillin, penicillin O, ampicillin, methicillin) and cephaloridine have been evaluated after being given intracerebrally to mice. Penicillin G was most neurotoxic; ampicillin and phenoxymethylpenicillin were one-fifth as toxic. Incubation with penicillinase markedly reduced or eliminated the convulsant effects of penicillin G, phenoxymethylpenicillin and ampicillin; this enzyme incubation did not alter the severity of seizures elicited by methicillin and cephaloridine. Diphenylhydantoin and phenobarbitone did not reduce penicillin G-induced seizures. A non-convulsant dose of leptazol potentiated the convulsions produced by the penicillins and cephaloridine. Injection volume, pH and the concentration of sodium and potassium did not produce behavioural changes within the ranges studied.

Direct administration of penicillin to the cerebral cortex of monkeys, cats, dogs, mice and man has been reported to produce electroencephalographic alterations and grossly observable convulsions (Walker & Johnson, 1945; Walker, Johnson & Kollros, 1944; Backus & Millichap, 1963). Encephalopathy has been demonstrated in patients after massive doses of penicillin G; in most, pre-existing renal insufficiency was present (Raichle, Kutt & others, 1971). Virtually all previous studies, in animals and man, have investigated the epileptogenic activity of penicillin G, employing a limited range of doses. We have sought to compare the convulsant activity of five derivatives of penicillin and cephaloridine (a penicillin-like drug of the cephalosporin class) in mice after intracerebral administration of these antibiotics.

METHODS

Intracerebral injections

Male Swiss albino mice, 19–23 g, were anaesthetized with ether and a hole bored with a 27 gauge needle just below the skull. This hole was located in the motor cortex, 3 mm anterior to bregma along the sagittal suture. Twenty-four h later, the mouse was again lightly anaesthetized with ether, and the test drug administered at a depth of 2 mm by 10 μl Hamilton syringe. Within 30 s of the injection, the animals regained complete consciousness. All solutions were freshly prepared in sterile water for injection, U.S.P., and injected in volumes of 0·4–4 μl.

Grades of seizure intensity

Seizures were graded according to a scale proposed by Millichap (1965). In brief, grade O, normal behaviour; grade 1, hyperkinesia; grade 2, stunning, head tremors

and catatonia; grade 3, generalized symmetrical clonic seizures, with loss of posture; grade 4, transient tonic flexion and extension of the forelimbs followed by prolonged generalized clonus; grade 5, tonic flexion and prolonged tonic extension of both fore and hind limbs, with often a fatal outcome, but sometimes followed by clonus and depression.

RESULTS AND DISCUSSION

Preliminary studies

Experiments were made with groups of five mice to ascertain the influence of the injection volume, pH and concentration of cations on seizure activity. Saline, injected in volumes up to 6 μl and intracerebral injection of 4 μl of phosphate buffer, pH 5 and 8 (the pH of the antibiotic solutions used), caused no behavioural aberrations. Doses of 2·0 mg of the antibiotics contain a maximum of 0·52 mg sodium and 0·40 mg potassium. Mice were pretreated with a non-convulsive dose of leptazol 15 min before the intracerebral administration of salt solutions (see below). No changes in behaviour were observed after injections of 1·32 mg NaCl or 0·76 mg KCl in a volume of 4 μl. Although penicillin's effects on seizure susceptibility might be in part the consequence of an interaction with other endogenous cations (e.g. calcium), this aspect was not investigated.

Convulsant effects of penicillins and cephaloridine

After intracerebral administration of the penicillin derivatives and cephaloridine, in general, seizures were seen within 2 min after injection, with maximal effects occurring within 15 min (Fig. 1); observations were continued for 2 h. Non-parallel slopes of the dose-response curves preclude a simple direct ranking of the relative neurotoxicity of the compounds for all grades of seizures. If grade 3 (clonus) is selected for comparative purposes, the rank order of toxicity in descending order, expressed as the approximate molar potency (10^{-6} mol), is: penicillin G (1·0) > methicillin (2·7) > cephaloridine (3·0) > penicillin O (4·4) > phenoxymethylpenicillin (5·2) > ampicillin (5·4). When evaluated in terms of the highest seizure grade obtained with a 2·0 mg dose of each compound, the relative order of toxicity is penicillin O > penicillin G > methicillin > cephaloridine > phenoxymethylpenicillin = ampicillin; grades of seizure severity ranged from 4·14 (penicillin O) to 3·00 (phenoxymethylpenicillin and ampicillin).

Penicillinase

To assess the ability of penicillinase to attenuate penicillin and cephaloridine-induced seizures, antibiotic-containing solutions were incubated with penicillinase at 25° for 5 min. The mixture was then administered intracerebrally, as described above. Penicillinase incubation totally eliminated the convulsant effects of penicillin G and ampicillin and reduced the seizure grade of phenoxymethylpenicillin by 70% at the concentration of enzyme employed (Table 1). By contrast, this enzyme was ineffective in reducing the seizures induced by methicillin and cephaloridine. These findings are in agreement with the literature concerning the susceptibility or resistance of these antibiotics to hydrolysis by penicillinase. In the presence of some penicillinase-producing staphlococci, cephaloridine is inactivated (Seneca, 1971). The ability of penicillinase to eliminate penicillin G convulsions has been previously reported (Gutnick & Prince, 1971).

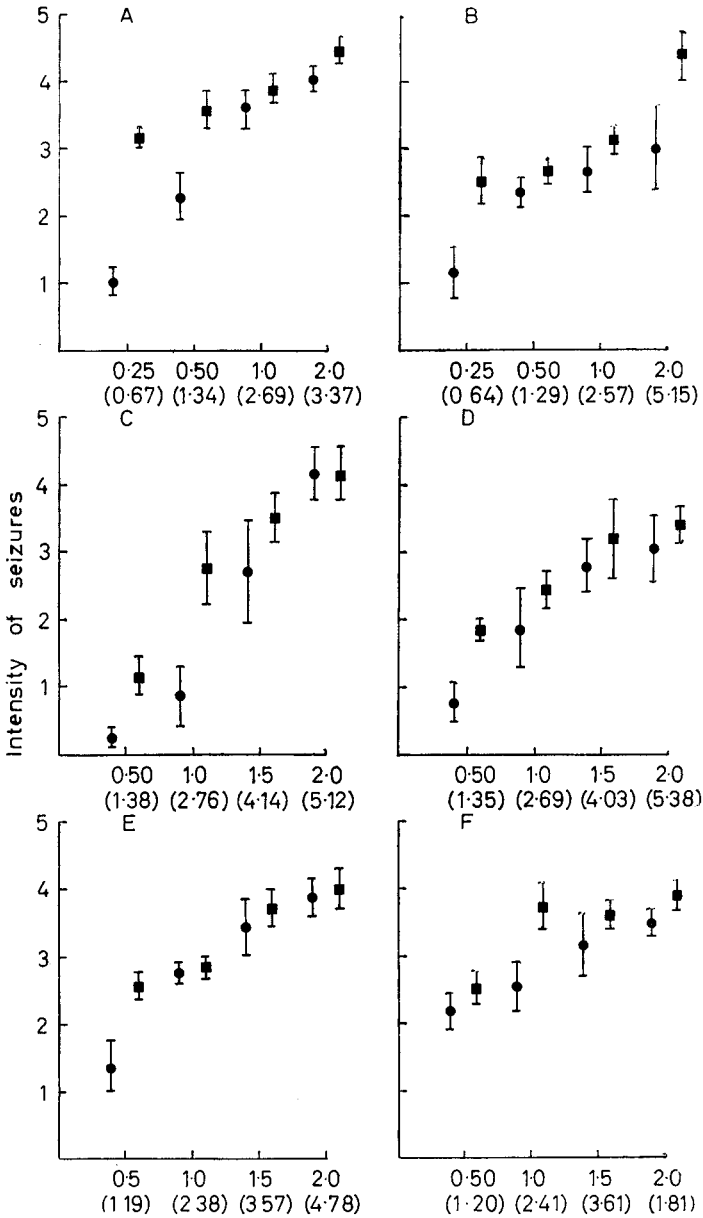

FIG. 1. The convulsant effects of intracerebrally administered penicillin derivatives and cephaloridine in mice. The numbers on the abscissa represent the total dose in mg administered to each animal; below, and in parentheses, are these doses expressed as mol (10^{-6}). To avoid clashing of the vertical bars which depict the standard error of the mean, data points are placed on each side of the actual dose of antibiotic administered. The scale on the ordinate corresponds to the intensity of the seizures as described in methods. A = sodium penicillin G; B = potassium phenoxymethylpenicillin (penicillin V); C = sodium penicillin O; D = sodium ampicillin; E = sodium methicillin; F = cephaloridine. Penicillin = (●); leptazol, 40 mg kg^{-1} s.c. administered 15 min before penicillin = (■). Each point represents the mean of 6–8 mice.

Table 1. *The effects of penicillinase on the convulsant effects of penicillins and cephaloridine.* Antibiotics were incubated with penicillinase at 25° for 5 min. This mixture was then administered intracerebrally as described in methods. Penicillinase, in concentrations of 4400 units produced no behavioural effects. The values are expressed as the mean ± standard error of the mean.

Antibiotic	Dose (mg)	Penicillinase (units)	Number of mice	Seizure grade
Penicillin G	2·0	—	7	4·00 ± 0·21
	2·0	3600	7	0
Phenoxymethylpenicillin	2·0	—	6	3·00 ± 0·63
	2·0	3300	7	0·87 ± 0·26
Ampicillin	2·0	—	6	3·00 ± 0·51
	2·0	4400	5	0
Methicillin	1·5	—	8	3·42 ± 0·42
	1·5	2500	5	3·40 ± 0·36
Cephaloridine	2·0	—	7	3·42 ± 0·20
	2·0	3300	6	3·50 ± 0·22

Anticonvulsant agents

Diphenylhydantoin and phenobarbitone were evaluated for their ability to modify penicillin G seizures in groups of 10 mice. One h before penicillin G (2·0 mg), animals were pretreated (s.c.) with diphenylhydantoin. Doses of 100–150 mg kg^{-1} did not alter penicillin's convulsant activity, whereas 200 mg kg^{-1} intensified and prolonged the duration of antibiotic seizures. Phenobarbitone, 50–125 mg kg^{-1} s.c., injected 2 h before central administration of penicillin G, has no protective action. These results are in agreement with those of Backus & Millichap (1963).

Leptazol-potentiated penicillin seizures

In an attempt to enhance the intensity of the penicillin-induced seizures, and yet limit the volume of centrally administered antibiotic, leptazol was administered in a non-convulsive dose (40 mg kg^{-1}, s.c.) 15 min before the antibiotic. At this time interval, antibiotic seizures were maximally potentiated. The effects of the leptazol-antibiotic interaction on seizure activity are presented in Fig. 1.

The magnitude of leptazol potentiation was not consistent, either among antibiotics or among the different doses of the same antibiotic. Evaluation of the relative molar potencies of compounds eliciting a grade 3 seizure pattern reveal that penicillin G was most toxic ($0{\cdot}50 \times 10^{-6}$ mol) and ampicillin least active ($3{\cdot}8 \times 10^{-6}$ mol). At a fixed dose of 2·0 mg of each antibiotic, after leptazol pretreatment, penicillin G produced the most intense seizure pattern (4·42) and ampicillin the least (3·37).

Recent studies with the squid stellate ganglion suggest that penicillin's convulsant activity may be the result of a drug-facilitated excitatory synaptic coupling within a pre-existing positive recurrent feed-back system (Ayala, Spencer & Gumnit, 1971). The mechanism underlying the differential neurotoxicity among the penicillin derivatives and cephaloridine in the present study remains to be elucidated. Because of the well-established neurotoxicity of penicillin G in man, only very small doses are recommended for intrathecal administration (Weinstein, 1970). We have observed ampicillin to be about one-fifth as toxic as penicillin G but its potential for the safe and effective treatment of cns infections requires further investigation.

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