Relationship between Structure and Convulsant Properties of Some β-Lactam Antibiotics following Intracerebroventricular Microinjection in Rats

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Received 24 March 1994/Returned for modification 24 June 1994/Accepted 10 October 1994

The epileptogenic activities of several β-lactam antibiotics were compared following their intracerebroventricular administration in rats. Different convulsant potencies were observed among the various β-lactam antibiotics tested, but the epileptogenic patterns were similar. The patterns consisted of an initial phase characterized by wet-dog shakes followed by head tremor, nodding, and clonic convulsions. After the largest doses of β-lactam antibiotics injected, clonus of all four limbs and/or the trunk, rearing, jumping, falling down, escape response, transient tonic-clonic seizures, and sometimes generalized seizures were observed, followed by a postictal period with a fatal outcome. At a dose of 0.033 μmol per rat, cefazolin was the most powerful epileptogenic compound among the drugs tested. It was approximately three times more potent than benzylpenicillin in generating a response and much more potent than other cephalosporins, such as ceftriaxone, cefoperazone, and cefamandole. No epileptogenic signs were observed with equimolar doses of cefotaxime, cefonicid, cefixime, and ceftizoxime in this model. The more convulsant compounds (i.e., cefazolin and cefazol) are both characterized by the presence of a tetrazole nucleus at position 7 and show a marked chemical similarity to pentyleneetetrazole. Imipenem and meropenem, the two carbapenems tested, also showed epileptogenic properties, but imipenem was more potent than meropenem, with a convulsant potency similar to those of ceftazol and benzylpenicillin. In addition, the monobactam aztreonam possessed convulsant properties more potent than those of cefoperazone and cefamandole. This suggests that the β-lactam ring is a possible determinant of production of epileptogenic activity, with likely contributory factors in the substitutions at the 7-aminocephalosporanic or 6-aminopenicillanic acid that may increase or reduce the epileptogenic properties of the β-lactam antibiotics. While the structure-activity relationship was also investigated, there seem to be no convincing correlations among the rank order of lipophilicities and the convulsant potencies of the compounds studied. The lack of marked convulsant properties of cefixime, cefonicid, cefuroxime, and cephradine suggests that these antibiotics may interact with a binding site which is different from that by which the β-lactam antibiotics exert their convulsant effects or may demonstrate a reduced affinity for the relevant site(s).

β-Lactam antibiotics (i.e., penicillins, cephalosporins, carbapenems, and monobactams) are antibiotics widely used in clinical practice because of their high antibacterial activity (20, 21). The administration of β-lactam antibiotics into the cerebral ventricles or subarachnoid space or directly to the cerebral cortex of chicks, cats, dogs, monkeys, mice, and rats has been reported to induce a focal or generalized epileptic state initially characterized by spike and wave discharges which are followed by clear tonic-clonic or clonic seizures (5, 7, 12, 13, 22, 23, 26, 29). More recently, seizure activity caused by imipenem-cilastatin has been also documented (11, 28, 30).

The convulsant action of penicillins and cephalosporins has been attributed to the inhibition of the γ-aminobutyric acid (GABA) system (1, 4, 5). The structural similarity of penicillin to the γ-aminobutyric acid antagonist bicuculline is of interest (3), since decreased inhibition in the hippocampus induced by penicillin contributes greatly to the epileptogenicity of penicillin (8).

The cephalosporins are a family of β-lactam antibiotics that contain the 7-aminopenicillosporanic acid nucleus. They differ in their basic structure from the penicillins in that the cephalosporins contain a six-membered dihydrothiazine ring instead of a five-membered thiazolidine ring fused to the β-lactam portion. The cephalosporins resemble penicillins in their actions as both antibiotics and convulsant compounds (27). Several investigators found that the ability of penicillin derivatives to produce a seizure focus was abolished after incubation with penicillinase (12, 15, 16). Since these studies support the idea that the β-lactam ring is an indispensable structural feature for epileptogenic activity of penicillins and cephalosporins, we decided to include in our study aztreonam, a monobactam, as well as two carbapenem compounds, imipenem and meropenem, and Ro 23-9424, made up of desacytetcemoxime linked to fleroxacin (14). In order to make a more accurate comparison of the convulsant activities of different β-lactam antibiotics, all compounds were administered intracerebroventricularly (i.c.v.). This route was utilized to reduce the differences in penetration of the central nervous system and in the distribution and rate of metabolism noted among the different β-lactam antibiotics when they are administered systemically (20). This route of administration does not eliminate the differences absolutely but does greatly reduce them.

In the present report, the convulsant effects of some new
Cephalosporins, penicillins, and carbapenems and a monobactam (Fig. 1 to 4) after acute injection into a lateral ventricle in freely moving rats are described. The results are compared and discussed with particular attention paid to the structure-activity relationship and the importance of lipophilicity.

MATERIALS AND METHODS

Animals and surgery. Adult male Wistar rats (200 to 250 g) were stereotaxically implanted with stainless steel guide cannulae under chloral hydrate anaesthesia, according to the atlas coordinates of Paxinos and Watson (25), to permit injection of drug into a lateral cerebral ventricle. After surgery, a minimum of 48 h was allowed for recovery before experiments were carried out. Freely moving rats were microinjected (0.2 \( \mu l/min \)) with an injector cannula which extended at least 1 mm below the tip of the guide cannula. The animals were placed individually in transparent cages (40 by 40 by 30 cm) and allowed to acclimatize to the new environment for 30 min. Preliminary studies to ascertain the influence of phosphate buffer solution (67 mM) in a volume of 2 or 6.6 \( \mu l \) injected intraventricularly showed no significant behavioral changes. A volume of 6.6 \( \mu l \) was used only when it was necessary to inject 3.3 \( \mu mol \) of some \( \beta \)-lactam antibiotics (i.e., cefonicid, cefuroxime, ceftizoxime, cefradine, ceftizoxime, piperacillin, meropenem, and Ro 23-9424).

The seizure response after microinjection of cephalosporins, penicillins, aztreonam, and carbapenems was graded according to the following scale: 0, normal behavior; 1, hyperkinesia, wet-dog shake, and sniffing; 2, head tremor and nodding; 3, clonus of one limb; 4, clonus of all four limbs and/or the trunk; 5, clonus of forelimbs, rearing, and falling; 6, transient tonic-clonic seizures and escape response; 7, generalized seizures followed by postictal period with a fatal outcome.

Lipophilicity measurements. The relative lipophilicity of the compounds was measured by reversed-phase thin-layer chromatography by a previously described method (2). Briefly, silanized silica gel plates (Merck 60 F254) were used for the nonpolar stationary phase. The polar mobile phase was a 30:70 (vol/vol) mixture of acetone and phosphate buffer at pH 7. Each compound was dissolved in chloroform (3.0 mg/ml), and 5 \( \mu l \) of solution was applied to the plate. The experiments were repeated five times with different dispositions of the compounds.
pounds on the plate. \( R_f \) values were expressed as the mean values of the five determinations. Lipophilicities (\( R_m \) values) were calculated from the experimental \( R_f \) values according to the formula

\[
R_m = \log \left( \frac{1}{R_f} \right) - 1
\]

Higher \( R_m \) values indicate greater lipophilicities.

**Statistical analysis.** The results of the treatment were analyzed statistically by nonparametric methods. A Kruskall-Wallis analysis of variance was first carried out, and if the result was significant, a Mann-Whitney U test was used to compare control and drug-treated animals. The convulsant doses required to induce clonic seizures (phase 3) in 50% of rats (CD50s) microinjected with \( \beta \)-lactam antibiotics and the relative confidence limits were determined by the method of Litchfield and Wilcoxon (19). At least 32 animals were used to calculate each CD50.

**Drugs.** The following drugs were used: benzylpenicillin, cephazolin, and cefitoxime (Farmitalia Carlo Erba Laboratories, Milan, Italy); cefotaxime and ceftotaxone (Schering, Milan, Italy); cefamandole (Eli Lilly & Co., Sesto Fiorentino, Florence, Italy); azlocillin and mezlocillin (Bayer Italia, Milan, Italy); cefonicid (I.S.F. Laboratories, Trezzano S/N Milan, Italy); ceftazidime (Sigma Tau Laboratories, Pomezia, Italy); cefoxitin and imipenem (Merck, Sharp & Dohme, Rome, Italy); cefotaxime (Hoechst, Frankfurt, Germany); piperacillin (Cyanamid Research Laboratories, Catania, Italy); ceferazone, aztreonam, and ce-

dinimicillin (Menarini Research Laboratories, Florence, Italy); sulbenicillin (Bracco S.p.A., Milan, Italy); amoxicillin (Zambeletti, Milan, Italy); ampicillin (Proter, Milan, Italy); ceftriaxone (Glaxo, Verona, Italy); Ro 23-9424 (Hoffmann-La Roche, Nutley, N.J.); cephradine (Hoffmann-La Roche, Basel, Switzerland); cephamet (Squibb, Rome, Italy); and meropenem trihydrate (Ici-Pharma S.p.A., Milan, Italy).

All drugs with the exceptions of ceftazidime, cephradine, aztreonam, imipenem, and meropenem are sodium salts. Drugs in powder form were dissolved in phosphate buffer solution (67 mM) and injected at pH 7.3 to 7.4.

**RESULTS**

**Behavioral epileptic changes.** The epileptogenic properties of \( \beta \)-lactam antibiotics varied according to the types of substituents present on the basic structures. The behavioral seizures consisted of an initial phase characterized by wet-dog shake episodes followed by head tremor, nodding, and clonus of limbs.

The test animals responded to the highest doses of \( \beta \)-lactam antibiotics with clonus of all four limbs and/or the trunk, rearing, jumping, falling down, transient tonic-clonic seizures, escape response, and generalized seizures followed by a postictal period with a fatal outcome.

All epileptic signs were dose related and were observed repeatedly, interrupted only with short pauses. Generally, the first behavioral signs were seen within 1 min after the infusion and the maximum effect was reached after a minimum lag time of 9 min after the injection. Data from dose-response curves of the epileptogenic effects induced by microinjection of various penicillins, cephalosporins, carbapenems, and aztreonam into the lateral cerebral ventricle are presented in Fig. 5 through 9 and Tables 1 and 2.

**Comparison of convulsant properties of penicillins, carbapenems, and aztreonam.** As shown in Fig. 5 and 6 and Table 1, benzylpenicillin was the most potent convulsant among the penicillins but was two to four times less potent than cefazolin, the most convulsant \( \beta \)-lactam antibiotic tested. The activity of benzylpenicillin was similar to that of imipenem, and the drug was more potent than aztreonam, ampicillin, meropenem, amoxicillin, mezlocillin, piperacillin, azlocillin, and sulbenicillin in inducing epileptic behavior. Of the two carbapenem
derivatives, imipenem was 4.4 times more potent than meropenem (Fig. 6 and Table 1).

Comparison of convulsant properties of cephalosporins. As shown in Fig. 7 through 9 and Table 2, cefazolin induced the strongest activity of all the drug tested. In addition, ceftezole, cefamandole, cefotetan, ceftriaxone, cefotaxime, ceftriaxone, cefotaxime, cefoxitin, cefuroxime, cephradine, cephradine, cefixime, and cefonicid, in this order, were less potent than cefazolin in inducing epileptic behavior (Table 2). By contrast, very high doses of up to 1.65 or 3.3 μmol of cefotaxime, ceftizoxime, and cefoxitin per rat were necessary in order to produce clonus of forelimbs, rearing, falling down, or transient tonic-clonic seizures and the escape response. No clear signs of behavioral seizures were noted even after administration of 1.0 and 3.3 μmol of cefuroxime, cephradine, cefixime, and cefonicid. In addition, Ro 23-9424 was also less convulsant than cefotaxime.

Physicochemical parameters. The relative lipophilicities

![TABLE 1. Convulsant doses and relative lipophilicities of various penicillins and other β-lactam derivatives after i.c.v. administration in rats](image)

**TABLE 2. Convulsant doses and relative lipophilicities of various cephalosporins and Ro 23-9424 after i.c.v. administration in rats**

![FIG. 6. Dose-response curves of seizure intensity score determined from behavioral changes after i.c.v. injection of β-lactam derivatives (see Materials and Methods for grading). □, azlocillin; ○, piperacillin; ■, imipenem; ▲, mezlocillin; ▲, meropenem.](image)

![FIG. 7. Dose-response curves of seizure intensity score determined from behavioral changes after i.c.v. injection of β-lactam derivatives (see Materials and Methods for grading). ▲, cefazolin; ●, cefoperazone; △, cefuroxime; ○, cephradine; ■, Ro 23-9424.](image)

![FIG. 8. Dose-response curves of seizure intensity score determined from behavioral changes after i.c.v. injection of β-lactam derivatives (see Materials and Methods for grading). ▲, cefazolin; ●, cefoperazone; △, cefuroxime; ○, cephradine; ▲, meropenem.](image)

![FIG. 9. Dose-response curves of seizure intensity score determined from behavioral changes after i.c.v. injection of β-lactam derivatives (see Materials and Methods for grading). ▲, cefazolin; ●, cefoperazone; △, cefuroxime; ○, cephradine; ▲, meropenem.](image)

DISCUSSION

Previous studies have indicated that systemic administration of cephalosporin to experimental animals produces epileptogenic effects (15, 16, 18, 22), and the present results further confirm these findings.

The present study used the i.c.v. route of administration to assess the convulsant effects of β-lactam antibiotics and assumed that the site of drug action is sufficiently close to the ventricular lumen that it may easily be reached by the drug. In fact, following microinjection of the highest doses, the first convulsant signs were observed within 1 min. In addition, the

(Rμs) and molecular weights of these β-lactam derivatives are summarized in Tables 1 and 2.
The present data clearly display a dose-dependent effect for several of the β-lactam antibiotics tested. Indeed, i.c.v. administration diminishes the roles played by peripheral metabolism, plasma protein binding, and drug penetration of the brain. Moreover, as the dose-response curves reflect the maximum effect but not the seizure duration, it is unlikely that the marked effects observed with some of the compounds could be attributed to their differential clearance. At the most, the latter point could be responsible for the long-lasting convulsant effects observed with some compounds.

In the present study, all β-lactam antibiotics except cefonicid, cefixime, ceftroxime, and cephradine showed clear epileptogenic activity. Cefazolin appeared the most convulsant of the compounds tested. In fact, cefazolin was two to four times more potent than benzylpenicillin. The substitution of a benzylidene hydrogen in the structure of benzylpenicillin with a sulfonic group (subbenicillin) or of an amino group (amoxicillin and amoxycillin) produces a marked reduction in convulsant potency. Azlocillin, mezlocillin, pipercillin, and cefoperazone, which are all characterized by the presence of a ureido group, showed epileptogenic activities which were similar but less than those of benzylpenicillin and cefazolin. Imipenem and meropenem, which differ structurally from other β-lactam classes by a carbapenem nucleus, also possess convulsant properties, but their epileptogenic potencies differed. In fact, the potency of imipenem appeared similar to that of ceftazidime, whereas the potency of meropenem was similar to that of cefoperazone. These findings are in agreement with the results of previous investigators (9, 24). Although meropenem is chemically related to imipenem, there are differences at position 2, which may account for the observed differences in convulsant liability. Aztreonam, which is characterized by having the only β-lactam ring with a heterocyclic ring at position 3, is much less convulsant than imipenem. The data suggest that the β-lactam ring is an important contributor to the epileptogenic properties observed with the β-lactam antibiotics.

Among the cephalosporins tested, compounds having a heterocyclic ring at position 3 and a (hetero)aromatic nucleus at position 7 of 7-aminocephalosporanic acid, such as cefotaxime, ceftazidime, cefotetan, cefamandole, and ceftaxone, showed some evidence of epileptogenic activity but less marked than that shown by benzylpenicillin. Only ceftazidime showed a potency more or less comparable to that of benzylpenicillin.

Compounds characterized by having a heterocyclic ring at position 7 of 7-aminocephalosporanic acid, such as cephadine, ceftoxime, cefoxitin, and cefixime, showed no epileptic activity, whereas cefotaxime and ceftizoxime demonstrated weak epileptic activity. Also, Ro 23-9424 showed very weak epileptogenic activity; it was 1.4 times less potent than cefotaxime.

Cefazolin and ceftazidime, which have a tetrazole ring at position 7, show a marked similarity to pentylenetetrazole, a well-known convulsant drug. Such a tetrazole group could be responsible for the greater convulsant activity of these two cephalosporins in comparison with those of the other antibiotics. In addition, it is interesting that cefamandole, cefoperazone, and cefotetan, which possess a tetrazole nucleus at position 3, showed weaker epileptogenic activity than cefazolin and ceftizole. Thus, it is reasonable to presume that the presence of a tetrazole ring at position 7 in cephalosporins is more effective in increasing the convulsant potency than the same substitution at position 3, as in part previously described by Kamei and coworkers (18). It is also interesting that cefonicid, which differs from cefamandole only by the presence of a sulfonic group on the tetrazole nucleus, showed no convulsant effect.

Ceftizoxime, cefotaxime, Ro 23-9424, ceftiraxone, cefixime, and ceftazidime are all characterized by the presence of a (2-aminothiazolyl)methoxyiminomoiety. However, the various degrees of convulsant activity of these antibiotics indicate that this group is not responsible for the observed effects.

There are at least two possible explanations for the lack of convulsant properties of cefixime, cefonicid, ceftroxime, and cephradine. First, perhaps these compounds cannot reach the site to induce convulsions; however, this is not very likely, inasmuch as the i.c.v. route of administration was utilized. In addition, these cephalosporins have, as mentioned above, structural characteristics different from those of the other β-lactam antibiotics which possess convulsant properties.

The second possibility is that these compounds may reach

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**FIG. 8.** Dose-response curves of seizure intensity score determined from behavioral changes after i.c.v. injection of β-lactam derivatives (see Materials and Methods for grading). △, ceftizole; ○, cefamandole; ▽, cefotetan; □, cefoxitin; ●, cefonicid.

**FIG. 9.** Dose-response curves of seizure intensity score determined from behavioral changes after i.c.v. injection of β-lactam derivatives (see Materials and Methods for grading). ■, ceftiraxone; ○, ceftazidime; △, cefotaxime; □, ceftizoxime; ●, cefixime.
some binding sites which are not the same as those by which the β-lactam antibiotics exert their convulsant effects. This leads to the suggestion that the β-lactam antibiotics may bind different sites which may or may not be responsible for epileptogenic activity in the brain. Though not very likely, this is an interesting idea that, if confirmed, may trigger a search for new β-lactam antibiotics without convulsant effects.

It should be noted that the failure to find an absolute correlation between the rank order of lipophilicity and the convulsant activities of these compounds may be due to several important possibilities. These include differences among β-lactam antibiotics in diffusion out of the cerebral ventricles and differences in mechanisms for producing convulsant effects. In regard to the first possibility, the different degrees of diffusion from the i.c.v. injection site were not measured for the β-lactam antibiotics examined. If such differences exist, they could account for the lack of correlation between lipophilicity and convulsant properties. In regard to the second possibility, we emphasize that β-lactam antibiotics could exert their convulsant effects by more than a simple interaction with the GABA receptor complex (3, 8). In fact, the convulsant actions of β-lactam antibiotics have been related to the reduction of GABA binding to its receptor sites (1, 4, 5, 17). However, the fact that compounds such as cefazolin, cefetazole, cefonicid, and β-lactam antibiotics have been related to the reduction of these effect by more than a simple interaction with the GABA receptor complex (3, 8). In fact, the convulsant actions of β-lactam antibiotics have been related to the reduction of GABA binding to its receptor sites (1, 4, 5, 17). However, the fact that compounds such as cefazolin, cefetazole, cefonicid, and cefixime, which possess very similar lipophilicities (R₂₃₀₆₈), showed different degrees of convulsant activity suggests the importance of other parameters. The concentrations of β-lactams able to interact with the GABA system are rather high and varied among the different β-lactam antibiotics but fit well in the concentration range used to demonstrate their convulsive or GABA-antagonistic properties in vitro (1, 15). Thus, it appears questionable whether a specific interaction of β-lactam antibiotics with GABA receptors alone can explain the convulsant activity of these compounds. An alternative mechanism, involving the increase of excitatory amino acid release in seizures induced by penicillin in cats, was suggested by Van Gelder et al. (29). Recently, we have demonstrated that some excitatory amino acid antagonists are able to counteract the seizures induced by imipenem, suggesting an involvement of excitatory amino acids in the genesis of seizures induced by this carbapenem derivative (6).

In conclusion, several factors, such as variable lipophilicity and differences in diffusion of these compounds, together with the pharmacodynamic mechanisms mentioned above deserve to be considered in explaining the convulsant properties of the various β-lactam antibiotics.

ACKNOWLEDGMENTS

Financial support from the Italian Ministry of University and Scientific and Technological Research (MURST) and the Italian Council for Research (CNR, Rome) is gratefully acknowledged.

We thank Antonino Giacopello for his skillful technical assistance.

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