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Intracerebroventricular injection of the antibiotic cefoselis produces convulsion in mice via inhibition of GABA receptors

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Abstract

A majority of β-lactam antibiotics (e.g., cephalosporins and penicillins) have convulsive activity to a greater or lesser extent. (6*R*,7*R*)-3-{[3-Amino-2-(2-hydroxyethyl)-2*H*-pyrazol-1-ium-1-yl]methyl}-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetylamino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate monosulfate (cefoselis), a newly developed injectable β-lactam antibiotic of fourth generation, might induce convulsions if cerebral concentrations become highly elevated. In the present study, we examined whether or not cefoselis had convulsive activity after direct brain administration, and we attempted to clarify the pharmacological mechanism of action. When cefoselis was injected into the lateral ventricle of the mouse brain at doses higher than 20 μg/animal, it produced convulsions dose-dependently. Cefoselis (50 μg/animal)-induced convulsions were prevented by pretreatment with 5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (MK-801), diazepam and phenobarbital (ED_{50} values (mg/kg) of 0.78, 1.59 and 33.0, respectively), but not by carbamazepine or phenytoin. When the effects of these anticonvulsants on the convulsions induced by intracerebral injection of bicuculline methiodide (BMI) or *N*-methyl-D-aspartate (NMDA) were investigated, the inhibitory profile of anticonvulsants on cefoselis-induced convulsions was similar to those induced by BMI (125 ng/animal) but differed markedly in their inhibitory activity on NMDA (100 ng/animal)-induced convulsions, which were not inhibited by diazepam. These results suggest that cefoselis may be convulsive at higher concentrations through a mechanism involving inhibition of γ-aminobutyric acid $(GABA)_A$ receptors.

Keywords: Cefoselis; Convulsion; GABA; NMDA; Mice

1. Introduction

Antibiotics have been used for the treatment of a variety of infectious diseases because of their wide-ranging and potent antimicrobial activity. However, overdosing or misuse of β-lactam antibiotics produces diverse adverse events, the most serious being seizures (Schliamser et al., 1991). When the cephalosporin antibiotic cefazolin was administered intravenously to patients with impaired renal function, its serum and cerebrospinal fluid concentrations became excessive and subsequently convulsions were observed (Yost et al., 1977; Bechtel et al., 1980). In addition to these clinical observations, experimental evidence has accumulated to suggest that certain cephalosporins have epileptogenic activity in animal models (Yu et al., 1984; De Sarro et al., 1989). Recent reports that carbapenems (e.g., imipenem and panipenem) and cephalosporins (e.g., cefazolin and cephaloridine) inhibit γ-aminobutyric acid (GABA) receptor binding (Shimada et al., 1992) and that β-lactams inhibit GABA-induced Cl^- currents in a concentration-dependent manner (Fujimoto et al., 1995), suggest that both classes of antibiotics might induce convulsions through the inhibition of central GABA receptors.

(6*R*,7*R*)-3-{[3-Amino-2-(2-hydroxyethyl)-2*H*-pyrazol-1-ium-1-yl]methyl}-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-methox-

Abbreviations: BMI, bicuculline methiodide; GABA, γ-aminobutyric acid; NMDA, *N*-methyl-D-aspartate; PCG, penicillin G.

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yiminoacetylamino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate monosulfate (cefoselis) was recently developed as an injectable antibiotic of a fourth-generation parenteral cephalosporin with antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) (Mine et al., 1993a,b). We have recently demonstrated, using electrophysiological and receptor binding studies, that cefoselis competitively inhibits the $GABA_A$ receptor in vitro (Sugimoto et al., 2002). Given this evidence, the drug could exhibit convulsive activity if cerebral concentrations become highly elevated due to high plasma concentrations or disruption of the blood–brain barrier.

Therefore, the aim of the present study was twofold; (1) to investigate whether or not cefoselis exhibits convulsive activity when directly administered into the brain; and (2) to clarify the mechanisms by which cefoselis may produce convulsions. To address these aims, we examined the convulsive potential of cefoselis after intracerebroventricular (icv) injection in mice. To determine the mechanism of cefoselis-induced convulsions, we examined the interaction of cefoselis with anticonvulsive agents possessing different mechanisms of action: diazepam (a benzodiazepine); MK-801 (dizocilpine, an *N*-methyl-D-aspartate (NMDA) receptor antagonist); phenobarbital (a barbiturate potentiating $GABA_A$ function); and carbamazepine and phenytoin (weak Na^+ channel blockers). We then compared the ability of these anticonvulsants to block convulsions induced by the $GABA_A$ receptor antagonist bicuculline methiodide (BMI) and by NMDA, a glutamate NMDA receptor agonist.

2. Materials and methods

2.1. Animals

Male ICR mice (6–7 weeks) were purchased from Nihon SLC (Shizuoka, Japan). Animals were housed at 23 ± 2 °C with humidity of 55 ± 5% under a 12-h light/dark cycle (lights on at 07:00 h) and were allowed access to food and water ad libitum. The head skin of mice was removed under light anesthesia with halothane. They were returned to their home cage and recovered from anesthesia within 10 min after the removal of head skin. At least 2 h after this procedure, experiments were begun. All animal procedures were carried out as approved by the Animal Care and Use Committee at Fujisawa Pharmaceuticals.

2.2. Drugs

Cefoselis was synthesized by Fujisawa Pharmaceutical (Osaka, Japan). 5-Methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]-cyclohepten-5,10-imine ((+)MK-801, dizocilpine) was purchased from RBI (Massachusetts, USA), and BMI, NMDA and carbamazepine were purchased from Sigma (Missouri, USA). Diazepam, phenobarbital and phenytoin were purchased from Yamanouchi Pharmaceutical (Tokyo, Japan), Sankyo (Tokyo, Japan) and Dainippon Pharmaceutical (Osaka, Japan), respectively.

2.3. Convulsive activities of cefoselis, BMI and NMDA

Cefoselis was dissolved in phosphate-buffered solution (in mM; KCl 2.67, KH_2PO_4 1.47, NaCl 138, Na_2HPO_4 8.1) without Ca^{2+} and Mg^{2+} (PBS(−)) using equimolar Na_2CO_3 as a solubilizing agent (pH 6.2–7.5). Cefoselis (5–100 μg/animal) was injected unilaterally into the lateral ventricle in conscious state by use of a procedure similar to that described by Haley and McCormick (1957). The injection needle (external diameter of 0.52 mm) of a Hamilton syringe fitted with a stainless stop to attain a depth of 2.0 mm was placed to the surface of the skull. The coordinates for microinjections were 2.0 mm lateral from the bregma, and total injection volume was 5 μl. Animals were observed for 60 min following intracerebroventricular injection to determine convulsive activity. Seizure episodes observed were as follows: wild running, jumping, clonic convulsions, tonic hindlimb extension and eventually death. Similarly, BMI was also dissolved in PBS(−) and was administered intracerebroventricularly at a dose of 15.625–250 ng/animal in a total volume of 5 μl and convulsions were observed for periods of 30 min. For NMDA-induced convulsions, the animals were treated with intracerebroventricular injection of NMDA dissolved in PBS(−) at a dose of 10–200 ng/animal in a volume of 5 μl, and they were observed for periods of 10 min.

2.4. Effects of anticonvulsants against cefoselis, BMI or NMDA-induced convulsions

Anticonvulsants were administered intraperitoneally 30 min prior to intracerebroventricular injection of cefoselis (50 μg/animal), BMI (125 ng/animal) or NMDA (100 ng/animal). Animals were observed for 60 min following intracerebroventricular injection for cefoselis, 30 min for BMI, or 10 min for NMDA for presence or absence of seizure. Antagonism by anticonvulsants was defined as complete inhibition of any of convulsive episodes. The pretreatment times and dose ranges of anticonvulsants were determined based on our preliminary studies and earlier studies by others (Kehne et al., 1992; Gasior et al., 1997; Fariello et al., 1998; Członkowska et al., 2000).

2.5. Data analysis

Dose–response relationships for each treatment were assessed by changes in the percentage of animals showing seizures. The CD_{50} values with 95% confidence limits

(CL) for convulsants and ED_{50} values with their 95% CLs for anticonvulsants on drug-induced convulsions were calculated by probit analysis (SAS software; SAS Institute, North Carolina, USA) (Fariello et al., 1998; Day et al., 1995). For statistical comparison of convulsion incidence between the control group and drug-treated group, Fisher's exact probability test was used. A P value less than .05 was considered statistically significant.

3. Results

3.1. Convulsive activities of cefoselis, BMI and NMDA

Cefoselis at doses of 5–100 μg/animal was injected into the lateral ventricle of mice brains. Whereas no convulsions were observed at doses of 5 and 10 μg/animal, 20 μg/animal induced convulsions. When cefoselis was administered at doses of 50 and 100 μg/animal, convulsions were observed in all animals, indicating that cefoselis induced convulsions in a dose-dependent manner (Table 1). Rolling, wild running and clonic type of convulsions were typically observed 2–3 min after the injection of cefoselis, and, thereafter, seizures increased in severity and eventually progressed to tonic convulsions and death. The CD_{50} value, the dose to produce convulsions in 50% of animals, for cefoselis-induced convulsion estimated by probit analysis was 20.4 μg/animal (95% CL = 17.9–23.5 μg/animal).

Similarly, both BMI (15.625–250 ng/animal icv) and NMDA (10–200 ng/animal icv) evoked convulsions in a dose-dependent manner (Table 1). Seizure episodes induced by BMI were similar to those by cefoselis: rolling, wild running and chronic convulsions followed by tonic convulsions were observed. In contrast, NMDA injection induced jumping and tonic convulsions. The CD_{50} value for BMI-induced convulsion estimated by probit analysis was 65.5 ng/animal (95% CL = 57.1–74.4 ng/animal). The CD_{50} value for NMDA-induced convulsion was calculated to be 50.1 ng/animal (95% CL = 42.6–57.7 ng/animal). The injection of PBS(−), a vehicle, in a volume of 5 μl, did not produce any abnormal behaviors including seizures.

For subsequent interaction studies, the minimum convulsant dose that produced convulsions in 100% of the animals was selected to standardize the intensity of convulsions. Thus, Cefoselis was administered at 50 μg/animal, BMI at 125 ng/animal and NMDA at 100 ng/animal to evaluate the effects of anticonvulsants.

Table 1
Incidence of convulsions following intracerebral administration of cefoselis, BMI or NMDA in mice

Cefoselis		BMI		NMDA	
μg/animal	Incidence (%)	ng/animal	Incidence (%)	ng/animal	Incidence (%)
5	0/20 (0)	15.625	0/10 (0)	10	0/10 (0)
10	0/20 (0)	31.25	0/10 (0)	20	0/10 (0)
20	12/20* (60)	62.5	3/10* (30)	50	3/10* (30)
50	20/20* (100)	125	10/10* (100)	100	10/10* (100)
100	20/20* (100)	250	10/10* (100)	200	10/10* (100)

Cefoselis, BMI or NMDA was administered intracerebroventricularly at the doses indicated in a total volume of 5 μl. Animals were observed for 60 min following intracerebroventricular injection for cefoselis (50 μg/animal), 30 min for BMI (125 ng/animal) or 10 min for NMDA (100 ng/animal) to determine if there was any convulsive activity. Each value represents the number of mice showing convulsions/total numbers of mice employed for each group (n = 20 per data point for cefoselis and n = 10 for BMI or NMDA). Numbers in parentheses indicate the percentage of animals showing convulsions.

* P < .05, statistically significant compared with vehicle control group, in which none of 20 animals showed convulsions following i.c.v. injection of PBS(−) buffer (by Fisher's exact probability test).

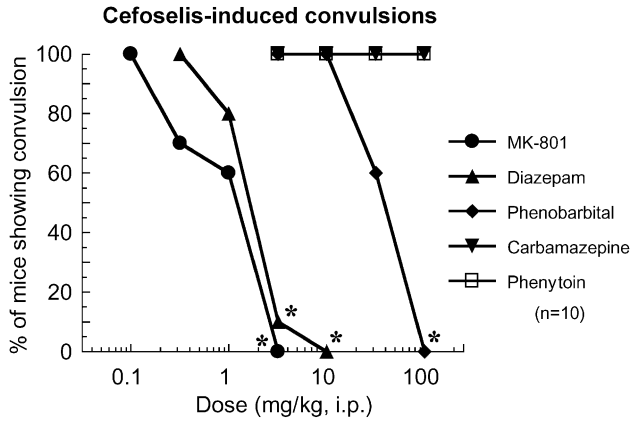

Fig. 1. Efficacy of anticonvulsants to prevent cefoselis-induced convulsions. Anticonvulsants were administered intraperitoneally, and 30 min later, cefoselis was administered intracerebroventricularly at a dose of 50 μg/animal. Inhibition of convulsive activity was observed for 60 min following cefoselis administration. Each data point represents the percentage of convulsions induced in each group of mice (n = 10 mice per data point). * P < .05, statistically significant compared with each corresponding control group (by Fisher's exact probability test).

3.2. Efficacy of anticonvulsants to prevent cefoselis-, BMI- or NMDA-induced convulsions

Administration of either MK-801 (0.1, 0.32, 1, 3.2 mg/kg ip), diazepam (0.32, 1, 3.2, 10 mg/kg ip) or phenobarbital (3.2, 10, 32, 100 mg/kg ip) 30 min prior to intracerebroventricular injection of cefoselis (50 μg/animal) produced a dose-dependent reduction in cefoselis-induced convulsions (Fig. 1). MK-801, diazepam and phenobarbital completely prevented cefoselis-induced convulsions at the highest doses tested. Calculated ED_{50} values were 0.78 (95% CL = 0.46–1.44), 1.59 (95% CL = 1.02–2.50) and 33.0 (95% CL = 26.4–40.9) mg/kg ip, respectively (Table 2). In contrast, pretreatment with carbamazepine or phenytoin scarcely affected cefoselis-induced convulsions up to doses of 100 mg/kg.

Table 2
Anticonvulsants ED_{50} values for preventing cefoselis-, BMI- and NMDA-induced convulsions

Anticonvulsant	ED_{50} value (mg/kg)		
	Cefoselis	BMI	NMDA
MK-801	0.78 (0.46–1.44)	0.45 (0.23–0.80)	0.71 (0.45–1.14)
Diazepam	1.59 (1.02–2.50)	1.79 (1.07–3.00)	>10
Phenobarbital	33.0 (26.4–40.9)	28.2 (22.8–35.6)	73.9 (59.8–102)
Carbamazepine	>100	>100	89.4 (69.1–142)
Phenytoin	>100	>100	>100

ED_{50} value is the dose of anticonvulsant required to prevent 50% of convulsions induced by cefoselis, BMI or NMDA. The ED_{50} values were estimated by probit analysis and were presented with 95% confidence limits in parenthesis.

The inhibitory actions of anticonvulsants on BMI (125 ng/animal)-induced convulsions were comparable to cefoselis-induced convulsions (Fig. 2). The ED_{50} values were 0.45 mg/kg (95% CL = 0.23–0.80) for MK-801, 1.79 mg/kg (95% CL = 1.07–3.00) for diazepam and 28.2 mg/kg (95% CL = 22.8–35.6) for phenobarbital (Table 2). Similar to the results observed for cefoselis, pretreatment with carbamazepine or phenytoin did not inhibit BMI-induced convulsions up to doses of 100 mg/kg.

In contrast, the effects of anticonvulsants on NMDA (100 ng/animal)-induced convulsions were quite different from cefoselis- and BMI-induced convulsions (Fig. 3 and Table 2). While MK-801 protected against NMDA-induced convulsions with an ED_{50} value of 0.71 mg/kg (95% CL = 0.45–1.14), diazepam failed to suppress NMDA-induced convulsions up to doses of 10 mg/kg.

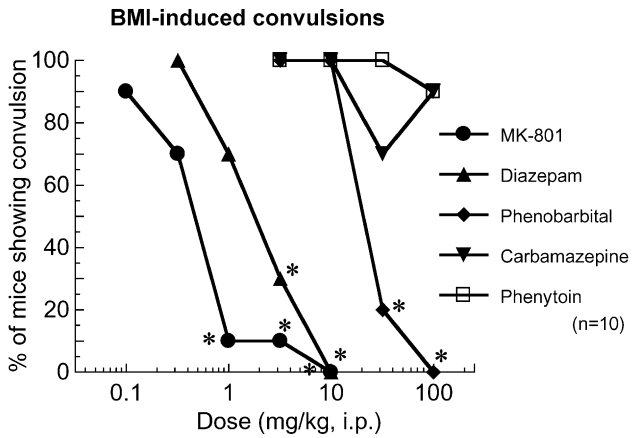

Fig. 2. Efficacy of anticonvulsants to prevent BMI-induced convulsions. Anticonvulsants were administered intraperitoneally, and 30 min later, BMI was administered intracerebroventricularly at a dose of 125 ng/animal. Inhibition of convulsive activity was observed for 30 min following BMI administration. Each data point represents the percentage of convulsions induced in each group of mice ($n = 10$ mice per data point). $*P < .05$, statistically significant compared with each corresponding control group (by Fisher's exact probability test).

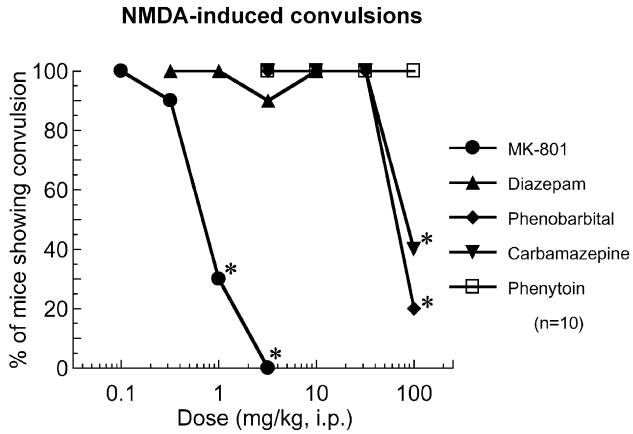

Fig. 3. Efficacy of anticonvulsants to prevent NMDA-induced convulsions. Anticonvulsants were administered intraperitoneally, and 30 min later, NMDA was administered intracerebroventricularly at a dose of 100 ng/animal. Inhibition of convulsive activity was observed for 10 min following NMDA administration. Each data point represents the percentage of convulsions induced in each group of mice ($n = 10$ mice per data point). $*P < .05$, statistically significant compared with each corresponding control group (by Fisher's exact probability test).

Phenobarbital and carbamazepine produced modest but statistically significant ($P < .05$ by Fisher's exact probability test) inhibition of NMDA-induced convulsions only at the highest dose (100 mg/kg ip).

4. Discussion

The present study clearly demonstrates that convulsions can be induced in mice following intracerebral injection of the novel antibiotic cefoselis. Data showing that convulsions can be induced by antibiotics in clinical use such as penicillin G (PCG) were reported as early as 1945 by Walker and Johnson. Since then, many studies have examined convulsions induced by PCG and β-lactam antibiotics in experimental animals (Kamei et al., 1991; Shimada et al., 1992; De Sarro et al., 1995). The present study provides the first evidence that cefoselis, one of a new generation of cephalosporin antibiotics, has similar intrinsic convulsive activity to other β-lactams (Shimada et al., 1992). When assessing the potential convulsive risk of these antibiotics in clinical use, consideration should be given to their convulsive activities in experimental animal studies. This should involve mechanistic studies in vitro, pharmacological studies in vivo and pharmacokinetic studies investigating drug distribution in the central nervous system (CNS). In this context, the method employed in the present study, where drug was injected directly into the brain, is a convenient screening method for convulsive potential, because it rules out the involvement of drug metabolism in peripheral organs, binding to serum protein and blood–brain barrier permeability, when compared to systemic drug administration. Cefoselis has been shown not to produce any convulsive activities at least in normal conscious mice and rats by

intravenous administration up to 1000 mg/kg and its permeability into the brain is quite low in normal rats and mice (unpublished observations). Given the present finding that intracerebral injections of cefoselis produces convulsions, attention should be given in its clinical use in situations where cerebral concentrations could become highly elevated. Further studies with animal models of compromised metabolism or blood–brain barrier permeability are warranted to clarify the clinical implications of cefoselis treatment in these patients.

Another important finding is that cefoselis-induced convulsions could be completely inhibited by pretreatment with anticonvulsive drugs, including the NMDA antagonist, MK-801, the benzodiazepine, diazepam, and the barbiturate, phenobarbital. In contrast, neither carbamazepine nor phenytoin could inhibit the convulsions. Thus, GABA and NMDA receptors could mediate this convulsive activity. Direct measurement of cefoselis-induced modulation of GABA or NMDA receptors using in vitro experimental models is one strategy being pursued in our laboratory. In the present study, however, we used an in vivo convulsion model to compare the pharmacological profile of cefoselis-induced convulsions with convulsions induced by the GABA antagonist, BMI, and the NMDA receptor agonist, NMDA.

Intracerebral injection of BMI dose-dependently induced convulsions in mice, in agreement with previously published findings on bicuculline and other $GABA_A$ antagonists such as picrotoxin (Clark and McMillian, 1990; Kehne et al., 1992; Fariello et al., 1998; Członkowska et al., 2000). $GABA_A$ antagonists are known to block GABA-induced Cl^- currents (Study and Barker, 1981; Barker et al., 1983; Akaike et al., 1985; Bormann and Clapham, 1985) and to depolarize the postsynaptic membrane potential (Jones, 1988; Hwa and Avoli, 1989). This depolarization leads to hyperexcitability of neurons in cortical and limbic areas, including the hippocampus, where seizure generating systems are located. Thus, both diazepam and phenobarbital potently prevented convulsions induced by BMI, as previously reported (Clark and McMillian, 1990; Kehne et al., 1992). In addition, BMI-induced convulsions were potently inhibited by MK-801, whereas carbamazepine and phenytoin had no effect. These findings are consistent with previous studies demonstrating that MK-801 showed potent anticonvulsive activities in bicuculline-induced convulsion models (Turski et al., 1990; Kehne et al., 1992; Fariello et al., 1998; Członkowska et al., 2000), whereas carbamazepine and phenytoin had little effect (Clark and McMillian, 1990; Fariello et al., 1998). Although BMI does not possess binding affinity for NMDA receptors, NMDA antagonists have been suggested to indirectly counteract bicuculline-induced convulsions (Turski et al., 1990). It has been reported that depolarization shifts and epileptiform bursts induced by application of bicuculline were sensitive to NMDA antagonists (Baldino et al., 1986; Jones, 1988; Hwa and Avoli, 1989). Furthermore, MK-801 markedly attenuates biochemical changes induced by bicuculline, such as phosphorylation of MAP kinase (Gass et al., 1993). It is conceivable that excessive activation of excitatory transmission mediated by NMDA receptors might occur secondary as consequence of impairment of the inhibitory GABAergic neurotransmission. Collectively, these findings suggest that NMDA receptors contribute indirectly to bicuculline-induced convulsions.

The effectiveness of anticonvulsants against NMDA-induced convulsions contrasted that observed against BMI-induced convulsions. Pretreatment with MK-801 completely prevented NMDA-induced convulsions, presumably via NMDA receptor blockade (Moreau et al., 1989; Laudrup and Klitgaard, 1993; Gasior et al., 1997). Interestingly, diazepam had no effect on NMDA-induced convulsions, in contrast to previous reports (Moreau et al., 1989). Although not entirely clear, possible underlying reasons for this discrepancy include differences in behavioral endpoints, the doses of NMDA administered, and in diazepam treatment protocols. Gasior et al. (1997) reported that diazepam attenuated NMDA-induced convulsions at a dosage higher than 10 mg/kg. We tried to investigate a higher dosage of diazepam (32 mg/kg) in our pilot study, however, the dose was found to be lethal due to its sedative actions and, therefore, in this context, it was a meaningless dose level. The finding that phenobarbital required almost two-fold ED_{50} value to prevent NMDA-induced convulsions compared to cefoselis- or BMI-induced convulsions partly support the failure of diazepam to inhibit NMDA-induced convulsions. Taken together, the lack of efficacy of diazepam on NMDA-induced convulsions leads us to conclude that GABA receptor-mediated responses are distinct from NMDA receptor-mediated responses.

Comparing anticonvulsant efficacies in three different convulsion models sheds further light on the mechanism of action of cefoselis-induced convulsions. Cefoselis behaved like BMI in terms of its sensitivity to anticonvulsants, suggesting that the convulsions induced by cefoselis are predominantly mediated by $GABA_A$ receptor blockade. The view was further supported by the fact that the form of convulsive episodes observed in animals treated with cefoselis was similar to that observed in BMI-injected animals rather than that in NMDA-treated ones. Dose-dependent and complete protection by diazepam and phenobarbital against cefoselis-induced convulsions agree with previous findings where β-lactam-induced convulsions were inhibited by diazepam and phenobarbital (Gartside, 1978; Kamei et al., 1983; Stone and Javid, 1985; Schneiderman and Evans, 1986). Numerous studies in vitro have also shown that β-lactam antibiotics act as antagonists of $GABA_A$ receptors. For instance, PCG and other β-lactams such as cefazolin, cephaloridine, cephalexin, imipenem and panipenem inhibit GABA-induced electrophysiological responses and inhibit $GABA_A$ receptor binding (Hori et al., 1985; Chow and Mathers, 1986; Williams et al., 1988; Shimada et al., 1992; Twyman et al., 1992; Fujimoto et al., 1995). Our recent

studies showed that cefoselis and bicuculine inhibited $GABA_A$ receptor binding with K_i values of 1310 ± 53 and 4.83 ± 0.51 μM, respectively (unpublished observations). Difference in the rank order of their affinities for $GABA_A$ receptors closely paralleled their potencies to induce convulsion in the present study, supporting strongly the involvement of $GABA_A$ receptors in the convulsions induced by cefoselis. Although MK-801 displayed anticonvulsive activity with similar potency in all three convulsion models, diazepam failed to inhibit NMDA-induced convulsions, suggesting that NMDA receptor-associated mechanisms function downstream of GABA-mediated transmissions in seizure development.

In conclusion, the present study clearly indicates that cefoselis has convulsive effects when injected directly into mouse brain, possibly mediated via inhibitory actions on $GABA_A$ receptors. Our findings suggest that, in clinical situations resulting in elevated CNS levels of cefoselis such as through overdosing or in patients with compromised blood–brain barriers, unfavorable CNS-related side effects might occur. Furthermore, these findings suggest that patients could be rescued from neurotoxicity by use of clinically available benzodiazepines as emergency treatment.

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