ELSEVIER

European Journal of Pharmacology 368 (1999) 149–159

ejp

Effects of some AMPA receptor antagonists on the development of tolerance in epilepsy-prone rats and in pentylenetetrazole kindled rats

Giovambattista De Sarro [a,*], Eugenio Donato Di Paola [a], Pietro Gareri [a], Luca Gallelli [a], Gisella Scotto [a], Angela De Sarro [b]

[a] *Department of Experimental Medicine, Faculty of Medicine, University of Catanzaro, Italy*
[b] *Institute of Pharmacology, Faculty of Medicine, University of Messina, Italy*

Received 14 October 1998; revised 28 December 1998; accepted 5 January 1999

Abstract

The non-selective α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) receptor antagonists, 2,3-benzodiazepine derivatives CFM-1 (3,5-dihydro-7,8-dimethoxy-1-phenyl-4*H*-2,3-benzodiazepin-4-one) and CFM-2 (1-(4′-aminophenyl)-3,5-dihydro-7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-one), following intraperitoneal (i.p.) administration, were studied against audiogenic seizures in genetically epilepsy-prone rats (GEPRs) or pentylenetetrazole induced kindling in rats. After acute i.p. administration the ED_{50} values of CFM-1 against the clonic and tonic phases of the audiogenic seizures 30 min after pretreatment were 40 (16–100) and 13 (8–25) μmol kg^{-1}, respectively. The animals used for chronic study were treated i.p. daily (at 10 h) for 4 weeks with CFM-1 (20 or 50 μmol kg^{-1}). Chronic treatment for 2 weeks with CFM-1 gave ED_{50} values against clonic and tonic seizures of 39 (22–69) and 16 (8–25) μmol kg^{-1}, respectively, whereas chronic treatment for 4 weeks gave ED_{50} values against clonic and tonic seizures of 42 (18–98) and 17 (7–41.3) μmol kg^{-1}, respectively. The duration of anticonvulsant activity observed between 0.5 and 4 h following administration of CFM-1 was similar for acute and chronic treatment. Two groups of Sprague–Dawley rats received CMF (20 or 50 μmol kg^{-1}) 30 min before a subconvulsant dose of pentylentetrazole (25 mg kg^{-1} i.p.) which is able to increase seizure severity in control animals (i.e., chemical kindling). Pretreatment with CFM-2 delayed the progression of seizure rank during repeated administration of pentylentetrazole. At the end of the period of repeated pentylentetrazole treatment (6 weeks) the mean seizure score was 0 in vehicle treated controls, 4.3 in animals treated with vehicle + pentylentetrazole, 2.2 in rats treated chronically with CFM-2 (20 μmol kg^{-1} i.p.) + pentylentetrazole and 1.0 in rats treated repeatedly with CFM-2 (50 μmol kg^{-1} i.p.) + pentylenetetrazole. CFM-2 was also able to antagonize the long-term increase in sensitivity of the convulsant effects of GABA function inhibitors in pentylentetrazole-kindled animals. Thus, the administration of a challenge dose of pentylentetrazole (15 mg kg^{-1} i.p.) or picrotoxin (1.5 mg kg^{-1} i.p.) 15 or 30 days after the end of the repeated treatment showed that animals treated with CFM-2 were significantly protected against seizures induced by pentylentetrazole or picrotoxin. The data suggest that, following repeated treatment, tolerance to the novel AMPA receptor antagonists does not develop (CFM-1 in genetically epilepsy-prone rats and CFM-2 in the pentylentetrazole kindling model of epilepsy). Thirteen minutes after drug injection on days 1, 14 and 28 of chronic treatment the motor impairment induced by these compounds was studied with a rotarod apparatus. The TD_{50} values for CFM-1 or CFM-2-induced impairment of locomotor performance were similar following acute and repeated treatment. The data also suggest that some novel 2,3-benzodiazepines may have clinical potential for some types of epilepsy.

Keywords: Epilepsy; 2,3-Benzodiazepine, AMPA receptor antagonist; Tolerance; Anticonvulsants; Audiogenic seizure; Genetically epilepsy-prone rat; Kindling; Pentylenetetrazole

1. Introduction

Excitatory amino acids, aspartate and glutamate, are the major excitatory neurotransmitters in the central nervous system. Compounds acting as antagonists at α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) re-

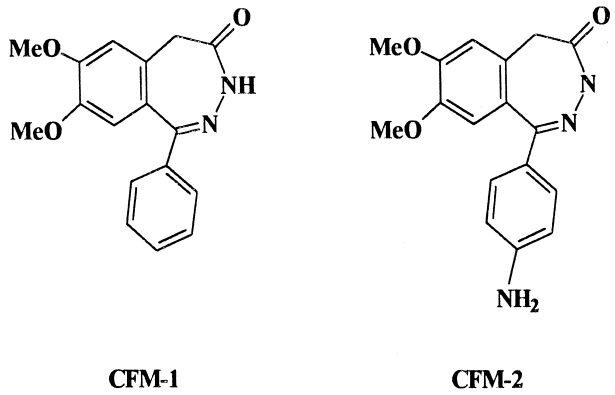
* Corresponding author. Department of Experimental and Clinical Medicine, School of Medicine, Catanzaro, Policlinico Mater Domini, Via T. Campanella, 88100, University of Cantazaro, Italy. Tel.: +39-961-712323; Fax: +39-961-774424; E-mail: desarro@unicz.thebrain.net

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00006-0

ceptors have been shown to possess anticonvulsant properties in various experimental models of epilepsy (De Sarro et al., 1987, 1995; Croucher et al., 1988; Patel et al., 1988; Chapman et al., 1991; Rogawski, 1993). In particular, 6-nitro-7-sulfamoylbenzo(*F*)quinoxaline-2,3-dione (NBQX), 1-(4-aminophenyl)-4-methyl-7,8-(methylenedioxy)-5*H*-2,3-benzodiazepine (GYKI 52466) and novel 2,3-benzodiazepines (3,5-dihydro-7,8-dimethoxy-1-phenyl-4*H*-2,3-benzodiazepin-4-one—CFM-1 and 1-(4′aminophenyl)-3,5-dihydro-7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-one—CFM-2) have recently been shown to exhibit anticonvulsant activity in DBA/2 mice and in genetically epilepsy-prone rats (Chapman et al., 1991; Smith et al., 1991; De Sarro et al., 1995, 1999; Chimirri et al., 1997). In contrast, 6,7-dinitroquinoxaline-2,3-dione (DNQX), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and a selective AMPA receptor antagonist, NBQX, do not have very strong and consistent anticonvulsant activity in the pentylenetetrazole model, being active against the tonic hind limb component (Velisek et al., 1995).

Tolerance to the anticonvulsant action of benzodiazepines and other classical anticonvulsant drugs has been studied in experimental animals (Lippa and Regan, 1977; File, 1983; Schmidt et al., 1986b; De Sarro et al., 1992, 1996a). Clinical reports suggest a similar situation, i.e., an especially rapid development of tolerance to benzodiazepines (Oxley, 1986; Schmidt et al., 1986a,b). Chronic administration of 2-amino-7-phosphonoheptanoic acid (AP7), *cis*-4-phosphonomethyl-2-piperidine-carboxylic acid (CGS 19755) and 3-(2-carboxypiperazine-4-yl) propenyl-1-phosphonic acid (CPPene) and DL-(*E*)-2-amino-4-methyl-5-phosphono-3-pentenoic acid (CGP 37849) and its carboxyethylester (CGP 39551) did not result in diminution of their anticonvulsant effects in rodents (Boast et al., 1988; Chapman, 1988; Smith and Chapman, 1993; De Sarro et al., 1996b). Since no data were available for tolerance to anticonvulsant effects of AMPA receptor antagonists in genetically epilepsy-prone rats, we now studied the development of tolerance to one of these 2,3-benzodiazepines acting as an excitatory amino acid antagonist. This experimental genetic model of epilepsy lends itself to following the development of tolerance over time. In this strain of rats, the seizures are relatively stable, so that drug effects on seizure activity can be measured repeatedly and the rate of tolerance development can be monitored. In contrast to experiments in which chemical convulsants are used, this model allows no possibility of drug interaction and there is no concern that reduced anticonvulsant effectiveness might result from the treated animal becoming more sensitive to the convulsant drug during chronic treatment.

Kindling is a model of epilepsy that has the advantages of both the epileptogen and spontaneous models (Moshé and Ludwig, 1988; Majkowski, 1989; McNamara, 1989; Mutani et al., 1991). On one hand, the initiation of seizures is definitely the result of epileptogenic manipulation and is highly time-connected to it. On the other hand, there is a close relation between the behavioural and electrocorticographic features of seizures and the intensity and duration of the kindling stimuli. Kindling is usually considered a drug-resistant model of epilepsy (Löscher, 1993). In these experiments, we studied the action of 1-(4′-aminophenyl)-3,5-dihydro-7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-one (CFM-2) on the development of kindling and on the post-kindling state in a chemically (pentylenetetrazole) kindled rat model. To allow better comparison of these different compounds, an attempt was made to choose anticonvulsant doses that would be approximately equieffective, based on dose–response activity of these drugs in preliminary experiments and on the results of a previous study (De Sarro et al., 1995, 1999; Chimirri et al., 1997). Since the solvent used for some experiments (30% dimethylsulfoxide (DMSO) with 70% sterile saline) can affect seizures induced by pentylentetrazole, an attempt was made to choose a hydrochloride salt of CFM-2, easily soluble in sterile saline for the pentylentetrazole kindling study. The CFM-1 derivative was used for chronic treatment in genetically epilepsy-prone rats because dimethylsulfoxide solution does not affect seizure response in genetically epilepsy-prone rats (De Sarro and De Sarro, 1993; De Sarro et al., 1990, 1996a, 1999).



CFM-1 CFM-2

Fig. 1. Chemical structure of compounds studied.

In addition, the effects on motor function in rats of both compounds CFM-1 and CFM-2 were assessed in the rotarod test. The resulting effects were assessed so as to demonstrate the clinical potential of these novel compounds. We also aimed to find whether these compounds had similar tolerance profiles in two different models of epilepsy (Fig. 1).

2. Materials and methods

2.1. Animals

Genetically epilepsy-prone rats, a strain derived from Sprague–Dawley rats were supplied from our breeding

stock (Institute of Pharmacology, Messina) from a colony kindly supplied by Dr. B.S. Meldrum, London University. The latter colony was obtained from the colony maintained by Dr. P.C. Jobe at Lousiana State University (Shreveport, USA). Progenitors of the latter were raised at the University of Arizona and named (UAZ:AGS (SD)). The rats were housed three or four per cage (350 × 530 mm long × 180 mm high) under stable conditions of humidity (60 ± 5%) and temperature (21 ± 2°C) and allowed free access to food and water until the time of the experiments. The animals were maintained on a 12 h light and 12 h dark cycle (lights on 19.00–07.00 h, off 1900–0700 h). Genetically epilepsy-prone rats were tested three times at weekly intervals between 6 and 8 weeks of age and only animals which showed an audiogenic seizure (score 9) in all three exposures to sound stimulation were used for these experiments.

One hundred Sprague–Dawley rats, weighing 250–280 g at the beginning of experiments were used for kindling studies. The animals were purchased from Harlan-Nossan (Corezzana, Milano, Italy). The experimental protocol and all the procedures involving animals and their care were conducted in conformity with the institutional guidelines and the European Council Directive of Cares and Policies.

2.2. Surgical procedure and kindling phase

Fronto-parietal steel screw electrodes were implanted bilaterally, while the ground electrode was implanted epidurally over the nasal bone under chloral hydrate anesthesia (400 mg kg^{-1} i.p.). The kindling phase of the experiment began at least 7 days after surgery. Pentylentetrazole was given i.p. to each rat at the dose of 25 mg kg^{-1} on Mondays, Wednesdays and Fridays (i.e., three times a week) at 09.00 h, for up to six consecutive weeks. When a rat treated with pentylentetrazole had a seizure score of 4 or 5 after three consecutive injections, it was defined as kindled, and it was used for the next study. In particular, when the animals showed behavioral and electrocortical seizures, CFM-2 was administered at doses of 10, 20, 30 or 50 μmol kg^{-1} i.p., 30 min before a 25 mg kg^{-1} dose of pentylentetrazole i.p. Control rats received an equivalent volume of solvent (sterile saline). Electrocortical activity was recorded with an 8-channel electrocortical EcoG machine (OTE Biomedica, Firenze, Italy).

The animals were immediately placed in a circular Plexiglass chamber (50 cm diameter) and the time from injection until response (onset latency for the myoclonic jerk, generalized clonus, and tonus) was recorded and scored according to the scale described below. The rats were observed for a maximum of 60 min following pentylentetrazole administration. Seizure severity was scored according to the following scale: 0 = no seizure; 1 = ear and facial twitching; 2 = myoclonic body jerks associated with behavioural rest; 3 = clonus of forelimb; 4 = generalized seizures with rearing and falling; 5 = generalized seizures with tonic hind limb extension and status epilepticus.

2.3. Anticonvulsant activity of CFM-1 in genetically epilepsy-prone rats

Seizures were induced in genetically epilepsy-prone rats, 190–250 g, 10 to 14-week old, male ($n = 96$) by exposing them to a mixed frequency sound of 12–16 kHz, 109 dB intensity under a hemispheric plexigas dome (58 cm diameter). Individual animals were initially tested 10 min before sound stimulation for assessment of locomotor activity and then placed in the dome for habituation and assessment of anticonvulsant activity. Auditory stimulation was applied for 60 s or up to the onset of convulsions. A full seizure response consisted of one or two running phases, followed by a convulsion (clonus of forelimbs, hindlimbs, head, pinnae, vibrissae and tail) and tonic extension to give a score of 9 (Jobe, 1981). The audiogenic seizure response was assessed on the following previously reported scale (De Sarro et al., 1990): 0 = no response, 1 = running only, 2 = two running phases, followed by a clonic convulsion (clonus of forelimbs, hindlimbs, head, pinnae, vibrissae and tail), 3 = one running phase, followed by a clonic convulsion (clonus of forelimbs, hindlimbs, head, pinnae, vibrissae and tail), 4 = two running phases followed by tonus of neck, trunk and forelimb and hindlimb clonus, 5 = one running phase followed by tonus of neck, trunk and forelimb and hindlimb clonus, 6 = two running phases followed by nearly complete tonic extension except hindfeet, 7 = one running phase followed by nearly complete tonic extension except hindfeet, 8 = two running phases followed by complete tonic extension, 9 = one running phase followed by complete tonic extension. The maximum response was recorded for each animal. Behavioural changes were observed during the period between drug administration and auditory testing.

2.4. Chronic treatment in genetically epilepsy-prone rats

The chronic treatment with CFM-1 was administered i.p. to genetically epilepsy-prone rats once daily, at 10 h. The doses used were: CFM-1 20 and 50 μmol kg^{-1}, these doses had decreased the tonic or clonic component of audiogenic seizures by 80–90%. Control rats received a solution containing 30% dimethylsulfoxide on 70% saline as appropriate. Treatments lasted 4 weeks. One day (i.e., 24 h) after the end of chronic treatment, the auditory stimulation was administered again to test for residual drug effects. The animals were usually tested 30 min following i.p. administration of the compound, while for the time course studies the genetically epilepsy-prone rats were

exposed to the sound stimulus at 0.5, 1, 1.5, 2, 3 and 4 h after oral administration of the compound.

2.5. Chronic treatment and behavioral observation on development of pentylentetrazole kindling

Pentylentetrazole was given i.p. at the dose of 25 mg kg^{-1} on Mondays, Wednesdays and Fridays (i.e., three times a week) at 09.00 h, for up to 6 consecutive weeks. CFM-2 was administered at the doses of 20 or 50 μmol kg^{-1} i.p., 30 min before each dose of pentylenetetrazole throughout the chronic treatment. These doses of CFM-2 were able to reduce the severity of kindled seizures to score 2 and score 1 of pentylenetetrazole-kindled seizures. Control rats received an equivalent volume of solvent (sterile saline). A fourth group of kindled animals were treated with CFM-2 (20 μmol kg^{-1} i.p.) three times daily (at 9, 15 and 21 h) for 4 weeks. Control animals received an equivalent volume of sterile saline at the same time.

The animals were immediately placed in a circular Plexiglass chamber and the time from injection until response (onset latency for the myoclonic jerk, generalized clonus, and tonus) was recorded and seizure severity was scored according to the scale described below (Section 2.2). The rats were observed for a maximum of 60 min following pentylenetetrazole administration. Rats which failed to display tonus in this time were assigned a tonic latency of 60 min.

The mean value of three seizure scores recorded for each rat in each week of treatment was used for statistical analysis. When a rat treated with pentylenetetrazole alone had a seizure score of 4 or 5 after three consecutive injections, it was defined as kindled, and its treatment was discontinued. On the same day, the treatment of one rat in each of the other experimental groups was discontinued in order to have comparable withdrawal periods across treatments. The rats whose treatment was discontinued upon fulfilment of the kindling criteria were assigned the score of their last week of treatment in each of the remaining weeks for the calculation of the results shown in Fig. 2. At the end of the repeated treatment, kindled rats receiving CFM-2 three times daily were treated one day (i.e., 24 h) after the last CFM-2 or vehicle injection, respectively, and the kindling stimulus was administered again to test for residual drug effects. All rats were coded and the persons involved in behavioural observations were not aware of which rats were kindled, i.e., experiments were done blind.

2.6. Challenge with pentylenetetrazole

Two independent challenge experiments were performed using pentylenetetrazole (15 mg kg^{-1}, i.p.) 15 days after the completion of the chronic treatment and the rats were observed for 1 h after the injection in order to determine the incidence of convulsions. Experiment 1 was carried out using 10 control rats chronically treated with

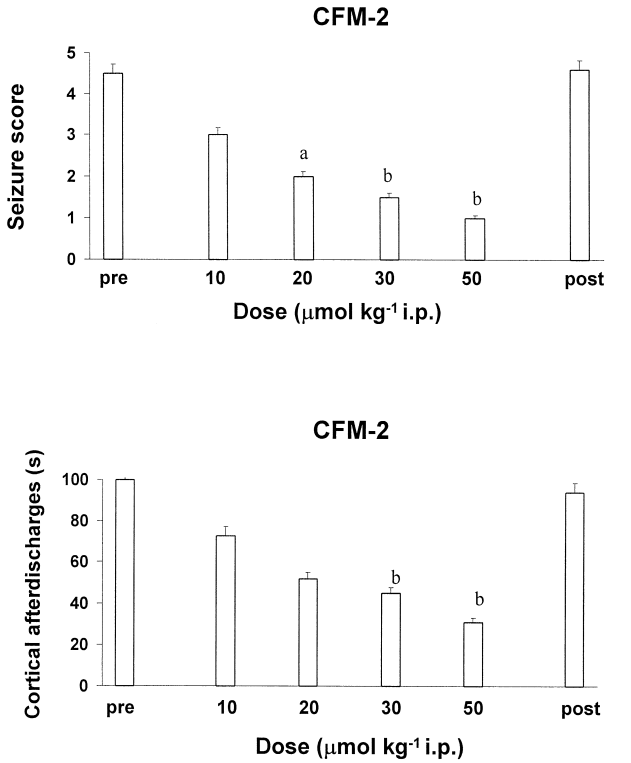


Fig. 2. Dose-dependent effects of CFM-2 (administered 30 min before) on severity and cortical afterdischarges elicited in pentylenetetrazole-kindled rats by i.p. administration of pentylenetetrazole 25 mg kg^{-1}. Ordinate shows seizure score, abscissa shows the dose expressed as μmol kg^{-1} i.p. Six to ten animals were used for the determination of each point. Significant differences are denoted by $^{a}P < 0.05$; $^{b}P < 0.01$ using Mann–Whitney *U*-test (seizure score) or Wilcoxon two sample test (cortical afterdischarges).

saline, 10 rats kindled with pentylenetetrazole (25 mg kg^{-1} i.p. three times a week and 8 rats treated with pentylenetetrazole plus CFM-2 (20 μmol kg^{-1} i.p.). Experiment 2 was done with 10 control rats, 10 pentylenetetrazole-kindled rats and 8 rats treated with pentylenetetrazole plus CFM-2 (50 μmol kg^{-1} i.p.).

2.7. Challenge with picrotoxin

One month after the challenge with pentylenetetrazole, the animals used in experiments 1 and 2 were divided into two groups of identical composition: 8 saline controls, 10 pentylenetetrazole-kindled rats and two groups of 8 rats each treated with pentylenetetrazole + CFM-2 (20 or 50 μmol kg^{-1} i.p.). All groups were challenged with picrotoxin (1.5 mg kg^{-1}, i.p.). The rats were observed for 1 h after picrotoxin in order to determine the incidence of seizures.

2.8. Effects on motor movements

Genetically epilepsy-prone rats or Sprague–Dawley treated with CFM-1 or CFM-2, respectively, were trained

just before anticonvulsant testing to do coordinated motor movements continuously for 5 min on a rotarod 4 cm in diameter 4.5 rpm. (U. Basile, Comerio, Varese, Italy). Impairment of coordinated motor movements was defined as inability of the animals to remain on the rotarod for a test period of 5 min according to Dunham and Miya (1957). On the morning of days 1, 14 and 28, the locomotor performance of the genetically epilepsy-prone rats or of Sprague–Dawley rats was usually assessed at 30 min after i.p. administration. The last day of the chronic treatment for the subgroups of 5–8 rats treated chronically with CFM-1, CFM-2 or vehicle for 14 or 28 days, were tested 30 min after the administration of the compounds to assess possible changes in the impairment of locomotor performance.

2.9. Statistical analysis

The maximum response for each animal was recorded. Comparisons were made between the results for the first and the final treatment day, using baseline values and results recorded 24 h after the final chronic dose, respectively. The effects of the treatment were analyzed statistically using non-parametric methods. Kruskall–Wallis Analysis of Variance was first carried out and if this showed significance a Mann–Whithey *U*-test was used to compare control and drug-treated genetically epilepsy-prone rats. The percentage of animals exhibiting tonic extension (seizure score = 6–7) or clonic phase (seizure score = 2–3) of the audiogenic seizure was determined for each dose and these values were plotted against corresponding doses for calculation of ED_{50} values (with 95% confidence limits). Latencies to fall(s) as a measure of locomotor performance were converted to percentages of the respective vehicle-treated groups and used to calculate the TD_{50} values (with 95% confidence limits). The ED_{50} and TD_{50} values for each compound were determined using the method of Litchfield and Wilcoxon (1949). At least 32 animals were used to calculate each ED_{50} values.

Pentylenetetrazole seizure latencies were compared using a two-way Analysis of Variance (ANOVA) with a Tukey's post hoc test and seizure severity scores were compared between groups using a Kruskall–Wallis nonparametric ANOVA followed by a Mann Whitney *U*-test. Data were compared with the Kruskall–Wallis analysis by ranks and Fisher's exact probability test. Electrocortical afterdischarges were analysed using the Wilcoxon two-sample test. All tests were used two-sided and $P < 0.05$ was considered significant. At least five animals were used for each dose level studied.

2.10. Drugs

Pentylenetetrazole and picrotoxin were purchased from Sigma (Milan, Italy), 3,5-dihydro-7,8-dimethoxy-1-phenyl-4*H*-2,3-benzodiazepin-4-one (CFM-1, molecular weight, MW = 296.15) and 1-(4′-aminophenyl)-3,5-dihydro-7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-one hydrochloride (CFM-2, MW = 345.07, now available from Tocris Cookson) were obtained from Prof. A. Chimirri (Dipartimento Farmaco Chimico, University of Messina, Italy). All compounds were easily dissolved in sterile saline, with the exception of CFM-1 which was dissolved in 30% DMSO and 70% sterile saline. For repeated administration, all compounds were administered i.p. (0.4 ml/100 g of body weight of the rat) as fresh solution.

3. Results

3.1. Anticonvulsant activity of CFM-1 in genetically epilepsy-prone rats

To allow a better evaluation of CFM-1 anticonvulsant effects, anticonvulsant doses were chosen that would be able to reduce the severity of audiogenic seizures, based on dose–response activity of these compounds in preliminary experiments (De Sarro et al., 1995, 1999; Chimirri et al., 1997). Significant protection ($P < 0.01$) against the audiogenic seizure phases in genetically epilepsy-prone rats was observed after CFM-1 (50, 66 and 100 μmol kg^{-1} i.p., Table 1). The dose of CFM-1 required to completely abolish audiogenic seizures (100 μmol kg^{-1} i.p.) was not associated with mild ataxia, sedation, reduction in locomotor activity or similar signs observed after i.p. injection of higher doses of CFM-1 (De Sarro et al., 1999, in press). The relative ED_{50} values (with 95% confidence limits) of CFM-1 are reported in Table 2. The duration of anticonvulsant protection observed after i.p. administration of CFM-1 (50 μmol kg^{-1}) following acute and repeated treatment is shown in Table 3. The anticonvulsant activity

Table 1
The effect of CFM-1 on audiogenic seizures in genetically epilepsy prone rats

Treatment	Concentration (μmol kg^{-1})	Vehicle	Drug
CFM-1	10	9.0 ± 0 (8)	8.5 ± 0.5 (8)
	21	9.0 ± 0 (8)	6.0 ± 1.0 (8)
	33	9.0 ± 0 (8)	3.0 ± 1.0 (8)[b]
	50	9.0 ± 0 (8)	2.0 ± 1.0 (8)[b]
	66	9.0 ± 0 (8)	1.0 ± 1.0 (8)[b]
	100	9.0 ± 0 (8)	0 ± 0 (8)[b]

Groups of genetically epilepsy-prone rats were administered i.p. the stated doses of the drugs or vehicle and exposed to auditory stimulation 30 min later.
Incidence of each seizure phase was recorded and median seizure score ± interquartile range for each dose is given.
The number of animals used are in parentheses.
Significant differences in the incidence of seizure phases between concurrent control and drug-treated group are denoted by [a]$P < 0.05$; [b]$P < 0.01$, Mann–Whitney *U*-test.

Table 2
Effects of CFM-1 (20 or 50 μmol kg^{-1}) on audiogenic seizures and rotarod performance in genetically epilepsy-prone rats

Treatment	CFM-1	
	20 μmol kg^{-1}	50 μmol kg^{-1}
Acute administration		
ED50 clonus	40 (16–100)	
ED50 tonus	13 (8–25)	
TD50	128 (98–167)	
TI	3.2	
Repeated treatment for 14 days		
ED_{50} clonus	39 (22–69)	34.1 (23.5–49.5)
ED_{50} tonus	16 (8–25)	17.2 (14.3–20.7)
TD_{50}	138 (106–180)	138 (106–180)
TI	3.5	4.0
Repeated treatment for 28 days		
ED_{50} clonus	42 (18–98)	35.7 (29.3–43.4)
ED_{50} tonus	17 (7–41.3)	15.9 (7.3–33.5)
TD_{50}	149 (101–220)	134 (96–187)
TI	3.6	3.8

ED_{50} or TD_{50} values (with 95% confidence limits) of CFM-1 administered i.p. (30 min before test) against the clonic and tonic phases of the audiogenic seizures in genetically epilepsy-prone rats were calculated according to the method of Litchfield and Wilcoxon (1949) and expressed as μmol kg^{-1}.
Therapeutic index (TI) = TD_{50}/ED_{50} clonus.

of this compound was evident after 30 min, with maximum protection 1–2 h after the administration. The antiseizure action was reduced after 3 h and had disappeared completely at 4 h after the i.p. administration of CFM-1. Similar anticonvulsant potency and duration of effects were observed following 4 weeks of treatment with CFM-1.

3.2. Anticonvulsant activity of CFM-1 after chronic administration

Fifty genetically epilepsy-prone rats were used for chronic treatment with CFM-1. Chronic treatment with i.p. administration of the vehicle (DMSO and saline, 20 rats) had no effect on any of the audiogenic seizure phases ($P > 0.13$) There was no statistically significant reduction of CFM-1 effects over time as measured from the occurrence of various phases of the audiogenic seizures in the chronic treatment groups (Tables 2 and 3). The effect of the various doses of CFM-1 on incidence of seizure phases was maintained for 28 days. The occurrence of phases of audiogenic seizures appeared almost similar to that observed on the first treatment day. No residual drug effects were observed in rats 24 h after chronic treatment for 4 weeks.

3.3. Effects on motor impairment

After doses of CFM-1 higher than those which showed full anticonvulsant activity, transient ataxia was observed in the majority of the genetically epilepsy-prone rats. This was reflected by an impairment of locomotor activity 30 min after acute or chronic administration of CFM-1. All animals which received vehicle stayed balanced for 5 min on the rotarod. After the acute administration of large doses of CFM-1 (140 or 180 μmol kg^{-1} i.p.) an impairment of locomotor performance was observed in genetically epilepsy-prone rats from 0.5 to 4 h. Since the TD_{50}/ED_{50} ratios were quite similar during the first 4 h we decided to measure the TD_{50} values at 30 min during chronic treatment. The doses of CFM-1 (20 and 50 μmol kg^{-1}) and CFM-2 (20 and 50 μmol kg^{-1}) used during chronic treatment were not able to induce ataxia, decreased muscle tone and reduction in locomotor activity or loss of righting reflex or any other adverse effects. The relative TD_{50} values (with 95% confidence limits) of CFM-1 during acute and repeated treatment are given in Table 2. The TD_{50} values (with 95% confidence limits) of CFM-2 during acute and repeated treatment are given in Table 6.

3.4. Effects of repeated treatment with CFM-2 on development of pentylenetetrazole kindling

To allow better evaluation of the anticonvulsant activity of CFM-2, anticonvulsant doses were chosen that would be able to reduce the severity of behavioural seizures induced by pentylenetetrazole kindling. Significant protection ($P < 0.01$) against the kindling seizures in rats was observed after CFM-2 (20 and 50 μmol kg^{-1} i.p.) (Fig. 2). The doses of CFM-2 required to significantly reduce kindling seizures (20 and 50 μmol kg^{-1} i.p.) were not associated with mild ataxia, sedation, reduction in locomotor activity or other similar signs observed after i.p. injection of higher doses of CFM-2 (De Sarro et al., 1999, in press). The

Table 3
Effects of CFM-1 (50 μmol kg^{-1}) on audiogenic seizures in genetically epilepsy-prone rats after acute and repeated treatment

TIME (h)	Treatment	
	Acute	Repeated
Control	9.0 ± 0 (8)	9.0 ± 0 (8)
0.5	2.0 ± 1.0 (8)[b]	2.0 ± 1.0 (8)[b]
1	0 ± 0 (8)[b]	0 ± 0 (8)[b]
1.5	1.0 ± 1.0 (8)[b]	1.0 ± 1.0 (6)[b]
2	5.0 ± 1.0 (8)[a]	4.0 ± 1.0 (8)[b]
3	6.0 ± 1.0 (5)	6.0 ± 1.0 (6)
4	9.0 ± 0 (8)	9.0 ± 0 (8)

Groups of genetically epilepsy-prone rats were administered i.p. the stated doses of CFM-1 and exposed to auditory stimulation after various intervals.
Incidence of each seizure phase was recorded and median seizure score ± interquartile range is reported.
The number of animals used is reported in brackets.
Significant differences in the incidence of seizure phases between concurrent control and drug-treated group are denoted by [a]$P < 0.05$; [b]$P < 0.01$ using Mann–Whitney *U*-test.

repeated treatment with pentylenetetrazole at a subconvulsant dose (25 mg kg^{-1} i.p.), three times a week, induced chemical kindling, as reflected by the progressive increase in the mean seizure score observed from the first week on. CFM-2 was able to reduce both the incidence and the severity of seizures occurring during the repeated treatment with pentylenetetrazole in a dose-dependent manner. As shown in Fig. 3, by the end of the repeated treatment with vehicle + pentylenetetrazole the mean seizure score was 4.1 and 18 out of 20 rats showed at least phase 4. When the animals were pretreated with CFM-2, 20 or 50 μmol kg^{-1} i.p., the mean seizure score was 2.2 and 1.0, respectively.

Another series of experiments studied whether or not previous treatment with CFM-2 was able to reduce or to delay the development of the long-term sensitization to the effects of pentylenetetrazole kindling which induced an impairment of GABAergic neurotransmission. As shown in Fig. 4 challenge with pentylenetetrazole, 15 days after the end of repeated treatment, elicited seizures in 2 out of 8 animals receiving CFM-2 (20 μmol kg^{-1} i.p.) + pentylenetetrazole in 1 out of 8 rats receiving CFM-2 (50 μmol kg^{-1} i.p.) + pentylenetetrazole, in 9 out of 10 rats treated with vehicle + pentylenetetrazole and in 0 out of 10 animals treated with vehicle. There was also a marked difference 30 days after completion of the repeated treatment in the incidence of seizures induced by a challenge dose of picrotoxin (1.5 mg kg^{-1} i.p.). Picrotoxin was able to induce seizures in all 10 vehicle + pentylenetetrazole kindled rats but not in rats chronically treated with CFM-2 (20 or 50 μmol kg^{-1} i.p.) or vehicle (Fig. 4). The duration of anticonvulsant protection observed after acute and repeated treatment with CFM-2 (50 μmol kg^{-1}) is given in Table 4.

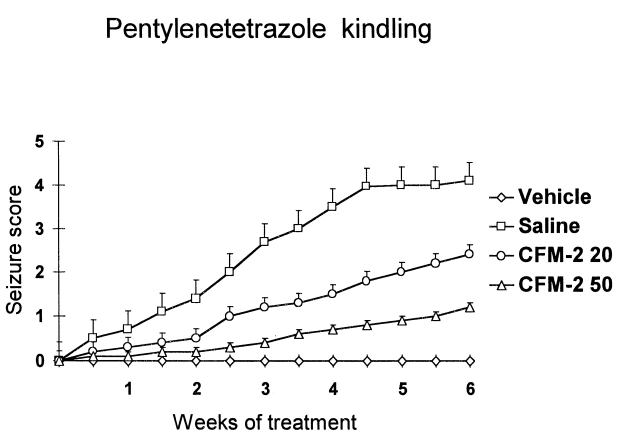


Fig. 3. Dose-dependent effects of CFM-2 on kindling induced by pentylenetetrazole in rats during repeated treatment for 6 weeks. CFM-2 (20 or 50 μmol kg^{-1} i.p.) was administered 30 min before each injection of pentylenetetrazole. Pentylenetetrazole was administered i.p. at the dose of 25 mg kg^{-1} three times a week. Control animals received an equivalent volume of the vehicle 30 min before pentylenetetrazole. The number of rats in each experimental group was as follows: Vehicle 20; Vehicle + pentylenetetrazole 24; CFM-2 (20 μmol kg^{-1} i.p.) + pentylenetetrazole 10; CFM-2 (50 μmol kg^{-1} i.p.) + pentylenetetrazole 10. A Kruskall–Wallis analysis by rank revealed some significant differences between the vehicle + pentylenetetrazole group and the other experimental groups ($^{a}P < 0.05$).

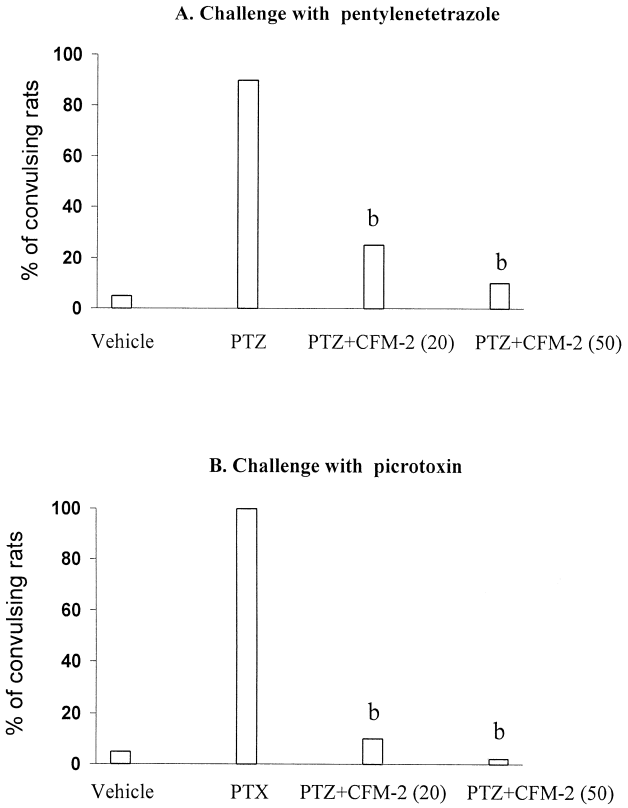


Fig. 4. Effects of challenge with pentylenetetrazole on rats receiving repeatedly vehicle, vehicle + pentylenetetrazole or CFM-2 (20 μmol kg^{-1} i.p.) + pentylenetetrazole or CFM-2 (50 μmol kg^{-1} i.p.) for 6 weeks. (A) Challenge with pentylenetetrazole (15 mg kg^{-1} i.p.) 15 days after the end of repeated treatment, when clonic seizures were seen. (B) Challenge with picrotoxin (PTX, 1.5 mg kg^{-1} i.p.) 30 days after the end of repeated treatment, when clonic seizures were seen. Significant differences are denoted by $^{b}P < 0.01$, Fisher's exact probability test.

Table 4
The effect of CFM-2 (50 μmol kg^{-1}) on pentylenetetrazole induced kindling seizures in rats after chronic treatment

TIME (h)	Treatment	
	Acute	Repeated
Control	5.0 ± 0 (8)	5.0 ± 0 (8)
0.5	1.0 ± 1.0 (8)^{b}	1.0 ± 1.0 (6)^{b}
1	0 ± 0 (6)^{b}	0 ± 0 (6)^{b}
1.5	0 ± 0 (6)^{b}	0 ± 0 (6)^{b}
2	3.0 ± 1.0 (6)^{b}	2.0 ± 1.0 (6)^{b}
3	4.0 ± 1.0 (6)	3.0 ± 1.0 (6)^{a}
4	5.0 ± 1.0 (6)	5.0 ± 1.10 (6)

Groups of rats were administered i.p. the stated doses of CFM-2 and exposed to pentylenetetrazole after various intervals.
Incidence of each seizure phase was recorded and median seizure score ± interquartile range is reported.
The number of animals used is given in brackets.
Significant differences in the incidence of seizure phases between concurrent control and drug-treated group are denoted by $^{a}P < 0.05$; $^{b}P < 0.01$ using Mann–Whitney *U*-test.

Table 5
Effects of CFM-2 (20 μmol kg^{-1}) on rotarod performance of Sprague–Dawley rats

Treatment	Time		
Acute administration	30	60	90
TD_{50}	57.4 (48–68.7)	46.9 (39.1–56.4)	94.3 (76.2–116.7)
Repeated treatment for 14 days			
TD_{50}	58.5 (41.6–82.3)	48.1 (38.7–59.8)	
Repeated treatment for 28 days			
TD_{50}	58.8 (47.2–73.2)	48.6 (38.4–61.5)	

TD_{50} values (with 95% confidence limits) of CFM-2 administered i.p. (30, 60 or 120 min before test) in Sprague–Dawley rats were calculated according to the method of Litchfield and Wilcoxon (1949) and expressed as μmol kg^{-1}.

3.5. Effects of repeated treatment with CFM-2 on development of pharmacological tolerance in pentylenetetrazole-kindled rats

In two other groups of kindled rats receiving three times daily either CFM-2 (20 μmol kg^{-1} i.p.) or vehicle, the kindling stimulus was discontinued 1 day after repeated treatments. The data reported in Table 5 show clearly that CFM-2 did not induce pharmacological tolerance to its anticonvulsant effects.

4. Discussion

The anticonvulsant potency and duration of anticonvulsant effects of CFM-1 appeared similar following acute and chronic administration to genetically epilepsy-prone rats. The main finding of present study was the lack of development of tolerance after repeated treatment with this AMPA receptor antagonist. This emphasises the marked difference between classical antiepileptic compounds and excitatory amino acid antagonists. Tolerance to the anticonvulsant action of antiepileptic compounds and in particular to the action of conventional 1,4- and 1,5-benzodiazepines has been reported in humans and in animals (Belleville and Fraser, 1957; Yanagita and Takahashi, 1970; Gastaut and Low, 1979; Oxley, 1986; Schmidt et al., 1986a,b; De Sarro et al., 1992, 1996a). Indeed, this characteristic of both *N*-methyl-D-aspartate (NMDA) (De Sarro et al., 1996b; Löscher et al., 1988, 1993) and AMPA (Löscher et al., 1993 and present study) receptor antagonists suggests a possible therapeutic use in forms of epilepsy which become resistant. With the kindled response, CFM-2 was able to reduce in a dose-dependent manner both the clinical signs of seizures and the afterdischarge duration, thus differing from competitive NMDA antagonists which appeared less efficacious in this respect (Holmes et al., 1990; Katayama et al., 1990; Sato et al., 1990; Dürmüller et al., 1994). It is important to note that the seizure score of rats treated repeatedly with pentylenetetrazole in combination with CFM-2 was greater than O during the last weeks of chronic treatment, suggesting that the protective effects of CFM-2 are not complete. One possible explanation for this is that the doses of CFM-2 used were insufficient to give complete protection throughout the chronic treatment. Higher doses of CFM-2 could be not studied because of the limited amount of CFM-2 available for the present study. An alternative explanation is that tolerance to the protective action of CFM-2 develops during the chronic treatment with pentylenetetrazole. This explanation appears to be unlikely in view of results obtained with the other 2,3-benzodiazepine derivative CFM-1 in genetically epilepsy-prone rats. In spite of this finding of a more potent activity on the kindled response, there was a slight but sometimes significant delay in the development of kindling when CFM-2 was administered daily prior to the kindling test. Our data also demonstrated that CFM-2 maintained its ability to reduce the seizures in kindled animals after chronic administration (Tables 4 and 6). The present results demonstrate that CFM-2 can delay the development of a progressive increase in the severity of seizures and the permanent sensitization to the convulsant effects of GABA function inhibitors observed in animals treated chronically with a subconvulsant dose of pentylenetetrazole. The results confirm the hypothesis that NMDA receptor activation is important for the induction of kindling (Croucher et al., 1988; Holmes et al., 1990; Sato et al., 1990; Dürmüller et

Table 6
Effects of chronic treatment with CFM-2 (20 μmol kg^{-1}) on development of pharmacological tolerance in pentylenetetrazole-kindled rats

Treatment	Experimental group	Seizure		Number of rats
		Latency	Score	
1 day after acute administration				
	Vehicle + Pentylenetetrazole	5.5 ± 1.8	4.3	10
	CFM-2 + Pentylenetetrazole	> 60[b]	2.2[b]	10
1 day after chronic administration				
	Vehicle + Pentylenetetrazole	5.8 ± 2.4	4.2	20
	CFM-2 + Pentylenetetrazole	> 60[b]	2.4[a]	20

Kindled animals were injected with CFM-2 (20 μmol kg^{-1}) or vehicle three times daily and pentylenetetrazole was administered i.p. (25 mg kg^{-1}) three times weekly for 4 weeks.
One day after the last injection of CFM-2 the animals were challenged with CFM-2 or vehicle followed 30 min later by the injection of pentylenetetrazole.
Rats were observed for 60 min after pentylenetetrazole. Significant differences in latency are denoted by [b]$P < 0.01$, ANOVA.
Significant differences in the incidence of seizure phases between concurrent control and drug-treated group are denoted by [a]$P < 0.05$; [b]$P < 0.01$, Mann–Whitney *U*-test.

al., 1994), whereas activation of AMPA receptors is important for the behavioural and electrical expression of kindled seizures (Bawin et al., 1993; Dürmüller et al., 1994; Namba et al., 1994). Earlier studies that addressed the role of AMPA/kainate receptors in kindling employed focal intracerebral microinjections of γ-D-glutamylaminomethylsulphonic acid (GAMS), a rather weak glutamate receptor antagonist acting preferentially on the AMPA/kainate receptors. GAMS increased the threshold in electrically kindled amygdala for both afterdischarge and behavioural seizure (Saitoh et al., 1991). In addition, Croucher et al. (1988) demonstrated that GAMS, microinjected repeatedly into the prepyriform cortex, delayed the kindling process. The differences between the study of Croucher et al. (1988) and ours might be due to partial selectivity of GAMS at AMPA/kainate receptors and/or be a feature of the injection site. The differences between our results and those reported by Löscher and Hönack (1994a) merit discussion. Löscher and Hönack (1994b) reported that, in mice, GYKI 52466 (10 and 15 mg/kg^{-1} i.p.) significantly increased the threshold for myoclonic seizures induced by intravenous pentylenetetrazole while no significant effect was observed following GYKI 52466 20 mg/kg^{-1} i.p. We used CFM-2, a novel 2,3-benzodiazepine which appears more potent and with a longer lasting effect than GYKI 52466 (Chimirri et al., 1997), and administered i.p. a subconvulsant dose of pentylenetetrazole (25 mg kg^{-1}) in a different species of rodent. In addition, the pharmacological activity that we characterized in the present study was the development of kindling and not the antagonism of myoclonic component of pentylenetetrazole seizures. Comparison of our experiments with those of Löscher and Hönack is thus extremely difficult, if not impossible. The same applies for the data for developing animals used by Velisek et al. (1995) and for those used here.

After i.p. administration of CFM-1 and CFM-2 there was no increase of neurological side-effects but the therapeutic indices ($TI = TD_{50}/ED_{50}$) of these compounds showed a gradual reduction of the CFM-1- and CFM-2-induced impairment of locomotor performance. This finding appears similar to that of previous studies demonstrating that the repetitive administration of excitatory amino acid antagonists acting as competitive NMDA receptor antagonists gradually reduced (Cain et al., 1988; Smith and Chapman, 1993; De Sarro et al., 1996b) or did no change (Croucher et al., 1988; Holmes et al., 1990) the adverse behavioural effects. Since we observed some differences in the TD_{50} values after acute and chronic treatment, we suggest that there might be tolerance to the adverse effects of 2,3-benzodiazepines. Reduction in locomotor activity, sedation, muscle relaxant activity and mild ataxia as side-effects of these derivatives have been reported by several authors (Honoré et al., 1988; Smith et al., 1991; Turski et al., 1992; Yamaguchi et al., 1993; De Sarro et al., 1995;) and might be important factors in the therapeutic window. In particular, the muscle relaxant effects of AMPA/kainate receptor antagonists are due to an action on spinal reflexes. GYKI 52466 blocks both mono- and polysynaptic spinal reflexes in cats and rats (Tarnawa et al., 1989; Block and Schwarz, 1994). In contrast to the amygdala kindling data obtained following competitive (CGP 37849) and uncompetitive (dizolcipine), NMDA receptor antagonists, CFM-2 plus pentylenetetrazole repeated treatment did not lead to enhanced susceptibility of kindled rats to stereotyped behaviours, confirming that AMPA/kainate and NMDA receptor antagonists do not produce the same functional brain changes. However, recently, Löscher (1998) reported that electrical kindling and pentylenetetrazole kindling do not involve the same brain changes. One possible explanation for this is that electrical kindling increases the number of NMDA receptors in the hippocampus (McNamara, 1994; Bradford, 1995) and induces long-lasting expression of a novel population of hippocampal NMDA receptors with altered sensitivity to NMDA receptor antagonists (Kraus et al., 1994). Psychotomimetic effects in man following ketamine and phencyclidine administration have also been reported (Domino and Luby, 1981; Lodge et al., 1988) with some potentially detrimental effects on learning (Morris et al., 1986; Collingridge and Bliss, 1987). In this respect, some authors (Muller et al., 1988; Bliss and Collingridge, 1993) demonstrated that long-term potentiation, which is dependent for its induction on the activation of NMDA receptors and is blocked in the presence of both competitive and non-competitive NMDA receptor antagonists, can proceed in presence of AMPA receptor antagonists. Thus, AMPA/kainate receptor antagonists may have significant advantages over NMDA ones. However, it is too early to draw conclusions with regard to efficacy and tolerability of these compounds in man: experience has shown that research in experimental animals can only give a partial estimation of the clinical efficacy and unwanted effect profile of experimental compounds. The observed anticonvulsant efficacy of these AMPA/kainate receptor antagonists proved to: (a) be a simple tool for animal experimental workers to study the effects of chronic AMPA receptor blockade, and (b) indicate the possibility of chronic administration in man for the treatment of some type of epilepsy. In addition, the present data confirmed that the activation of AMPA receptors is important for the behavioural and electrical expression of kindled seizures (Bawin et al., 1993).

Acknowledgements

Financial support from the Italian Ministry of University and Scientific and Technological Research (MURST, Roma) is gratefully acknowledged. Our thanks to Mr. Antonio Giacopello for his skillful technical assistance.

References

Bawin, S.M., Satmary, W.M., Sheppard, A.R., Adey, W.R., 1993. Roles of the NMDA and quisqualate/kainate receptors in the induction and expression of kindled bursts in rat hippocampal slices. Epilepsy Res. 15, 7–13.

Belleville, R.E., Fraser, H.F., 1957. Tolerance to some effects of barbiturates. J. Pharmacol. Exp. Ther. 120, 469–474.

Bliss, T.V.P., Collingridge, G.L., 1993. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361, 31–39.

Block, F., Schwarz, M., 1994. The depressant effect of GYKI 52466 on spinal reflex transmission in rats is mediated via non-NMDA and benzodiazepine receptors. Eur. J. Pharmacol. 256, 149–153.

Boast, C.A., Pastor, G., Gerhardt, S.C., Hall, N.R., Liebman, J.M., 1988. Behavioral tolerance and sensitization to CGS 19755, a competitive *N*-methyl-D-aspartate antagonist. J. Pharmacol. Exp. Ther. 147, 556–561.

Bradford, H.F., 1995. Glutamate, GABA and epilepsy. Prog. Neurobiol. 47, 477–511.

Cain, D.P., Desorough, K.A., Mckitric, D.J., 1988. Retardation of amygdala kindling by antagonism of NMD-aspartate and muscarinic cholinergic receptors: evidence for the summation of excitatory mechanisms in kindling. Exp. Neurol. 100, 179–187.

Chapman, A.G., 1988. Anticonvulsant activity of excitatory amino acid antagonists. In: Cavalheeiro, E.A., Lehmann, J., Turski, L. (Eds.), Frontiers in Excitatory Amino Acid Research. Alan Liss, New York, pp. 203–210.

Chapman, A.G., Smith, S.E., Meldrum, B.S., 1991. The anticonvulsant effect of the non-NMDA antagonists, NBQX and GYKI 52466 in DBA/2 mice. Epilepsy Res. 9, 92–96.

Chimirri, A., De Sarro, G.B., De Sarro, A., Gitto, R., Grasso, S., Quartarone, S., Zappalà, M., Giusti, P., Libri, V., Costanti, A., Chapman, A.G., 1997. 1-Aryl-3,5-dihydro-4*H*-2,3-benzodiazepin-4-ones: novel AMPA receptor antagonists. J. Med. Chem. 40, 1258–1269.

Collingridge, G.L., Bliss, T.V.P., 1987. NMDA receptor: their role in long-term potentiation. TINS 10, 288–294.

Croucher, M.J., Bradford, H.F., Sunter, D.C., Watkins, J.C., 1988. Inhibition of the development of electrical kindling of the prepyriform cortex by daily focal injections of excitatory amino acid antagonists. Eur. J. Pharmacol. 152, 29–38.

De Sarro, G.B., De Sarro, A., 1993. Anticonvulsant properties of non-competitive antagonists of the *N*-methyl-D-aspartate receptor in genetically epilepsy-prone rats: comparison with CPPene. Neuropharmacology 32, 51–58.

De Sarro, G.B., Patel, S., Meldrum, B.S., 1987. Anticonvulsant action of a kainate antagonist γ-glutamylaminomethylsulphonic acid (GAMS) injected focally into the substantia nigra and entopeduncular nucleus. Eur. J. Pharmacol. 132, 229–236.

De Sarro, G.B., De Sarro, A., Federico, F., Meldrum, B.S., 1990. Anticonvulsant properties of some calcium antagonists on sound-induced seizures in genetically epilepsy-prone rats. Gen. Pharmacol. 21, 769–778.

De Sarro, G.B., Rotiroti, D., Gratteri, S., Sinopoli, S., Iuliano, M., De Sarro, A., 1992. Tolerance to anticonvulsant effects of clobazam, diazepam and clonazepam in genetically epilepsy-prone rats. In: Biggio, G., Concas, A., Costa, E. (Eds.), GABAergic synaptic transmission. Adv. in Biochemical Pharmacology, Vol. 47. Raven Press, New York, pp. 249–254.

De Sarro, G.B., Chimirri, A., De Sarro, A., Gitto, R., Grasso, S., Giusti, P., Chapman, A.G., 1995. GYKI 52466 and related 2,3-benzodiazepines as anticonvulsant agents in DBA/2 mice. Eur. J. Pharmacol. 294, 411–422.

De Sarro, G.B., Donato Di Paola, E., Aguglia, U., De Sarro, A., 1996a. Tolerance to anticonvulsant effects of some benzodiazepines in genetically epilepsy-prone rats. Pharmacol. Biochem. Behav. 55, 39–48.

De Sarro, G.B., De Sarro, A., Ammendola, D., Patel, S., 1996b. Lack of development of tolerance to anticonvulsant effects of two excitatory amino acid antagonists, CGP 37849 and CGP 39551 in genetically epilepsy-prone rats. Brain Res. 734, 91–97.

De Sarro, G.B., Rizzo, M., Sinopoli, V.A., Gitto, R., De Sarro, A., Zappalà, M., Chimirri, A., 1999. Relationship between anticonvulsant activity and plasma level of some 2,3-benzodiazepines in genetically epilepsy-prone rats. Pharmacol. Biochem. Behav. 60, 123, in press.

Domino, E.F., Luby, E.D., 1981. Abnormal mental states induced by phencyclidine as a model of schizophrenia. In: Domino, E.F. (Ed.), PCP (Phencyclidine): Historical and Current Perspectives. NPP Books: Ann Arbor, pp. 401–418.

Dunham, N.W., Miya, T.S., 1957. A note on a simple apparatus for detecting neurological deficit in rats and mice. J. Am. Pharm. Ass. 46, 208–209.

Dürmüller, N., Craggs, M., Meldrum, B.S., 1994. The effect of the non-NMDA receptor antagonists GYKI 52466 and NBQX and the competitive NMDA receptor antagonist D-CPPene on the development of amygdala kindling and on amygdala-kindled seizures. Epilepsy Res. 17, 167–174.

File, S.E., 1983. Tolerance to the anti-pentylenetetrazole effects of diazepam in the mouse. Psychopharmacology 79, 284–286.

Gastaut, H., Low, M.D., 1979. Antiepileptic properties of clobazam, a 1,5 benzodiazepine in man. Epilepsia 20, 437–446.

Holmes, K.H., Bilkey, D.K., Laverty, R., Goddard, G.V., 1990. The *N*-methyl-D-aspartate antagonists aminophosphonovalerate and carboxypiperazinephosphonate retard the development and expression of kindled seizures. Brain Res. 506, 227–235.

Honoré, T., Davies, S.N., Drejer, J., Fletcher, E.J., Jacobsen, P., Lodge, D., Nielsen, F.E., 1988. Quinoxalinediones: potent competitive non-NMDA glutamate receptor antagonists. Science 241, 701–703.

Jobe, P.C., 1981. Pharmacology of audiogenic seizures. In: Brown, R.D., Daigneault, E.A. (Eds.) Pharmacology of Hearing. Experiment and Clinical Bases. Wiley, New York, pp. 271–304.

Katayama, K., Morimoto, K., Sato, K., Ohnishi, M., Okamoto, M., Otsuki, S., 1990. The role of NMDA receptors in epilepsy. 1: Effects of NMDA receptor antagonists (CPP and MK-801) on amygdala kindling in rats. Jpn. J. Psychiatry Neurol. 44, 451–452.

Kraus, J.E., Yeh, G.C., Bonhaus, D.W., Nadler, J.V., McNamara, J.O., 1994. Kindling induces the long-lasting expression of a novel population of NMDA receptors in hippocampal region CA3. J. Neurosci. 14, 4196–4205.

Lippa, A.S., Regan, B., 1977. Additional studies on the importance of glycine and GABA in mediating the actions of benzodiazepines. Life Sci. 21, 1779–1784.

Litchfield, J.T., Wilcoxon, F., 1949. A simplifield method of evaluating dose–effect experiments. J. Pharmacol. Exp. Ther. 96, 99–113.

Lodge, D., Aram, J.A., Church, J., Davies, S.N., Martin, D., Millar, J., Zeman, S., 1988. Sigma opiates and excitatory amino acids. In: Lodge, D. (Ed.). Excitatory Amino Acids in Health and Disease. Wiley, Chichester, pp. 237–259.

Löscher, W., 1993. Which animal models should be used in the search for new antiepileptic drugs. Epilepsia 34, 188.

Löscher, W., 1998. Pharmacology of glutamate receptor antagonists in the kindling model of epilepsy. Prog. Neurobiol. 54, 721–741.

Löscher, W., Hönack, D., 1994a. Over-additive anticonvulsant effect of memantine and NBQX in kindled rats. Eur. J. Pharmacol. 259, R3–R5.

Löscher, W., Hönack, D., 1994b. Effects of the non-NMDA antagonists NBQX and the 2,3-benzodiazepine GYKI 52466 on different seizure types in mice: comparison with diazepam and interactions with flumazenil. Br. J. Pharmacol. 113, 1349–1357.

Löscher, W., Nolting, B., Hönack, D., 1988. Evaluation of CPP, a selective NMDA antagonists, in various rodent models of epilepsy. Comparison with other NMDA antagonists,and with diazepam and phenobarbital. Eur. J. Pharmacol. 152, 9–17.

Löscher, W., Rundfeldt, C., Hönack, D., 1993. Low doses of NMDA receptor antagonists synergistically increase the anticonvulsant effect

of the AMPA receptor antagonist NBQX in the kindling model of epilepsy. Eur. J. Neurosci. 5, 1545–1550.

Majkowski, J., 1989. Kindling and memory. In: Trimble, M.R., Boldwig, T.G. (Eds.), The Clinical Relevance of Kindling. Wiley, Chichester, pp. 211–230.

McNamara, J.O., 1989. Development of new pharmacological agents for epilepsy; lessons from the kindling model. Epilepsia 30, S13–S18.

McNamara, J.O., 1994. Cellular and molecular basis of epilepsy. J. Neurosci. 14, 3413–3425.

Morris, R.G.M., Anderson, E., Lynch, G.S., Baudry, M., 1986. Selective impairment of learning and blockade of long-term potentiation by and *N*-methyl-D-aspartate receptor antagonist. Nature 319, 774–776.

Moshé, S., Ludwig, N., 1988. Kindling. In: Pedley T., Meldrum B.S. (Eds.), Recent Advances in Epilepsy. Churchill Livingstone, Edinburgh, pp. 21–44.

Muller, D., Joly, M., Lynch, G., 1988. Contribution of quisqualate and NMDA receptors to the induction and expression of LTP. Science 242, 1694–1697.

Mutani, R., Cantello, R., Gianelli, M., Betticcu, D., 1991. Rational basis for the development of new antiepileptic drugs. Epilepsy Res. 19, 23–28.

Namba, T., Morimoto, K., Sato, K., Yamada, N., Kuroda, S., 1994. Antiepileptogenic and anticonvulsant effects of NBQX, a selective AMPA receptor antagonist, in the rat kindling model of epilepsy. Brain Res. 638, 36–44.

Oxley, J., 1986. Tolerance to the antiepileptic effect of clobazam in patients with severe epilepsy. In: Koella, W.P., Frey, H.H., Froscher, W., Meinardi, H. (Eds.), Tolerance to Beneficial and Adverse Effects of Antiepileptic Drugs. Raven Press, New York, pp. 119–125.

Patel, S., De Sarro, G.B., Meldrum, B.S., 1988. Regulation of seizure threshold by excitatory amino acids in the striatum and entopeduncular nucleus of rats. Neuroscience 23, 837–850.

Rogawski, M.A., 1993. Therapeutic potential of excitatory amino acid antagonists: channel blockers and 2,3-benzodiazepines. TIPS 14, 325–331.

Saitoh, H., Osonoe, K., Osonoe, M., Mori, N., Kumashiro, H., 1991. An important role of non-NMDA receptors in the expression of kindled amygdaloid seizure in rats. Jpn. J. Psychiatry Neurol. 45, 386–387.

Sato, M., Racine, R.J., McIntyre, D.C., 1990. Kindling: basic mechanisms and clinical validity. Electroencephalogr. Clin. Neurophysiol. 76, 459–472.

Schmidt, D., Löscher, W., Oxley, J., 1986a. Clinical development of benzodiazepines and other antiepileptic drugs for intractable epilepsy. In: Schmidt, D., Morselli, P.L. (Eds.), Intractable Epilepsy, Raven Press, New York, pp. 219–225.

Schmidt, D., Rohde, M., Wolf, P., Roeder-Vanner, U., 1986b. Tolerance to the antiepileptic effect of clobazam. In: Koella, W.P., Frey, H.H., Froscher, W., Meinardi, H. (Eds.), Tolerance to Beneficial and Adverse Effects of Antiepileptic Drugs. Raven Press, New York pp. 109–115.

Smith, S., Chapman, A.G., 1993. Acute and chronic anticonvulsant effects of D(−)CPPene in genetically epilepsy-prone rats. Epilepsy Res. 15, 193–199.

Smith, S.E., Durmuller, N., Meldrum, B.S., 1991. The non-*N*-methyl-D-aspartate antagonists, GYKI 52466 and NBQX are anticonvulsant in two animal models of reflex epilepsy. Eur. J. Pharmacol. 201, 179–183.

Tarnawa, I., Farkas, S., Berzsenyi, P., Pataki, A., Andrasi, F., 1989. Electrophysiological studies with a 2,3-benzodiazepine muscle relaxant: GYKI 52466. Eur. J. Pharmacol 167, 193–199.

Turski, L., Jacobsen, P., Honorè, T., Stephens, D.N., 1992. Relief of experimental spasticity and anxiolytic/anticonvulsant actions of the alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionate antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(*F*)quinoxaline. J. Pharmacol. Exp. Ther. 260, 742–747.

Velisek, L., Kubova, H., Mares, P., Vachova, D., 1995. Kainate/AMPA receptor antagonists are anticonvulsant against the tonic hindlimb component of pentylenetetrazol-induced seizures in developing rats. Pharmacol. Biochem. Behav. 51, 153–158.

Yamaguchi, S., Donevan, S.D., Rogawski, M.A., 1993. Anticonvulsant activity of AMPA/kainate antagonists: comparison of GYKI 52466 and NBQX in maximal electroshock and chemoconvulsant seizure models. Epilepsy Res. 15, 179–184.

Yanagita, T., Takahashi, S., 1970. Development of tolerance to and physical dependence on barbiturates in rhesus monkeys. J. Pharmacol. Exp. Ther. 172, 163–169.