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Comparative anticonvulsant activity of some 2,3-benzodiazepine derivatives in rodents

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Abstract

The anticonvulsant activities of some 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA)/kainate receptor antagonists, noncompetitive (2,3-benzodiazepines) and a competitive 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(*F*)-quinoxaline (NBQX), were compared in different experimental seizure models. In particular, compounds were evaluated against audiogenic seizure in DBA/2 mice, maximal electroshock seizure (MES) test and various chemoconvulsant models; both groups showed a protective action against audiogenic seizure, MES- and pentylenetetrazole (PTZ)-induced seizures. All 2,3-benzodiazepines were also protective against clonic and tonic seizures and lethality induced by 4-aminopyridine, kainate, AMPA and 3-mercaptopropionic acid but were ineffective against NMDA-induced seizures. NBQX was unable to affect 4-aminopyridine-, mercaptopropionic acid- and NMDA-induced seizures. The duration of anticonvulsant action of 33 μmol/kg of some 2,3-benzodiazepine in DBA/2 mice, genetically susceptible to audiogenic seizures, was also investigated. The derivatives possessing a thiocarbonyl group at the C-4 position of heptatomic ring showed higher anticonvulsant activities and longer lasting protective effects. We conclude that all 2,3-benzodiazepines studied are effective against various models of experimental epilepsy and the presence of thiocarbonyl groups at the C-4 position of heptatomic ring is able to increase the anticonvulsant effect of these compounds.

Keywords: Epilepsy; AMPA/kainate receptor antagonists; NBQX; GYKI 52466; GYKI 53655; GYKI 53773; CFM-2; 2,3-Benzodiazepines; Audiogenic seizures; DBA/2 mice; Maximal electroshock; Chemoconvulsions

1. Introduction

The antagonists of 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA)/kainate receptor possess some advantages compared to *N*-methyl-D-aspartate (NMDA) receptor antagonists, including higher neuroprotective potency after ischaemic attacks, higher anticonvulsant potency in temporal lobe epilepsy and reduced side-effects (Buchan et al., 1993; Rogawski, 1993; Löscher and Hönack, 1992; Löscher and Schmidt, 1994; Lees, 1996; Chimirri et al., 1997, 1998, 1999; De Sarro et al., 1995, 1998, 1999a,b; Zappalà et al., 2000). Therefore, AMPA/kainate receptor antagonists could be of interest in the therapies of neurodegenerative disorders (Buchan et al., 1993; Rogawski, 1993; Lees, 1996; De Sarro et al., 1998, 1999a,b). In various models of epileptic seizures, both 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(*F*)-quinoxaline (NBQX) and the prototype noncompetitive AMPA/kainate receptor antagonist 1-(4-aminophenyl)-4-methyl-7,8-methylendioxy-5*H*-2,3-benzodiazepine (GYKI 52466) were unable to exert anticonvulsant effects at doses below those inducing sedation and motor impairment (Scheme 1). This indicates that the therapeutic index of the current generation of AMPA/kainate receptor antagonists may be lower than initially thought (Yamaguchi et al., 1993; Löscher and Hönack, 1994).

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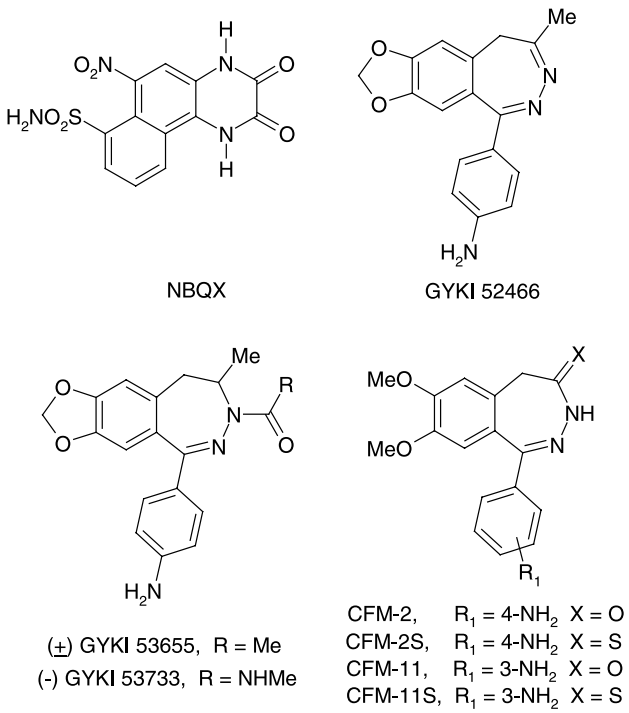

Scheme 1.

Ionotropic AMPA/kainate receptors can be divided into two distinct receptor complexes: AMPA receptors and kainate receptors (Bettler and Mulle, 1995). Because of the lack of potent and selective agonists and antagonists for kainate receptors, the physiological role of kainate receptors has so far remained obscure (Henley, 1994; Bettler and Mulle, 1995; Jorgensen et al., 1995; Fletcher and Lodge, 1996; Lerma et al., 1997). To date, most AMPA/kainate receptor antagonists show a preference for AMPA receptors although one compound, 5-nitro-6,7,8,9-tetrahydrobenzo(*G*)indole-2,3-dione-3-oxime (NS-102), has a moderate seven-fold preference for the low affinity kainate receptor (Lees, 1996). AMPA receptor types are composed of combinations of four (GluR1–4) subunits, existing as "flip and flop" splice variants which mediated fast excitatory potentials by the flux of Na^+ and Ca^{2+} (Sutcliffe et al., 1996). The AMPA receptor complex has at least three distinct binding sites at which antagonists can act: (a) the glutamate (Glu) binding sites for competitive antagonists, (b) an allosteric site at which noncompetitive antagonists can bind and (c) a polyamine site, within the ion channel (Chimirri et al., 1999). In addition, on these receptor proteins, five proteins (GluR5–7, KA1 and KA2) have a ligand binding site at which kainate is far more potent than AMPA (Bettler and Mulle, 1995; Jorgensen et al., 1995; Lees, 1996). Since most currently available AMPA/kainate receptor antagonists have little specificity for the various subunits or combinations of subunits, new and more selective compounds should be developed. These drugs may not only be of great value in discriminating the function of kainate and AMPA receptors and their subunits, but may also have a potential as novel therapeutic agents with less adverse effects than the currently available AMPA/kainate antagonists.

The seven new noncompetitive AMPA/kainate receptor antagonists described in this study were selected from a series of 7,8-dimethoxy-3,5-dihydro-4*H*-2,3-benzodiazepine-4-(thi)ones (CFMs) and 3-*N* substituted 7,8-methylenedioxy-4,5-dihydro-2,3-benzodiazepines (GYKI 53655 and GYKI 53773) (Scheme 1). The basis of selection was high potency and selectivity for AMPA and/or kainate receptors, high in vivo potency after systemic administration, and an acceptable ratio between neuroprotective or anticonvulsant properties and adverse effects, i.e., doses exerting neuroprotective and anticonvulsant effects being clearly below those inducing "neurotoxic" effects e.g. motor impairment or sedation. Pharmacological characteristics of these novel compounds are described in this study with special emphasis on their effects in genetic or chemical models of (convulsive) epilepsy.

In addition, a K^+ channel antagonist 4-aminopyridine is able to elicit convulsion in different animal species including humans (Yamaguchi and Rogawski, 1992; Spyker et al., 1980); it produces seizure-like events and interictal epileptiform discharges in the entorhinal and temporal neocortex and short recurrent discharges in the hippocampus. Epileptiform discharges in the hippocampus are considered a model of drug resistant epilepsy and are sensitive to retigabine (Yonekawa et al., 1995a; Armand et al., 1999) but not to conventional anticonvulsants such as carbamazepine, phenytoin and valproic acid (Dreier and Heinemann, 1990; Zhang et al., 1991; Yonekawa et al., 1995b). For this reason, novel 2,3-benzodiazepines, NBQX and a conventional antiepileptic, diazepam, were evaluated in the 4-aminopyridine model. Diazepam has been chosen as a conventional antiepileptic drug being considered a very active compound against seizures induced by convulsant agents impairing the GABAergic neurotransmission.

2. Materials and methods

2.1. Animals

Male DBA/2 mice weighing 6–12 g (22–26 days old) and ICR CD-1 mice weighing 20–30 g (42–48 days old) were used in the present study (Harlan Italy Correzzana, Milano, Italy). The animals were housed in groups of 8–10 under a 12-h light/dark cycle (lights on at 7:00 a.m.) with food and water available ad libitum. The experimental protocol was approved by the University of Catanzaro Ethical Committee. All procedures are in compliance with the Nationals Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) and European Communities Council Directive of 24 November 1986 (86/609 EEC).

2.2. Audiogenic seizures in DBA/2 mice

The experiments were performed according to the method previously described by De Sarro et al. (1984). Test compounds or vehicle (controls) were given intraperitoneally to groups of 10 mice per dose. Thirty minutes later each mouse was placed under a hemispheric perspex dome (58 cm in diameter) challenged with a 12–16 kHz sinusoidal tone at 109 dB in a covered plexiglass cylinder. Seizure response was assessed by two independent observers. The sound evoked behaviour was coded using the following scale: 0 = *no response*, 1 = *wild running*, 2 = *clonus*, 3 = *tonic flexor* and/or extensor, 4 = *respiratory arrest*, on the basis of the concordant opinion of the observers. Sound stimulus was applied for 60s, but it was interrupted earlier, when the observed animal showed tonic extensor seizure. The maximum response was recorded for each animal. Rectal temperature was recorded immediately prior to auditory testing using an Elektrolaboratoriet thermometer type T.E.3. Behavioral changes were observed during the period between drug administration and auditory testing.

In paudiogenic seizure test, the duration of the anticonvulsant action of 33 μmol/kg GYKI 52466, CFM-2, CFM-2S, CFM-11, CFM-11S, GYKI 53655 and GYKI 53773 (LY300164, talampanel) were also examined. Percentage of mice showing clonic or tonic phase and the duration of anticonvulsant activity were compared to control group and, eventually, statistically analyzed. In these experiments, separate vehicle- and drug-treated groups were used at each pretreatment time (15–240 min).

2.3. Anticonvulsant effects in the maximal electroshock seizure (MES) test

The tested compounds were administered intraperitoneally to groups of 10 male ICR CD-1 mice (body weight 20–26 g) per dose in a volume of 0.1 ml/10 g. Tonic seizures were induced by electroshock via ear electrodes 30 min (NBQX: 5 min) after drug administration. The stimulus, which was applied for 0.2 s, consisted of rectangular impulses of 4.64 ms duration and 14.7 mA amplitude at a frequency of 100 Hz. The incidence of tonic (hind limb) seizures was counted.

2.4. Pentylenetetrazole (PTZ)-induced seizures in ICR CD-1 mice

Male ICR CD-1 mice (20–26 g, 42–48 days old) were pretreated with vehicle or drug 45 min (groups of 10 mice per dose) before the subcutaneous administration of PTZ. For systemic injections, all tested compounds were given intraperitoneally (0.1 ml/10 g of mouse body weight). The convulsive dose 97 (CD_{97}) of PTZ (85 mg/kg) was applied and the animals were observed for 30 min. A threshold convulsion was an episode of clonic spasms lasting for at least 5 s. The absence of this threshold convulsion over 30 min indicated that the tested substance had the ability to elevate the PTZ seizure threshold (Swinyard and Woodhead, 1982).

2.5. Seizures induced by administration of kainate

Kainate was administered subcutaneously at a dose of 32 mg/kg (previously determined CD_{97} value) 15 min after intraperitoneal administration of 2,3-benzodiazepine derivatives. ICR CD-1 (22–30 g, 42–48 days old) mice showing 5 s or more of clonic activity were scored as nonprotected according to Donevan et al. (1994). The period of observation was 60 min.

2.6. Seizures induced by administration of 4-aminopyridine

4-Aminopyridine was administered subcutaneously to ICR CD-1 (22–30 g, 42–48 days old) mice (groups of 10 mice per dose) at a dose of 13.3 mg/kg (previously determined CD_{97} value) 15 min after intraperitoneal administration of 2,3-benzodiazepine derivatives. Animals showing tonic extension or death were scored as nonprotected according to Yamagucki and Rogawski (1992). The period of observation was 60 min.

2.7. Seizures induced by administration of 3-mercaptopropionic acid

3-Mercaptopropionic acid was administered intravenously at a dose of 110 mg/kg (previously determined CD_{97} value) 15 min after intraperitoneal administration of 2,3-benzodiazepine derivatives (groups of 10 mice per dose). ICR CD-1 (22–30 g, 42–48 days old) mice showing clonic or tonic activity or death were scored as nonprotected according to Horton and Meldrum (1973). The period of observation was 60 min.

2.8. Seizures induced by intracerebroventricular administration of NMDA or AMPA

Seizures were induced by intracerebroventricular injections of NMDA or AMPA. In particular, animals (groups of 10 mice per dose) were pretreated intraperitoneally with AMPA/kainate receptor antagonists and later for intracerebroventricular injections, mice were anesthetized with ether and injections were made in the left or right lateral ventricle (coordinates 1 mm posterior and 1 mm lateral to the bregma; depth 2.4 mm) using a 10-μl Hamilton microsyringe (type 701N) fitted with a nylon cuff on the needle as previously described (De Sarro et al., 1994). Injections of drugs by this procedure led to a uniform distribution throughout the ventricular system within 10 min (De Sarro et al., 1988). The animals were placed singly in a 30 × 30 × 30 cm box and the observation time was 20 min after NBQX injection and 30 min after the administration of AMPA or NMDA. The occurrence of clonic and tonic seizure signs and their latency were recorded. For statistical analysis, clonus, tonus

or death within the 30-min observation period was used as a parameter and inhibition of seizure phases or death as criterion of drug effect. The relationship between the doses (μmol/kg) and the relative rate of protection compared to the vehicle controls (nearly 100% mortality) was assessed.

2.9. Statistical analysis

Statistical comparison between groups of control and drug-treated DBA/2 or ICR CD-1 mice was made using Fisher's exact probability test (incidence of the seizure phases) or ANOVA with Dunnett's t test (rectal temperatures). The percentage incidence of each phase of the audiogenic seizure was determined for each drug. These values were plotted against the corresponding doses by a computer construction of the dose–effect curves for calculation of ED_{50} (with 95% confidence limits). The ED_{50} values for each compound were calculated using a computer program of the method of Litchfield and Wilcoxon (1949). At least 32 animals were used to calculate each ED_{50} value. Statistical evaluation for the time course of anticonvulsant effects was carried out using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison when appropriate.

2.10. Drugs

NBQX (molecular weight, MW = 336.3) was kindly supplied by Novo Nordisk (Malov, Denmark). AMPA was purchased from Tocris Cookson (Bristol, UK), NMDA, kainate, 4-aminopyridine and 3-mercaptopropionic acid from Sigma (St. Louis, MO, USA). GYKI 52466 dihydrochloride (MW = 366.25) was purchased from Tocris Cookson. Talampanel (GYKI 53773, LY 300164, MW = 337.4) was kindly supplied by Lilly Research Labs (Indianapolis, IN, USA) and GYKI 53655 (MW = 333.4) was kindly supplied by EGIS Pharmaceuticals (Budapest, Hungary). All the 2,3-benzodiazepine (CFM-2, MW = 311.3; CFM-2S, MW = 327.4; CFM-11, MW = 311.3; CFM-11S, MW = 327.4) derivatives studied were synthesized in our laboratories.

For systemic injections, all compounds were given intraperitoneally (0.1 ml/10 g of body weight of the mouse) as a freshly prepared solution in 50% dimethylsulphoxide and 50% sterile saline (0.9% NaCl). Doses and time of administration are reported in the tables. Previous experiments have shown that this vehicle, when administered intraperitoneally, does not affect either behavior or response to auditory stimulation of DBA/2 mice or seizures induced by the chemoconvulsants used in the present study in ICR CD-1 mice.

All drugs administered intracerebroventricularly were dissolved in sodium phosphate buffer 67 mM, microinjected in a volume of 5 μl per mouse. Doses and time of administration are reported in the tables. NBQX was dissolved in a minimum quantity of 1 N NaOH, the final volume was made up with sodium phosphate buffer. When necessary, pH was adjusted to 7.3–7.4 by adding 0.2 N HCl. In order to avoid the light sensitivity of some compounds, weighing and handling were carried out under sodium vapor lamps and the substances were protected from light during the experiments.

3. Results

3.1. Anticonvulsant activity in DBA/2 mice

All 2,3-benzodiazepine derivatives and NBQX administered 30 min before auditory stimulation were able to protect against sound-induced clonic and tonic seizures in a dose dependent manner. Tonic fit and death were completely prevented by CFM-2S at dose over 3.3 μmol/kg, GYKI 53655, GYKI 53773 and CFM-11S at doses over 10 μmol/kg, CFM-2, CFM-11 and NBQX at doses over 21 μmol/kg, respectively, while the corresponding value for GYKI 52466 was 33 μmol/kg (Fig. 1). The ED_{50} values for the inhibition of clonic and tonic seizures are reported in Table 1. Diazepam was able to antagonize the clonic and tonic phases of the seizures in DBA/2 mice at dose over 0.30 μmol/kg. All

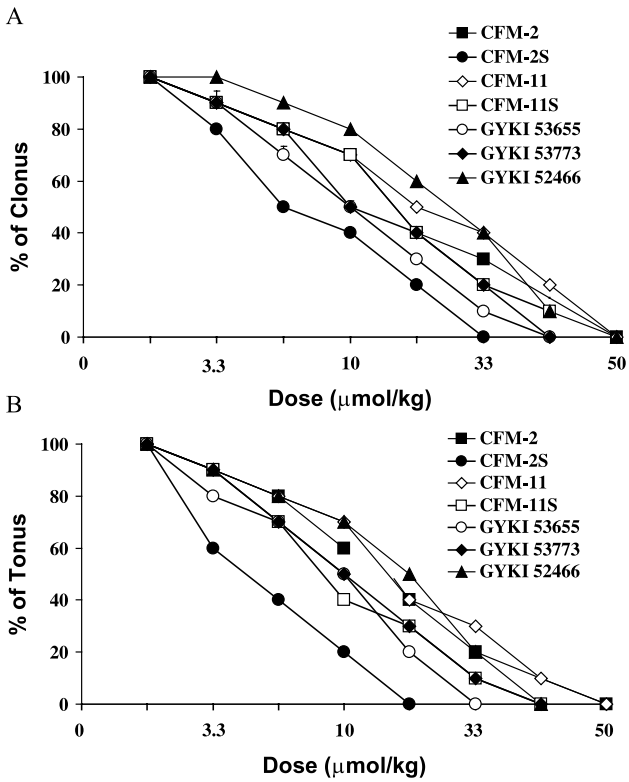

Fig. 1. The dose response curves of the anticonvulsant effects of some AMPA/kainate receptor antagonists administered intraperitoneally 1–100 μmol/kg at 60 min CFM-2 (■–■), CFM-2S (●–●), CFM-11 (◇–◇), CFM-11S (□–□), GYKI 53655 (○–○), GYKI 53773 (◆–◆) and GYKI 52466 (▲–▲). Abscissa shows the doses, ordinate shows (A) percentage of clonic seizures, (B) percentage of tonic seizures. Ten animals were used for the determination of each point. $n = 40–60$ mice for each compound tested.

Table 1
Anticonvulsant effect in audiogenic sensible DBA/2 mice and in the maximal electroshock seizure test in ICR CD-1 mice

Compound	ED_{50} (μmol/kg ip)			
	Audiogenic seizures		MES	PTZ
	Clonus	Tonus		
GYKI 52466	35.8 (24.4–52.4)	25.3 (16–40)	35.7 (29.3–43.4)	68.3 (56.2–83.1)
CFM-2	15 (9–24)	12.6 (8–19)	15.9 (7.3–33.5)	22.6 (11.7–43.8)
CFM-11	19.3 (16.9–22)	18.3 (16–20.8)	22.5 (13.4–37.7)	40.7 (21.9–56.9)
CFM-2S	6.3 (2.6–15.4)	3.3 (1.3–8.3)	7.75 (3.89–15.4)	15.4 (7.0–33.9)
CFM-11S	18.8 (8.7–36.5)	9.1 (3.7–22.3)	18.5 (14.1–23.7)	34.9 (28.4–43.1)
NBQX	18.3 (9.45–35.3)	11.9 (6.15–22.5)	39.6 (26.9–58.3)	85.9 (71.5–103.3)
Diazepam	0.28 (0.20–0.39)	0.24 (0.15–0.39)	>30	0.43 (0.27–0.68)
GYKI 53655	10.3 (7.4–14.3)	8.3 (6.1–11.3)	15.5 (11.5–20.9)	42.5 (25.8–70.1)
GYKI 53773	13.4 (10.1–17.8)	9.7 (7.0–13.4)	28.8 (23.5–35.3)	56.3 (34.2–92.7)

All data above reported are expressed in μmol /kg and were calculated according to the method of Litchfield and Wilcoxon (1949).

2,3-benzodiazepines were much less potent than diazepam (Table 1).

3.2. Anticonvulsant effects in the MES test

All AMPA/kainate receptor antagonists, including NBQX, blocked generalized tonic seizures in the MES test after intraperitoneal administration in mice (Table 1). Similarly, to earlier reports (Donevan et al., 1994; Vizi et al., 1996; Chimirri et al., 1997, 1998), the compounds protected mice against tonic extension seizures in the MES test in a dose-dependent fashion. The ED_{50} values (+95% confidence limits) of studied compounds are shown in Table 1.

The most potent compound was CFM-2S (ED_{50}: 7.75 μmol/kg), followed by GYKI 53655 (ED_{50}: 15.5 μmol/kg) and CFM-2 (ED_{50}: 15.9 μmol /kg), whereas all other 2,3-benzodiazepines had ED_{50} values between 18.5 and 35.7 μmol /kg. NBQX was the less potent compound; it was active at a dose of 39.6 μmol/kg.

3.3. Anticonvulsant effects against PTZ-induced seizures

All 2,3-benzodiazepines studied demonstrated anticonvulsant activity against PTZ-induced seizure at doses double than those able to possess anticonvulsant activity against audiogenic seizures or maximal electroshock test. The compounds showed the following rank order of potency: CFM-2S>CFM-2>CFM-11S>CFM-11>GYKI 53665> GYKI 53773>GYKI 52466. The anticonvulsant activity of NBQX against PTZ-induced seizures was observed following doses 4.69 times larger than those able to exert anticonvulsant activity against the clonic phase of audiogenic seizures (Table 1).

3.4. Anticonvulsant effects against NMDA- and AMPA-induced seizures

All compounds tested were ineffective against NMDA-induced seizures, while all drugs, with exception of diazepam, were able to protect against seizures induced by AMPA (Table 2). NBQX was the most potent compound against tonus induced by intracerebroventricular administration of AMPA. The 2,3-benzodiazepines studied showed the following rank order of potency against clonus induced by intracerebroventricular administration of AMPA: CFM-11S>CFM-2S>GYKI 53655>CFM-11>CFM-2>GYKI 53773>GYKI 52466.

3.5. Anticonvulsant activity against 4-aminopyridine-, kainate- or 3-mercaptopropionic acid-induced seizures

All 2,3-benzodiazepines studied were able to protect against seizures induced by 4-aminopyridine, kainate or 3-mercaptopropionic acid, while diazepam was ineffective against 4-aminopyridine and kainate, and NBQX was ineffective against 4-aminopyridine and 3-mercaptopropionic acid (Table 3). The compounds showed the following rank order of potency against kainate-induced seizures: CFM-2S>GYKI 53773>GYKI 53665>CFM-2>GYKI 52466> CFM-11S>CFM-11>NBQX.

Table 2
Effects of compounds studied against AMPA- or NMDA-induced seizures in DBA/2 mice

Compound	ED_{50} (mg/kg ip) for prevention of seizures induced by			
	NMDA		AMPA	
	Tonus	Clonus	Tonus	Clonus
GYKI 52466	NA	NA	40.5 (26.3–60.8)	57.5 (43.5–76.0)
CFM-2	NA	NA	25 (16.5–30.0)	32 (23.2–44.3)
CFM-11	NA	NA	27.8 (21.5–35.9)	30.9 (23.9–39.9)
CFM-2S	NA	NA	11.9 (4.60–30.8)	17.1 (7.70–38.0)
CFM-11S	NA	NA	14.2 (11.1–18.17)	16.3 (12.54–21.2)
NBQX	NA	NA	7.4 (4.7–11.6)	16.5 (10.8–25.2)
Diazepam	NA	NA	NA	NA
GYKI 53655	NA	NA	16.7 (12.8–21.8)	25.9 (18.1–37.1)
GYKI 53773	NA	NA	29.1 (22.6–37.5)	44.2 (34.6–56.5)

All data above reported are expressed in μmol/kg and were calculated according to the method of Litchfield and Wilcoxon (1949). NA = not active until 100 μmol/kg.

Table 3
Anticonvulsant effect against 4-aminopyridine-kainate- or 3-mercaptopropionic acid-induced seizure in ICR CD-1 mice

Compound	4-Aminopyridine	Kainate	3-Mercaptopropionic acid
GYKI 42566	143 (129–169)	27.8 (18.8–40.9)	47 (32.9–67.2)
CFM-2	27.1 (19.7–37.3)	23.06 (19.6–27.13)	28.3 (18.2–44.1)
CFM-11	32.7 (26.2–40.8)	33.49 (27.18–40.03)	37.8 (29–49.3)
CFM-2S	12.6 (9.1–17.4)	11.5 (8.85–14.95)	18.1 (12.8–25.6)
CFM-11S	29.3 (21.9–39.2)	28.9 (22.4–37.29)	32.8 (26.2–40.9)
Diazepam	NA	NA	0.90 (0.63–1.28)
NBQX	NA	35.6 (24.3–52.1)	NA
GYKI 53655	28.2 (18.8–42.3)	15.4 (9.7–24.5)	57.1 (39.7–82.1)
GYKI 53773	28.8 (23.5–35.3)	15.3 (12.3–19)	53.4 (36.9–77.3)

All data above reported are expressed in mg/kg and were calculated according to the method of Litchfield and Wilcoxon (1949). NA = not active until 100 μmol/kg.

3.6. Time course of anticonvulsant activity in the audiogenic sensible DBA/2 mice

As for the time course of anticonvulsant activity (Fig. 2), the effect of GYKI 52466 decreased to 50% after approx. 90 min, while the other compounds showed more than 70% seizure suppression even after 120 min. The strong anticonvulsive effect of CFM-2S and GYKI 53733 gradually disappeared from 180 to 240 min, while the effect of CFM-2, CFM-11, CFM-11S and GYKI 53655 rapidly decreased between 150 and 180 min and then it slowly disappeared. However, after 240 min, all compounds investigated were ineffective.

In exit inhibition (Fig. 2), GYKI 52466 was completely protective for only 30 min, while the anticonvulsant effects of CFM-2, CFM-11, CFM-11S, GYKI 53655 and GYKI 53733 lasted between 90 and 105 min, and those of CFM-2S lasted approx. 120 min.

4. Discussion

The present study demonstrated the anticonvulsant efficacy of seven 2,3-benzodiazepine derivatives in various seizure models of experimental epilepsy. GYKI 52466 and related compounds were found to protect against audiogenic seizures, maximal electroshock, PTZ-, AMPA/kainate-, 4-aminopyridine- and mercaptopropionic acid-induced seizures. It is very difficult to extrapolate a possible more selective action of one of these 2,3 benzodiazepines on AMPA or kainate subtype receptors and a conclusion could be purely speculative. Reduction in locomotor activity, sedation, muscle relaxant activity and mild ataxia as side-effects of these derivatives have been reported by several authors (Honorè et al., 1988; Smith et al., 1991; Turski et al., 1992; Yamaguchi et al., 1993; De Sarro et al., 1995, 1998, 1999a,b) 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; Abraham et al., 2001).

The studied 2,3-benzodiazepines showed an excellent, broad spectrum anticonvulsant properties against seizures evoked by physical (sound and electroshock) or chemoconvulsive agents indicating an antiepileptic efficacy superior to NBQX and diazepam against MES-test and 4-aminopyridine-induced seizures (Tables 1 and 3).

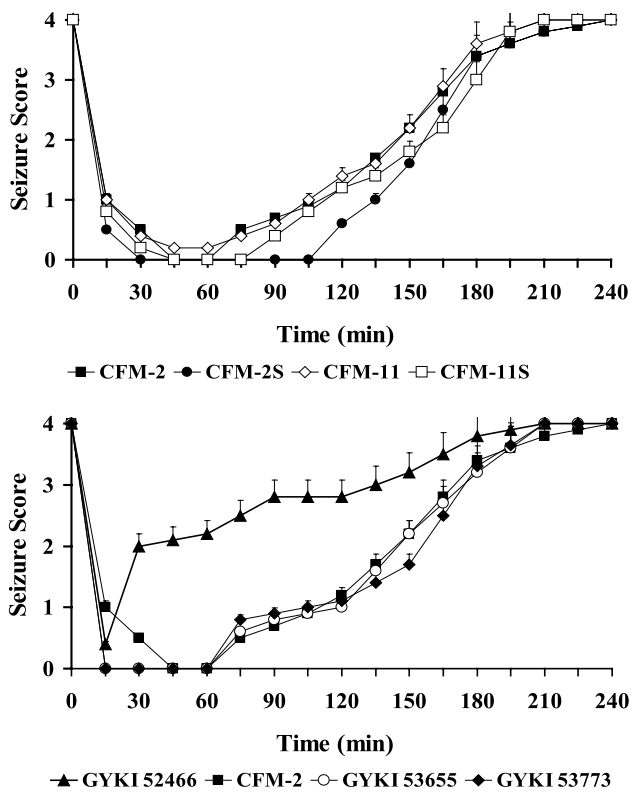

Fig. 2. Anticonvulsant effects of CFM-2 (■–■), CFM-2S (●–●), CFM-11 (◇–◇), CFM-11S (□–□), GYKI 53655 (○–○), GYKI 53773 (◆–◆) and GYKI 52466 (▲–▲) against audiogenic seizures in DBA/2 mice. The ordinate shows seizure score, the abscissa shows the time after intraperitoneal administration of drug in minutes. Ten animals were used for the determination of each point.

In particular, as can be seen in Tables 1 and 3, CFM-2S was the most active compound within the series of 2,3-benzodiazepine derivatives, probably due to its physicochemical characteristics. It was also more effective than competitive AMPA receptor antagonist NBQX both in preventing MES and against some chemoconvulsant-induced seizures (Tables 1 and 3).

Table 2 shows that no compound studied proved to prevent seizures induced by NMDA, whereas all 2,3-benzodiazepine derivatives and NBQX generally showed interesting activity against AMPA-induced seizures. If we compare the rank order of potency against AMPA or against kainate-induced seizures, CFM-2S, GYKI 53773, GYKI 53655 and CFM-2 appear the most interesting compounds.

It is also interesting to note that CFM-2S shows the longest time-course with respect to the other studied compounds (Fig. 2). This behaviour can be explained considering its capacity to cross the blood–brain barrier and to interact with AMPA receptors as such, but it could also be due to its biotransformation to CFM-2 by changing thiocarbonyl group into carbonyl moiety (De Sarro et al., 1999a,b).

In conclusion, CFM-2S proved to be most potent and longer-lasting of the tested AMPA/kainate receptor antagonists in all seizures models of experimental epilepsy; only diazepam showed more marked effects against audiogenic seizures, PTZ- and mercaptopropionic acid-induced convulsions. The latter result is obvious since diazepam is the most active compound in enhancing GABAergic neurotransmission among drugs tested in the present study. The fact that 2,3-benzodiazepine tested showed a broader spectrum of anticonvulsant efficacy when compared with NBQX might suggest that they act on some specific AMPA/kainate receptor subtypes that are not affected by NBQX. It is also possible to conclude that 2,3-benzodiazepine but not NBQX are able to interfere with convulsant phenomena elicited by 4-aminopyridine and 3-mercaptopropionic acid. Since previous findings demonstrated that kainate receptors, containing the GluR5 subtype of kainate receptor, regulate synaptic inhibition in the hippocampus and could provide a selective and new target for antiepileptic therapy (Clarke et al., 1997; Löscher et al., 1999), it could be interesting to evaluate the effects of some 2,3-benzodiazepines in some form of temporal epilepsy.

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