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Relationship Between Anticonvulsant Activity and Plasma Level of Some 2,3-Benzodiazepines in Genetically Epilepsy-Prone Rats

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DE SARRO, G. M. RIZZO, V. A. SINOPOLI, R. GITTO, A. DE SARRO, M. ZAPPALA AND A. CHIMIRRI. *Relationship between anticonvulsant activity and plasma level of some 2,3-benzodiazepines in genetically epilepsy-prone rats.* PHARMACOL BIOCHEM BEHAV **61**(3) 215–220, 1998.—The anticonvulsant effects of some novel 2,3-benzodiazepines acting as α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid/kainate (AMPA/KA) antagonists were evaluated in genetically epilepsy prone rats. The ED_{50} values against clonic and tonic seizures (in μmol/kg) revealed that the rank order of anticonvulsant activity was: GYKI 52466 > 2,3BZ-2 > 2,3 MBZ-2 > NBQX. Maximal anticonvulsant protection was observed 15–45 min after the IP administration of NBQX and GYKI 52466, 30–90 min after the IP administration of 2,3BZ-2, and 45–120 min after the IP administration of 2,3MBZ-2. The time course of plasma levels of rats treated with GYKI 52466 showed that peak plasma concentration was observed 15 min after IP administration, 2,3BZ-2 revealed that peak plasma concentration was achieved 45 min after IP administration, whereas following 2,3MBZ-2 administered IP, two curves were detected; one is referred to the parent compound and the other to its demethylate metabolite that corresponds to 2,3BZ-2. The therapeutic index (ratio of TD_{50} values for impaired rotarod performance and ED_{50} values for anticonvulsant activity) revealed that NBQX and GYKI 52466 were slighly more toxic than 2,3BZ-2 and 2,3MBZ-2. The present data suggest that 2,3-benzodiazepines acting at AMPA/kainate receptors play an important role in the generation and/or propagation of the audiogenic seizures in genetically epilepsy-prone rats. © 1998 Elsevier Science Inc.

AMPA/kainate antagonists 2,3-Benzodiazepine Genetically epilepsy prone rats Plasma level Epilepsy

THE discovery of selective and nonselective α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid/kainate (AMPA/KA) receptor antagonists such as 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(F)-quinoxaline (NBQX), and 1-(4-aminophenyl)-4-methyl-7,8-methylene-dioxy-5H-2,3-benzodiazepine (GYKI 52466) have facilitated extensive studies of the physiological roles of AMPA/kainate receptors in the central nervous system (CNS). Several actions of selective AMPA/kainate receptor antagonists, for example, neuroprotective actions against ischemia (1,14,23,25), glutamate-induced neurotoxicity (29), and anti-Parkinsonism actions (17,28) have been reported. Systemic administration of 2,3-benzodiazepines was able to attenuate in a dose-dependent manner the audiogenic seizures in DBA/2 mice (4). Furthermore, 2,3-benzodiazepines antagonized in DBA/2 mice seizures induced by AMPA but not those induced by NMDA (2,5). Although the neurological activity of these AMPA/kainate receptor antagonists have been studied in acute models of epilepsy and neurodegenerative diseases in vivo and/or in vitro (2,4,9,15,20,24), very little is known about their action in a chronic model of generalized epilepsy. In addition, no correlations exist between anticonvulsant potency and plasma levels of these 2,3-benzodiazepine derivatives.

The genetically epilepsy-prone rats in a Sprague–Dawley-derived strain that was initially discovered at the University of Arizona (16) has been widely used to demonstrate the anti-

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convulsant and proconvulsant properties of novel and conventional compounds (6–8,10–12,19). The present study was aimed to determine the anticonvulsant properties of 3,5-dihydro-7,8-dimethoxy-1-phenyl-4H-2,3-benzodiazepin-4-one (2,3BZ-2) and its methyl-derivative (2,3MBZ-2) in this genetic model of generalized epilepsy. In addition, we have also designed to correlate plasma concentrations of these compounds with their anticonvulsant activity. In addition, the anticonvulsant properties of NBQX, a selective AMPA/KA receptor antagonist, and GYKI 52466, a nonselective AMPA/KA receptor antagonist, were compared with those of 2,3-benzodiazepines. The chemical structures of compounds studied are reported in Fig. 1.

METHOD

Animals

Genetically epilepsy prone rats (GEPR-9s), a strain derived from Sprague–Dawley rats, were kindly supplied by our breeding stock (Institute of Pharmacology, University of Messina) from a colony originally instituted at the Louisiana State University at Shreveport, LA, by Dr. P. C. Jobe and obtained from Prof. B. S. Meldrum (University of London). Progenitors of this latter were raised at the University of Arizona and named [UAZ: AGS (SD)]. The rats were housed three or four per cage in 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. Animals were maintained on a 12 L:12 D cycle (lights on 0700–1900 h, off 1900–0700 h). GEPRs were tested three times at weekly intervals between 6 and 8 weeks of their life, and only animals that showed an audiogenic seizure in all three exposures to sound stimulation were used for these experiments. 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 laws and policies.

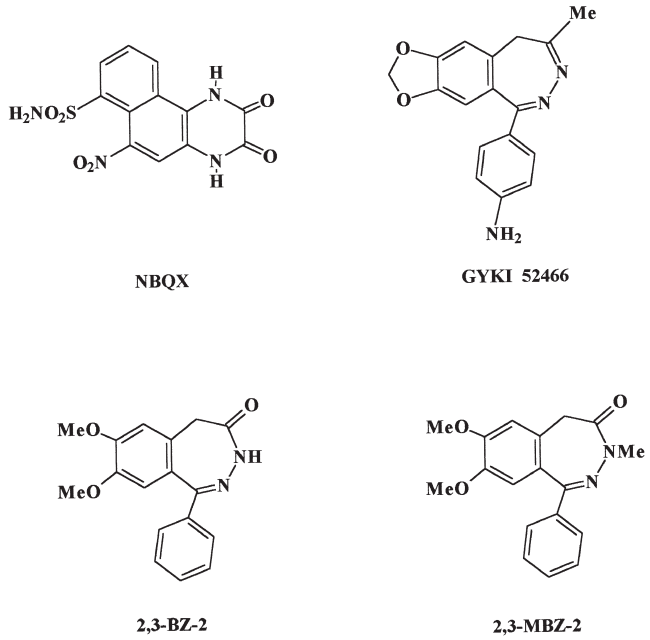

FIG. 1. Chemical structures of compounds studied.

Anticonvulsant Activity

Seizures were induced in GEPR-9s, 180–260 g, 12–18-week-old, males (n = 140) by exposing them to a mixed frequency sound of 12–16 kHz, 109 dB intensity under a hemispheric plexiglas dome (58 cm diameter). Individual animals were initially tested 10 min before sound stimulation for assessment of locomotor activity and then placed into the dome cage for habituation and assessment of anticonvulsant activity. Auditory stimulation was applied for 60 s or until the onset of convulsions occurred. A full seizure response (S.R.) 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. In particular, the audiogenic seizure response was assessed on the following scale previously reported (7): 0 = no response; 1 = running only; 2 = one running phases, followed by a clonic convulsion (clonus of forelimbs, hindlimbs, head, pinnae, vibrissae, and tail); 3 = two running phases, 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. Behavioral changes were observed during the period between drug administration and auditory testing.

Effects on Motor Movement

Genetically epilepsy-prone rats were trained just before anticonvulsant testing to do coordinated motor movements, continously for 5 min on a rotarod 4 cm in diameter, at 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 (13).

Determination of Plasma Levels of 2,3-Benzodiazepines

2,3-BZs plasma levels were analyzed by high-performance liquid chromatography (HPLC) as recently described by Rizzo et al. (21). Briefly, the method included a double step extraction and the use of internal standard (I.S.) for quantitation.

The HPLC system consisted of a Beckman System Gold 125 solvent module with a 20 μl loop injection valve and a variable wavelength ultraviolet 166 Detector set a 254 nm, and a Epson Endeavoir 4DX2/50 L integrator. A Beckman Ultrasfere ODS 25 cm × 4.6 mm i.d. reverse-phase column were used with a ODS guard 4.5 cm × 4.6 mm. The column was eluted with methanol–water (65:35, v/v) at the flow rate of 1.5 ml/min. Because the presence of buffer (pH 4–8) and organic modifier (CH_3-CN) reduced the limit of detection, we used the previous described mobile phase. The chromatograph was operated at room temperature. A Millex G. V. Millipore filter (0.2 μm) with a syringe kit were used to filter sample extracts. Methanol and water HPLC grade were used. Prazepam (Parke Davies, Milano, Italy) was used as I.S. Saturated phosphate buffer (pH = 7.0) was prepared. Stock solution (1 mg/ml) of 2,3-benzodiazepines (GYKI 52466, 2,3BZ-2, and 2,3MBZ-2) were done in methanol. Working solution were made by appropriate dilution with methanol and used to

prepare blood and acqueous standards. Under 4% chloral hydrate anesthesia, rats were decapitated, blood cells were removed by centrifugation, and the separated plasma was stored at 70°C until use. Those samples were utilized as quality control specimens. The dose calibration curves were constructed from four replicate measurements of four concentrations of each compound over the range from 62.5 to 500 ng/ml. A linear response was observed over the examined concentration range. The regression coefficient was 0.998 for 2,3BZ-2, 0.999 for 2,3MBZ-2, and 0.997 for GYKI 52466. The relative retention times were 3.1 min for 2,3BZ-2, 4.3 min for GYKI 52466, 5.06 min for 2,3MBZ-2, and 10.9 min for I.S. The time of each analysis was 15 min. The lower limit of detection was 9.5 for GYKI 52466, 6.5 ng/ml for 2,3 BZ-2, and 8 ng/ml for 2,3MBZ-2. The sensitivity of the method allowed for easy quantitation of 10 ng/ml of these drugs in a 0.5 ml blood sample.

Drugs

All the 2,3-benzodiazepines were synthesized in our laboratory as previously described (4) and dissolved in a solution containing 50% of dimethylsulfoxide and 50% of sterile saline. NBQX (Tocris Cookson, Bristol, UK) and GYKI 52466 (Tocris Cookson) were dissolved in sterile saline. For systemic administration, all compounds were administered intraperitoneally (IP) (0.4 ml/100 g/b.wt. of the rat), as a freshly ultrasonicated solution. At least six animals were used for each dose level studied.

Statistical Analysis

The effects of treatment were analysed statistically, using nonparametric methods. A Kruskall–Wallis analysis of variance (*H*-test) was first carried out, and if this was significant, a Mann–Whitney *U*-test was used to compare control and drug-treated animals. The percentage of animals exhibiting tonic extension (seizure response = 4–5) or clonic phase (seizure response = 2–3) of the audiogenic seizure was determined for each dose of compound administered, and these values were plotted against corresponding doses by a computer construction of the dose–effect curves for calculation of the ED_{50} (with 95% confidence limits). Median neurotoxic dose (TD_{50} with 95% confidence limits), the dose which made 50% of animals fall from the rotarod, was calculated as the ED_{50}. The ED_{50} and TD_{50} values for each compound were determined, using the method of Litchfield and Wilcoxon (18). At least 32 animals were used to calculate each ED_{50} and TD_{50} value. The values of blood samples determined by HPLC are expressed as means ± SEM.

RESULTS

Anticonvulsant Properties of NBQX and GYKI 52466

Both compounds tested reduced the occurrence and the severity of audiogenic seizure phases in a dose dependent manner. In particular, NBQX (33, 50, 66, and 100 μmol/kg IP) was able to significantly ($Hs = 7.78$, $U = 35.5$, $p < 0.01$) reduce the median seizure scores of the audiogenic seizures in genetically epilepsy prone rats (Fig. 2). The time course studies revealed that NBQX (33 μmol/kg IP) showed the maximum activity from 15 to 45 min (Fig. 3). Lower doses of NBQX (10 and 25 μmol/kg IP) had a weak and nonsignificant anticonvulsant activity. In addition, GYKI 52466 (33, 50, and 66 μmol/kg IP) was able to significantly ($Hs = 8.16$, $U = 38.5$, $p < 0.01$) reduce the median seizure scores of the audiogenic seizures (Fig. 2). The time course studies revealed that GYKI 52466 (33 μmol/kg IP) showed the maximum activity from 15 to 45 min (Fig. 3). Lower doses of GYKI 52466 (10 and 25 μmol/kg IP) had a weak and nonsignificant anticonvulsant effect. The ED_{50} values (with 95% confidence limits) for suppression of clonic and tonic phases of the audiogenic seizures in genetically epilepsy prone rats are reported in Table 1. The group of GEPR-9s receiving vehicle did not affect any component of the audiogenic seizures ($Hs < 0.124$, $p > 0.13$) (Fig. 3).

Anticonvulsant Properties of 2,3 BZ-2 and 2,3 MBZ-2

2,3BZ-2 (10–100 μmol/kg IP) and 2,3MBZ-2 (21–100 μmol/kg IP) dose dependently reduced the median seizure scores of audiogenic seizures in genetically epilepsy prone rats (Fig. 2). In particular, 2,3BZ-2 (33, 50, 66, and 100 μmol/kg IP) was able to significantly ($Hs = 8.34$, $U = 39.5$, $p < 0.01$) reduce the tonic and clonic component of the audiogenic seizures. Lower doses of 2,3BZ-2 (10 and 21 μmol/kg IP) had a weak and nonsignificant anticonvulsant activity. In addition, 2,3MBZ-2 (50, 66, and 100 μmol/kg IP) significantly ($p <$

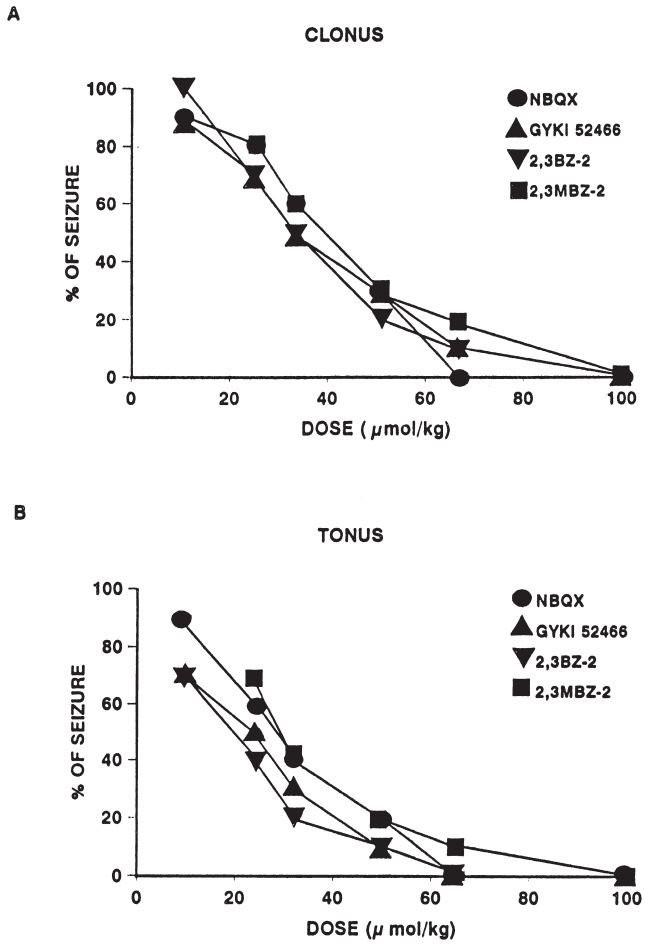

FIG. 2. Dose–response curves of the anticonvulsant activity of NBQX, GYKI 52466, 2,3BZ-2, and 2,3MBZ-2 against audiogenic seizures in genetically epilepsy prone rats observed 30 min after drug administration. Ordinate shows seizure score, abscissa shows the dose expressed as μmol/kg IP. For the determination of each point six to eight animals were used.

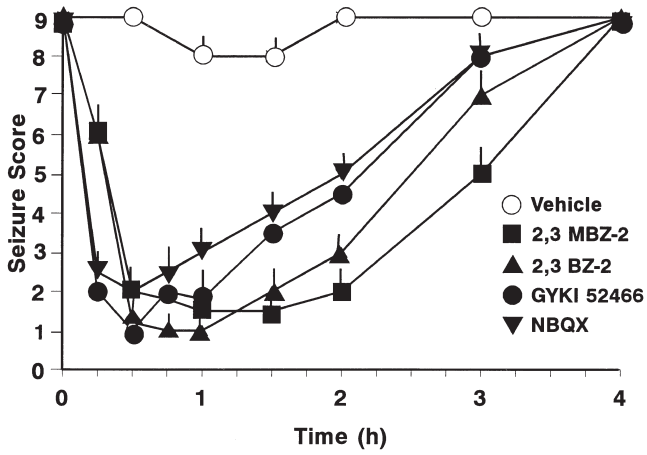

FIG. 3. Anticonvulsant effects observed after IP administration of an equimolar dose (33 μmol/kg) of GYKI 52466, NBQX, 2,3BZ-2, and 2,3MBZ-2 against audiogenic seizures in genetically epilepsy prone rats. Ordinate shows seizure score, abscissa shows the time after intraperitoneal administration of drug in hours. For the determination of each point six to eight animals were used.

0.01) reduce the occurrence of tonic and clonic component of the audiogenic seizures. Lower doses of 2,3MBZ-2 (21 and 33 μmol/kg IP) had a weak and nonsignificant anticonvulsant activity. In addition, 2,3MBZ-2 (50, 66, and 100 μmol/kg IP) significantly ($Hs = 7.12$, $U = 33.5$, $p < 0.01$) reduced the occurrence of tonic and clonic seizures. Lower doses of 2,3MBZ-2 (21 and 33 μmol/kg IP) had a weak and nonsignificant anticonvulsant effect. The time course studies revealed that 2,3BZ-2 (33 μmol/kg IP) showed the maximum activity from 30 to 90 min, while 2,3MBZ-2 (33 μmol kg IP) had the maximum activity from 45 to 120 min (Fig. 3). The ED_{50} values (with 95% confidence limits) for suppression of clonic and tonic phases of the audiogenic seizures in genetically epilepsy-prone rats are shown in Table 1. The data reported in this table clearly indicated that after various time pretreatment the anticonvulsant properties of 2,3BZ-2 become evident before than those of 2,3MBZ-2. The latter compound demonstrated a longer lasting anticonvulsant activity than the 2,3 BZ-2.

Effects on Motor Impairment

In rats, no adverse side effects were observed with doses of AMPA/kainate antagonists ranging from 10 to 50 μmol/kg IP. Higher doses of all compounds tested induced reduction of locomotor activity and sedation. An impairment of locomotor performance was observed in some rats from 15 to 120 min following the higher doses of compounds studied. The TD_{50}/ED_{50} ratio of the AMPA/kainate antagonists is reported in Table 1. No loss of righting reflex or other neurological adverse effects were evident.

Time Course of Plasma Concentrations

In Fig. 4 we report the time courses of plasma concentrations of rats treated with GYKI 52466 and 2,3BZ-2. Peak plasma concentration was achieved after 15 and 45 min from IP administration of GYKI 52466 and 2,3BZ-2, respectively.

TABLE 1

EFFECTS OF GYKI 52466, NBQX, 2,3BZ-2, AND 2,3MBZ-2 ON SOUND-INDUCED SEIZURES AND ON ROTAROD PERFORMANCE IN GENETICALLY EPILEPSY-PRONE RATS

Compound	Pretreatment Time (min)	Clonus (μmol/kg)	Tonus (μmol/kg)	TD_{50} Locomotor Deficit (μmol/kg)	TD_{50}/ED_{50}
GYKI 52466	15	42 (18–98)	18 (10–32)	97 (77–122)	2.3
	30	31 (16–60)	16 (9–28)	68 (46–100)	2.2
	60	65 (33–128)	33 (15–73)	149 (101–220)	2.3
	120	ND	ND		
NBQX	15	48 (26–89)	23 (14–38)	102 (83–125)	2.1
	30	39 (22–69)	18 (8–40)	78 (54–113)	2
	60	77 (38–156)	35 (21–58)	168 (114–248)	2.2
2,3BZ-2	15	ND	ND		
	30	40 (16–100)	13 (8–23)	128 (89–167)	3.2
	60	33 (15–73)	17 (7–17.3)	112 (88–142)	3.4
	90	42 (18–98)	21 (8–55.1)		
	120	63 (24–165.4)	30 (16–56.2)	220 (169–286)	3.5
2,3MBZ-2	15	ND	ND		
	30	48 (18–128)	25 (14–47)	149 (101–220)	3.2
	60	42 (18–98)	22 (13–37.2)	138 (106–180)	3.3
	90	35 (20–61.3)	18 (8–40)		
	120	49 (22–109.1)	25 (14–47)	171 (136–215)	3.5

Groups of 8–10 rats received GYKI 52466, NBQX, 2,3BZ-2, or 2,3MBZ-2, and the percentage of animals displaying clonic or tonic seizures were calculated. The table shows the ED_{50} values (with 95% confidence limits) representing the dose of compound that protected 50% of rats from clonic or tonic component of the audiogenic seizures. All data are calculated according to the method of Litchfield and Wilcoxon (18). Groups of 8–10 rats received GYKI 52466, NBQX, 2,3BZ-2, and 2,3MBZ-2, and were tested on the rotarod for assessment of possible motor impairment. TD_{50} (with 95% confidence limits) were calculated according to the method of Litchfield and Wilcoxon (18). All data are expressed as μmol/kg IP. TD_{50}/TE_{50}, therapeutic index represents the ratio between TD_{50} and ED_{50} (from the clonic phase of the audiogenic seizures).

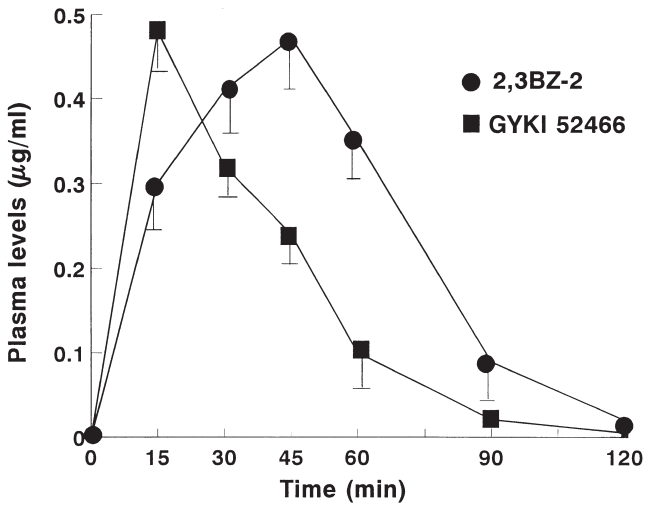

FIG. 4. Time course of plasma levels of GYKI 52466 and 2,3BZ-2 in rats. Ordinate shows the plasma level; abscissa shows the time after intraperitoneal administration of GYKI 52466 and 2,3BZ-2.

Time course of plasma concentrations of rats treated with 2,3MBZ-2 shows two curves (Fig. 5), the first is referred to the IP injected compound and the second to its demethylate metabolite, the 2,3BZ-2. Peak plasma concentration of 2,3MBZ-2 was achieved 15 min after IP administration, and 45 min after IP injection the plasma concentration decreased significantly. Whereas for its metabolite (2,3BZ-2) peak plasma concentrations was achieved 45 min after drug administration. It should be noted as 2,3MBZ-2 plasma concentrations decreased, the 2,3BZ-2 plasma concentration increased. The 2,3BZ-2 and 2,3MBZ-2 plasma levels were particularly low, and the drugs desappeared from plasma 90 min following IP administration.

This analytical study showed the main metabolic pathway for the compound of a methylated series: a demethylation in N-3. The other metabolites were separated by chromatographic method and their structure studied by liquid chromatography-mass spectometry. Their chemical structures will be confirmed by comparison with synthesized compounds. Our HPLC analytic method does not permit to detect NBQX.

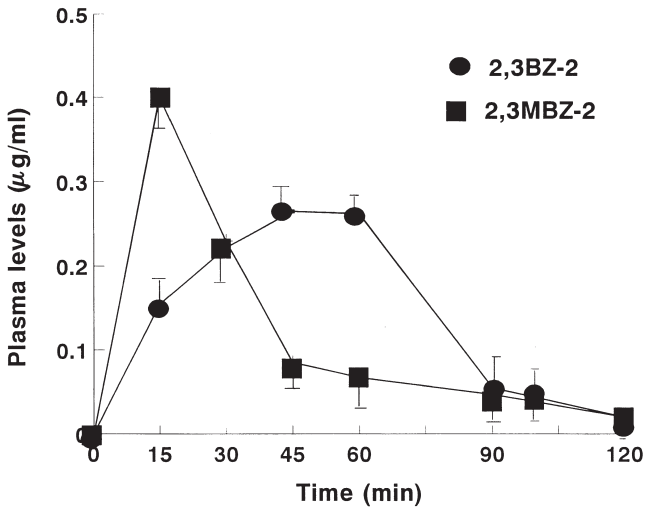

FIG. 5. Time course of plasma levels of 2,3MBZ-2 in rats. Ordinate shows the plasma level; abscissa shows the time after intraperitoneal administration of 2,3MBZ-2.

DISCUSSION

The recent availability of centrally active antagonists of nonNMDA (AMPA/kainate) excitatory amino acid receptors has made it possible to evaluate the potential of such compounds as antiepileptic agents in animal seizure models. Tarnawa and co-worker have previously described a novel 2,3 benzodiazepine, named GYKI 52466 (1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3 benzodiazepine), which inhibits spinal reflexes in cats but does not potentiate the inhibitory action of GABA and acts as non-NMDA antagonists (26,27). In addition, GYKI 52466 showed a selective anticonvulsant activity against seizures induced by ICV administration of AMPA and kainate in mice (2,24,30). GYKI 52466 has also demonstrated to possess anticonvulsant properties in two genetic models of audiogenic seizures the DBA/2 mice and the genetically epilepsy-prone rats as well as in the photosensitive baboons *Papio papio* (24).

The present study demonstrated that the novel 2,3-benzodiazepines (2,3BZ-2 and 2,3MBZ-2) have a potent anticonvulsant activity in genetically epilepsy-prone rats comparable to that of GYKI 52466 and NBQX. Because the effects of these novel 2,3-benzodiazepines were similar to those of NBQX and GYKI 52466 (3–5), it is suggested that AMPA/kainate receptors play an important role in the generation or/and propagation of the audiogenic seizures in genetically epilepsy-prone rats.

The time course for the anticonvulsant effects for GYKI 52466 in mice, rats, and baboons was very short (from 5 to 60 min) after intraperitoneal or intravenous administration (2,24). One possible explanation for the presence of anticonvulsant activity of 2,3BZ-2 and 2,3MBZ-2 even if the plasma levels are particularly low is the possibility of accumulation of these 2,3-benzodiazepines in the brain or a slow clearance of them from the brain.

The present study demonstrated that the two novel 2,3-benzodiazepines that show some chemical similarities to GYKI 52466 have a longer lasting time course than GYKI 52466.

This analytical study also showed the main methabolic pathway for the compound of a methylated series: a demethilation in N-3.

We have previously demonstrated that 2,3-benzodiazepines possess anticonvulsant activity against AMPA- or kainate-induced seizures but not against NMDA induced seizures (2–5,24). In addition, 2,3-benzodiazepines have shown to be more potent against maximal electroshock than pentylenetetrazole or 4-aminopyridine induced seizures (3,4,30). The genetically epilepsy prone rat is a model of gene-linked generalized epilepsy which exhibits seizures in response to stimuli (i.e., sound and hyperthermia) with a lower threshold and more intense seizure response for a given stimulus (electrical or chemical) than other strains of rat (6,8,16). The differences observed in anticonvulsant effects between different experimental seizures might be due to the fact that the audiogenic seizures particularly seem to be more sensitive to the anticonvulsant activity of antagonists of excitatory amino acid receptors than other traditional seizure tests as previously suggested by our group (7,8). This was also confirmed by the fact that GYKI 52466 and the present 2,3-benzodiazepines seem

to act as noncompetitive antagonist and to antagonize the AMPA/kainate receptor-mediated responses by an allosteric blocking mechanism (22) and do not affect $GABA_A$ receptor-mediated responses as previously suggested (3,4).

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REFERENCES

1. Buchan, A. M.; Li, H.; Cho, S.; Pulsinelli, W. A.: Blockade of the AMPA receptor prevents CA1 hippocampal injury following severe but transiet forebrain ischemia in adult rats. Neurosci. Lett. 132:255–258; 1991.
2. Chapman, A. G.; Smith, S. E.; Meldrum, B. S.: The anticonvulsant effect of the non-NMDA antagonists, NBQX and GYKI 52466 in mice. Epilepsy Res. 9:92–96; 1991.
3. Chimirri, A.; De Sarro, G. B.; De Sarro, A.; Gitto, R.; Grasso, S.; Quartarone, S.; Zappalà, M.; Giusti, P.; Libri, V.; Constanti, A.; Chapman, A. G.: 1-Aryl-3,5-dihydro-4H-2,3-benzodiazepin-4-ones: Novel AMPA receptor antagonists. J. Med. Chem. 40:1258–1269; 1997.
4. De Sarro, G. B.; Chimirri, A.; De Sarro, A.; Gitto, R.; Grasso, S.; Giusti, P.; Chapman, A. G.: GYKI 52466 and related 2,3-benzodiazepines as anticonvulsant agents in DBA/2 mice. Eur. J. Pharmacol. 294:411–422; 1995.
5. De Sarro, G. B.; Chimirri, A.; De Sarro, A.; Gitto, R.; Grasso, S.; Zappalà, M.; Rizzo, M.; Chapman, A.: Anticonvulsant properties of new 2,3-benzodiazepines in mice. Fundam. Clin. Pharmacol. 10:184; 1996.
6. De Sarro, A.; De Sarro, G. B.: Responsiveness of genetically epilepsy prone rats to aminophylline-induced seizures and interactions with quinolones. Neuropharmacology 30:169–176; 1991.
7. De Sarro, G. B.; De Sarro, A.: Anticonvulsant activity of competitive antagonists of NMDA receptor in genetically epilepsy prone rats. Eur. J. Pharmacol. 215:221–229; 1992.
8. De Sarro, G. B.; De Sarro, A.: Anticonvulsant properties of non competitive antagonists of NMDA receptor in genetically epilepsy prone rats. Comparison with CPPene. Neuropharmacology 32:51–58; 1993.
9. De Sarro, G. B.; Ongini, E.; Bertorelli, R.; Aguglia, U.; De Sarro, A.: Excitatory amino acid neurotransmission through both NMDA and nonNMDA receptors is involved in the anticonvulsant activity of felbamate in DBA/2 mice. Eur. J. Pharmacol. 262:11–19; 1994.
10. De Sarro, G. B.; Donato Di Paola, E.; Aguglia, U.; De Sarro, A.: Tolerance to anticonvulsant effects of clobazam, diazepam and clonazepam in genetically epilepsy prone rats. Pharmacol. Biochem. Behav. 55:39–48; 1996.
11. De Sarro, A.; Trimarchi, G. R.; Ammendola, D.; De Sarro, G. B.: Repetitive treatment with quinolones potentiates the seizures induced by aminophylline in genetically epilepsy-prone rats. Gen. Pharmacol. 23:853–859; 1992.
12. De Sarro, G. B.; Trimarchi, G. R.; Sinopoli, S.; Masuda, Y.; De Sarro, A.: Anticonvulsant effects of U-54494A and U-50488H in genetically epilepsy-prone rats and DBA/2 mice: A possible involvement of glycine/NMDA receptor complex. Gen. Pharmacol. 24:439–447; 1993.
13. Dunham, N. W.; Miya T. S.: A note on a simple apparatus for detecting neurological deficit in rats and mice. J. Am. Pharm. Assoc. 46:208–209; 1957.
14. Frank, L.; Bruhn, T.; Diemer, N. H.: Regional cerebral protein synthesis after transient ischemia in the rat: Effect of the AMPA antagonist NBQX. Neurosci. Lett. 140:108–112; 1992.
15. Gean, P. W.: The epileptiform activity induced by 4-aminopyridine in rat amygdala slices: Antagonism by non-*N*-methyl-D-aspartate receptor antagonists. Brain Res. 530:251–256; 1990.
16. Jobe, P. C.: Pharmacology of audiogenic seizures. In: Brown, R. D.; Daigneault, E. A., eds. Pharmacology of hearing. Experiment and clinical bases. New York: John Wiley; 1981:271–304.
17. Klockgether, T.; Turski, L.; Honore, T.; Zhang, Z. M.; Gash, D. M.; Kurlan, R.; Greenamyre, J. T.: The AMPA receptor antagonist NBQX has antiparkinsonian effects in monoamine-depleted rats and MPTP-treated monkeys. Ann. Neurol. 30:717–723; 1991.
18. Litchfield, J. F.; Wilcoxon, F.: A simplified method of evaluating dose–effects experiments. J. Pharmacol. Exp. Ther. 96:99–113; 1949.
19. Meldrum, B. S.; Millan, M.; Patel, S.; De Sarro, G. B.: Anti-epileptic effects of focal micro-injection of excitatory amino acid antagonists. J. Neural Transm. 72:191–198; 1988.
20. Rainnie, D. G.; Asprodini, E. K.; Shinnick-Gallagher, P.: Kindling-induced long-lasting changes in synaptic transmission in the basolateral amygdala. J. Neurophysiol. 67:443–454; 1992.
21. Rizzo, M.; Sinopoli, V. A.; Gitto, R.; Zappalà, M.; De Sarro, G. B.; Chimirri, A.: High-performance liquid chromatographic determination of new 2,3-benzodiazepines. J. Chromatogr. B. 705:149–153; 1998.
22. Rogawski, M.: Therapeutic potential of excitatory amino acid antagonists: Channel blockers and 2,3-benzodiazepines. Trends Pharmacol. Sci. 14:325–331; 1993.
23. Sheardown, M. J.; Nielsen, E. O.; Hansen, A. J.; Jacobsen, P.; Honore, T.: 2,3-Dihydroxy-6-nitro-7-sulfamoyl-benzo(F)-quinoxaline: A neuroprotectant for cerebral ischemia. Science 247:571–574; 1991.
24. Smith, S. E.; Durmuller, N.; Meldrum, B. S.: 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; 1991.
25. Smith, S. E.; Meldrum, B. S.: Cerebroprotective effect of a non-*N*-methyl-D-aspartate antagonist, GYKI 52466, after focal ischemia in the rat. Stroke 23:861–864; 1992.
26. Tarnawa, I.; Farkas, S.; Berzsenyi, P.; Pataki, A.; Andreasi, F.: Electrophysiological studies with 2,3-benzodiazepine muscle relaxant: GYKI 52466. Eur. J. Pharmacol. 167:193–199; 1989.
27. Tarnawa, I.; Engberg, I.; Flatman, J. A.: GYKI 52466 an inhibitor of spinal reflexes is a potent quisqualate antagonist. In: Lube, G.; Resental, G. A., eds. Amino acids: Chemistry, biology and medicine. Escona: Leiden; 1990:538–546.
28. Wachtel, H.; Kunow, M.; Loeschman, P. A.: NBQX (6-nitro-sulfamoyl-benzo-quinoxaline-dione) and CPP (3-carboxy-piperazin-propyl phosphonic acid) potentiate dopamine agonist induced rotations in substantia nigra lesioned rats. Neurosci. Lett. 142:179–182; 1992.
29. Westergren, I.; Johansson, B. B.: NBQX, an AMPA antagonist, reduces glutamate-mediated brain edema. Brain Res. 573:324–326; 1992.
30. Yamaguchi, S.; Donevan, S. D.; Rogawski, M. A.: Anticonvulsant activity of AMPA/kainate antagonists: Comparison of GYKI 52466 and NBQX in maximal electroshock and chemoconvulsant seizure models. Epilepsy Res. 15:179–184; 1993.