Effects of Some Excitatory Amino Acid Antagonists and Drugs Enhancing \( \gamma \)-Aminobutyric Acid Neurotransmission on Pefloxacin-Induced Seizures in DBA/2 Mice

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The behavioral and convulsant effects of pefloxacin (PEFLO), a quinolone derivative, were studied after intraperitoneal (i.p.) administration to Dilute Brown Agouti DBA/2J (DBA/2) mice, a strain genetically susceptible to sound-induced seizures. The anticonvulsant effects of some excitatory amino acid (EAA) antagonists acting at \( N \)-methyl-D-aspartate (NMDA) or \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate (KA) receptors and of some compounds enhancing \( \gamma \)-aminobutyric acid (GABA)-ergic transmission against seizures induced by PEFLO were also evaluated. The present study demonstrated that both groups of compounds administered i.p. or intracerebroventricularly were able to protect against seizures induced by PEFLO. However, ifenprodil and (±)-\( \alpha \)-(chlorophenyl)-4-[[4-fluorophenyl]methyl]-1-piperidine-ethanol (SL 82.0715), two compounds acting on the polyamine site of the NMDA receptor complex, were unable to provide any protection. The relationship between the different sites of action and the anticonvulsant activities of these derivatives were discussed. Although the main mechanism of PEFLO-induced seizures cannot be easily determined, potential interactions with the receptors of EAA exist. In fact, antagonists of EAA, and in particular, those acting at NMDA receptors, were able to increase the threshold for the seizures or to prevent the seizures induced by PEFLO, while compounds acting at the polyamine site did not provide any protection. The AMPA-KA receptor antagonists were also able to exert anticonvulsant activity, but with minor potency in comparison to those of NMDA antagonists. In addition, the fact that compounds enhancing GABA-ergic neurotransmission were also able to protect the mice against seizures induced by PEFLO suggests an involvement of GABA system.

Because of their excellent antibacterial activities, quinolone agents have been widely adopted for use in clinical practice. In particular, pefloxacin (PEFLO) shows a very broad spectrum of activity against gram-positive and gram-negative bacteria and is widely distributed in tissues. This explains why PEFLO is successful against various systemic infections. Although recently developed quinolones are less toxic than earlier compounds, they still produce a very few incidences of various adverse effects on the central nervous system such as dizziness, headache, seizures, and hallucinations (2, 17, 20, 25, 26). We have previously reported the proconvulsant effects of several quinolones when administered together with aminophylline in rats and with \( \beta \)-lactam derivatives in mice (9–12). The convulsant actions of quinolones have been attributed to the inhibition of \( \gamma \)-aminobutyric acid (GABA) binding to its receptor (1, 31, 39, 41). However, the concentrations of quinolones able to interact with the GABA system are rather high and varied among the different quinolones tested by a factor of about 100. Thus, it appears questionable whether a specific interaction of quinolones with GABA receptors alone can explain the convulsant activities of these compounds (16). In addition, some in vivo studies indicated that dopamine, opioid, and glutamergic receptors may also be involved in the effects of quinolones on the central nervous system (16, 42). Because quinolones possess some chemical similarities to kynurenic acids, which may be endogenous ligands for excitatory amino acid (EAA) recep-

tors (35), we may suppose a possible interaction of quinolones with glutamate receptor binding sites.

The genetically epilepsy-prone mouse, or the Dilute Brown Agouti DBA/2J (DBA/2) mouse, has been known since 1947 to be a strain of mice susceptible to audiogenic seizures (21). In fact, mice of this strain undergo an age-dependent (between 16 and 30 days) sequence of generalized convulsions when they are exposed to a loud mixed-frequency sound (12 to 16 kHz; 90 to 120 dB) such as a doorbell (4, 6). The audiogenic seizures in DBA/2 mice is a genetic form of reflex epilepsy; the counterpart in humans may be brain stem or centroencephalic epilepsy, e.g., “absence” or “musiconic” seizures (4–6, 19, 33). In addition, it has been reported that DBA/2 mice develop resistance to sound-induced seizures with maturity but still have increased susceptibility to seizures resulting from a variety of nonaudiogenic convulsant treatments including chemical and physical stimuli (4, 5, 19). This change in seizure incidence with age is one of the more striking similarities between the human and the mouse forms of epilepsy. Thus, DBA/2 mice have been considered an excellent animal model for the study of certain kinds of human epilepsy and for testing convulsant or anticonvulsant drugs (4, 5, 7). In fact, we have previously demonstrated that the convulsant properties of antibiotics and chemotherapies can be more easily evaluated with this model than with models with other strains of mice (9, 12, 14). This mouse model was chosen for the examination of the effects of PEFLO since it might mirror PEFLO treatment of patients with predisposing epileptic factors. In previous studies we evaluated the proconvulsant effects of some quinolones on seizures induced by imipenem and cefazolin administration to DBA/2 mice.

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mice and found that PEFLO was the most potent of the quinolones tested (9, 12). Thus, with these concepts in mind, in this experimental study we used PEFLO to treat DBA/2 mice with the intention of gaining information on the possible interference of EAA antagonists and of drugs enhancing GABA-ergic transmission with the mechanisms responsible for PEFLO-induced seizures.

**MATERIALS AND METHODS**

**Subjects.** DBA/2 mice (weight, 16 to 24 g; age, 42 to 48 days) were purchased from Charles River (Calco, Como, Italy). Upon arrival at the laboratory, they were housed at 10 mice per cage in a controlled environment (temperature, 22 ± 1°C; light-dark cycle, lights on from 0800 to 2000 h), with food and water available ad libitum.

**TABLE 1. EAA antagonists used in the present study**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Abbreviation</th>
<th>Mol wt</th>
<th>Dose range</th>
<th>Pretreatment time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-[(-)-2-Phenyl]propionic acid</td>
<td>CPP</td>
<td>252.2</td>
<td>2–80 nmol/g</td>
<td>30</td>
</tr>
<tr>
<td>3-[(-)-2-Benzyloxypropionic acid</td>
<td>CPPene</td>
<td>268.2</td>
<td>2–30 nmol/g; 5–60 pmol/mouse</td>
<td>30</td>
</tr>
<tr>
<td>(+)-5-Methyl-10,11-dihydro-5H-dibenzo(a,d)-cyclohepten-5,10-imine</td>
<td>MK-801</td>
<td>337.48</td>
<td>0.15–0.75 nmol/g; 100–800 pmol/mouse</td>
<td>15</td>
</tr>
<tr>
<td>Maleic acid or dioxilone</td>
<td>CGP 39511</td>
<td>245.1</td>
<td>1–8 nmol/g</td>
<td>30</td>
</tr>
<tr>
<td>Ifendopril tartrate</td>
<td>IFE</td>
<td>400.5</td>
<td>1–2.5 nmol/g</td>
<td>30</td>
</tr>
<tr>
<td>[(±)-4-Chlorophenyl]-1-(4-fluorophenyl)ethyl-1-piperidinethanol</td>
<td>SL 82.0715</td>
<td>384.32</td>
<td>1.2–2.6 nmol/g</td>
<td>30</td>
</tr>
<tr>
<td>2,3-Dihydroxy-6-nitro-7-sulfamoyl-benzof(A)-quinoxaline</td>
<td>NBQX</td>
<td>342</td>
<td>7.3–87.7 nmol/g</td>
<td>5</td>
</tr>
<tr>
<td>Methyleneedioxy-5H-2,3-benzoazepine hydrochloride</td>
<td>GYKI 52466</td>
<td>329.79</td>
<td>10–100 nmol/g</td>
<td>5</td>
</tr>
<tr>
<td>3,5-Dihydroxy-7,8-dimethoxy-1-phenyl-4H-2,3-benzoazepine-4-one</td>
<td>2,3-BZ-2</td>
<td>296.15</td>
<td>16.9–101 nmol/g</td>
<td>30</td>
</tr>
<tr>
<td>Kynurenic acid</td>
<td>AP5</td>
<td>225.2</td>
<td>0.1–0.5 nmol/mouse</td>
<td>15</td>
</tr>
<tr>
<td>gamma-Glutamylaminomethylsulfonic acid</td>
<td>KYNA</td>
<td>189.2</td>
<td>0.5–5 nmol/mouse</td>
<td>15</td>
</tr>
<tr>
<td>6-Cyano-7-nitroquinoxaline-2,3-dione</td>
<td>CNQX</td>
<td>232.16</td>
<td>1–10 nmol/mouse</td>
<td>15</td>
</tr>
</tbody>
</table>

* CCP and CPPene were kindly supplied by P. L. Herrling (Sandoz Ltd., Berne, Switzerland). MK-801 (dioxilone) was kindly supplied by A. Wilkins (Merck Sharp & Dohme, Rho, Italy). CGP 39511 was kindly supplied by M. Schmutz (CIBA-GEIGY, Basel, Switzerland). IFE and SL 82.0715 were kindly supplied by J. Alexander (LERS-Synthelabo, Parix, France). NBQX was kindly supplied by T. Honore (Novo Nordish, Malov, Denmark). GYKI 52466 was purchased from RBI (Natick, Mass.). 2,3-BZ-2 was kindly supplied by A. Chimini (Department of Medicinal Chemistry, University of Messina, Messina, Italy). AP5 and KYNA were purchased from Sigma (St. Louis, Mo.), and gamma-GAMS and CNQX were purchased from Toceis (Buckhurst Hill, United Kingdom).

* Competitive NMDA receptor antagonists.
* Administered i.p.
* Administered i.c.v.
* Noncompetitive NMDA receptor antagonists.
* AMPA-KA receptor antagonists.
* Broad-spectrum EAA antagonist.

**TABLE 2. Drugs enhancing GABA-ergic transmission used in the present study**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Abbreviation</th>
<th>Mol wt</th>
<th>Range of dose</th>
<th>Pretreatment time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscimol</td>
<td>MSC</td>
<td>114.1</td>
<td>0.125–0.5 nmol/mouse</td>
<td>15</td>
</tr>
<tr>
<td>gamma-Vinyl-GABA</td>
<td>GVGA</td>
<td>129.18</td>
<td>7.5–15.0 nmol/g</td>
<td>120</td>
</tr>
<tr>
<td>Baclofen</td>
<td>BAC</td>
<td>213.7</td>
<td>11.7–93.6 nmol/g</td>
<td>30</td>
</tr>
<tr>
<td>Diazepam</td>
<td>DIA</td>
<td>284.76</td>
<td>1.75–14 nmol/g</td>
<td>30</td>
</tr>
<tr>
<td>Tiagabine hydrochloride</td>
<td>TIA</td>
<td>412</td>
<td>1.2–9.7 nmol/g</td>
<td>30</td>
</tr>
</tbody>
</table>

* GVGA was kindly supplied by M. Seiler (Merrell-Down, Strasbourg, France).
* Administered i.c.v.
* Administered i.p.
* GABA transaminase inhibitor.
* GABA, benzodiazepine receptor complex agonist.
* GABA uptake inhibitor.

**Testing of anticonvulsant activity.** We administered PEFLO and anticonvulsant compounds intraperitoneally (i.p.) in a volume of 0.1 ml/10 g of body weight; however, some compounds that do not cross the blood-brain barrier were administered intracerebroventricularly (i.c.v.) under ethyl ether anesthesia in a volume of 5 μl/mouse by the method of De Sarro et al. (13). Briefly, for i.c.v. administration, the injection needle of a Hamilton type 75N syringe fitted with a nylon stop to attain a depth of 3.2 mm was placed perpendicular to the surface of the skull. The coordinates for microinjections were as follows: 1.0 mm lateral from the midline and 1.5 mm posterior from the bregma. Eight to 10 animals were given each dose of the compound being tested. The mice were divided into four groups, as follows: Group 1 consisted of mice injected i.p. with several doses of PEFLO (0.1 to 1.0 mg/g of body weight given i.p.). Group 2 consisted of animals that were pretreated i.p. with several doses of both EAA receptor antagonists and drugs enhancing GABA-ergic transmission and that were then injected with a stated dose of PEFLO (0.78 mg/g, which approximates the i.p. 95% convulsant dose [CD95] for clonus). Group 3 consisted of animals that were pretreated i.c.v. with several doses of both receptor antagonists of EAA and drugs enhancing GABA-ergic transmission and that were injected i.p. with a stated dose of PEFLO (0.78 mg/g, which approximates the i.p. CD95 for clonus). Group 4 consisted of animals that were pretreated i.p. with a single dose of MK-801, CPPene, CGP 39511, NBQX, 2,3-BZ-2, DIA, and GVGA or vehicle at appropriate times before the injection of different doses of PEFLO (0.2 to 2.0 mg/g given i.p.) (see Tables 1 and 2 for definitions of the abbreviations).

After the administration of PEFLO the mice were observed for 120 min through a Plexiglass box (40 by 40 by 30 cm), and the occurrence of clonic and tonic seizures or death of animals was scored on the following scale by the

**FIG. 1. Site of action of NMDA receptor antagonists.**
method of De Sarro et al. (9): 0, no response; 1, wild running; 2, clonus; 3, tonus; and 4, respiratory arrest. The compounds used and their doses and times of administration are reported in Tables 1 and 2. Figures 1, 2, and 3 show the sites of action of the different compounds tested.

Administration of compounds. The compounds were prepared at an appropriate concentration. The compounds administered i.p., PEFLO (pseudoxacin mesylate; Rhone-Pulenc Labs, Milan, Italy), CCP, CPPene, MK-801, CGP 39551, IFE, SL 82,0715, NBOX, GVG, BAC, TIA, and DIA, were dissolved in sterile saline. GYKI 52466 and 2,3-BZ-2 were also administered i.p. but were dissolved in 50% dimethylsulfoxide and 50% sterile saline.

The compounds administered i.c.v., CPPene, MK-801, NBOX, AP7, KYNA, GNOX, γ-δ-GAMS, and MSC, were dissolved in a minimum quantity of 1 N NaOH, and the final volume was made up with sodium phosphate buffer (67 mM). When necessary the pH was adjusted to 7.3 to 7.4 by adding 0.2 N HCl.

Electrocortical activity. Electrocortical activity was recorded (eight-channel electrocorticograph machine; OTE Biomedica, Florence, Italy) through four chronically implanted steel-screw electrodes inserted bilaterally on the frontoparietal area. At least three mice, treated with CPPene, MK-801, DIA, GVG, or 2,3-BZ-2–PEFLO (0.78 mg/g given i.p.) were studied to evaluate changes in ECoG activity.

Statistical analysis. Statistical comparisons between control (vehicle) and drug-treated groups were made by using Fisher’s exact probability test (incidence of the seizure phases). The percent incidence of each phase of the seizure was determined for each dose of PEFLO administered. CD50 with 95% confidence limits for each phase of the seizure response were estimated by the method of Litchfield and Wilcoxon (24) adapted for analysis with an International Business Machines computer. The CD95 of PEFLO by i.p. administration was also evaluated by the same method (24). The CD50 of PEFLO was used to assess the possible anticonvulsant effects shown by the several compounds tested. The lethality following PEFLO-induced seizures was scored within 120 min, and 50% lethal doses (LD50) were calculated by the same method (24). The 50% effective doses (ED50s), which were the doses of the compounds tested that were able to suppress seizures in 50% of the animals treated with PEFLO, were also estimated by the method of Litchfield and Wilcoxon (24).

RESULTS

Effects of systemic administration of PEFLO. Systemic (i.p.) administration of PEFLO (0.1 to 1.0 mg/g) consistently resulted in clonic-tonic seizures which appeared within 14 to 20 min following the administration of the drug to the mice and which lasted from 25 to 90 min, depending on the dose. After administration of the lowest doses of PEFLO, mice showed an increase in locomotor activity, rare episodes of wild running or escape response, nodding, and clonus of the forelimbs and hind limbs. With higher doses (0.6 and 0.8 mg/g) tonus of the forelimbs and hind limbs was also observed, and in some animals the tonic extension of the hind limbs was followed by respiratory arrest and death (Table 3). The CD50 of PEFLO for producing clonus was 0.35 mg/g (0.26 to 0.47 mg/g), while that for triggering tonus was 0.44 mg/g (0.34 to 0.56 mg/g) (Fig. 4 and Table 4). Furthermore, PEFLO induced lethality, with a LD50 of 0.62 mg/g (0.53 to 0.74 mg/g).

Effects of systemic administration of EAA antagonists on seizures induced by PEFLO. The i.p. administration of the various EAA antagonists, MK-801 (0.05, 0.1, 0.2, and 0.3 μg/g, equivalent to 0.15, 0.30, 0.60, and 0.75 nmol/g, respectively), CPP (2.0, 5.0, 10.0, and 20.0 μg/g, equivalent to 8, 20, 40, and 80 nmol/g, respectively), CPPene (2.0, 4.0, 6.0, and 8.0 μg/g, equivalent to 7.9, 19.8, 39.6, and 79.3 nmol/g, respectively), CGP 39551 (0.25, 0.5, 1.0, and 2.0 μg/g, equivalent to 1, 2, 4,

<table>
<thead>
<tr>
<th>PEFLO dose (mg/g body weight)</th>
<th>Clonic seizures</th>
<th>Tonic seizures</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.2</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>0.4</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>0.5</td>
<td>7</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>0.6</td>
<td>9</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>0.8</td>
<td>10</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>1.0</td>
<td>10</td>
<td>10</td>
<td>9</td>
</tr>
</tbody>
</table>

* Male DBA/2 mice were randomly assigned to experimental groups, each containing 10 animals, and were injected i.p. with various doses of PEFLO. The data are presented as the number of animals showing seizure phases among the 10 mice tested.
ing them against PEFLO-induced convulsions were as follows: CPPene (0.02, 0.04, and 0.06 nmol/mouse), MK-801 (0.2, 0.4, and 0.8 nmol/mouse), γ-D-GAMS (0.4, 0.5, and 0.6/mouse), KYNA (1.2, 2.5, and 5 nmol/mouse), CNQX (3.3, 6.6, and 10.0 nmol/mouse), NBQX (1.0, 3.3, and 6.6 nmol/mouse), and the GABAB agonist MSC (0.25, 0.375, and 0.5 nmol/mouse) was able to antagonize the seizures induced by PEFLO (0.78 mg/g given i.p.). The relative potencies of these compounds in protecting against PEFLO-induced convulsions were as follows: CPPene > MK-801 > MSC > AP7 > γ-D-GAMS > NBQX > KYNA > CNQX (Table 6).

Electrocortical activity. In the animals used to study electrocortical activity, we observed that the electrocorticographic epileptic discharges appeared within 12 to 20 min in mice pretreated i.p. with vehicle-PEFLO (0.78 mg/g). Epileptic discharges appeared within 12 to 20 min in mice pretreated with vehicle and the largest doses of some EAA antagonists and DIA or GVG administered i.p. to DBA/2 mice.

<table>
<thead>
<tr>
<th>Compound</th>
<th>CD50 (μg/g [95% confidence limits])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle + PEFLO</td>
<td>0.35 (0.26–0.47)</td>
</tr>
<tr>
<td>CGP 39551 + PEFLO</td>
<td>1.23 (1.02–1.48)</td>
</tr>
<tr>
<td>CPPene + PEFLO</td>
<td>1.35 (1.21–1.51)</td>
</tr>
<tr>
<td>NBQX + PEFLO</td>
<td>1.02 (0.90–1.16)</td>
</tr>
<tr>
<td>2,3-BZ-Z + PEFLO</td>
<td>1.17 (0.92–1.49)</td>
</tr>
<tr>
<td>MK-801 + PEFLO</td>
<td>1.12 (0.95–1.32)</td>
</tr>
<tr>
<td>DIA + PEFLO</td>
<td>1.43 (1.18–1.73)</td>
</tr>
<tr>
<td>GVG + PEFLO</td>
<td>1.57 (1.28–1.92)</td>
</tr>
</tbody>
</table>

- **Vehicle + PEFLO** was administered at 0.2 to 2.0 mg/g i.p. All data were calculated by the method of Litchfield and Wilcoxon (24).
- Significant differences (P < 0.05) between CD50s for groups treated with vehicle-PEFLO and groups treated with EAA antagonists-PEFLO and DIA- or GVG-PEFLO are denoted.

TABLE 5. ED50s of some EAA antagonists and drugs enhancing GABA-ergic transmission administered i.p. against seizures induced by PEFLO in DBA/2 mice

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose range (nmol i.p.)</th>
<th>ED50 (μg/g [95% confidence limits])</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPP</td>
<td>2–80</td>
<td>19.98 (9.29–42.96)</td>
</tr>
<tr>
<td>CPPene</td>
<td>2–30</td>
<td>7.68 (4.63–12.75)</td>
</tr>
<tr>
<td>CGP 39551</td>
<td>1–8</td>
<td>2.08 (1.1–3.93)</td>
</tr>
<tr>
<td>MK-801</td>
<td>0.15–0.75</td>
<td>0.27 (0.15–0.49)</td>
</tr>
<tr>
<td>GYKI 52466</td>
<td>2,3-BZ-Z</td>
<td>27.67 (14.63–49.99)</td>
</tr>
<tr>
<td>NBQX</td>
<td>7.3–87.7</td>
<td>37.89 (19.43–73.88)</td>
</tr>
<tr>
<td>IFE</td>
<td>1–2.5</td>
<td>&gt;2.5 (NA*)</td>
</tr>
<tr>
<td>SL 82.0715</td>
<td>1–2.6</td>
<td>&gt;2.6 (NA*)</td>
</tr>
<tr>
<td>DIA</td>
<td>1.75–14</td>
<td>1.93 (1.16–3.22)</td>
</tr>
<tr>
<td>TIA</td>
<td>1.2–9.7</td>
<td>2.5 (1.31–4.77)</td>
</tr>
<tr>
<td>BAC</td>
<td>11.7–93.6</td>
<td>33.08 (19.23–56.9)</td>
</tr>
<tr>
<td>GVG</td>
<td>9,700–15,500</td>
<td>9,529 (7,875–11,530)</td>
</tr>
</tbody>
</table>

- **PEFLO** was administered at 0.78 mg/g i.p. All data are for antagonism of clonic and tonic seizures and were calculated by the method of Litchfield and Wilcoxon (24).
- **NA**, not active.
charges did not appear in animals pretreated with CPPene (8 μg/g given i.p.)–PEFLO or DIA (1 μg/g given i.p.)–PEFLO or were less evident in those animals pretreated with MK-801 (0.3 μg/g given i.p.)–PEFLO, 2,3-BZ-2 (20 μg/g given i.p.)–PEFLO, or GVG (1.5 mg/g given i.p.)–PEFLO (Fig. 5, 6, and 7).

**TABLE 6.** ED\textsubscript{50} of some EAA antagonists and MSC, a GABA\textsubscript{A} agonist, administered i.c.v. against seizures induced by PEFLO in DBA/2 mice

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose range (nmol/mouse)</th>
<th>ED\textsubscript{50} (mg/g [95% confidence limit])</th>
<th>Clonic phase</th>
<th>Tonic phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP7</td>
<td>0.3–0.5</td>
<td>0.25 (0.17–0.38)</td>
<td>0.20 (0.13–0.29)</td>
<td></td>
</tr>
<tr>
<td>CPPene</td>
<td>0.02–0.06</td>
<td>0.013 (0.004–0.043)</td>
<td>0.008 (0.005–0.013)</td>
<td></td>
</tr>
<tr>
<td>KYNA</td>
<td>1.2–5</td>
<td>1.74 (0.93–3.28)</td>
<td>1.16 (0.58–2.32)</td>
<td></td>
</tr>
<tr>
<td>MK-801</td>
<td>0.2–0.8</td>
<td>0.23 (0.15–0.35)</td>
<td>0.17 (0.11–0.26)</td>
<td></td>
</tr>
<tr>
<td>γ-D-GAMS</td>
<td>0.4–0.6</td>
<td>0.45 (0.36–0.55)</td>
<td>0.37 (0.30–0.44)</td>
<td></td>
</tr>
<tr>
<td>NBOX</td>
<td>1.0–6.6</td>
<td>1.68 (0.93–3.05)</td>
<td>0.64 (0.25–1.18)</td>
<td></td>
</tr>
<tr>
<td>CNQX</td>
<td>3.3–10</td>
<td>4.21 (2.35–7.57)</td>
<td>2.49 (1.35–4.61)</td>
<td></td>
</tr>
<tr>
<td>MSC</td>
<td>0.125–0.5</td>
<td>0.25 (0.15–0.38)</td>
<td>0.19 (0.13–0.27)</td>
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PEFLO was administered at 0.78 mg/g i.p. All data are for antagonism of clonic and tonic seizures and were calculated by the method of Litchfield and Wilcoxon (24).

**DISCUSSION**

The mechanisms of the convulsant activities of quinolones are largely unresolved, despite suggestions from in vitro data that quinolones may be acting through inhibition of GABA binding to its receptor sites (1, 31, 39, 41). In particular, since the epileptogenic activities of some quinolones were suppressed by compounds that enhance GABA-ergic neurotransmission, i.e., GVG and TIA, or by MSC and DIA, which are agonists for the GABA\textsubscript{A}-benzodiazepine receptor complex (22), while it was influenced at neurotoxic doses by baclofen, a GABA\textsubscript{B} receptor agonist, the epileptogenic activities of quinolones likely involve the GABA\textsubscript{A}-benzodiazepine receptor complex. This conclusion is in agreement with the results of previous studies on the interaction of quinolones with the benzodiazepine-GABA receptor complex (1, 31, 39, 41). In addition, Enginar and Eroglu (18) demonstrated that the quinolone ofloxacin is able to reduce the threshold of convulsions induced by pentylentetrazole, which is believed to block the actions of GABA through its effects at the chloride ionophore coupled to the GABA\textsubscript{A} receptor complex, and therefore, it has been considered a specific DIA antagonist (29, 36, 37). Concerning the anticonvulsant potencies of the different compounds tested, differences exist among the groups. The most important finding of this study was that N-methyl-D-aspartate (NMDA) antagonists such as CPP, CGP 39551, CPPene, AP7, and MK-801 blocked seizures induced by i.p. administration of

![FIG. 5. Electrocorticographic patterns after saline–PEFLO (0.78 mg/g given i.p.) administration (A, B, C, and D) and GVG (1.5 mg/g given i.p.)–PEFLO (0.78 mg/g given i.p.) administration (A\textsubscript{1}, B\textsubscript{1}, C\textsubscript{1}, and D\textsubscript{1}) in DBA/2 mice. rCxs: right cortex; lCxs: left cortex. (A and A\textsubscript{1}) Electrocorticographic recordings 15 min after the injection of saline or GVG. (B and B\textsubscript{1}) Electrocorticographic recordings 20 min after PEFLO injection. (C and C\textsubscript{1}) Electrocorticographic recordings 60 min after PEFLO injection. (D and D\textsubscript{1}) Electrocorticographic recordings 120 min after PEFLO injection.](http://aac.asm.org/Downloaded from May 2, 2016 by guest)
PEFLO with potencies higher than or equal to those of the GABA 
agonist MSC, the benzodiazepine DIA, and the inhibitor of GABA 
uptake TIA. \( \gamma \)-D-GAMS, GYKI 52466, 2,3-
BZ-2, and NBQX, which are preferential \( \alpha \)-amino-3-hydroxy-
5-methyl-4-isoxazolepropionic acid (AMPA)-kainate (KA) 
receptor antagonists (8, 14, 23, 34), also antagonized the 
seizures induced by PEFLO, but with minor potencies compared 
with those of the NMDA antagonists. The doses of AMPA-KA 
agonists used are in the range of those which have been 
shown to block excitatory neurotransmission at non-NMDA 
receptors, thus demonstrating that AMPA-KA-dependent 
mechanisms are activated in the course of PEFLO-induced 
seizures as well. The broad-spectrum antagonist KYNA was 
also found to be a potent anticonvulsant, and a trend similar to 
that seen for KYNA was seen following the systemic admin-
istration of some selective NMDA receptor antagonists. It is 
remarkable that the doses of MK-801, CPPene, CGP 39551, 
and CPP required to block the clonic and tonic components of 
PEFLO-induced convulsions were less than or similar to those 
used in other experimental models to antagonize clonic- tonic 
seizures in mice (27, 38), while the doses of drugs enhancing 
GABA-ergic transmission are usually higher than those able to 
block audiogenic seizures in DBA/2 mice (4–6). IFE and SL 
82.0715, two compounds that act on the polyamine site of the 
NMDA receptor complex (3, 30), were unable to protect 
against seizures induced by PEFLO, suggesting that the poly-
amine site does not play a principal role in the genesis of 
seizures induced by PEFLO. The results of this study demon-
strate that the excitation mediated by dicarboxylic amino acids 
plays a crucial role in the pathogenesis of seizures caused by 
PEFLO in mice, similar to that observed following pretreat-
ment with EAA antagonists in seizures induced by bicuculline, 
pilocarpine, and imipenem (14, 15, 28, 40). It is most likely that 
excessive activation of EAA receptors occurs secondarily to or 
concomitantly with the impairment of the inhibitory GABA-
ergic neurotransmission caused by PEFLO and is essential for 
the propagation of seizures. The NMDA receptors seem to 
play a particularly pivotal role in PEFLO-induced seizures, 
since NMDA antagonists were potent anticonvulsants in the 
present study. This observation is in line with those from a 
previous study showing that the proconvulsive activities of 
some quinolones may be antagonized by EAA antagonists 
(42). The present study also demonstrated that the seizure 
intensity was consistently reduced in animals pretreated with 
most EAA antagonists or the latter phases of seizures failed to 
occur. In addition, a different incidence of seizures was ob-
served in mice after pretreatment with EAA antagonists. A 
quantitative comparison of the anticonvulsant efficacies of the 
EAAs in antagonizing PEFLO-induced seizures and audi-
ogenic seizures in DBA/2 mice seems to demonstrate some 
similarities among the EAAs (e.g., the anticonvulsant poten-
cies of CPP, CPPene, CGP 39551, and NBQX). However, IFE 
and SL 82.0715 showed no or weak anticonvulsant activity or 
less of a protective effect against PEFLO-induced seizures, 
while they were potent anticonvulsants against audiogenic sei-
zures (3). The possibility that PEFLO possesses agonistic or 
modulatory properties at receptors activated by EAAs might 
be an alternative explanation to the one involving GABA-ergic

FIG. 6. Electrocorticographic patterns after CPPene (8 \( \mu \)g/g given i.p.)–PEFLO (0.78 mg/g given i.p.) administration (A, B, C, and D) and MK-801 (0.3 \( \mu \)g/g given i.p.)–PEFLO (0.78 mg/g given i.p.) administration (A1, B1, C1, and D1) in DBA/2 mice. rCx, right cortex; lCx, left cortex. (A and A1) Electrocorticographic recordings 15 min after the injection of CPPene or MK-801. (B and B1) Electrocorticographic recordings 20 min after PEFLO injection. (C and C1) Electrocorticographic recordings 60 min after PEFLO injection. (D and D1) Electrocorticographic recordings 120 min after PEFLO injection.
transmission, which requires experimental confirmation. Since previous studies have indicated that systemic administration of quinolones to experimental animals or humans may sometimes produce proconvulsant or epileptogenic effects (9–12, 17, 18, 20, 25, 32, 42), we conclude that physicians should consider the possible epileptogenic activity of PEFLO when treating patients with predisposing epileptic factors.

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