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Involvement of Inhibitory and Excitatory Neurotransmitters in Levofloxacin- and Ciprofloxacin-Induced Convulsions in Mice

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We studied the effects of γ-aminobutyric acid (GABA)–benzodiazepine receptor agonists and glutamate receptor antagonists on levofloxacin (LVFX)- and ciprofloxacin (CPFX)-induced convulsions using intrathecal (i.t.) injections in mice. We also studied the effects of these agonists and antagonists on exacerbated convulsions induced by coadministration of the quinolone with 4-biphenylacetic acid (BPAA). The agonists or antagonists were injected i.t. 5 min and BPAA was administered orally 30 min before a single i.t. injection of the quinolone (10 μl per animal). The animals were observed for clonic convulsion and death, and latency times to the appearance of convulsion were determined. Among the agonists, baclofen showed marked inhibition of both LVFX- and CPFX-induced convulsions, while other compounds such as GABA, muscimol, diazepam, and 3-aminopropylphosphonic acid had slight effects. Among the antagonists, kynurenic acid showed the strongest inhibition of convulsions caused by all doses of LVFX and CPFX and prolonged latency times; γ-glutamyl-aminomethylsulfonic acid (GAMS) also markedly inhibited convulsions. The antagonists D-AP-5, AP-7, and 6,7-dinitroquinoxaline-2,3-dione (DNQX) had slight effects. Additionally, GAMS, DNQX, and MK-801 significantly lowered the incidence of death in the groups treated with CPFX. The enhanced convulsive activities of LVFX or CPFX by pretreatment with BPAA were clearly blocked by baclofen, kynurenic acid, GAMS, and DNQX. D-AP-5 and AP-7 also showed clear effects on the activity of LVFX. These results suggest that LVFX has fewer effects on the brain than CPFX and that convulsions induced by these quinolones alone and by these quinolones administered with BPAA may be mediated largely through glutamate and $GABA_B$ rather than $GABA_A$ receptors in mice.

Because of their excellent activities, quinolone antibacterial agents have been widely adopted for use in clinical practice. Although recently developed quinolones are less toxic than earlier compounds, they still produce very few incidences of various adverse effects on the central nervous system. Among these, convulsions remain a serious problem; enoxacin (ENX) (29), norfloxacin (NFLX) (2, 39), and ciprofloxacin (CPFX) (3) have been reported to induce convulsions in humans. Furthermore, these convulsions have been reported to be enhanced by coadministration with nonsteroidal anti-inflammatory drugs (NSAIDs) (2, 3, 29, 31). The mechanisms underlying these convulsions have therefore been investigated in animal models.

It is well-known that quinolones competitively inhibit in vitro the binding of [^{3}H]γ-aminobutyric acid ([^{3}H]GABA), [^{3}H]muscimol, and [^{3}H]diazepam to GABA-benzodiazepine (BDZ) receptors in postsynaptic membranes and are thought to have antagonistic actions on these receptors (1, 6, 14, 26–28, 33, 40). Moreover, their enhanced convulsive activity in the presence of NSAIDs is thought to be mediated through the augmentation of interactions between quinolones and $GABA_A$ by NSAIDs, as evidenced by electrophysiological studies (10, 18) and binding assays (7). In contrast, other studies have reported the failure of GABA-benzodiazepinergic drugs to inhibit completely convulsions caused by coadministration of a quinolone and an NSAID (11, 20, 30, 31), the lack of any clear correlation between the activities of quinolones in inhibiting GABA binding to the receptor and their convulsive activities (23), and the inhibition of quinolone-induced convulsions by glutamate receptor antagonists (38). Therefore, a GABA-ergic mechanism is thought to be an essential but not the sole component of the mechanism that induces convulsions; glutamate is also suspected of being involved (11).

Fewer in vivo studies have been performed; each has involved systemic administration and only a few kinds of compounds. In clarifying the actions of quinolones on neurotransmitter receptors, the intrathecal (i.t.) injection of the compounds is considered an effective means of observing directly their effects on the CNS. We therefore conducted the present in vivo study using 11 kinds of GABA-BDZ receptor agonists and glutamate receptor antagonists and observed their effects on convulsions induced by levofloxacin (LVFX), an optically active isomer of ofloxacin (OFLX), and CPFX in mice. We also investigated their effects on the enhanced convulsive activities of the quinolones when administered concomitantly with 4-biphenylacetic acid (BPAA), the active metabolite of the NSAID fenbufen.

MATERIALS AND METHODS

Male ddY mice were purchased at 4 weeks of age from Japan SLC Co., Ltd. (Slc:ddY), housed in wire-mesh cages, and kept in an air-conditioned room (temperature, 25 ± 2°C; humidity, 55 ± 15%; light cycle, 12 h/day). They were allowed free access to commercial chow (F II; Funabashi Farm, Funabashi, Japan) and tap water and were acclimated to laboratory conditions until they were 4 weeks old. Six and

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seven animals were assigned to each experimental group for intravenous (i.v.) and i.t. administration, respectively.

i.v. injection study. LVFX and CPFX were synthesized at Daiichi Pharmaceutical Co. Ltd., dissolved in saline, sterilized with a membrane filter (pore size, 0.02 μm; Millex, Millipore), and injected once into the tail vein at doses of 139, 167, 200, 240, 288, and 346 mg/kg of body weight at a fixed volume and speed of 20 ml/kg and 0.5 ml/min, respectively.

i.t. injection study. LVFX was dissolved in saline at 0.063, 0.13, 0.25, 0.5, and 1%, and CPFX was dissolved in saline at 0.031, 0.063, 0.13, 0.25, and 1%. The GABA-BDZ receptor complex agonists GABA, muscimol, baclofen, and 3-aminopropylphosphonic acid (APA) were purchased from Sigma and were also dissolved in saline at 1, 0.001, 0.25, and 1%, respectively, while another agonist, diazepam, was used from a 0.5% injection solution (Takeda, Osaka, Japan). The glutamate receptor antagonists D-AP-5, AP-7, γ-glutamylaminomethylsulfonic acid (GAMS), and 6,7-dinitroquinoxaline-2,3-dione (DNQX; Sigma) were used as 0.5% solutions in saline. MK-801 (Funakoshi, Tokyo, Japan) and kynurenic acid (Tokyo Kasei Kogyo, Tokyo, Japan) were dissolved in saline (maximum solubility, 1.25%) and saline (0.2%) to which 0.01 N NaOH was added. Concentrations were determined in preliminary experiments to induce sedation or loss of the righting reflex for 5 to 10 min by muscimol and MK-801, 15 to 40 min by APA and AP-7, and 30 min or more by others after a single i.t. injection of 10 μl per animal. All test solutions were sterilized just before use.

To estimate convulsive activities, 10 μl of LVFX or CPFX was injected alone into the subarachnoid space of the lumbar spinal column in conscious animals. To examine inhibitory effects on quinolone-induced convulsion, one of the agonists or antagonists mentioned above was injected at 10 μl into the same site 5 min before the injection of a quinolone. In this experiment, doses of LVFX and CPFX were 0.25 and 1% and 0.063, 0.25, and 1%, respectively; these concentrations were found to induce a high incidence of convulsions, usually shortly after their i.t. injection.

Coadministration study with BPAA. To choose a suitable dose, BPAA was suspended in 0.5% sodium carboxymethyl cellulose and was administered orally (p.o.) at 400 mg/kg to the animals 30 min before a single i.t. injection of LVFX at 0.063 or 0.13%. After confirming the exacerbation of convulsions by the combination of BPAA and LVFX at 0.13%, GABA, muscimol, diazepam, baclofen, D-AP-5, AP-7, GAMS, DNQX, or kynurenic acid at the same doses described above was also injected i.t. 5 min before the injection of LVFX. In a second experiment, CPFX was administered in the same way as LVFX as a 0.031% solution.

Clinical signs in all animals were examined for at least 35 min after treatment. Incidences of clonic convulsion and death were determined, and latency times to the appearance of convulsions were measured.

Statistical analysis. Differences in the incidences of convulsions were statistically analyzed by the χ^2 test between the quinolone alone and the quinolone plus other compound groups.

RESULTS

i.v. injection study. The incidences of convulsion and death after a single i.v. injection of LVFX or CPFX to mice are shown in Table 1. Incidences of clonic convulsion were zero of six, one of six, three of six, three of six, six of six, and six of six mice for LVFX and one of six, one of six, four of six, five of six, six of six, and six of six mice for CPFX in the groups treated with drug at 139, 167, 200, 240, 288, and 346 mg/kg, respectively. The doses that induced convulsions in 50% of mice were 200 or 240 mg/kg for LVFX and between 167 and 200 mg/kg for CPFX, suggesting a slightly greater neurotoxicity for the latter compound. Almost all animals in both the LVFX and CPFX groups which experienced convulsions died. Convulsions and death occurred immediately after injection in the low-dose group and during injection in the high-dose group.

TABLE 1. Incidence of clonic convulsion and death in mice after a single i.v. injection of LVFX or CPFX

Dose (mg/kg)	No. of animals experiencing the indicated event/total no. of animals tested			
	LVFX		CPFX	
	Convulsion	Death	Convulsion	Death
0[a]	0/6	0/6	—[b]	—
139	0/6	0/6	1/6	1/6
167	1/6	1/6	1/6	1/6
200	3/6	3/6	4/6	4/6
240	3/6	2/6	5/6	5/6
288	6/6	5/6	6/6	6/6
346	6/6	6/6	6/6	6/6

[a] Saline was administered.
[b] —, not examined.

i.t. injection study. Table 2 shows the incidences of clonic convulsions, latency times to the appearance of convulsions, and death after a single i.t. injection of LVFX or CPFX. LVFX injection induced convulsions at doses of 0.13% (incidence, three of seven mice) or more, with incidences gradually increasing (0.25%, six of seven mice; 0.5%, seven of seven mice; 1%, seven of seven mice) and latency times decreasing as the doses were increased. One animal each in the 0.25 and 1% groups died after experiencing convulsions. The 0.063% dose caused no abnormalities. With CPFX, a clear demarcation was seen between doses that caused convulsions in all mice and those that had no effect; a dose of 0.031% caused no abnormality, but a dose of 0.063% or greater induced convulsions in seven of seven animals in each group. Only six of seven animals in the 1% group died.

TABLE 2. Incidence of clonic convulsion and death in mice after a single i.t. injection of LVFX or CPFX[a]

Dose (%)[b]	LVFX			CPFX		
	LT (s)	Incidence[c]		LT (s)	Incidence	
		CC	D		CC	D
0[d]	—[e]	0/7	0/7			
0.031	—	—	—	—	0/7	0/7
0.063	—	0/7	0/7	326	7/7[f]	0/7
0.13	655	3/7	0/7	229	7/7[f]	0/7
0.25	159	6/7[f]	1/7	64	7/7[f]	0/7
0.5	88	7/7[f]	0/7	—	—	—
1.0	51	7/7[f]	1/7	31	7/7[f]	6/7[f]

[a] LT, latency time; CC, clonic convulsion; D, death.
[b] A total of 10 μl of each solution was injected i.t.
[c] Incidence is number of animals with the indicated event/total number of animals tested.
[d] Saline was injected.
[e] —, not examined.
[f] Significantly different ($P < 0.05$) from the saline group.

TABLE 3. Effect of inhibitory-neurotransmitter agonists on convulsion and death induced by LVFX or CPFX[a]

Compound (dose [%])	Quinolone dose (%)	LVFX			CPFX		
		LT (s)	Incidence[b]		LT (s)	Incidence	
			CC	D		CC	D
Control (none)	0.063				326	7/7	0/7 (0)[c]
	0.25	159	6/7	1/7 (0)	64	7/7	0/7 (0)
	1	51	7/7	1/7 (0)	31	7/7	6/7 (0)
GABA (1)	0.063				—[d]	0/7[e]	0/7 (0)
	0.25	301	5/7	1/7 (0)	209	6/7	3/7 (3)
	1	115	7/7	3/7 (0)	87	7/7	7/7 (2)
Muscimol (0.001)	0.063				220	2/7[e]	0/7 (0)
	0.25	329	5/7	0/7 (0)	184	7/7	4/7 (2)
	1	124	7/7	4/7 (2)	63	7/7	7/7 (2)
Diazepam (0.5)	0.063				—	0/7[e]	0/7 (0)
	0.25	—	0/7[e]	0/7 (0)	394	4/7	0/7 (0)
	1	186	4/7	0/7 (0)	134	7/7	5/7 (0)
Baclofen (0.25)	0.063				—	0/7[e]	0/7 (0)
	0.25	—	0/6[e]	1/7 (1)	—	0/5[e]	2/7 (2)
	1	—	0/7[e]	6/7[e] (5)	1,016	3/4	6/7 (5)
APA (1)	0.063				435	2/7[e]	0/7 (0)
	0.25	207	4/6	1/7 (1)	607	3/3	6/7[e] (5)
	1	111	4/4	6/7[e] (5)	152	6/6	7/7 (3)

[a] A total of 10 μl of each compound was injected i.t. 5 min before the injection of LVFX or CPFX. LT, latency time; CC, clonic convulsion; D, death.
[b] Incidence is number of animals with the indicated event/total number of animals tested.
[c] Values in parentheses are number of animals showing pulmonary edema.
[d] —, not examined.
[e] Significantly different ($P < 0.05$) from the corresponding LVFX or CPFX group.

A difference in the convulsive effects between LVFX and CPFX was more clearly seen with i.t. injection than with i.v. injection.

The effects of GABA-BDZ receptor agonists on LVFX- or CPFX-induced convulsions are presented in Table 3. Pretreatment with baclofen or APA induced deaths from pulmonary edema, particularly with the higher quinolone doses, although such edema was never seen when quinolones were injected alone. Some deaths occurred without any preceding convulsion; these were excluded from the calculation of convulsion incidences. Similar deaths also occurred sporadically in other groups, but no clear dose dependence was seen. The incidence of death was increased in the baclofen (1%) and APA (0.25%) groups. GABA and muscimol inhibited significantly the convulsions induced by CPFX at 0.063%, but not at 0.25 or 1%. No effect was seen on LVFX-induced convulsions. Diazepam completely inhibited convulsions by both LVFX at 0.25% and CPFX at 0.063%. Baclofen showed the most remarkable effects among the agonists used, completely inhibiting convulsions in the groups treated with LVFX at 0.25 and 1% and CPFX at 0.063 and 0.25%. APA significantly decreased the incidence of convulsions in the CPFX (0.063%) group, but not in the LVFX group. Latency times to convulsion were increased in all groups.

The effects of glutamate receptor antagonists on the quinolone-induced convulsions are shown in Table 4. D-AP-5 and AP-7 completely inhibited convulsions induced by LVFX at 0.25% and CPFX at 0.063%. In addition, D-AP-5 inhibited convulsion by CPFX at 0.25%. MK-801 had no effect on the incidence of convulsions in any group. GAMS significantly inhibited LVFX (0.25 and 1%)-induced and CPFX (0.063 and 0.25%)-induced convulsions, while DNQX also inhibited convulsions when mice were treated with lower doses of LVFX and CPFX. Kynurenic acid completely or significantly inhibited the induction of convulsions by all doses of LVFX and CPFX and was the most effective antagonist used in the present study. MK-801, GAMS, and DNQX significantly reduced the incidence of death in the CPFX (1%) group. Latency times were more markedly prolonged in almost all groups than in those of the GABA-BDZ agonist groups mentioned above.

Coadministration study with BPAA. A single oral pretreatment with BPAA at 400 mg/kg increased the incidence of clonic convulsions after i.t. injection of LVFX at 0.063 and 0.13%, from zero of seven and three of seven animals to two of seven and seven of seven animals, respectively. LVFX at 0.13% was therefore chosen for coadministration with BPAA. Injection of CPFX alone caused neither convulsions nor death when it was used at 0.031%, whereas the combination of CPFX and BPAA induced convulsions in seven of seven animals and death in six of seven animals. CPFX was therefore used at 0.031%; results are presented in Table 5.

Baclofen, GAMS, DNQX, and kynurenic acid completely inhibited convulsions induced by coadministration of LVFX or CPFX with BPAA and were associated with a marked reduction in death when they were administered with CPFX. Convulsions induced by LVFX plus BPAA were also completely blocked by D-AP-5 and AP-7, the former showing similar effects on convulsions induced by CPFX plus BPAA. In addition, diazepam significantly decreased the incidence of convulsions induced by LVFX plus BPAA. GABA and muscimol had no effect on convulsions induced by either quinolone.

TABLE 4. Effect of excitatory-neurotransmitter antagonists on convulsion and death induced by LVFX or CPFX[a]

Compound (dose [%])	Quinolone dose (%)	LVFX			CPFX		
		LT	Incidence[b]		LT	Incidence	
			CC	D		CC	D
Control (none)	0.063				326	7/7	0/7 (0)[c]
	0.25	159	6/7	1/7 (0)	64	7/7	0/7 (0)
	1	51	7/7	1/7 (0)	31	7/7	6/7 (0)
D-AP-5 (0.5)	0.063				—[d]	0/7[e]	0/7 (0)
	0.25	—	0/7[e]	0/7 (0)	1,350	1/6[e]	1/7 (1)
	1	793	3/6	1/7 (1)	752	6/6	2/7 (1)
AP-7 (0.5)	0.063				—	0/7[e]	0/7 (0)
	0.25	—	0/7[e]	0/7 (0)	1,326	5/7	0/7 (0)
	1	1,133	3/6	1/7 (1)	472	7/7	2/7 (0)
MK-801 (1.25)	0.063				2,162	3/7	0/7 (0)
	0.25	1,373	3/7	0/7 (0)	443	7/7	0/7 (0)
	1	420	7/7	0/7 (0)	154	7/7	0/7[e] (0)
GAMS (0.5)	0.063				—	0/7[e]	0/7 (0)
	0.25	—	0/5[e]	2/7 (2)	1,890	1/6[e]	1/7 (1)
	1	2,340	1/5[e]	2/7 (2)	1,217	3/7	0/7[e] (0)
DNQX (0.05)	0.063				—	0/7[e]	0/7 (0)
	0.25	116	2/7[e]	0/7 (0)	1,106	4/6	1/7 (1)
	1	398	4/6	1/7 (1)	1,020	6/7	1/7[e] (0)
Kynurenic acid (0.2)	0.063				—	0/7[e]	0/7 (0)
	0.25	—	0/7[e]	1/7 (0)	—	0/7[e]	0/7 (0)
	1	1,785	2/6[e]	1/7 (1)	1,665	2/6[e]	3/7 (1)

[a] A total of 10 μl of each compound was injected i.t. 5 min before the injection of LVFX or CPFX. LT, latency time; CC, clonic convulsion; D, death.
[b] Incidence is number of animals with indicated event/total number of animals tested.
[c] Values in parentheses are number of animals showing pulmonary edema.
[d] —, not examined.
[e] Significantly different ($P < 0.05$) from the corresponding LVFX or CPFX group.

In this experiment, no death resulted from pulmonary edema.

DISCUSSION

Almost all quinolones have been assessed for their convulsive activities; OFLX and CPFX are ranked as having weak convulsive activities (5, 7, 13, 19, 23). The LVFX used in this study is the *S*-(−)-isomer of OFLX and has acute toxicity and convulsive activity similar to those of OFLX in mice after i.v. administration (15, 24). Naora et al. (22) reported that CPFX penetrates into the brains and cerebrospinal fluid of rats at 0.1 to 0.5 μg/g or μg/ml after a single i.v. injection of 10 mg/kg. Concentrations of LVFX in the brain and cerebrospinal fluid after i.v. injection of the same dose to rats were several times those of CPFX (data not shown), however, suggesting that LVFX crosses the blood-brain and/or blood-cerebrospinal fluid barriers more easily than CPFX does. In spite of this, LVFX showed no greater convulsive activity than CPFX after i.v. injection and showed significantly less convulsive activity than CPFX after i.t. injection in the present study. The lowest dose of LVFX that induced convulsions and the dose that induced convulsions in all animals were two and eight times greater than those of CPFX, respectively. Additionally, the incidence of convulsions increased in a dose-dependent manner in the groups treated with LVFX, in contrast to the clear demarcation between doses of CPFX that induced convulsions in 0 and 100% of animals. Thus, a clear difference in the convulsive activities between the two quinolones was revealed when they were administered by i.t. injection, with LVFX proving to be less neurotoxic than CPFX in mice.

Pretreatment with diazepam, which acts selectively on the BDZ receptor of the GABA-BDZ receptor complex and enhances the function of the $GABA_A$ receptor (17), com-

TABLE 5. Effect of agonists and antagonists of inhibitory and excitatory neurotransmitters on convulsion induced by coadministration of LVFX or CPFX with BPAA[a]

Compound (dose [%])	Incidence of convulsions[b]	
	LVFX	CPFX
BPAA	7/7	7/7
BPAA + GABA (1)	7/7	7/7
BPAA + muscimol (0.001)	7/7	7/7
BPAA + diazepam (0.5)	2/7[c]	7/7
BPAA + baclofen (0.25)	0/7[c]	0/7[c]
BPAA + D-AP-5 (0.5)	0/7[c]	2/7[c]
BPAA + AP-7 (0.5)	0/7[c]	5/7
BPAA + GAMS (0.5)	0/7[c]	0/7[c]
BPAA + DNQX (0.05)	0/7[c]	0/7[c]
BPAA + kynurenic acid (0.2)	0/7[c]	0/7[c]

[a] BPAA (400 mg/kg) was administered orally at 30 min before and each compound was injected i.t. 5 min before a single i.t. injection of LVFX (0.13%) or CPFX (0.031%).
[b] Incidence is number of animals with convulsions/total number of animals.
[c] Significantly different ($P < 0.05$) from the BPAA plus quinolone group.

pletely inhibited the convulsions induced by i.t. injection of low doses of LVFX or CPFX. GABA, a $GABA_A$ plus $GABA_B$ receptor agonist, inhibited only convulsions induced by low-dose CPFX. Muscimol, a $GABA_A$ receptor agonist, showed the same effects as GABA. Various reports have described the antagonistic actions of CPFX and/or OFLX on the GABA-BDZ receptor complex as follows; the drug(s) inhibited GABA and/or muscimol binding to the receptor (1, 6, 8, 33), shortened the pentobarbital-induced sleeping time in mice (8), and showed central nervous system-stimulating effects in electroencephalograms, which were reversed by concurrent administration with a BDZ agonist (34). The convulsive actions of other quinolones including lomefloxacin, pipemidic acid, NFLX, and ENX have also been studied with regard to their inhibitory effects on [^{3}H]muscimol binding to rat synaptic membranes (1). In accordance with the results presented in those reports, our results also showed similar effects of both LVFX and CPFX on the GABA-BDZ receptor complex. Enginar and Eroglu (9) have reported that OFLX administered intraperitoneally (i.p.) to mice increased the incidence of pentylenetetrazol-induced convulsions and that this was inhibited by diazepam administered i.p., whereas CPFX had no effect in this model. These results for OFLX indicate that it has effects similar to those of LVFX on the mouse central nervous system; a significant difference, however, exists between their results and ours for CPFX. This discrepancy may be due to the difference in the route of drug administration. In the experiment of Enginar and Eroglu (9), CPFX and OFLX were administered i.p. to mice at the same doses (20 and 80 mg/kg, respectively). There is a possibility that CPFX could not reach the brain at concentrations that were sufficient to exacerbate the pentylenetetrazol-induced convulsion because the level of CPFX in the brain is speculated to be markedly less than that of OFLX after i.p. administration, considering the levels of 0.1 to 0.5 and 5 μg/g for CPFX (22) and OFLX (25), respectively, after a single i.v. injection to rats at 10 and 20 mg/kg, respectively.

We tested other GABA receptor agonists which are selective for the $GABA_B$ receptor, namely, baclofen and APA (4). Baclofen inhibited both LVFX- and CPFX-induced convulsions more effectively than the $GABA_A$ receptor agonists did. Additionally, APA inhibited only those CPFX convulsions induced at the low dose. These results suggest that both quinolones induce convulsions mainly through interaction with $GABA_B$ receptors rather than through interaction with the $GABA_A$ receptors.

Glutamate receptors are found in the mammalian brain; these include *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), and kainic acid (KA), metabotropic and presynaptic types (12). The present study showed that compounds which antagonize these receptors inhibit quinolone-induced convulsions in mice. D-AP-5 and AP-7, which are competitive antagonists of the NMDA receptor, inhibited convulsions induced by LVFX (low dose) or CPFX (low and/or middle doses), whereas the noncompetitive antagonist MK-801 did not. MK-801 did, however, reduce the incidence of death in the group treated with a high dose of CPFX. In contrast, GAMS and DNQX, which antagonize both AMPA and KA receptors, markedly inhibited convulsions by LVFX or CPFX, respectively, only at the low doses of LVFX or CPFX. Both compounds also reduced deaths by high-dose CPFX. Kynurenic acid is reported to be a nonselective glutamate receptor antagonist (26) which possesses particular affinity for the NMDA and AMPA receptors (16). This compound markedly inhibited convulsions induced by LVFX and CPFX at all doses. The inhibition is speculated to be caused by the preexistence of kynurenic acid at the receptor, which prevents the binding of quinolones to the site because kynurenic acid has a quinolone ring-like structure. Considering these results with findings for interactions with GABA-BDZ receptors, the activation of glutamate receptors appears to contribute to the induction of convulsions by both LVFX and CPFX; non-NMDA receptors are thought to play a slightly more important role than NMDA receptors in the case of LVFX. Although naladixic acid (NA) and oxolinic acid have no appreciable effect on quisqualate, kainate, or NMDA binding to the receptor in vitro (38), glutamate receptor antagonists including MK-801 have been shown to completely block both NA- and oxolinic acid-induced convulsions in mice (38). Possible mechanisms include the in vivo biotransformation of quinolones into chemical entities which then have the potential to interact with glutamate receptors; the ability of glutamate receptor antagonists to block quinolone-induced seizures may relate to an indirect rather than a direct antagonism of quinolone activity via glutamate receptors (38). Although the details of such activity on the receptors remain unclear, the results of both the present and previous studies suggest that LVFX and CPFX induce convulsions in mice through direct or indirect accelerating and inhibiting actions on both glutamate and GABA-BDZ receptors, respectively; the proposed mechanisms are complex (5, 11, 23, 32, 38). Additionally, quinolones are thought to interact more strongly with glutamate receptors than with GABA-BDZ receptors, because treatment of mice with glutamate receptor antagonists resulted in a decreased incidence of death and a more marked prolongation of the latency time.

The mechanisms by which NSAIDs exacerbate quinolone-induced convulsions in relation to the inhibition of $GABA_A$ binding to the receptor have been investigated by a number of researchers. The coexistence of a quinolone and an NSAID has been shown to facilitate inhibition of [^{3}H]muscimol binding to the receptor (1, 18), increase the amplitude of electrically evoked field potentials in the CA_1 region of the rat hippocampus in vitro (5), and reduce the GABA-evoked whole-cell current (10). In the present study, however, baclofen, D-AP-5, AP-7, GAMS, DNQX, and kynurenic acid clearly blocked the enhanced convulsive actions induced by concomitant administration of LVFX with BPAA in mice, with all compounds except AP-7 having the same or similar effects in the groups treated with CPFX. Furthermore, diazepam slightly decreased the action of LVFX. These data indicate that $GABA_B$ and glutamate contributed to the exacerbated convulsions induced by the quinolone and BPAA. In the case of LVFX, BDZ was additionally involved in the enhanced convulsions. Among the glutamate receptors, non-NMDA subtypes are thought to act mainly on the enhanced convulsions induced by CPFX plus BPAA because the inhibitory effects caused by GAMS and DNQX were more remarkable than those caused by D-AP-5 and AP-7. However, GABA and diazepam both completely inhibited convulsions induced by CPFX alone, whereas neither had any effect on those induced by CPFX plus BPAA. These discrepancies may be due to a difference in drug concentration in the brain when CPFX is administered alone and when CPFX is coadministered with BPAA. By reducing the renal clearance of CPFX (21), coadministered fenbufen may facilitate entry of the quinolone into the central nervous system in rats not only by elevating levels in serum but also by enhancing permeability across the blood-

brain or blood-cerebrospinal fluid barrier (22). However, no difference in metabolism and the concentration of other quinolones, including OFLX, in tissue, between administration of a quinolone alone and a quinolone plus an NSAID has been reported (13, 40).

MK-801 and ketamine, both NMDA receptor antagonists, have also been shown to interact with GABA-ergic transmission by their suppressive effects on picrotoxin-, pentylenetetrazol-, or bicuculline-induced tonic-clonic seizures during ontogenesis in rats aged 7 through 90 days (35–37). The results of the present study may also indicate the existence of interactions between glutamate- and GABA-mediated transmissions, but not clearly so. We therefore conclude that, except for some additional effects for CPFX, both LVFX and CPFX induce convulsions in mice through complex interactions mainly with glutamate and $GABA_B$ receptors after i.t. injection alone and after coadministration with BPAA.

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