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Interaction of Felbamate and Diazepam Against Maximal Electroshock Seizures and Chemoconvulsants in Mice

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GORDON, R., M. GELS, W. DIAMANTIS AND R. D. SOFIA. *Interaction of felbamate and diazepam against maximal electroshock seizures and chemoconvulsants in mice.* PHARMACOL BIOCHEM BEHAV **40**(1) 109–113, 1991.—The anticonvulsant effects of felbamate alone or in combination with diazepam were investigated against maximal electroshock-, pentylenetetrazol-, isoniazid- and bicuculline-induced seizures in mice. A single subprotective dose of felbamate, a dose which offers no protection to animals when combined with diazepam, enhanced the protective effects of diazepam against seizures induced by electroshock, pentylenetetrazol and isoniazid, as measured by significant reduction of ED_{50} values. However, felbamate failed to significantly affect the protective action of diazepam against bicuculline. Felbamate does not interact directly with the GABA-benzodiazepine-ionophore complex. Thus the enhancement of anticonvulsant activity of diazepam by felbamate against maximal electroshock and pentylenetetrazol may involve an indirect effect at benzodiazepine receptors. The anticonvulsant action of felbamate against isoniazid does not seem to involve benzodiazepine receptors and may be due to reversing the inhibitory effect of isoniazid on glutamate decarboxylase (GAD) activity. The interaction between felbamate and diazepam may also involve other mechanisms.

Felbamate Diazepam Maximal electroshock seizures Pentylenetetrazol Bicuculline Isoniazid Benzodiazepine GABA receptor

IT is generally accepted that gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the central nervous system. Benzodiazepines have been shown to bind to a specific allosteric site in the benzodiazepine-GABA receptor-ionophore complex (18,24). Thus the anticonvulsant mechanism of action of benzodiazepines appears to be due to facilitation of GABAergic transmission (3, 4, 10). The anticonvulsant activity of barbiturates may also be mediated by interaction with the GABA system (15, 19, 20). Recent studies on the interaction of benzodiazepines with barbiturates have shown that when subprotective doses of diazepam were combined with subprotective doses of barbiturates the anticonvulsant activity of diazepam was potentiated in a variety of experimental seizures (16, 22, 23). Phenytoin, a drug which is ineffective against chemically induced seizures (25), enhanced the protective effects of diazepam against seizures produced by pentylenetetrazol (6,8). The ability of phenytoin to increase the total number of benzodiazepine binding sites (9) and interact with $GABA_A$ receptors (2) may account for these results.

Felbamate (2-phenyl-1,3-propanediol dicarbamate) is an anticonvulsant compound effective in nontoxic doses against maximal electroshock seizures, pentylenetetrazol, and picrotoxin, but ineffective against bicuculline and strychnine in mice (26). Felbamate is currently undergoing clinical trials for the treatment of partial seizures. The present study evaluates the interaction of felbamate when administered in a subprotective dose, i.e., a dose which offers no protection to mice, and diazepam against maximal electroshock seizures and convulsions induced by pentylenetetrazol, isoniazid and bicuculline. The anticonvulsant potencies of felbamate alone or combined with diazepam were expressed as ED_{50} values.

METHOD

Animals

Male CD-1 mice weighing 25–35 g were purchased from Charles River Breeding Laboratories, Kingston, NY. Animals were housed in standing wire cages with free access to food and water. Experiments were initiated only after an acclimation period of at least five days to the animal room environment which consisted of automatically controlled illumination with a 12-hour light/dark cycle and controlled temperature and relative humidity.

Anticonvulsant Effect

Maximal electroshock seizures were induced in mice via corneal electrodes (30 mA, 60 Hz, 0.2 s). Abolition of hindlimb tonic extension was considered as protection (25). Preliminary studies were conducted to determine the dose of each convulsant

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agent producing clonic and tonic seizures in 100% of the animals.

The anticonvulsant effects of felbamate and diazepam were evaluated against pentylenetetrazol (100 mg/kg, SC), bicuculline (3.5 mg/kg SC), and isoniazid (450 mg/kg IP) in mice (5). Abolition of a minimal threshold seizure was considered as protection. Felbamate and diazepam were administered intraperitoneally, felbamate at 60 min and diazepam at either 60 or 30 min prior to convulsive tests. Animals were observed for 30 min following administration of convulsants.

Drug Interaction

In order to study the interaction between felbamate and diazepam, a subprotective dose of felbamate was combined with diazepam in mice and the animals subjected to either electrically or chemically induced seizures. The number of animals protected in each group was recorded.

Drugs

Felbamate was developed and synthesized at Wallace Laboratories. Pentylenetetrazol, bicuculline, and isoniazid were purchased from Sigma Chemical Co. (St. Louis, MO). Diazepam was a gift from Hoffmann-La Roche (Nutley, NJ).

Felbamate was suspended in a 50% solution of polyethylene glycol 400 in sterile water. Diazepam was administered in 0.9% NaCl. Pentylenetetrazol and isoniazid were dissolved in 0.9% NaCl. Bicuculline was dissolved in a few drops of 0.1 M HCl, and the final volume was made up with 0.9% NaCl. All drugs were administered in a volume of 10 ml/kg body weight.

Statistics

ED_{50} values and confidence limits were calculated by the probit method of Lieberman (11). Significance between ED_{50} values was determined by the method of Litchfield and Wilcoxon (12). The drug interaction experiments were analyzed using the Fisher Exact Probability Test. A value of $p<0.05$ was considered statistically significant.

RESULTS

Effects of Felbamate and Diazepam Against Maximal Electroshock Seizures

The effect of felbamate against maximal electroshock seizures in mice is shown in Table 1. Felbamate offered dose-dependent protection with an ED_{50} value of 30.0 mg/kg. A single subprotective dose of felbamate (15 mg/kg) combined with diazepam significantly increased the potency of diazepam (3 and 10 mg/kg) from 20 to 30% protection to 100 and 90% protection, respectively, against seizures (Fig. 1).

Effects of Felbamate and Diazepam Against Pentylenetetrazol-Induced Seizures

Felbamate protected mice against pentylenetetrazol in a dose-related manner with an ED_{50} value of 98.4 mg/kg (Table 1). The anticonvulsant potency of diazepam (0.1, 0.2 and 0.4 mg/kg doses) was enhanced when combined with a subprotective dose of felbamate (30 mg/kg) which increased the percent protection from 0, 10 and 30% to 50, 70 and 80%, respectively (Fig. 2).

Effects of Felbamate and Diazepam Against Isoniazid-Induced Seizures

Felbamate effectively protected mice against isoniazid-induced seizures; the ED_{50} value was 129.9 mg/kg (Table 1). A subprotective dose of felbamate (30 mg/kg) increased the potency of diazepam (0.1, 0.3 and 1 mg/kg doses) against isoniazid-induced seizures, with significant enhancement when felbamate was combined with 0.3 mg/kg of diazepam (Fig. 3).

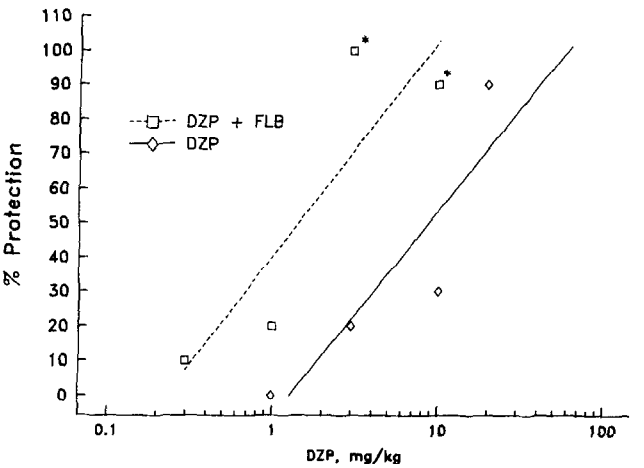

FIG. 1. Protective effects of diazepam alone and in combination with 15 mg/kg of felbamate against maximal electroshock seizures. Felbamate and diazepam were injected IP 60 min prior to testing. Results were obtained from 10–20 animals. $*p<0.05$, as compared to diazepam alone by Fisher exact probability test.

Effects of Felbamate and Diazepam Against Bicuculline-Induced Seizures

Felbamate, in doses up to 100 mg/kg, had no influence on bicuculline-induced threshold (clonic) seizures (Table 1). The interaction of a subprotective dose of felbamate (50 mg/kg) with diazepam (0.3, 1 and 3 mg/kg doses) enhanced the protective effect of the benzodiazepine against bicuculline-induced seizures.

TABLE 1

PROTECTIVE EFFECTS OF FELBAMATE IN SEVERAL ANTICONVULSANT TESTS IN MICE

Felbamate IP Dose (mg/kg)	Test Procedure	No. Mice Protected / No. Mice Tested	ED_{50} (mg/kg)
20		1/10	
40	MES	8/10	30.0 (24.5–36.9)
60		10/10	
50		1/10	
100	SC PTZ	5/10	98.4 (75.5–128.3)
150		8/10	
30		0/10	
100	IP ISO	5/10	129.9 (80.8–208.9)
300		7/9	
30		0/10	
50	SC BIC	0/10	>100
100		0/10	

MES, maximal electroshock seizure; SC PTZ, subcutaneous pentylenetetrazol; IP ISO, intraperitoneal isoniazid; SC BIC, subcutaneous bicuculline.

Ninety-five percent confidence limits are in parentheses.

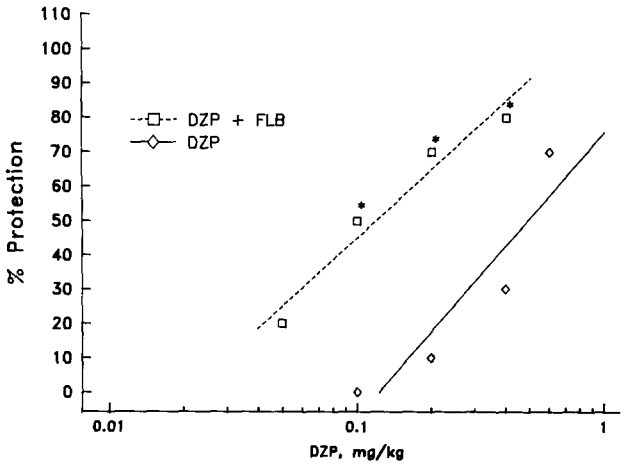

FIG. 2. Protective effects of diazepam alone and in combination with 30 mg/kg of felbamate against pentylenetetrazol-induced seizures. Felbamate and diazepam were injected IP 60 min prior to testing. Results were obtained from 10 animals. *$p<0.01$, as compared to diazepam alone by Fisher exact probability test.

Effect of Felbamate on the Potency of Diazepam

The effect of a subprotective dose of felbamate on the anticonvulsant potency of diazepam is depicted in Table 2. In the maximal electroshock seizure test, the ED_{50} value of diazepam was significantly reduced 85%, from 11.2 to 1.7 mg/kg, by felbamate (Fig. 1). In addition, felbamate significantly enhanced the potency of diazepam against pentylenetetrazol- and isoniazid-induced seizures with respective ED_{50} values reduced from 0.48 to 0.12 mg/kg (75%) (Fig. 2) and from 2.8 to 0.18 mg/kg (94%) (Fig. 3). Although felbamate was ineffective against bicuculline, it reduced the ED_{50} value of diazepam against bicuculline-induced seizures approximately 50% from 3.7 to 1.8 mg/kg (Fig. 4).

DISCUSSION

Seizures induced by pentylenetetrazol and bicuculline are thought to occur by impairment of GABAergic neurotransmission (5, 14, 15, 29), whereas isoniazid-induced convulsions result from inhibition of glutamic acid decarboxylase (GAD) and reduction in brain GABA content (17,29). Felbamate, when combined with diazepam in subeffective doses, enhanced the protective effects of diazepam against maximal electroshock seizures, pentylenetetrazol, and isoniazid-induced convulsions but not against bicuculline-induced threshold (clonic) seizures. Moreover, the combination of felbamate and diazepam resulted in significant reductions in the ED_{50} value of diazepam against maximal electroshock seizures, pentylenetetrazol, and isoniazid. Felbamate displayed broad anticonvulsant activity in animal studies but showed little or no measurable inhibition of [^{3}H]flunitrazepam and [^{3}H]GABA receptor binding (26). Recent studies have confirmed the failure of felbamate to interact with benzodiazepine and $GABA_A$ binding sites and, in addition, showed that felbamate did not alter the specific binding of [^{35}S]t-butylbicyclophosphorothionate to picrotoxin receptor sites of rat brain cerebral cortex or cerebellum (28). Thus felbamate does not interact with the $GABA_A$ receptor complex, and felbamate's anticonvulsant effects are not related to modulation of GABAergic transmission.

The mechanism of action for the anticonvulsant activity of felbamate is unknown at this time. However, felbamate may act indirectly to enhance the protective effects of diazepam at benzodiazepine and $GABA_A$ sites. Interestingly, phenytoin, which showed little or no affinity for the [^{3}H]diazepam binding site in rat brain (1), potentiated the anticonvulsant effect of diazepam against pentylenetetrazol (6–8) and electroshock (6), but not against bicuculline and isoniazid (6). The protective effects of phenytoin against pentylenetetrazol and electroshock could be interpreted in terms of the ability of phenytoin to increase the total number of benzodiazepine receptors (9). In support of this idea, Paul et al. (21) demonstrated a direct correlation between benzodiazepine receptor occupancy by diazepam and protection against pentylenetetrazol-induced seizures. Furthermore, they showed that only a small fraction (less than 30%) of benzodiazepine receptors need to be occupied to produce a complete anti-

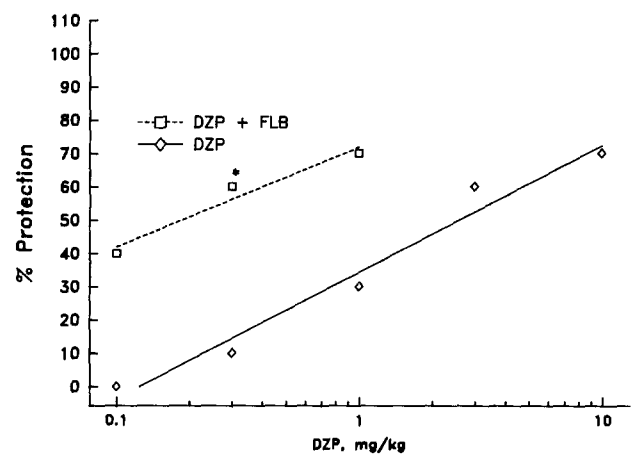

FIG. 3. Protective effects of diazepam alone and in combination with 30 mg/kg of felbamate against isoniazid-induced seizures. Felbamate and diazepam were injected IP 60 min and 30 min, respectively, prior to testing. Results were obtained from 10 animals. *$p<0.05$, as compared to diazepam alone by Fisher exact probability test.

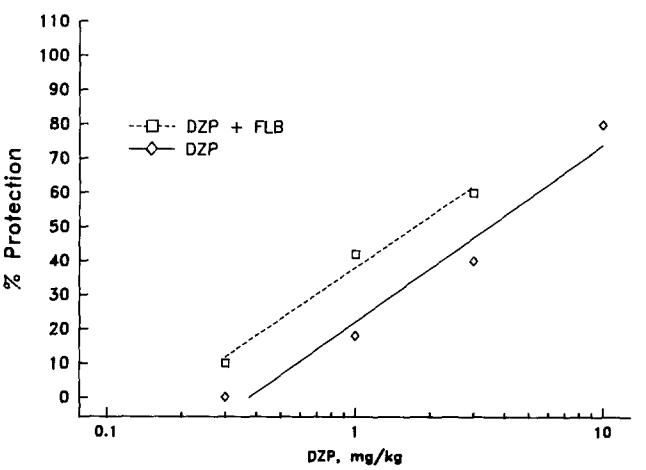

FIG. 4. Protective effects of diazepam alone and in combination with 50 mg/kg of felbamate against bicuculline-induced seizures. Felbamate and diazepam were injected IP 60 min prior to testing. Results were obtained from 10–20 animals.

TABLE 2

INTERACTION OF A SUBPROTECTIVE DOSE OF FELBAMATE WITH THE ANTICONVULSANT POTENCY OF DIAZEPAM

Treatment	IP ED_{50} (mg/kg)*			
	MES	SC PTZ	IP ISO	SC BIC
Diazepam	11.2 (7.3–17.2)	0.48 (0.3–0.7)	2.8 (1.3–6.0)	3.7 (2.0–6.6)
Diazepam + Felbamate†	1.7 (1.1–2.6)‡	0.12 (0.07–0.2)‡	0.18 (0.04–0.85)‡	1.8 (0.9–3.4)

*The 95% confidence limits are shown in parentheses.

†Felbamate was administered intraperitoneally in doses of 15, 30, 30 and 50 mg/kg in the MES, SC PTZ, IP ISO and SC BIC tests, respectively.

‡$p<0.05$ versus diazepam alone.

convulsant effect. Thus felbamate may act in a manner similar to phenytoin to increase the number of benzodiazepine binding sites. However, it would not explain the enhancement of the anticonvulsant activity of diazepam by felbamate against isoniazid. Also, the convulsant activity of bicuculline involves GABAergic mechanisms (5, 27, 29). Loscher and Frey (13) found the activity of GAD was inhibited by isoniazid, whereas bicuculline had no effect on the enzyme. Phenytoin prevented the depletion of GABA by isoniazid but did not protect animals from convulsion induced by isoniazid and other chemoconvulsants. Thus a mere increase in GABA levels was not sufficient for an anticonvulsant effect (13). In contrast, sodium valproate reversed the isoniazid-induced inhibition of GAD activity, significantly elevated GABA concentrations, and was twice as potent against isoniazid-induced seizures than against bicuculline-induced convulsions (13).

Felbamate, like valproate, has broad anticonvulsant activity; the drug increases seizure threshold and prevents seizure spread (26). Furthermore, from the present data, felbamate is more effective against isoniazid than against bicuculline. Thus the antagonism of isoniazid-induced seizures by felbamate may be due to reversing the inhibitory effect of isoniazid on the activity of GAD which is reported to be more relevant to seizure excitability than GABA concentration (29). Investigations are being conducted to more fully characterize the mechanism of action of felbamate.

In conclusion, felbamate in subprotective doses was capable of significantly enhancing the protective effects of diazepam against maximal electroshock seizures, pentylenetetrazol, and isoniazid but not against bicuculline as measured by ED_{50} values. The mechanism of action of felbamate appears to be unique in that it is not due to direct interaction with the $GABA_A$ receptor-ionophore complex but may be attributed to indirect effects and probably involves other mechanisms.

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