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Anticonvulsant Actions of Fominoben: Possible Involvement of Benzodiazepine Receptors

FRANK BALDINO, JR.,* BARBARA KRESPAN AND HERBERT M. GELLER

**Central Research and Development Department, E. I. du Pont de Nemours and Company 500 S. Ridgeway Avenue, Glenolden, PA 19036*
Department of Pharmacology, UMDNJ-Rutgers Medical School Box 101, Piscataway, NJ 08854

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BALDINO, F., JR., B. KRESPAN AND H. M. GELLER. *Anticonvulsant actions of fominoben: Possible involvement of benzodiazepine receptors.* PHARMACOL BIOCHEM BEHAV 21(1) 137–143, 1984.—The purpose of this study was to examine the benzodiazepine-like activity of fominoben-HCl, a compound with prominent antitussive and respiratory stimulant actions. Towards this end we examined the anticonvulsant actions of fominoben as well as its ability to displace benzodiazepine (BDZ) binding from brain membranes. Scatchard analysis of binding data demonstrated that fominoben displaced ^{3}H-flunitrazepam binding from rat cortical membrane preparations. Furthermore when tested against ^{3}H-ethyl-β-carboline-3-carboxylate, the addition of GABA resulted in a mean (±SE) shift of the IC_{50} from 4.05±0.10 μM to 2.2±0.05 μM, a characteristic of benzodiazepine agonists. Seizures were induced in male, Swiss Webster mice with pentylenetetrazol (PTZ) or 3-mercaptoproprionic acid (3-MP). Fominoben (50 and 100 mg/kg) completely protected mice from seizures induced by 50 mg/kg PTZ and elevated the seizure latency against 75 mg/kg of PTZ. The anticonvulsant effects of fominoben were less pronounced against 3-MP-induced seizures. The benzodiazepine antagonist Ro 15-1788 antagonized the anticonvulsant action of fominoben against both convulsants. Taken together, these data suggest that the anticonvulsant action of fominoben may be mediated by agonistic actions at benzodiazepine binding sites.

Fominoben Anticonvulsant Benzodiazepines

FOMINOBEN (3'chlor-2'-[N-methyl-N-[(morpholino-carbonyl) methyl]-aminomethyl] benzanilide is a centrally acting antitussive agent which also produces a profound respiratory stimulation [23]. This unusual combination of antitussive and respiratory stimulant activity differs markedly from the prototype antitussive agent codeine, whose profound antitussive effects are coupled with severe respiratory depression [2], suggesting a CNS mechanism for fominoben which is distinct from that of conventional antitussive agents. Furthermore, unlike codeine, the antitussive action of fominoben is not reduced by the opiate antagonist levallorphan [10]. Recent evidence also indicates that fominoben (at concentrations up to 10 μM) does not displace the non-narcotic antitussive ^{3}H-dextromethorphan from its specific binding site [6]. Other less well characterized CNS actions have been attributed to fominoben including an anticonvulsant effect which differs qualitatively from that produced by other CNS depressants [31].

A previous report that fominoben displaces ^{3}H-flunitrazepam from putative benzodiazepine binding sites in the central nervous system [1] with an IC_{50} of 1.5 μM suggested that fominoben might have actions as a BDZ "agonist" or "antagonist" compound. The present study was designed to extend these observations using both neurochemical and behavioral paradigms. Ligands for the benzodiazepine binding site are differentiated by the ability of GABA to enhance the affinity of benzodiazepine agonists (e.g., anticonvulsants) without affecting the affinity of antagonists (e.g., blockers of BDZ-anticonvulsant action such as Ro 15-1788 or ethyl-β-carboline-3-carboxylate (βCCE)) [4,27]; the affinity of proconvulsant benzodiazepine ligands such as methyl β-carboline-3-carboxylate (β-CCM) and methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) are reduced by GABA [4]. We thus conducted experiments to investigate whether the interaction of fominoben with the benzodiazepine binding site is competitive or non-competitive, and to determine whether the affinity of fominoben for benzodiazepine binding sites is affected by GABA. The three apparent classes of benzodiazepine ligands can also be differentiated by behavioral tests: benzodiazepine agonists display prominent anticonvulsant and anxiolytic activity, antagonists such as Ro 15-1788 are ineffective alone and block agonist activity [9,22], whereas proconvulsant benzodiazepine ligands have been shown not only to induce seizures and decrease the potency of other anticonvulsants, but also to be anxiogenic [5, 14, 26]. We tested the action of fominoben on the seizure latency and effective dose of two convulsant agents (pentylenetetrazol

and 3-mercaptoproprionic acid). Finally we examined whether fominoben's interaction with these compounds is affected by the BDZ antagonist, Ro 15-1788 [9]. Additionally, since both the benzodiazepines [25] and fominoben [33] have been shown to influence the purinergic system, it was of interest to investigate the interaction of the putative purinergic antagonist, aminophylline, with fominoben. The results of these studies support the hypothesis that fominoben is a compound with benzodiazepine-like activity.

METHOD

Biochemistry

Neurochemical methods were performed as described by Paul and Skolnick [18]. Briefly, adult Sprague Dawley rats (Taconic Farms, Germantown, NY) were killed by decapitation, and the cerebral cortices removed [32]. Tissue was disrupted with polytron (setting 6; 15 seconds) (Brinkmann Instruments, Westbury, NY) in 50 mM Tris-HCl buffer, pH 7.4. For Scatchard analysis the tissue was centrifuged at 20,000 × g for 20 min, and the pellet resuspended and recentrifuged a total of five times. The pellet was resuspended, frozen on solid CO_2, and stored at −20°C until used. Incubation volumes were 1500 μl consisting of 1000 μl of tissue (approximately 0.05 mg protein), 437.5 μl of buffer and/or drugs, and 62.5 μl of ligand. Diazepam (3 μM) was used to determine nonspecific binding. Specific binding was defined as the difference in binding obtained in the presence and absence of diazepam. In experiments examining the effects of GABA on the potency of fominoben and flurazepam, Sprague Dawley rats (Charles River) were used; the cortex was removed as previously described. The tissue was homogenized and centrifuged 3× at 19000 rpm for 30 min and the pellet was resuspended in 50 mM Tirs-citrate buffer (pH 7.4). Incubations were carried out for 30 min at 0°C, and terminated by filtration through GF/B filters using three 5 ml washes of ice-cold buffer. The filters were suspended in Scintiverse (Fisher) and radioactivity measured in a Packard 460 liquid scintillation spectrometer. Protein was determined using the Miller (1959) modification of the Lowry technique.

The radiolabelled chemicals used were (^{3}H) Ro 15-1788 (Sp. Act 87.5 Ci/mmol), (^{3}H) flunitrazepam (Sp. Act. 72.4 Ci/mmol) and (^{3}H) β-CCE (Sp. Act. 58 Ci/mmol) purchased from New England Nuclear, Boston, MA. For Scatchard analysis fominoben (1.5 nM) was used to displace multiple concentrations of ^{3}H-flunitrazepam. Multiple concentrations of flurazepam (6.85, 12.5, 25, 50, and 100 nM) and fominoben (0.625, 1.25, 2.5, 5, and 10 μM) were used to obtain IC_{50} values for these compounds against ^{3}H-β-CCE (0.5 nM) in the presence and absence of GABA (50 μM).

Anticonvulsant Studies

Drug naive male Swiss Webster mice (Charles River) weighing between 15 and 30 g were utilized. They were housed in groups of 12 in a room maintained at 23±1°C having a 12 hr light-dark cycle. Food and water were available ad lib.

The chemicals used were pentylenetetrazol (Sigma), fominoben hydrochloride (Karl Thomae GmbH, Biberach an der Riss, West Germany), Ro 15-1788 (Hoffmann La Roche), 3-mercaptoproprionic acid (Sigma), and aminophylline (Sigma). Ro 15-1788 was sonicated and suspended in physiological saline (0.9% NaCl). The remaining compounds were dissolved in physiological saline. All compounds were injected intraperitoneally in a constant volume of 1 ml/kg. Drug administration was randomized. All experiments were done between 10:30 a.m. and 4 p.m.

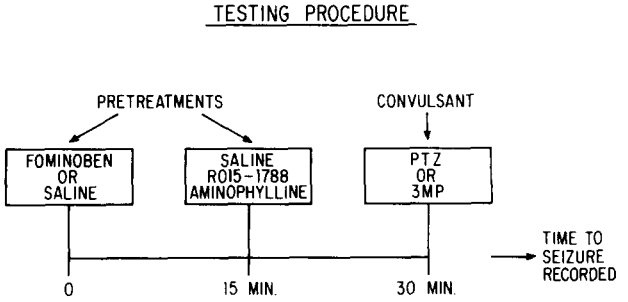


FIG. 1. Schematic representation of the paradigm utilized to assess the anticonvulsant effects of fominoben. Time=0: Various doses of fominoben or saline were administered intraperitoneally. Time=15 minutes: mice were injected (IP) with saline (1 ml/kg), Ro 15-1788 (1, 5, or 10 mg/kg), or aminophylline (2.5 or 5 mg/kg). Time=30 minutes: The convulsant agent 3-MP (75 mg/kg) or PTZ (50 or 75 mg/kg) was given (IP) and the time to seizure recorded.

On the day of the experiment the mice were allowed to acclimatize for 60 min prior to testing. At the beginning of the experiment, the mice were weighed and each mouse placed in a 4000 ml beaker with the top open to permit ventilation. The floor of the beaker was covered with absorbent filter paper, and an opaque screen was placed between beakers for isolation purposes. Thirty minutes before the injection of PTZ, fominoben hydrochloride was administered at 0.1, 0.5, 10, 25, 50, 100 mg/kg IP. Control animals received an injection of 1 ml/kg saline. Fifteen minutes after the injection of the test compound either saline, the putative benzodiazepine antagonist Ro 15-1788 (1, 5, 10 or 20 mg/kg IP) or aminophylline (0.5, 1.0, 2.5, 5 mg/kg IP) was administered. Thirty minutes after the first injection, PTZ (50, 60, or 75 mg/kg IP) or 3-MP (75 mg/kg IP) were administered and the time to tonic-clonic seizure was recorded by an observer who was not aware of the drug treatment. Mice that did not develop tonic-clonic convulsions within 15 minutes of PTZ or 3-MP were considered maximally protected. Delayed seizures were not observed in this study. The time sequence of this test paradigm is illustrated in Fig. 1. At the end of the experiment the animals were sacrificed by exposure to CO_2 gas.

Data were analyzed with student's *t*-test. An analysis of variance and the Dunnets *t*-test were used to compare several experimental groups with the same control. An effect was considered significant if $p \leq 0.05$.

RESULTS

Fominoben and Benzodiazepine Binding

Fominoben inhibited the specific binding of (^{3}H) flunitrazepam to synaptosomal membranes prepared from rat cortex (Fig. 2). A significant increase in the apparent K_d of flunitrazepam was observed by inclusion of fominoben in the incubation mixture, while the B_{max} values obtained were not significantly different from control (Student's *t*-test).

Figure 3 illustrates the inhibition of (^{3}H) β-CCE binding by either fominoben or flurazepam. Fominoben inhibited the

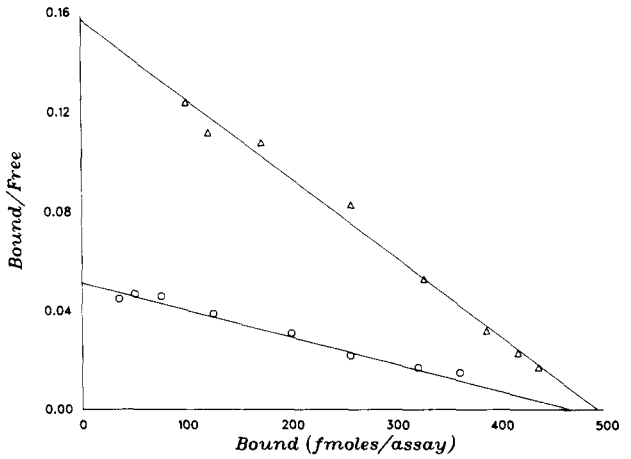
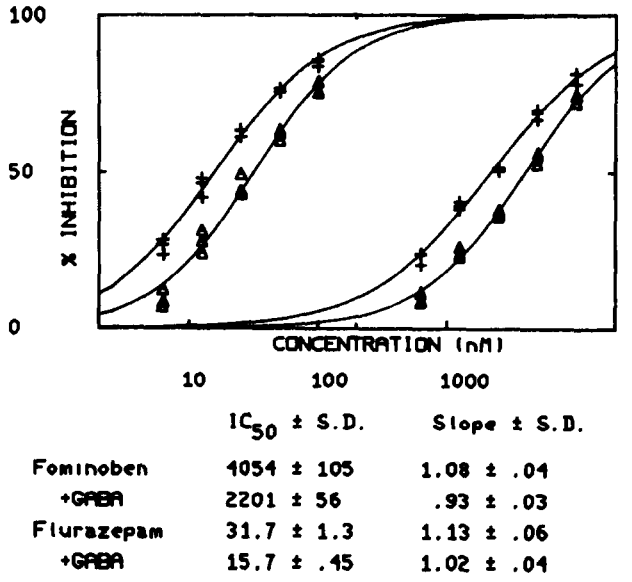


FIG. 2. Scatchard analysis of the effect of fominoben on (^{3}H) flunitrazepam binding to washed rat cerebral cortical membranes. Scatchard analysis was determined with 0.25–16 nM (^{3}H) flunitrazpam. (△), (^{3}H) flunitrazepam binding. Mean (±SE) K_d, 2.1 (±0.5) nM: Mean (±SE) B_{max}, 492.4 (±8.6) fmol/assay; r=−0.995; (○), (^{3}H) flunitrazepam + fominoben (1.5 μM) mean (±SE) K_d, 6.1 (±0.7) nM; B_{max}, 470.2±9.4 fmol/assay; r=−0.998. Each assay contained 0.501 mg protein. n=4.



	IC_{50} ± S.D.	Slope ± S.D.
Fominoben	4054 ± 105	1.08 ± .04
+GABA	2201 ± 56	.93 ± .03
Flurazepam	31.7 ± 1.3	1.13 ± .06
+GABA	15.7 ± .45	1.02 ± .04

FIG. 3. Effect of GABA on the potencies of flurazepam and fominoben in displacing ^{3}H β-CCE (0.5 nM) from washed rat cerebral cortical membranes. GABA (50 μM) did not significantly alter the binding of (^{3}H) β-CCE. Right curve: fominoben (+), fominoben + GABA (△); left curve: flurazepam (+), flurazepam + GABA (△). The mean (±SE) IC_{50} values for flurazepam and fominoben in the presence and absence of GABA are indicated in the figure. The IC_{50} values for both compounds in the absence of GABA were significantly different from those obtained with the addition of GABA ($p<0.01$; Student's t-test). Each assay contained a mean (±SE) of 167±15 μg protein/ml.

binding of (^{3}H) β-CCE (0.5 nM) with a mean (±SE) IC_{50} of 4054±105 nM. In the presence of GABA (50 μM) the mean (±SD) IC_{50} of fominoben was decreased to 2201 (±56) nM. A similar increase in the potency of flurazepam was observed in the presence of GABA, the IC_{50} value decreasing from 31.7 to 15.7 nM. Preliminary studies in our laboratory have shown similar increases in the potency of both fominoben and flurazepam in inhibiting (^{3}H) Ro 15-1788 (1 nM) binding to brain membranes by the addition of 30 μM GABA (fominoben, K_i=1.4 μM; fominoben + GABA, K_i=0.75 μM). GABA does not significantly affect the binding of either 0.5 nM β-CCE or 1 nM Ro 15-1788 [4].

Fominoben vs. PTZ Seizures

The anticonvulsant effect of increasing doses of fominoben against PTZ (50 mg/kg) precipitated seizures is presented in Fig. 4. Administration of PTZ (at doses of 50 mg/kg IP and greater) produced a tonic-clonic convulsion in all control mice tested. The mean (±SE) latency of the response to 50 mg/kg PTZ in control animals (saline-pretreated) was 193 (±25) sec. Treatment with very low doses of fominoben (0.1 and 0.5 mg/kg) were without effect on seizure latency in these animals. Groups pretreated with fominoben, 10 and 25 mg/kg, responded with a non-significant increase in latency of seizures to 313 (±26) and 254 (±28) sec, respectively. However, pretreatment with 50 and 100 mg/kg of fominoben completely protected (>900 sec) these animals from seizures.

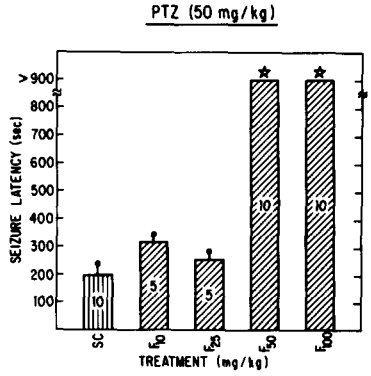


FIG. 4. Dose-response relationship for fominoben against seizures induced with 50 mg/kg PTZ. The bars depict the mean (±SE) latency (sec) to seizure after administration of fominoben (F10, F25, F50, and F100 mg/kg) or saline control (SC). The n for each group as indicated by the number within each bar. *$p<0.01$ (Dunnet's t-test).

The time of onset of seizure activity after 75 mg/kg of PTZ (133±38 sec) was reduced and the seizure duration prolonged relative to that seen at the lower PTZ dose. Fominoben (50 mg/kg and 100 mg/kg IP) increased the time to seizure to 728 (±171) and 696 (±263) sec, respectively. At 100 mg/kg of fominoben, the actual number of animals seizing was reduced. This reduction was related to the dose of PTZ, with complete protection being observed against 50 mg/kg, while partial protection was observed against 60 mg/kg (Fig. 5). This consistent anticonvulsant activity of high doses of fominoben (50 and 100 mg/kg) was present without any obvious alterations in spontaneous motor activity as determined

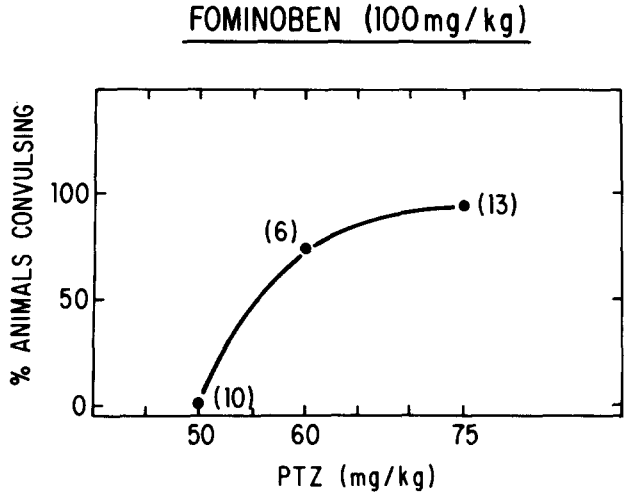


FIG. 5. Increase in the number of animals having a seizure with increasing doses of PTZ. PTZ_{50}, none of the fominoben (100 mg/kg) pretreated animals seized; PTZ_{60}, 66.7% of the animals seized; PTZ_{75}, 92.3% of animals seized. The n for each group is indicated within the brackets associated with each data point. The effects of fominoben with PTZ 50 and 60 mg/kg are significantly different from saline controls (ANOVA; $p>0.01$). All 3 effects are significantly different from each other (ANOVA; $p>0.01$; Newman-Keuls $p<0.01$).

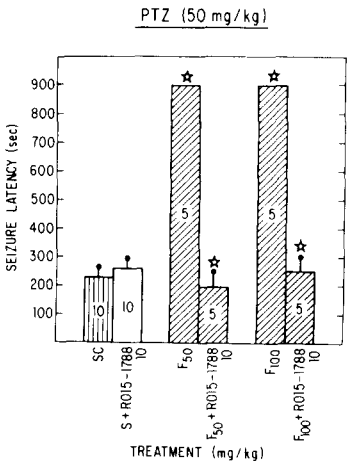


FIG. 6. Antagonism of the anticonvulsant effects of fominoben by the benzodiazepine antagonist Ro 15-1788 (10 mg/kg, IP). The mean (±SE) latency to seizure (PTZ 50 mg/kg) is indicated by the bars. Saline control group (SC; saline (S) + Ro 15-1788 (open bar); fominoben 50 and 100 mg/kg (hatched bars); fominoben + Ro 15-1788 (hatched bars). The n for each group is indicated within each bar. *$p<0.01$ (ANOVA) F_{50} and F_{100} different from SC (Newman-Keuls $p<0.01$). F_{50} + Ro 15-1788 different from F_{50} and F_{100} + Ro 15-1788 different from F_{1-0} (Newman-Keuls $p<0.01$).

by general arousal behavior (e.g., postural position, grooming, jumping, exploring).

Interactions with Ro 15-1788

In other groups of mice we examined the interaction of the putative benzodiazepine antagonist, Ro 15-1788, with fominoben and PTZ (Fig. 6). Ro 15-1788 administered 15 min after fominoben (either 50–100 mg/kg, IP) abolished the anticonvulsant effects of fominoben on seizure latency. The latency to PTZ-induced seizure, determined in mice pretreated with both fominoben (50 or 100 mg/kg) and Ro 15-1788 (10 mg/kg), was not significantly different from the saline-treated group. The administration of Ro 15-1788 at 10 mg/kg in the control group was without effect on the seizure threshold of these animals. The ability of Ro 15-1788 to antagonize the protection afforded by fominoben (50 mg/kg) against PTZ (50 mg/kg) induced seizures increases with the dose of Ro 15-1788. The lowest dose of Ro 15-1788 (1 mg/kg) did not alter the protective effect of fominoben on seizure latency (>900 sec), but 5 and 10 mg/kg of Ro 15-1788 reduced the mean seizure latency of fominoben protected mice to 266 (±82) and 196 (±29) sec respectively (Fig. 7A). The administration of 1, 5, and 10 mg/kg of Ro 15-1788 to the saline control group did not influence the seizure threshold of these mice, nor did it produce any observable behavioral changes in these animals. However, when the dose of Ro 15-1788 was increased to 20 mg/kg, the "antagonist" was anticonvulsant against 50 mg/kg pentylenetetrazol in saline pretreated animals (Fig. 7B).

Fominoben and Aminophylline

In order to determine if the anticonvulsant action of fominoben is mediated through an interaction with adenosine-dependent mechanisms, the putative adenosine antagonist aminophylline was administered 15 minutes after fominoben (Table 1). Aminophylline at a dose of 0.5, 1.0 and 2.5 mg/kg (IP) did not influence the anticonvulsant action of fominoben, nor did it alter the latency to seizure threshold in the control group. However, a higher dose of aminophylline (5 mg/kg) reduced the mean latency to seizure by 15% suggesting a proconvulsant action of aminophylline alone. Therefore this active dose of aminophylline was not tested with fominoben.

Fominoben vs. 3-MP Induced Seizures

Administration of 3-MP (75 mg/kg IP) produced a tonic-clonic seizure in all animals tested. The animals developed a prominent tonic-clonic episode with complete fore- and hindlimb flexion and extension.

The mean (±SE) latency of saline pretreated animals to seizure development with 3-MP was 206 (±8) sec. This seizure latency was greater than that obtained with 75 mg/kg of PTZ. Utilizing a paradigm identical to that used previously with PTZ, mice were pretreated with fominoben and time to tonic-clonic seizure was recorded as seizure latency. Pretreatment with fominoben, 50 and 100 mg/kg (IP), significantly increased the mean seizure latency to 250 (±32) and 322 (±23) sec, respectively, as seen in Fig. 8. The effect of fominoben against 3-MP induced seizures is dose related. However, this anticonvulsant activity of fominoben against 3-MP was less than that observed against both 50 and 75 mg/kg of PTZ. None of the animals were completely protected (>900 sec) from 3-MP induced seizures even at the highest dose of fominoben used (100 mg/kg).

The putative benzodiazepine antagonist Ro 15-1788 (5 mg/kg) blocked the anticonvulsant effects of fominoben in 3-MP precipitated seizures, as seen in Fig. 8. The mean

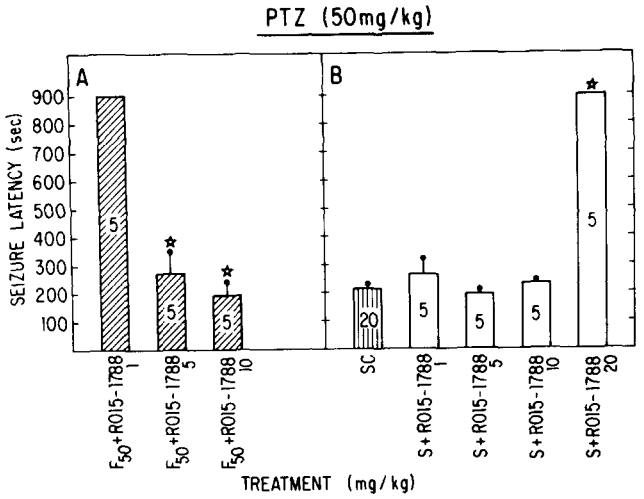


FIG. 7. A. Dose related antagonism of the anticonvulsant effects of fominoben (50 mg/kg) by Ro 15-1788 (1, 5 and 10 mg/kg). Bars indicate the mean (±SE) latency to seizure induced by PTZ (50 mg/kg). *F<0.05 (ANOVA). The n for each group is indicated within each bar. B. Effect of various doses of Ro 15-1788 (1, 5 and 10 mg/kg) on mean (±SE) seizure latency of the control group (SC). Seizures were induced by PTZ (50 mg/kg). The n for each group is indicated within each bar. *$p<0.01$ (ANOVA). F_{50} + Ro 15-1788$_5$ and F_{50} + Ro 15-1788$_{10}$ different from F_{50} + Ro 15-1788, (Newman-Keuls $p<0.01$).

(±SE) seizure latency in the presence of 50 and 100 mg/kg of fominoben was reduced to 178 (±16) and 187 (±15) respectively. These values were not significantly different from control. Ro 15-1788 (5 mg/kg) administered to saline pretreated animals did not alter the latency to 3-MP-induced seizure.

DISCUSSION

The results of this study demonstrate that fominoben is a competitive antagonist of ^{3}H-Ro 15-1788 binding to rat brain membranes and that this antagonism is enhanced by GABA. In addition, fominoben increases seizure latency for both PTZ- and 3-MP induced convulsions in the mouse, and this anticonvulsant action of fominoben is effectively antagonized by the benzodiazepine antagonist Ro 15-1788. Taken together these results support the hypothesis that the anticonvulsant actions of fominoben are mediated through occupancy of a benzodiazepine receptor.

In previous reports, it had been noted that fominoben could displace the benzodiazepine ^{3}H-flunitrazepam from a brain membrane preparation with an IC_{50} of 1.5 μM [1]. The present study confirms this BDZ-displacing ability, and demonstrated that the mean IC_{50} for displacement of ^{3}H-β-CCE is 4.05 μM. Addition of GABA to the reaction mixture reduced the mean IC_{50} to 2.2 μM. The increase in affinity of fominoben in the presence of GABA has been proposed as a procedure to discriminate between compounds which act as BDZ "agonists" or "antagonists." The affinity of agonists is increased by GABA, whereas the affinity of antagonists is unchanged [4, 13, 27]. The increase in affinity of fominoben by GABA (in 0.5 nM ^{3}H-β-CCE) would predict that fominoben would possess activity as a benzodiazepine agonist in behavioral tests. The fact that the IC_{50} of fominoben was 100 times larger than the IC_{50} of the anticonvulsant BDZ flurazepam would predict that fominoben would be less potent than flurazepam in behavioral tests.

Fominoben was indeed found to be active as an anticonvulsant at doses which have been reported to prolong barbiturate-induced sleeping time in the mouse, and which are 10% of the LD_{50} in mice [23]. Fominoben's action was displayed against both PTZ- and 3-MP induced seizures, a characteristic of benzodiazepine-like compounds. However, the anticonvulsant action of fominoben against doses of PTZ and 3-MP that induced seizures with comparable latency of onset was disparate. Fominoben at 50 and 100 mg/kg completely protected animals from developing seizures with 50

TABLE 1

EFFECT OF AMINOPHYLLINE ON FOMINOBEN ENHANCEMENT OF SEIZURE LATENCY*

Drug Treatment	Seizure Latency (sec)
Saline control	56 ± 6.1
Fominoben 100 mg/kg	552 ± 158
Aminophylline 0.5 mg/kg	58 ± 8
Aminophylline 0.5 mg/kg and Fominoben 100 mg/kg	483 ± 150
Aminophylline 1.0 mg/kg	48 ± 3
Aminophylline 1.0 mg/kg and Fominoben 100 mg/kg	469 ± 123
Aminophylline 2.5 mg/kg	43 ± 2
Aminophylline 2.5 mg/kg and Fominoben 100 mg/kg	454 ± 185

*All seizures were induced by PTZ (75 mg/kg, IP). Values shown are mean ± S.E.M.

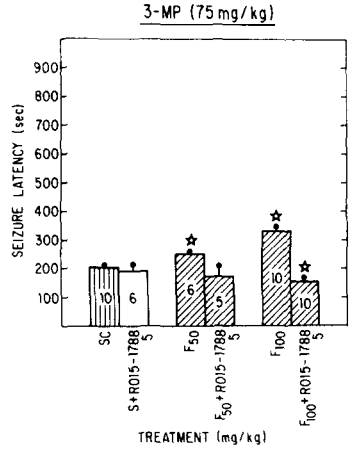


FIG. 8. Anticonvulsant effects of fominoben against 3-MP (75 mg/kg) induced seizures. Bars indicate the mean (±SE) latency to seizure for each group. The saline group (SC) is depicted by the vertically lined bars. The fominoben enhanced latency to seizure (50 and 100 mg/kg) and its antagonism by Ro 15-1788 (5 mg/kg) is indicated by the hatched bars. *$p<0.05$ (ANOVA), the n for each group is indicated by the number within each bar. F_{50} and F_{100} are significantly different from control F_{100} + Ro 15-1788 (5 mg/kg) is significantly different from F_{100}. (Newman-Keuls $p<0.05$).

mg/kg PTZ. On the other hand, animals treated with 75 mg/kg 3-MP were not protected from developing seizures by these doses of fominoben, although fominoben increased the latency of onset of seizures. This difference in anticonvulsant potency against 3-MP and PTZ-induced seizures is most likely a result of the different mechanisms of action of these agents in producing convulsions. The enzyme glutamic acid decarboxylase is competitively inhibited by 3-MP, thus resulting in a reduction of GABA levels in the CNS [24]. PTZ has been reported not to interact with GABA metabolism [17]. Recent studies have suggested that the mechanism of PTZ is through an action at a postsynaptic site distinct from, but associated with, the GABA receptor, perhaps directly on the neuronal membrane or the Cl-ionophore complex [16,29]. This association between PTZ and benzodiazepines is supported by the high correlation between benzodiazepine binding site occupancy and potency against PTZ seizures [19,22]. Thus agents which specifically interact with the GABA system (e.g., muscimol, amino-oxyacetic acid) are more effective against 3-MP-induced seizures than PTZ [28], whereas benzodiazepines are more effective against PTZ than 3-MP [30]. The anticonvulsant actions of fominoben in this study are consistent with those observed for anticonvulsant benzodiazepines.

The antagonism of fominoben's actions by Ro 15-1788 provides further evidence that the anticonvulsant actions of fominoben are mediated through a benzodiazepine receptor. Ro 15-1788 completely blocked the anticonvulsant actions of fominoben. The demonstrated ability of Ro 15-1788 to antagonize the actions of benzodiazepines in both behavioral and physiological tests [9, 15, 22] has been correlated with an action of Ro 15-1788 at the benzodiazepine binding site [9]. Ro 15-1788 has been demonstrated to reverse the proconvulsant effects of the BDZ antagonists β-CCE and 6,7 dimethoxy-4 ethyl-β-carboline-3-carboxylate in the mouse seizure threshold model [4,15] at doses which were ineffective against seizure threshold when Ro 15-1788 was administered alone.

A dose of 20 mg/kg of Ro 15-1788 produced an anticonvulsant action of its own, completely protecting mice from seizures induced by 50 mg/kg PTZ. Furthermore, elevated doses of Ro 15-1788 similar to those utilized in this study were found to possess intrinsic activity in the rat sympathetic ganglion preparation [15]. These effects are reminiscent of the agonist-like actions of benzodiazepines and suggests that Ro 15-1788 may possess efficacy as a partial agonist at the benzodiazepine receptor.

There has been considerable debate concerning the role of adenosine and other purines in the pharmacology of benzodiazepines in the CNS. It has been suggested that benzodiazepines bind to the purine uptake site in brain and inhibit the uptake of adenosine into synaptosomes [3]. Furthermore, caffeine and theophylline, putative purine antagonists, have been suggested to antagonize the anticonvulsant and CNS depressant actions of benzodiazepines [20,21] while the purines, inosine and hypoxanthine, antagonize PTZ seizures, an action similar to benzodiazepine agonists [25]. However, the low potency of benzodiazepines in inhibiting adenosine uptake (EC_{50}=200 μM) is not consistent with the high potency with which these agents produce their effects [25]. Accordingly, the inability of theophylline to antagonize the anticonvulsant actions of diazepam [7] makes it unlikely that the effects of benzodiazepines are mediated through the purinergic system. Since fominoben has been reported to act as an inhibitor of purine uptake [33], it was of interest to determine whether the anticonvulsant effects of fominoben could be antagonized by aminophylline. The inability of aminophylline to reduce the anticonvulsant actions of fominoben in this study is consistent with its inability to antagonize effects of benzodiazepines and suggests that the purinergic system is not involved in the anticonvulsant action of fominoben.

The question arises concerning the actual molecule responsible for the effects of systemically injected fominoben. Fominoben rapidly undergoes extensive metabolism by the liver, and little of the parent compound is excreted [11]. Coupled with the rather low affinity displayed by fominoben for the benzodiazepine binding site, it was originally postulated that the effects observed 30 minutes after intraperitoneal administration are due to the presence of active metabolites, rather than the parent compound. *In vitro* electrophysiological studies in our laboratory, showed that fominoben added to a superfusion medium where drug metabolism is essentially nil, reverses the reduction in spontaneous neuronal activity produced by GABA and glycine [8]. These electrophysiological results, although consistent with the analeptic and respiratory stimulant actions reported for fominoben [23], are inconsistent with its anticonvulsant actions and could easily be interpreted to suggest that the anticonvulsant effects of fominoben could have resulted from active metabolites as well as the parent compound. However, our *in vitro* neurochemical studies demonstrating an IC_{50} shift to lower values with the inclusion of GABA in the incubation mixture suggests that the "agonist like" anticonvulsant properties of fominoben reported in this study are not likely the result of active metabolites. Perhaps the physiological data represent an action of fominoben at alternative BDZ sites or non-BDZ sites in cultured tissue. Further studies are necessary to resolve these electrophysiological findings.

In conclusion, these data presented in this paper suggest that anticonvulsant effects of fominoben may be mediated by a benzodiazepine receptor.

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REFERENCES

1. Antoniadis, A., W. E. Muller and U. Wollert. Central nervous system stimulating and depressing drugs as possible ligands of the benzodiazepine receptor. *Neuropharmacology* **19:** 121–124, 1980.

2. Belleville, J. W., L. A. Escarraga, S. C. Wallenstein and R. W. Houde. The respiratory effects of codeine and morphine in man. *Clin Pharmacol Ther* **9:** 435, 1968.

3. Bender, A. S., J. W. Phillis and P. H. Wu. Diazepam and flurazepam inhibit adenosine uptake by rat brain synaptosomes. *J Pharm Pharmacol* **32:** 293–294, 1980.
4. Braestrup, C., R. Schmiechen, G. Neef, M. Nielsen and E. H. Petersen. Interaction of convulsive ligands with benzodiazepine receptors. *Science* **216:** 1241–1243, 1982.
5. Cowen, P. J., A. R. Green, D. J. Nutt and I. L. Martin. Ethyl-β-carboline carboxylate lowers seizure threshold and antagonized flurazepam-induced sedation in rats. *Nature* **290:** 54–55, 1981.
6. Craviso, G. L. and J. M. Musacchio. High affinity dextromethorphan binding sites in guinea-pig brain II. Competition experiments. *Mol Pharmacol* **23:** 629–640, 1983.
7. Dunwidde, T. V. and T. Worth. Sedative and anticonvulsant effects of adenosine analogs in mouse and rat. *J Pharmacol Exp Ther* **22:** 70–76, 1982.
8. Geller, H. M. and F. Baldino, Jr. Fominoben: A novel amino acid antagonist. *Soc Neurosci Abstr* **7:** 110, 1981.
9. Hunkeler, W., H. Mohler, L. Pieri, P. Polc, E. P. Bonetti, R. Cumin, R. Schoffner and W. Haefely: Selective antagonists of benzodiazepines. *Nature* **290:** 514–516, 1981.
10. Kito, G., Y. Kuse, T. Yakushiji, T. Miyata, Y. Okano, M. Suto and T. Ishihara: Pharmacology of 3'-chloro-2'[N-methyl-N[(morpholino carbonyl) methyl]aminomethyl] benzanilide (PB-89, fominoben), a new antitussive possessing respiratory stimulating action. *Jpn J Pharmacol* **8:** 1491–1513, 1974.
11. Kopitar, Z. Pharmakokinetik von 14 C-markiertem fominoben bei ratten and trachtigen Mausen. *Arzneimittelforsch* **23:** 311–315, 1973.
12. Miller, G. L. Protein determination for large numbers of samples. *Anal Chem* **31:** 964, 1959.
13. Möhler, H. and J. G. Richards. Agonist and antagonist benzodiazepine receptor interaction *in vitro*. *Nature* **294:** 763–765, 1981.
14. Ninan, P. T., T. M. Insel, R. M. Cohen, J. M. Cook, P. Skolnick and S. M. Paul. Benzodiazepine receptor mediated experimental "anxiety" in primates. *Science* **218:** 1132–1134, 1982.
15. Nutt, D. J., P. J. Cowen and H. J. Little. Unusual interactions of benzodiazepine receptor antagonists. *Nature* **295:** 436–438, 1982.
16. Olsen, R. W., F. Leeb-Lundberg and C. Napias. Picrotoxin and convulsant binding sites in mammalian brain. *Brain Res Bull* **5:** Suppl 2, 1–5, 1980.
17. Olsen, R. W., M. K. Ticku, P. C. Van Ness and D. Greenlee. Effects of drugs on γ-aminobutyric acid receptors, uptake, release and synthesis *in vitro*. *Brain Res* **139:** 277–294, 1978.
18. Paul, S. M. and P. Skolnick. Rapid changes in brain benzodiazepine receptors after experimental seizures. *Science* **202:** 892–894, 1978.
19. Paul, S. M., P. J. Syapin, B. A. Paugh, V. Moncada and P. Skolnick. Correlation between benzodiazepine receptor occupation and anticonvulsant effects of diazepam. *Nature* **281:** 688–689, 1979.
20. Phillis, J. W., J. P. Edstrom, S. W. Ellis and J. R. Kirkpatrick. Theophylline antagonizes flurazepam-induced depression of cerebral cortical neurons. *Can J Physiol Pharmacol* **57:** 917–920, 1979.
21. Polc, P., E. P. Bonetti, L. Pieri, R. Cumin, R. M. Angioi, H. Möhler and W. E. Haefely. Caffeine antagonizes several central effects of diazepam. *Life Sci* **28:** 2265–2275, 1981.
22. Polc, P., J. P. Laurent, R. Scherschlicht and W. Haefely. Electrophysiological studies on the specific benzodiazepine antagonist Ro 15-1788. *Naunyn Schmiedebergs Arch Pharmacol* **316:** 317–325, 1981.
23. Puschmann, S. and R. Engelhorn. Pharmakologische Unterschungen iber eine substanz mit antitussiven und atmungs anregenden Eigenschaften. *Arzneimittelforsch* **23:** 296–304, 1973.
24. Roberts, F., P. V. Taberner and R. G. Hill. The effect of 3-mercaptoproprionate, an inhibitor of glutamine decarboxylase, on the levels of GABA and other amino acids, and on presynaptic inhibition in the rat cuneate nucleus. *Neuropharmacology* **17:** 715–720, 1978.
25. Skolnick, P., S. M. Paul and P. J. Marangos. Brain benzodiazepine levels following intravenous administration of [^{3}H] diazepam: Relationship to the potentiation of purinergic depression of central nervous system neurons. *Can J Physiol Pharmacol* **57:** 1040–1042, 1979.
26. Skolnick, P., S. Paul, J. Crawley, K. Rice, S. Barker, R. Weber, M. Cain and J. Cook: 3-Hydroxymethyl-β-carboline antagonizes some pharmacological actions of diazepam. *Eur J Pharmacol* **69:** 505–527, 1981.
27. Skolnick, P., M. M. Schweri, E. F. Williams, V. Y. Moncada and S. M. Paul. An *in vitro* binding assay which differentiates benzodiazepine "agonists" and "antagonists." *Eur J Pharmacol* **78:** 133–136, 1982.
28. Stone, W. E. and M. J. Javid. Muscimol as an antagonist of chemical convulsants. *Arch Int Pharmacodyn* **253:** 294–300, 1981.
29. Stone, W. E. and M. J. Javid. Quantitative evaluation of the actions of anticonvulsants against different chemical convulsants. *Arch Int Pharmacodyn Ther* **240:** 66–78, 1979.
30. Stone, W. E. and M. J. Javid. Benzodiazepines and phenobarbital as antagonists of dissimilar chemical convulsants. *Epilepsia* **19:** 361–368, 1978.
31. Takahama, K., C. Kito, T. Miyata, Y. Okano and Y. Kasé. Central effects of 3'chloro-2'-[N-methyl-N-[(morpholino carbonyl)methyl] aminomethyl]benzanilide (fominoben, PB-89) a new antitussive possessing respiratory stimulating action. *Pharmacometrics (Jpn)* **15:** 365–374, 1978.
32. Williamson, M. H., S. M. Paul and P. Skolnick. Demonstration of 3-H diazepam binding to benzodiazepine receptors *in vivo*. *Life Sci* **23:** 1935–1940, 1978.
33. Wu, P. H., J. W. Phillis and A. S. Bender. Do benzodiazepines bind at adenosine uptake sites in CNS. *Life Sci* **28:** 1023–1031, 1981.