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Effect of felbamate and its combinations with conventional antiepileptics in amygdala-kindled rats

K.K. Borowicz[a,*], N. Ratnaraj[b], P.N. Patsalos[b], S.J. Czuczwar[a,c]

[a]Department of Pathophysiology, Lublin Medical University, Jaczewskiego 8, Lublin 20-090, Poland
[b]Pharmacology and Therapeutics Unit, Department of Clinical and Experimental Epilepsy, Institute of Neurology, Queen Square, London, UK
[c]Isotope Laboratory, Institute of Agricultural Medicine, 20-090 Lublin, Jaczewskiego 2, Poland

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Abstract

We investigated the effect of felbamate, administered singly and in combination with carbamazepine, phenobarbital, phenytoin or clonazepam, on various behavioral and electrographic correlates of seizures in amygdala-kindled rats. Felbamate (5 or 10 mg/kg) significantly increased afterdischarge threshold, shortened seizure and afterdischarge durations but remained without effect on seizure severity. Furthermore, the combination of felbamate (2.5 mg/kg) with carbamazepine (7.5 mg/kg; both drugs at their subeffective doses), was associated with the reduction in seizure severity and afterdischarge duration. In relation to the afterdischarge duration, the antiseizure potency of felbamate and carbamazepine, in combination, was comparable with that of carbamazepine (10 mg/kg) administered alone. Neither carbamazepine (7.5 and 10 mg/kg) nor felbamate (2.5–10 mg/kg) affected seizure severity, whereas the combined administration of felbamate (2.5 mg/kg) with carbamazepine (7.5 mg/kg) led to significant reduction in seizure severity from the fifth to the third stage of Racine's scale. Among the conventional antiepileptic drugs evaluated in this study, only valproate (100 mg/kg) and clonazepam (0.1 mg/kg) exerted similar action on seizure severity. However, the combinations of felbamate (2.5 mg/kg), with subeffective doses of valproate, phenobarbital, phenytoin or clonazepam, were not associated with any protective action. As blood and brain felbamate and carbamazepine concentrations were unaffected, a pharmacokinetic interaction can be excluded and a pharmacodynamic interaction concluded. These data suggest that felbamate and carbamazepine, administered in combination, may be useful in patients with drug-resistant partial epilepsy.

Keywords: Felbamate; Carbamazepine; Phenobarbital; Phenytoin; Clonazepam; Amygdala-kindled seizures; Drug interaction

1. Introduction

Felbamate is a propanediol derivative structurally similar to the antianxiety drug meprobamate (Frey and Bartels, 1997). It is associated with a broad spectrum of activity in three basic models of experimental epilepsy, namely, the maximal electroshock test for generalized tonic–clonic convulsions, pentetrazole-induced seizures for generalized absence or myoclonic seizures and the kindling model for partial and secondary generalized seizures (McNamara, 1984). Furthermore, this spectrum of activity has been confirmed clinically (Theodore et al., 1995). In addition, felbamate is associated with significant analgesic properties, providing an effective alternative to carbamazepine and phenytoin in the treatment of neuropathic pain (Hunter et al., 1997). Another beneficial action of felbamate is associated with its efficacy in hemifacial spasm, a motor analogue of trigeminal neuralgia (Mellick, 1995).

Felbamate was licensed for clinical use in 1995. Subsequently, 33 cases of aplastic anemia were reported, seven of these patients died; although prior blood dyscrasias were reported in 45% of the affected patients, 32% had a history of preexisting immune disorders (Arroyo and de la Morena, 2001; Pellock, 1999). Felbamate remains, however, as one of the few antiepileptic drugs that possess a low incidence of central nervous system side effects (Harty and Rogawski, 2000). Therefore, despite its severe peripheral undesired effects, felbamate is recommended by the FDA Peripheral and Central Nervous System Drugs Advisory Committee for

* Corresponding author. Tel.: +48-81-7425837; fax: +48-81-7425828.
E-mail address: kornel@asklepios.am.lublin.pl (K.K. Borowicz).

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use in patients in whom the potential benefit may outweigh the risk. Such cases include partial refractory epilepsy and the Lennox–Gastaut syndrome (Mellick, 1995; Winkler and Luerm, 1998).

Although approximately 60–70% of newly diagnosed epileptic patients become seizure-free with monotherapy antiepileptic drugs, the remainder does not respond satisfactorily, and these patients require combination therapy. However, the prescribing of two or more antiepileptic drugs is undertaken empirically, is essentially a hit-and-miss situation and, commonly, such combinations are associated with undesirable side effects due to pharmacokinetic and/or pharmacodynamic interactions (Patsalos and Perucca, 2003a,b). Experimental data may be helpful for predicting which drug combinations may prove particularly effective in epileptic patients. When two antiepileptic drugs are involved in a pharmacodynamic interaction, such an interaction can present as antagonistic, additive or supraadditive (synergistic) in type. The synergistic effect would be particularly valuable in clinical practice. However, when synergism in anticonvulsant efficacy is associated with pronounced undesirable effects, the therapeutic index may be unchanged or even lowered. Sometimes, increased antiseizure effect may be the consequence of a pharmacokinetic interaction and be associated with an increased drug plasma and or brain concentration. Thus, to determine the exact nature of a drug interaction, pharmacokinetic correlates should also be ascertained (Czuczwar and Borowicz, 2002).

It has been suggested that two drugs with distinct mechanism of action may be associated with synergistic anticonvulsant effects. In addition, additivity may be observed when drugs with similar pharmacological profiles are combined, and there have been numerous studies to investigate these possibilities (Czuczwar and Borowicz, 2002). Previously, we observed that felbamate at subprotective doses does not affect the protective activity of conventional antiepileptic drugs (valproate, carbamazepine, phenytoin and phenobarbital) against maximal electroshock in mice (Borowicz et al., 2000). However, when these drugs were investigated at low doses, they enhanced the anticonvulsant activity of felbamate (Gordon et al., 1993). Although felbamate increased the potency ratio of valproate and lamotrigine against 4-aminopyridine, it was without effect on pentylenetetrazole-evoked convulsions in mice (Cuadrado et al., 2003a,b). Moreover, felbamate decreased the protective index of valproate, but increased that of lamotrigine. It is interesting to note that in these studies, both valproate and lamotrigine decreased the felbamate brain concentrations (Cuadrado et al., 2003a,b). In the clinical setting, add-on therapy with valproate and felbamate in patients with the Lennox–Gastaut syndrome led to a 40% reduction in drop attacks. However, efficacy may, in part, be related to the increased plasma valproate concentrations (Deckers et al., 2000).

To date, there are no suitable experimental models for the Lennox–Gastault syndrome. Consequently, we used amygdala-kindled seizures in rats, an established animal model of drug-resistant epilepsy, to investigate the action and interaction of felbamate and various conventional antiepileptic drugs (valproate, carbamazepine, phenobarbital, clonazepam, and phenytoin). Therefore, only phenytoin-sensitive rats were used in this study (Ebert and Löscher, 1999; Ebert et al., 2000). Additionally, drug-related neurotoxic effects were determined by use of the chimney test (motor coordination) and the passive-avoidance task (long-term memory).

2. Material and methods

2.1. General

The experiments were carried out on male Wistar rats weighing 200–250 g (authorized breeder: Górzkowska, Warsaw, Poland). the Rats used for the kindled seizures were phenytoin sensitive. The animals were housed in colony cages, with free access to food (chow pellets) and tap water. The experimental temperature was 21 ± 1 °C, and rats were on a natural light–dark cycle. The experimental groups, consisting of eight rats, were chosen by means of a randomized schedule. All experiments were undertaken between 10:00 a.m. and 2:00 p.m. The animal procedures were conducted in accordance with the regulations of the Bioethical Committee of Lublin, Poland. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Drugs

Phenytoin (Sigma, St. Louis, USA), carbamazepine (Polfa, Starogard Gdañski, Poland), clonazepam (Polfa, Cracow, Poland) and felbamate (Schering-Plough, USA) were suspended in a 1% solution of Tween 81 (Loba Chemie, Vienna, Austria). The solvent (at the concentration used) did not affect the seizure-related variables. Valproate magnesium (Polfa, Rzeszów, Poland) and phenobarbital sodium (Polfa, Cracow, Poland) were dissolved in sterile water. All drugs were administered intraperitoneally in a volume of 3 ml/kg and, at times, experimentally assessed as reflecting their maximal anticonvulsant activity (phenytoin 120 min; phenobarbital and felbamate 60 min; valproate, clonazepam and carbamazepine 30 min before tests).

2.3. Surgery and kindling procedure

The rats were anesthetized with chloral hydrate (350 mg/kg ip) and stereotactically implanted with a bipolar electrode in the right basolateral amygdala. Coordinates for electrode implantation, with bregma as reference, were anteroposterior (AP) = − 2.4, mediolateral (ML) = 4.6 and dorsoventral (DV) = 8.5 (Paxinos and Watson, 1986). Scull screws served as the indifferent reference electrode. The electrode assembly was attached to the scull by

dental acrylic cement. After electrode implantation, the animals were treated with an antibiotic for one week to prevent infection.

After a postoperative period of 2 weeks, the stimulation of amygdala was initiated. Kindling was performed by constant current stimulations (500 μA, 1 ms, biphasic square-wave pulses, 50 Hz for 1 s), applied once daily until at least 10 sequential fully kindled Stage 5 seizures were elicited. Animals required approximately 10–15 stimulations to become fully kindled. Then, the threshold for induction of afterdischarges in the stimulated amygdala was determined by a staircase procedure (series of stimulations at intervals of 1 min, increasing in steps of about 20% of the previously applied current) until an afterdischarge of at least 3 s duration was evoked. The afterdischarges from the amygdala were recorded prior to and after the stimulation. The seizure activity was assessed according to a modified Racine's system (Racine, 1972). The respective scores were 0 = no seizure response, 1 = immobility, eye closure, ear twitching, twitching of vibrissae, sniffing, facial clonus, 2 = head nodding associated with more severe facial clonus, 3 = clonus of one forelimb, 3.5 = bilateral forelimb clonus without rearing, 4 = bilateral forelimb clonus with rearing, 4.5 = falling on a side (without rearing), loss of righting reflex accompanied by generalized clonic seizures and 5 = rearing and falling on the back accompanied by generalized clonic seizures. Seizure duration was the duration of limbic seizures (Stages 1–2) and motor seizures (Stages 3–5). Afterdischarges were defined as spikes with a frequency of at least 1 Hz and amplitude at least twice greater than the prestimulation baseline present in the EEG recorded from the site of stimulation. Control experiments, on animals receiving only vehicle, were undertaken 2 days before and 2 days after respective treatments.

2.4. Chimney test

The effects of antiepileptic drugs on motor coordination were quantified by use of the chimney test of Boissier et al. (1960). In this test, animals had to climb backwards up a plastic tube (7 cm inner diameter, 50 cm length). Motor impairment was indicated by the inability of rats to climb backwards up the tube within 60 s.

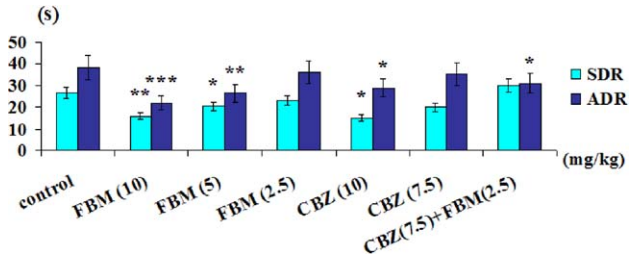

Fig. 1. Effect of felbamate (FBM) and carbamazepine (CBZ), singly and in combination, on seizure duration (SDr) and afterdischarge duration (ADr) in fully kindled rats. SDr and ADr data are means ± S.D. (seconds). Control readings were made 2 days before and after the respective treatments. * $P<.05$, ** $P<.01$ and *** $P<.001$ vs. averaged control (Wilcoxon signed rank test).

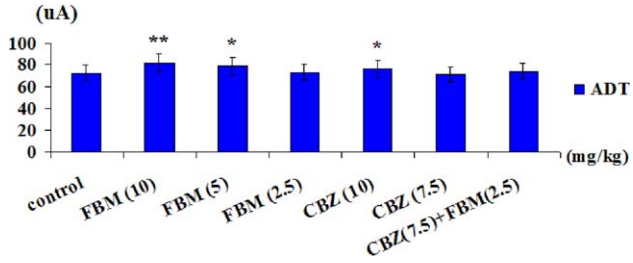

Fig. 2. Effect of felbamate (FBM) and carbamazepine (CBZ), singly and in combination, on afterdischarge threshold (ADT) in fully kindled rats. ADT data are means ± S.D. (in μA). * $P<.05$, ** $P<.01$ vs. averaged controls (Wilcoxon signed rank test). See also legend of Fig. 1.

2.5. Passive avoidance task

The step-through passive avoidance task, which is recognized as a measure of long-term memory, was used (Venault et al., 1986).The drug-treated rats were placed in an illuminated box (40 × 40 × 40 cm) connected to a dark box (40 × 40 × 40 cm), which was equipped with an electric grid floor. Entrance to the dark box was punished by an electric footshock (0.7 mA for 2 s; facilitation of acquisition). The animals that did not enter the dark compartment within 60 s were excluded from the experiment. Twenty-four hours later, the same rats, but without drug administration, were placed into the illuminated box and observed up to 180 s. The mean time to enter the dark box was subsequently calculated. The controls (saline-treated animals) were similarly evaluated.

2.6. Determination of plasma and brain carbamazepine and felbamate concentrations

Blood and brain samples for the analysis of carbamazepine and felbamate content were collected after decapitation. Sampling times were based on the time of convulsive tests and were at 30 and 60 min after the administration of carbamazepine and felbamate, respectively. Blood (approximately 1 ml) was collected into Eppendorf tubes containing

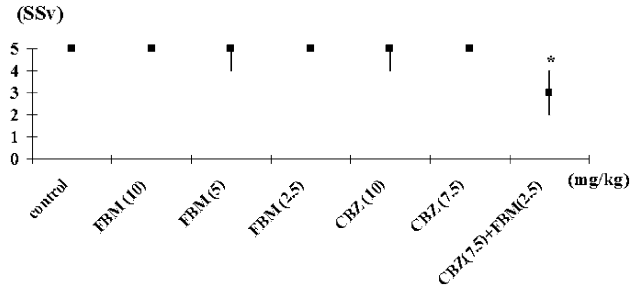

Fig. 3. Effect of felbamate (FBM) and carbamazepine (CBZ), singly and in combination, on seizure severity (SSv) in fully kindled rats. Data are shown as medians with 25, 75 percentiles of six rats per group. Mann–Whitney U test was used for statistical analysis of the data. * $P<.05$ vs. respective control. See also legend of Fig. 1.

Table 1
Effect of valproate (VPA), both singly and in combination with felbamate (FBM), on amygdala-kindled seizures

Treatment (mg/kg)	C	ADT	C	SSv	C	SDr	C	ADr
VPA (100)	71.4 ± 5.7	85.0 ± 9.1**	5 (5;5)	3.5 (3;4)*	23.7 ± 5.3	14.1 ± 8.4*	42.8 ± 8.3	25.2 ± 6.0**
VPA (75)	71.9 ± 5.5	81.9 ± 5.9**	5 (5;5)	4.5 (4;5)	22.1 ± 4.1	16.2 ± 3.9	35.7 ± 2.8	29.6 ± 5.6
VPA (50)	76.1 ± 6.2	78.3 ± 5.7	5 (4.5;5)	4.5 (4;5)	23.7 ± 4.5	24.3 ± 5.0	35.5 ± 2.7	33.5 ± 3.0
VPA (50) + FBM (5)	66.0 ± 4.2	74.9 ± 6.8**	5 (5;5)	4.5 (5;5)	26.3 ± 3.7	25.9 ± 3.7	35.6 ± 4.2	33.0 ± 4.9
VPA (50) + FBM (2.5)	68.2 ± 4.5	69.5 ± 4.6	5 (5;5)	5 (5;5)	30.0 ± 3.1	29.2 ± 3.2	34.5 ± 3.7	34.9 ± 3.8

The data represent means ± S.D. or medians with 25, 75 percentiles of eight rats per group. Control readings (from rats receiving only vehicle) were made 2 days before and after the respective treatments. FBM, felbamate; C, cintrol; ADT, afterdischarge thershold; SSv, seizure severity; SDr, seizure duration; Adr, afterdischarge duration. VPA was administered 30 min, and FBM 60 min before the seizure test.

* $P < .05$ vs. respective controls (Student's t test for paired replacement).

** $P < .01$ vs. respective controls (Student's t test for paired replacement).

1 drop of heparin. Brains were homogenized in TDx buffer (Abbott, Irving, TX, USA) using Eppendorf tubes. Subsequently, blood and brain samples were centrifuged at 10 000 rpm (Abbott centrifuge) for 3 min. Plasma (70 μl) was transferred into Amicon Centrifree Micropartion System tubes (Amicon, Danvers, USA) for the separation of free from protein bound component. The tubes were subsequently centrifuged at 3 000 rpm (MPW-360 centrifuge; Mechanika Precyzyjna, Warsaw, Poland) for 10 min. Free-plasma carbamazepine and brain concentrations were determined by fluorescent polarization immunoassay using an Abbott TDx analyzer (Abbott).

The concentration of felbamate in the plasma and brain was determined by high-performance liquid chromatography (HPLC). The instrument was an automated Gilson (Anachem) HPLC system and comprised of a Gilson 234 autosampler, Gilson 306 Pumps and Gilson UV 155 variable wave-length detector. The mobile phase comprised of 50 mmol phosphate buffer and acetonitrile. Chromatographic separation was achieved using a LiChrospher 60 RP-select B 5 μm column (BDH-Merck). Samples were prepared for analysis as follows: 50 μl plasma or brain supernatant were pipetted into a 1.5-ml plastic tube, to which was added 100 μl acetonitrile, and the sample was vortex-mixed for 1 min and was centrifuged (5 min). Ninety microliters of the supernatant were transferred into an autosampler vial, from which 10 μl were injected automatically into the column.

2.7. Statistics

The statistical significances between afterdischarge threshold values, seizure and afterdischarge durations were calculated by the Student's t test for paired replicates. The statistical significances between seizure scores were evaluated by the Wilcoxon signed rank test. The results from the chimney test were compared using Fisher's exact probability test, while those from the passive avoidance task were compared using the Kruskal–Wallis test followed by Dunn's post hoc test. Plasma and brain concentrations of felbamate and carbamazepine were evaluated using the unpaired Student's t test.

3. Results

3.1. Effects of felbamate on seizure activity in fully kindled rats

Felbamate, at the dose of 10 and 5 mg/kg, exerted a protective effect by considerably increasing the afterdischarge threshold and shortening both the seizure and afterdischarge durations. In contrast, 2.5 mg/kg felbamate was devoid of any significant effect on any of the seizure parameter studied (Figs. 1–3).

3.2. Effect of conventional antiepileptic drugs and their combinations with felbamate on amygdala-kindled seizures

Carbamazepine (10 mg/kg) significantly increased the afterdischarge threshold (from 70 to 76.2 μA), as well as shortened seizure and afterdischarge durations (from 22.7 to 15.4 s and from 36.6 to 29.3 s, respectively; Fig. 1). Valproate (75 mg/kg) significantly increased the afterdischarge threshold from 71.9 to 81.9 μA (Table 1). In

Table 2
Effect of phenobarbital (PB) and its combinations with felbamate (FBM) against amygdala-kindled seizures in rats

Treatment (mg/kg)	C	ADT	C	SSv	C	SDr	C	ADr
PB (15)	71.9 ± 4.2	74.36 ± 3.4	5 (5;5)	4 (3;4.5)	23.9 ± 5.6	13.1 ± 6.9*	42.6 ± 6.6	24.1 ± 5.9*
PB (10)	71.6 ± 4.0	74.0 ± 3.2	5 (5;5)	4.75 (4;5)	25.4 ± 5.0	23.7 ± 5.3	42.7 ± 4.8	37.2 ± 5.8
PB (15) + FBM (5)	70.0 ± 2.2	73.3 ± 4.0*	5 (5;5)	4 (3.5;4.5)	26.5 ± 3.5	18.6 ± 2.8*	36.2 ± 4.6	24.1 ± 3.2*
PB (15) + FBM (2.5)	68.4 ± 4.3	69.3 ± 5.0	5 (5;5)	5 (5;5)	24.3 ± 3.6	22.6 ± 3.4	44.2 ± 3.9	44.0 ± 4.0

For abbreviations and additional details, see Table 1 legend. PB and FBM were administered 60 min before the seizure test.

* $P < .05$ vs. respective controls.

Table 3
Effect of phenytoin (PHT), both singly and in combination with felbamate (FBM), on amygdala-kindled seizures

Treatment (mg/kg)	C	ADT	C	SSv	C	SDr	C	Adr
PHT (20)	70.7 ± 5.3	73.6 ± 6.3	5 (5;5)	5 (4;5)	29.2 ± 6.7	23.7 ± 4.5 *	48.9 ± 5.9	41.4 ± 6.1 *
PHT (17.5)	70.0 ± 5.2	73.3 ± 4.1	5 (5;5)	5 (4.5;5)	27.6 ± 3.6	22.6 ± 5.6	40.2 ± 6.3	33.5 ± 7.9
PHT (17.5) + FBM (5)	68.7 ± 4.9	73.3 ± 7.5 *	5 (5;5)	4.75 (3;5)	27.1 ± 2.9	24.3 ± 2.5	42.8 ± 4.8	34.3 ± 3.7 *
PHT (17.5) + FBM (2.5)	72.3 ± 4.2	73.6 ± 4.3	5 (5;5)	5 (5;5)	31.2 ± 3.2	30.1 ± 3.1	47.6 ± 4.1	46.3 ± 4.3

For abbreviations and additional details see Table 1 legend. PHT was administered 120 min, and FBM 60 min before the seizure test.
* $P < .05$ vs. respective controls.

addition, 100 mg/kg valproate reduced seizure activity (from 5 to 3 μA) and shortened the seizure and afterdischarge durations (from 23.7 to 14.1 s, and from 42.6 to 25.2 s, respectively). Phenobarbital (15 mg/kg) shortened the seizure duration from 23.9 to 13.1 s, and the afterdischarge duration from 42 to 24.1 s (Table 2). Phenytoin (20 mg/kg) reduced the seizure and afterdischarge durations, from 29.2 to 23.7 s, and from 48.9 to 41.4 s, respectively (Table 3). Clonazepam at 0.1 mg/kg increased the afterdischarge threshold (from 70.6 to 77 μA), while when administered at 0.25 mg/kg, it significantly reduced all seizure parameters (Table 4). Thus, the afterdischarge threshold was increased from 70.3 to 74.3 μA, and the seizure severity was reduced from 5 to 3. In addition, the seizure duration was shortened from 26 to 20.7 s, while the afterdischarge duration from 36.9 to 25.4 s. The administration of felbamate (2.5 mg/kg) and carbamazepine (7.5 mg/kg; both compounds at their subeffective doses), in combination, resulted in significant reduction in seizure severity (from 5 to 3). Moreover, the afterdischarge duration was shortened from 44.8 to 26.7 s. When felbamate (2.5 mg/kg) was coadministered with phenobarbital, clonazepam or phenytoin, antiseizure activity was unaffected. However, when felbamate, at its lowest effective dose of 5 mg/kg, was coadministered with these antiepileptic drugs, used at their highest subprotective doses (carbamazepine 7.5 mg/kg, clonazepam 0.05 mg/kg, phenobarbital 10 mg/kg, phenytoin 17.5 mg/kg and valproate 50 mg/kg), an antiseizure effect was observed. With all drug combinations, the afterdischarge threshold was increased. Combining felbamate (5 mg/kg) with carbamazepine (7.5 mg/kg), phenobarbital (10 mg/kg) and clonazepam (0.05 mg/kg) led to the reduction of seizure and afterdischarge durations. The combination of felbamate (5 mg/kg) and carbamazepine (7.5 mg/kg) resulted in a decrement of seizure severity in addition (Tables 1–4, Figs. 1–3).

3.3. Chimney test and passive avoidance task

Felbamate (up to 10 mg/kg), carbamazepine (7.5, 10 mg/kg), valproate (50, 75 mg/kg), phenobarbital (10, 15 mg/kg), phenytoin (17.5, 20 mg/kg) and clonazepam (0.05, 0.1 mg/kg) was not associated with any significant motor- or long-term memory impairment (Table 5). Similarly, the combinations of the antiepileptic drugs, at their lower doses, with felbamate (5 mg/kg) did not result in any undesired effects (Table 5).

3.4. Carbamazepine and felbamate plasma and brain concentrations

The free-plasma concentration of the carbamazepine (7.5 mg/kg) + saline administered rats was 1.89 ± 0.22 μg/ml (mean ± S.D.; $n = 6$), while the concurrent brain concentration was 2.27 ± 0.26 μg/g of wet brain tissue (mean ± S.D.; $n = 6$). For the rats administered with carbamazepine (7.5 mg/kg) + felbamate (2.5 mg/kg), the free-plasma concentration of carbamazepine was 1.72 ± 0.23 μg/ml (mean ± S.D.; $n = 6$), whereas the concurrent brain concentration was 2.31 ± 0.29 μg/g (mean ± S.D.; $n = 6$). There was no statistical difference between the two groups. Plasma felbamate concentrations were 0.61 ± 0.11 μg/ml ($n = 6$) after the administration of 2.5 mg/kg felbamate and 0.93 ± 0.09 μg/ml ($n = 6$) after the administration of 2.5 mg/kg felbamate + 7.5 mg/kg carbamazepine. The concurrent brain

Table 4
Effect of clonazepam (CZP), both singly and in combination with felbamate (FBM), on amygdala-kindled seizures

Treatment (mg/kg)	C	ADT	C	SSv	C	SDr	C	ADr
CZP (0.25)	70.3 ± 5.3	74.3 ± 3.5 *	5 (5;5)	3 (2;4) *	26.0 ± 4.9	20.7 ± 3.2 *	36.9 ± 4.0	25.4 ± 3.4 * *
CZP (0.1)	70.6 ± 4.2	77.0 ± 6.4 *	5 (5;5)	5 (4.5;5)	23.4 ± 6.7	21.5 ± 3.7	35.7 ± 4.2	35.5 ± 5.3
CZP (0.05)	70.6 ± 3.9	71.9 ± 3.7	5 (5;5)	4.75 (4.5;5)	27.6 ± 5.7	24.0 ± 5.2	48.1 ± 6.0	44.6 ± 6.2
CZP (0.05) + FBM (5)	71.1 ± 3.6	76.6 ± 2.8 * *	5 (5;5)	4 (3;4.5)	29.4 ± 2.6	24.1 ± 2.3 *	44.6 ± 5.7	39.3 ± 4.2 *
CZP (0.05)+ FBM (2.5)	72.1 ± 3.8	74.0 ± 4.1	5 (5;5)	5 (4.5;5)	30.5 ± 3.2	28.9 ± 4.4	46.7 ± 4.8	46.1 ± 5.0

For abbreviations and additional details, see Table 1 legend. CZP was administered 30 min, and FBM 60 min before the seizure test.
* $P < .05$ vs. respective controls.
* * $P < .01$ vs. respective controls.

Table 5
Effect of felbamate (FBM), both singly and in combination with various conventional antiepileptic drugs, on retention (passive avoidance task) and motor coordination (chimney test) in rats

Treatment (mg/kg)	Median (25, 75 percentile)	Rats impaired (%)	
		30 s	60 s
Vehicle	180 (180; 180)	0	0
FBM (5)	180 (180; 180)	12.5	0
FBM (2.5)	180 (180; 180)	0	0
CBZ (10)	180 (180; 180)	12.5	0
CBZ (7.5)	180 (180; 180)	0	0
CBZ (7.5) + FBM (5)	180 (180; 180)	0	0
VPA (75)	180 (180, 180)	12.5	12.5
VPA (50)	180 (180, 180)	0	0
VPA (50) + FBM (5)	180 (180, 180)	0	0
PHT (20)	167 (62, 180)	37.5	25
PHT (17.5)	180 (152, 180)	25	12.5
PHT (17.5) + FBM (5)	173 (78; 180)	37.5	12.5
PB (15)	180 (180; 180)	25	12.5
PB (10)	180 (180; 180)	12.5	0
PB (10) + FBM (5)	180 (180; 180)	25	12.5
CZP (0.1)	174 (69; 180)	12.5	12.5
CZP (0.05)	180 (180; 180)	0	0
CZP (0.05) + FBM (5)	180 (180; 180)	12.5	0

The results from the passive avoidance task are presented as medians with 25, 75 percentile values of eight determinations. The retention was quantified as the time period (in seconds) that the animals avoided the dark compartment. Motor impairment is expressed as a percentage of animals that failed to perform the chimney test during a 30- and a 60-s observation period. Statistical analysis of data obtained from the passive avoidance task was performed using the Kruskal–Wallis test and with a post hoc Dunn's test. Data obtained from the chimney test were compared using the Fisher's exact probability test. CBZ, carabamazepine; CZP. clonazepam; FBM, felbamate; PHT, phenytoin, PB, phenobarbital; VPA, valproate.

concentrations were, respectively, 0.13 ± 0.13 μg/ml ($n = 6$) and 0.24 ± 0.07 μg/ml ($n = 6$).

4. Discussion

The present study demonstrates that felbamate not only exerts significant anticonvulsant activity in amygdala-kindled rats, but also positively interacts with carbamazepine in this model of intractable epilepsy. The protective effect observed after the combined treatment of felbamate and carbamazepine, applied at their subeffective doses (2.5 and 7.5 mg/kg, respectively), may indicate synergistic interaction between the two antiepileptic drugs. It should be also noted that felbamate, at its lowest effective dose of 5 mg/kg, significantly increased the afterdischarge threshold and reduced both seizure and afterdischarge durations. In addition, combinations of felbamate (5 mg/kg) with remaining conventional antiepileptic drugs resulted in moderate anti-seizure action in kindled rats. Nevertheless, observed effect, probably due to the efficacy of felbamate alone, indicates a relatively weak ability of this drug to interact with other antiepileptics. However, the concomitant administration of carbamazepine (7.5 mg/kg) and felbamate (5 mg/kg) may be to some extend superior over that of felbamate (5 mg/kg) administered alone because the drug combination was associated with an additional reduction in seizure severity. The effect of a drug on seizure severity, seizure duration and afterdischarge duration can be interpreted as to reflect the ability of a drug to limit seizure spread in the central nervous system. Furthermore, the increment of afterdischarge threshold may be considered to indicate the effect of a drug on the seizure focus (Löscher et al., 1989). Therefore, the present data would suggest that felbamate, alone and in combination with carbamazepine, acts to control seizures both at the level of the focus and also during the subsequent spread of the seizure.

Measurement of free plasma carbamazepine and brain concentrations of carbamazepine showed that there was no statistically significant difference between the different groups. Similarly, no significant difference was observed in relation to total plasma and brain concentrations of felbamate, although there was a tendency for the values to be lower in the group administered with carbamazepine and felbamate in combination. Thus, the observed enhancement of the anticonvulsant effect of felbamate by carbamazepine is unlikely to be the consequence of a pharmacokinetic interaction. The rationale for measuring the free-plasma concentration of carbamazepine as opposed to the total concentration of felbamate is that carbamazepine is highly protein bound (75–82%; Karunanayake et al., 1990), while felbamate is not (22–34%; Adusumalli et al., 1991) and is consequently more amenable to a pharmacologically relevant plasma protein binding interaction.

Clinically, it is well documented that felbamate is susceptible to hepatic enzyme induction (Hachad et al., 2002; Patsalos and Perucca, 2003a) and that carbamazepine, when administered chronically, induces its metabolism, resulting in lower felbamate blood concentrations (Rambeck et al., 1996). Conversely, felbamate can induce the metabolism of carbamazepine, resulting in a reduction of carbamazepine plasma concentrations. As expected, in the present acute studies, such pharmacokinetic interactions were not observed.

Felbamate is associated with numerous mechanisms of action, but its ability to block the NR2B subunit of NMDA receptor is considered to be prominent (Harty and Rogawski, 2000). However, it also significantly enhances $GABA_A$ receptor (Rho et al., 1994), interrupts glutamatergic neurotransmission through AMPA/kainate receptors (DeSarro et al., 1994) and inhibits epileptiform activity induced by kainate in CA1 rat hippocampal slices, suggesting that felbamate may exert indirect antagonistic action at kainate receptors (Domenici et al., 1996). Additionally, felbamate blocks voltage-dependent sodium channels (White et al., 1992) and the sodium channel α subunit.

To date, NMDA receptor antagonists have been found to exhibit a broad spectrum of anticonvulsant activity in various animal seizure models and also to protect against kindling development (Rogawski, 1992). However, felbamate is the only marketed antiepileptic drug that exhibits

NMDA receptor blocking activity at clinically relevant doses (Correadetti and Pugliese, 1998). In fact, NR2B selectivity may, at least, partially contribute to felbamate's low neurobehavioral toxicity in relation to other NMDA receptor antagonists (for review, see Czuczwar et al., 1996). What is interesting is that the utility of felbamate in the Lennox–Gastaut syndrome may be related to the abundance of NR2B subunits in the developing brain (Harty and Rogawski, 2000). In our opinion, the heterogeneous mechanisms of action by felbamate may be responsible for its pharmacodynamic interaction with carbamazepine in amygdala-kindled rats. The lack of such interaction with the antiepileptic drugs, phenytoin, valproate and clonazepam may be the consequence of their affinity to different types of the α subunit of the sodium channel (for review, see Deckers et al., 2000).

Previously, we reported that felbamate, at its highest ineffective dose, failed to affect the protective activity of valproate, carbamazepine, phenytoin or phenobarbital against maximal electroshock-induced seizures in mice (Borowicz et al., 2000). When felbamate was administered at its lowest protective dose, it enhanced the action of only phenytoin. In contrast, Gordon et al. (1993) reported that all four antiepileptic drugs, applied at their subprotective doses (about half of their ED_{50} values), potentiated the antielectroshock activity of felbamate. In light of the above data, our present data support the concept that the effectiveness of drug combinations may be dependent not only on the drug-to-drug ratio, but also on the variability that will occur depending on the experimental model of epilepsy that may be used. This supports our previous observation in relation to combinations of topiramate with conventional antiepileptic drugs in two other models of experimental epilepsy-pentylenetetrazole-induced convulsions in mice and amygdala-kindling in rats (Borowicz et al., 2003).

In conclusion, we have observed a significant pharmacodynamic interaction between felbamate and carbamazepine in the amygdala-kindled rat model of drug-resistant epilepsy. Such interaction was not observed with valproate, phenytoin, phenobarbital and clonazepam. Although the anticonvulsant effect of the carbamazepine and felbamate, in combination, was not spectacular, it was also associated with a significant reduction in seizure severity, and this observation would be particularly relevant clinically. In contrast, neither low-dose carbamazepine nor felbamate alone affected this parameter. This interaction, if applicable to the clinical setting, may serve to enhance the treatment of patients with refractory epilepsy.

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