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# Synergistic interaction between felbamate and lamotrigine against seizures induced by 4-aminopyridine and pentylenetetrazole in mice

Antonio Cuadrado, Jesús Bravo, Juan A. Armijo*

*Department of Physiology and Pharmacology, Clinical Pharmacology Service, Marqués de Valdecilla University Hospital, University of Cantabria School of Medicine, Avda. de Valdecilla, s/n, E-39008 Santander, Spain*

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**Abstract**

We compared the effects of adding a nonprotective dose of felbamate to increasing single doses of lamotrigine with those of monotherapy and vice versa in CD1 mice. Anticonvulsant effects were evaluated against seizures induced by both 14 mg/kg of 4-aminopyridine and 110 mg/kg of pentylenetetrazole, and neurotoxic effects were evaluated by the rotarod test. Changes in anticonvulsants, γ-aminobutyric acid (GABA) and glutamate concentrations in the whole brain were also assessed. Lamotrigine increased the potency ratio of felbamate against 4-aminopyridine (1.80, 95% confidence interval (CI) 1.23–2.65, $P<0.05$) but not against pentylenetetrazole nor on rotarod, the protective index being increased from 12.0 to 17.1 for 4-aminopyridine, with a reduction in brain felbamate, and with an increase in brain GABA. Felbamate increased the potency ratio of lamotrigine against 4-aminopyridine (4.35, 95% CI 2.05–9.25, $P<0.05$) but not on rotarod, the protective index being increased from 4.4 to 15.7; there were no changes in brain lamotrigine, and changes in brain GABA and/or glutamate were unrelated to the pharmacodynamic effects. In conclusion, a nonprotective dose of lamotrigine increased the therapeutic index of felbamate and vice versa, and these effects appeared to be pharmacodynamic.


*Keywords:* Felbamate; Lamotrigine; Pentylenetetrazole; 4-Aminopyridine; Drug interaction

## 1. Introduction

Polytherapy with antiepileptic drugs is necessary in clinical practice due to the limited efficacy of monotherapy (Leach, 1997; Czuczwar and Przesmycki, 2001). Clinical trials are the definitive way to identify those combinations that may be worthwhile in the human setting but they have methodological and ethical difficulties. Therefore, studies in animals can help to select the most promising combinations to be studied in the clinical setting and provide evidence on clinically relevant drug combinations (Leach, 1997; Czuczwar and Przesmycki, 2001). In fact, a relationship has been found between the greater efficacy of some combinations of anticonvulsants in animals and humans. For example, a beneficial interaction between valproate and ethosuximide has been found both in animals (Bourgeois, 1988a) and against refractory absences in humans (Rowan et al., 1983). Similarly, a beneficial interaction between lamotrigine and valproate has been shown in mice (De Sarro et al., 1996) and both against absences (Panayiotopoulos et al., 1993; Pisani et al., 1993; Ferrie et al., 1995) and against refractory seizures (Pisani et al., 1993, 1999; Ferrie and Panayiotopoulus, 1994; Brodie and Yuen, 1997; McCabe et al., 1998, 2001; Privitera and Welty, 1998; Arzimanoglou et al., 2001). Consequently, some authors made claims for the suitability of performing conclusive clinical trials based on the results obtained from antiepileptic drug combinations in animals (Leach, 1997; Czuczwar and Przesmycki, 2001).

Felbamate and lamotrigine are two new broad-spectrum antiepileptic drugs that are effective not only against partial seizures but also against seizures particularly refractory to conventional therapy such as those caused by Lennox–Gastaut and/or West syndromes (Colucci et al., 1996; Pellock, 1999; Cilio et al., 2001). Although a severe restriction has been placed on felbamate use, it remains a useful agent as a third or fourth option in patients with Lennox–Gastaut syndrome or refractory partial epilepsy

* Corresponding author. Tel.: +34-942-203370; fax: +34-942-347411.
*E-mail address:* facasj@humv.es (J.A. Armijo).

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(Pellock, 1999; French et al., 1999). Both anticonvulsants act on excitatory and inhibitory neurotransmission through different and additional mechanisms that may contribute to increasing their anticonvulsant activity. The antiepileptic effect of felbamate is attributed to the blocking of the glycine recognition site on the *N*-methyl-D-aspartate subtype glutamate receptor and potentiation at the γ-aminobutyric acid (GABA) type A receptor (Pellock, 1999; Fraser et al., 1999; Cilio et al., 2001), whereas lamotrigine inhibits voltage-dependent $Na^+$ channels, stabilizing neuronal membranes and prolonging depolarisation (Pisani et al., 1993; Davis et al., 1994; Meldrum and Leach, 1994; Fariello et al., 1995; Leach, 1997; Macdonald, 1999). Although lamotrigine inhibits veratrine-induced glutamate release from rat brain slices at therapeutically relevant concentrations, experiments in conscious rats led to the conclusion that lamotrigine in relevant doses does not inhibit physiological glutamate release in vivo (Waldmeier et al., 1996). Colucci et al. (1996), after discarding a pharmacokinetic interaction, have proposed a possible pharmacodynamic interaction to explain the increase in side effects observed when both drugs are used together. However, we have not found any specific study of the interaction between both drugs either in animals or in humans.

The aim of this study was to analyse the effects that adding a nonprotective dose of felbamate to increasing single doses of lamotrigine and vice versa has on the anticonvulsant effects against hind limb extension induced by 4-aminopyridine and clonus induced by pentylenetetrazole, and on the neurotoxic effects evaluated on rotarod in mice taking into account the possibility of pharmacokinetic interactions and changes in GABA and glutamate in the whole brain.

## 2. Materials and methods

### 2.1. Animals

Adult male albino mice (CD No. 1 strain, Charles River) weighing 20–28 g were housed for at least 7 days before experiments at controlled temperature (19–24 °C) and under natural light/dark conditions; food and water were available ad libitum. All procedures were performed between 9 a.m. and 2 p.m. Protocol complies with the European Community guidelines for the use of experimental animals and was approved by the Physiology and Pharmacology Department of our University.

### 2.2. Anticonvulsants and neurotoxic models

Anticonvulsant efficacy was tested against seizures induced by the 97% convulsant dose ($CD_{97}$) of subcutaneous 4-aminopyridine or pentylenetetrazole both purchased from Sigma (St. Louis, MO, USA). The $CD_{97}$ established in our laboratory for these mice was 14 mg/kg for 4-aminopyridine and 110 mg/kg for pentylenetetrazole. 4-Aminopiridine and pentylenetetrazole were dissolved in 0.9% $Na^+$ chloride and administered into a loose fold of skin in the midline of the neck at a volume of 0.01 ml/g of body weight. Fresh drug solutions were prepared each day. The main end-points were the abolition of the hind limb tonic extension induced by 4-aminopyridine or the abolition of the clonus lasting at least 5 s induced by pentylenetetrazole. Periods of observation were 30 min both for 4-aminopyridine and pentylenetetrazole models. Periods of latency until hind limb extension or clonus were evaluated as secondary end-points.

Minimal neurotoxicity was assessed by the rotarod test (Dunham and Miya, 1957). Before the experiment, mice were placed on a 3-cm rod rotating (Rota-rod Treadmill 7600, Ugo-Basile) at 6 revolutions per minute in two training sessions lasting 10 and 5 min, respectively. After anticonvulsant administration, mice were tested again on the rotarod. The end-point to evaluate minimal neurotoxicity was the inability of the mouse to maintain its equilibrium for 1 min in each of three trials.

Felbamate (Schering-Plough) and lamotrigine (Glaxo-Wellcome) were administered by transesophageal gavage in 1% methylcellulose at a volume of 0.01 ml/g of body weight. Felbamate and lamotrigine were administered 120 and 60 min prior to testing, respectively.

### 2.3. Groups of study and experimental design

Anticonvulsant efficacy and toxicity were evaluated on groups of at least 12 mice for each dose of each drug in monotherapy and in association. A minimum of three percentage points between the limits of 0% to 100% was required. Control groups were included in each experiment.

The anticonvulsant effects of felbamate in monotherapy were studied at doses of 50, 65, 100, 125, 160, 200 and 250 mg/kg against the hind limb extension induced by 4-aminopyridine, and at doses of 12.5, 25, 35, 50, 75, 150, 250 and 500 mg/kg against clonus induced by pentylenetetrazole. The anticonvulsant effects of lamotrigine in monotherapy were studied at doses of 3, 5, 12, 20, 30, 40, 60 and 80 mg/kg against the hind limb extension induced by 4-aminopyridine, and at doses of 0.75, 1.5, 3.5, 5, 8, 16, 32, 50 and 65 mg/kg against the clonus induced by pentylenetetrazole.

For each antiepileptic drug in monotherapy, the highest nonprotective dose was established in our laboratory with the following criteria: (i) it was the highest dose that did not produce a statistically significant effect, and (ii) it was the highest dose unable to protect 10% of mice against 4-aminopyridine or pentylenetetrazole seizures. The highest nonprotective dose of lamotrigine in the 4-aminopyridine model was 5 mg/kg (7% of mice protected from hind limb extension, ns). In the pentylenetetrazole model, it was 1.5 mg/kg (6% of mice protected from clonus, ns, data not

shown). The highest nonprotective dose of felbamate in the 4-aminopyridine model was 65 mg/kg (8% of mice protected from hind limb extension, ns). The neurotoxic effects of felbamate in monotherapy were studied at doses of 160, 600, 800, 1200, 1600 and 2000 mg/kg and the neurotoxic effects of lamotrigine in monotherapy at doses of 20, 40, 50, 60 and 80 mg/kg.

The anticonvulsant interaction between felbamate and lamotrigine was assessed bi-directionally: (i) the influence of a nonprotective dose of lamotrigine (5 mg/kg) on the effects of 50, 65, 100 and 125 mg/kg of felbamate against 4-aminopyridine and the influence of a nonprotective dose of lamotrigine (1.5 mg/kg) on the effects of 12.5, 35 and 75 mg/kg of felbamate against pentylenetetrazole, and (ii) the influence of a nonprotective dose of felbamate (65 mg/kg) on the effects of 3, 5, 12 and 20 mg/kg of lamotrigine against 4-aminopyridine. The influence of felbamate on the anticonvulsant effects of lamotrigine in the pentylenetetrazole model could not be analysed because lamotrigine in monotherapy was only partly effective in this model.

The interaction between felbamate and lamotrigine on neurotoxic effects was also assessed bi-directionally: (i) the influence of 5 mg/kg of lamotrigine (the highest dose associated with felbamate in the anticonvulsant studies) on the neurotoxic effects of 800, 1200 and 1600 mg/kg of felbamate, and (ii) the influence of 65 mg/kg of felbamate on the neurotoxic effects of 40, 50 and 60 mg/kg of lamotrigine.

Pharmacokinetic interactions and neurochemical changes were studied in groups of at least five mice. These groups were different from those used in the anticonvulsant and neurotoxic experiments. Again, the study was bi-directional: (i) the influence of 5 mg/kg of lamotrigine on whole brain concentrations of felbamate, GABA and glutamate achieved 120 min after the administration of 25, 65 and 125 mg/kg of felbamate, and (ii) the influence of 65 mg/kg of felbamate on whole brain concentrations of lamotrigine, GABA and glutamate achieved 60 min after the administration of 5, 12 and 20 mg/kg of lamotrigine. Mice were killed by cervical dislocation, and the whole brain was immediately removed, weighed and homogenized in 2 ml of methanol. Homoge-

Table 1
Influence of a nonprotective dose of lamotrigine on the effects that increasing single doses of felbamate have on the incidence and latency of hind limb extension induced by 4-aminopyridine and clonus induced by pentylenetetrazole in CD1 mice

| FBM dose (mg/kg) | FBM monotherapy | | | FBM + LTG | | | $P'<$ | $P''<$ |
|---|---|---|---|---|---|---|---|---|
| | $N$ | Protection (%) | Latency (min), mean (S.D.) | $N$ | Protection (%) | Latency (min), mean (S.D.) | | |
| *(A) Effects on hind limb extension induced by 14 mg/kg of subcutaneous 4-aminopyridine* | | | | | | | | |
| 0 | 29 | 0 | 10.7 (4.8) | 27 | 7 | 12.9 (5.7) | NS | NS |
| 50 | 12 | 25[a] | 16.2 (8.8) | 12 | 42[b,c] | 24.3 (7.6)[c,d] | NS | NS |
| 65 | 12 | 8 | 13.6 (7.1)[e] | 12 | 67[c,d] | 25.8 (6.8)[c,d] | 0.01 | 0.001 |
| 100 | 12 | 33[b] | 21.5 (8.1)[d,e,f] | 12 | 92[c,d,f] | 29.5 (1.7)[c,d] | 0.01 | 0.01 |
| 125 | 12 | 75[d,f] | 27.6 (4.7)[d,f] | 24 | 92[c,d,f] | 29.0 (3.6)[c,d,f] | NS | NS |
| 160 | 12 | 92[d,f] | 28.7 (4.6)[d,f] | – | – | – | – | – |
| 200 | 12 | 100[d,f] | 30.0 (0.0)[d,f] | – | – | – | – | – |
| 250 | 12 | 100[d,f] | 30.0 (0.0)[d,f] | – | – | – | – | – |
| $P$ | – | $<0.001$ | $<0.001$ | – | $<0.01$ | $<0.05$ | – | – |
| *(B) Effects on clonus induced by 110 mg/kg of subcutaneous pentylenetetrazole* | | | | | | | | |
| 0 | 20 | 0 | 7.0 (5.0) | 18 | 6 | 13.7 (8.5) | NS | $<0.01$ |
| 12.5 | 12 | 8 | 11.9 (9.2) | 12 | 17 | 16.6 (9.8)[b] | NS | NS |
| 25 | 12 | 17 | 13.6 (10.4) | – | – | – | – | – |
| 35 | 12 | 25[a] | 19.8 (10.5)[b] | 12 | 25[a] | 22.9 (5.7)[c,d] | NS | NS |
| 50 | 12 | 42[b] | 18.7 (12.1)[a] | – | – | – | – | – |
| 75 | 12 | 58[d,f] | 25.3 (6.5)[d,f] | 12 | 50[b,c] | 22.0 (10.2)[c,d] | NS | NS |
| 150 | 12 | 67[d,f] | 25.3 (8.3)[d,f] | – | – | – | – | – |
| 250 | 12 | 83[d,f] | 28.0 (5.5)[d,f] | – | – | – | – | – |
| 500 | 12 | 58[d,f] | 21.8 (11.5)[d] | – | – | – | – | – |
| $P$ | – | $<0.001$ | $<0.001$ | – | NS | NS | – | – |

FBM: felbamate, LTG: lamotrigine, $N$: number of mice in each group, NS: nonsignificant, Protection: percentage of mice protected from convulsions, $P$: significance between doses of felbamate (excluded control), $P'$: significance vs. the effect on protection of the same dose in monotherapy, $P''$: significance vs. the effect on latencies of the same dose in monotherapy, S.D.: standard deviation.

[a] $P<0.05$ vs. control (mice untreated).

[b] $P<0.01$ vs. control (mice untreated).

[c] Statistically significant vs. the effect of the nonprotective dose of lamotrigine (5 mg/kg in the 4-aminopyridine and 1.5 mg/kg in the pentylenetetrazole model).

[d] $P<0.001$ vs. control (mice untreated).

[e] Statistically significant vs. the following higher dose.

[f] Statistically significant vs. lower doses.

nates were centrifuged at 2500 × *g* for 10 min at 4 °C and the upper phase stored at −20 °C until assay.

### 2.4. Assays

Brain felbamate and lamotrigine concentrations were assayed by the modified liquid chromatographic method used by Fraser et al. (1995) modified by Cuadrado et al. (2002). Within-assay coefficients of variation in two control samples with 0.75 and 3.0 mg/l of lamotrigine were 6.0% (S.D. = 5.6) and 7.5% (S.D. = 8.8), respectively, and between-assay coefficients of variation were 9.4% and 10.1%, respectively. Within-assay coefficients of variation in two control samples with 0.75 and 3.0 mg/l of felbamate were 8.1% (S.D. = 6.0) and 10.4% (S.D. = 8.1), respectively, and between-assay coefficients of variation were 8.4% and 6.6%, respectively.

Brain GABA concentration was assayed by the liquid chromatographic method of Turnell and Cooper (1982) modified as described by Valdizán and Armijo (1992). Mean brain GABA concentration in control groups was 1.99 (S.D. = 0.1) μmol/g of tissue. Within-assay and between-assay coefficients of variation in a control sample with 0.75 mmol/l of GABA were 4.3% (S.D. = 3.2) and 17%, respectively.

Brain glutamate concentration was assayed by the modified liquid chromatographic method used by Löscher et al. (1993) modified by Cuadrado et al. (2002). Within-assay coefficients of variation in two control samples with 1.25 and 2.75 mmol/l of glutamate were 3.3% (S.D. = 3.4) and

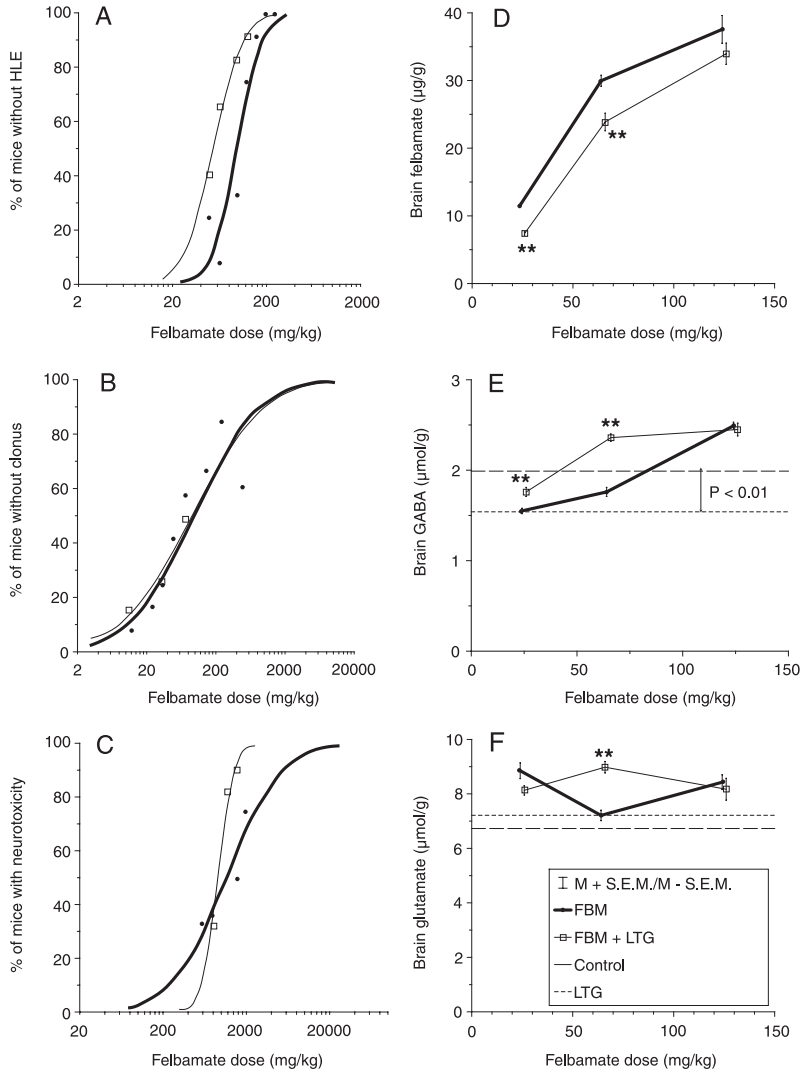


Fig. 1. Felbamate dose–response curves against hind limb extension (HLE) induced by 4-aminopyridine (A) or against clonus induced by pentylenetetrazole (B), and dose–neurotoxic response evaluated by rotarod (C) after single increasing doses of felbamate both in monotherapy (FBM) and after adding a nonprotective dose of lamotrigine of 5 mg/kg in the 4-aminopyridine model and of 1.5 mg/kg in the pentylenetetrazole model (FBM + LTG). Influence of adding 5 mg/kg of lamotrigine to increasing single doses of felbamate on whole brain felbamate concentration (D), and on whole brain GABA (E) and glutamate (F) concentrations in CD1 mice. $^{**}P < 0.01$ vs. the same dose in monotherapy. Data are mean (M) ± standard error of the mean (S.E.M.). *P*: significance between the control group and the nonprotective dose of lamotrigine.

Table 2
Influence of a nonprotective dose of lamotrigine on anticonvulsant and neurotoxic effects of increasing single doses of felbamate and vice versa in CD1 mice

| Groups | $ED_{50}$ (mg/kg) | | $TD_{50}$ (mg/kg) | PI ($TD_{50}/ED_{50}$) | |
|---|---|---|---|---|---|
| | 4-AP | PTZ | | 4-AP | PTZ |
| *(A) Influence of a nonprotective dose of lamotrigine on increasing single doses of felbamate* | | | | | |
| FBM | 96.0 (76.1–121.1) | 99.7 (58.2–171.0) | 1150.0 (697.4–1896.5) | 12 | 11.5 |
| FBM+LTG | 53.2 (39.1–72.2) | 90.7 (31.1–264.5) | 907.9 (735.6–1120.7) | 17.1 | 10 |
| Potency ratio FBM/(FBM+LTG) | 1.80[a] (1.23–2.65) | 1.09 (0.33–3.64) | 1.27 (0.74–2.18) | – | – |
| *(B) Influence of a nonprotective dose of felbamate on increasing single doses of lamotrigine* | | | | | |
| LTG | 12.3 (9.5–15.8) | – | 54.1 (43.3–67.7) | 4.4 | – |
| LTG+FBM | 2.8 (1.4–5.7) | – | 44.3 (39.7–49.4) | 15.7 | – |
| Potency ratio LTG/(LTG+FBM) | 4.35[a] (2.05–9.25) | – | 1.22 (0.95–1.57) | – | – |

Data are median and 95% confidence interval in brackets. $ED_{50}$: median effective dose, $TD_{50}$: median neurotoxic dose, FBM: felbamate, LTG: lamotrigine, PI: protective index, PTZ: pentylenetetrazole, 4-AP: 4-aminopyridine.
[a] $P<0.05$ by the Litchfield and Wilcoxon method.

4.8% (S.D. = 5.4), respectively, and between-assay coefficients of variation were 1.6% and 5.7%, respectively.

### 2.5. Statistical analysis

Median effective doses ($ED_{50}$), median neurotoxic doses ($TD_{50}$), potency ratio between monotherapy and association (i.e. $ED_{50}$ monotherapy/$ED_{50}$ association or $TD_{50}$ monotherapy/$TD_{50}$ association) and 95% confidence interval (95% CI) were calculated by Litchfield and Wilcoxon's (1949) log-probit method using the Pharm/PCS v. 4.0 software. The protective index ($TD_{50}/ED_{50}$) for felbamate and lamotrigine in monotherapy and in association was also calculated. Qualitative variables were compared by the Fisher's exact test, and quantitative variables by means of one-way analysis of variance followed by the Newman–Keuls test or by the non-parametric Mann–Whitney *U*-test using SPSS for Windows V 9.0 software. A two-sided $P<0.05$ was considered significant throughout. Data are expressed as mean and standard deviation (S.D.) in text and tables and as mean and standard error of the mean (S.E.M.) in figures.

## 3. Results

### 3.1. Influence of lamotrigine on felbamate

#### 3.1.1. Influence on the anticonvulsant and neurotoxic effects

The effects of single increasing doses of felbamate in monotherapy and in association with a nonprotective dose of lamotrigine on the incidence and latency of hind limb extension induced by 4-aminopyridine and clonus induced by pentylenetetrazole are shown in Table 1. Lamotrigine

Table 3
Influence of a nonprotective dose of felbamate on the effects that increasing single doses of lamotrigine have on the incidence and latency of hind limb extension induced by 14 mg/kg of subcutaneous 4-aminopyridine in CD1 mice

| LTG dose (mg/kg) | LTG monotherapy | | | LTG+FBM | | | *P′*< | *P″*< |
|---|---|---|---|---|---|---|---|---|
| | *N* | Protection (%) | Latency (min), mean (S.D.) | *N* | Protection (%) | Latency (min), mean (S.D.) | | |
| 0 | 29 | 0 | 10.7 (4.8) | 12 | 8 | 13.6 (7.1) | NS | NS |
| 3 | 12 | 0 | 11.4 (2.4) | 12 | 50[a] | 22.3 (8.4)[a,b] | 0.05 | 0.001 |
| 5 | 27 | 7 | 12.9 (5.7)[c] | 12 | 67[a,b] | 25.8 (6.8)[a,b] | 0.001 | 0.001 |
| 12 | 12 | 33[c,d] | 22.0 (6.9)[a,c,e] | 12 | 92[a,b] | 29.0 (3.5)[a,b,e] | 0.01 | 0.01 |
| 20 | 24 | 79[a,e] | 27.4 (5.6)[a,e] | 12 | 92[a,b] | 29.8 (0.6)[a,b,e] | NS | NS |
| 30 | 12 | 100[a,e] | 30.0 (0.0)[a,e] | – | – | – | – | – |
| 40 | 12 | 100[a,e] | 30.0 (0.0)[a,e] | – | – | – | – | – |
| 60 | 12 | 100[a,e] | 30.0 (0.0)[a,e] | – | – | – | – | – |
| 80 | 12 | 100[a,e] | 30.0 (0.0)[a,e] | – | – | – | – | – |
| *P* | – | <0.001 | <0.001 | – | <0.05 | <0.01 | – | – |

FBM: felbamate, LTG: lamotrigine, *N*: number of mice in each group, NS: nonsignificant, Protection: percentage of mice protected from convulsions, *P*: significance between doses of lamotrigine (excluded control), *P′*: significance vs. the effect on protection of the same dose in monotherapy, *P″*: significance vs. the effect on latencies of the same dose in monotherapy, S.D.: standard deviation.
[a] $P<0.001$ vs. control (mice untreated).
[b] Statistically significant vs. the effect of the nonprotective dose of felbamate (65 mg/kg).
[c] Statistically significant vs. the following higher dose.
[d] $P<0.01$ vs. control (mice untreated).
[e] Statistically significant vs. lower doses.

shifted the dose–response curve of felbamate against the incidence of hind limb extension induced by 4-aminopyridine to the left, this effect being more pronounced at low than at high doses of felbamate (Fig. 1A). The $ED_{50}$ of felbamate decreased from 96.0 mg/kg in monotherapy to 53.2 mg/kg in association with lamotrigine, the anticonvulsant potency ratio of felbamate being 1.80 ($P < 0.05$) (Table 2). In contrast, lamotrigine did not modify the effects of felbamate against the incidence of clonus induced by pentylenetetrazole (Fig. 1B), the anticonvulsant potency ratio of felbamate being 1.09 (Table 2). Furthermore, lamotrigine prolonged the periods of latency until hind limb extension induced by 4-aminopyridine observed with felbamate in monotherapy, but latencies until clonus induced by pentylenetetrazole increased less than expected, taking into account the effect of lamotrigine in monotherapy, or even decreased (Table 1).

With regard to neurotoxicity, lamotrigine increased the slope of the dose–neurotoxic curve and, therefore, increased the toxicity of felbamate at doses of 600 and 800 mg/kg which are higher than those at which an increase of the anticonvulsant effects in the 4-aminopyridine model was observed (250 mg/kg or lower) (Fig. 1C) but the neurotoxic potency ratio of felbamate was not significantly changed.

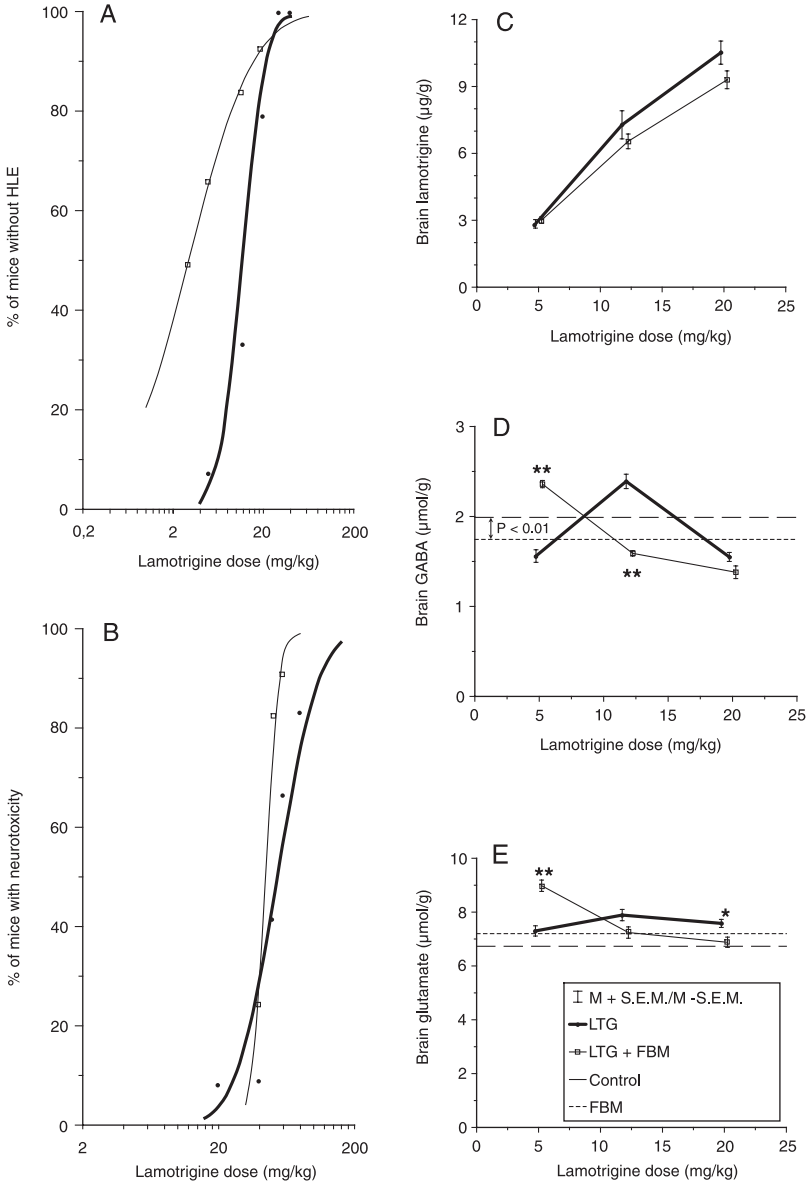


Fig. 2. Lamotrigine dose–response curves against hind limb extension (HLE) induced by 4-aminopyridine (A) and dose–neurotoxic response evaluated by rotarod (B) after single increasing doses of lamotrigine both in monotherapy (LTG) and after adding a nonprotective dose of felbamate of 65 mg/kg (LTG + FBM). Influence of adding 65 mg/kg of felbamate to increasing single doses of lamotrigine on whole brain lamotrigine concentration (C), and on whole brain GABA (D) and glutamate (E) concentrations in CD1 mice. *$P < 0.05$ and **$P < 0.01$ vs. the same dose in monotherapy. Data are mean (M) ± standard error of the mean (S.E.M.). *P*: significance between control and the nonprotective dose of felbamate.

Consequently, the protective index of felbamate increased from 12.0 in monotherapy to 17.1 in association with lamotrigine in the 4-aminopyridine model and decreased from 11.5 to 10 in the pentylenetetrazole model (Table 2).

*3.1.2. Influence on brain felbamate and on brain GABA and glutamate concentrations*

Lamotrigine statistically reduced felbamate concentration in the whole brain at doses of 25 and 65 mg/kg of felbamate (Fig. 1D). On the other hand, lamotrigine significantly increased the effects of felbamate on brain GABA at doses of 25 mg/kg (from 1.55 to 1.76 μmol/g, $P<0.01$) and 65 mg/kg (from 1.76 to 2.36 μmol/g, $P<0.01$) but not at doses of 125 mg/kg of felbamate. This synergistic effect on brain GABA was unexpected because 5 mg/kg of lamotrigine in monotherapy reduced brain GABA in relation to the control group (from 1.99 to 1.56 μmol/g, $P<0.01$) (Fig. 1E). With regard to glutamate, lamotrigine either did not change or even raised the increase in brain glutamate produced by felbamate alone (Fig. 1F).

*3.2. Influence of felbamate on lamotrigine*

*3.2.1. Influence on the anticonvulsant and neurotoxic effects*

The effects of single increasing doses of lamotrigine in monotherapy and in association with a nonprotective dose of felbamate on incidence and latency of hind limb extension induced by 4-aminopyridine are shown in Table 3. Felbamate shifted the dose–response curve of lamotrigine against the incidence of hind limb extension induced by 4-aminopyridine to the left, this effect being more pronounced at low than at high doses of lamotrigine (Fig. 2A). The $ED_{50}$ of lamotrigine decreased from 12.3 mg/kg in monotherapy to 2.8 mg/kg in association with felbamate, the anticonvulsant potency ratio of lamotrigine being 4.35 ($P<0.05$) (Table 2). Felbamate also increased the periods of latency in relation to those observed with lamotrigine alone (Table 3).

Felbamate increased the slope of the dose–neurotoxic response curve and, therefore, increased the toxicity of lamotrigine at doses of 40 and 50 mg/kg, which are higher than those at which the increase of the anticonvulsant effects was observed (20 mg/kg or lower) (Fig. 2B), but the neurotoxic potency ratio was not significantly changed. Consequently, the protective index of lamotrigine increased from 4.4 in monotherapy to 15.7 in association with felbamate (Table 2).

*3.2.2. Influence on brain lamotrigine and on brain GABA and glutamate concentrations*

Felbamate did not statistically modify lamotrigine concentrations in the whole brain (Fig. 2C), increased brain GABA in relation to the effects of lamotrigine alone at doses of 5 mg/kg of lamotrigine (from 1.56 to 2.36 μmol/g, $P<0.01$) and decreased it at doses of 12 mg/kg of lamotrigine (from 2.39 to 1.59 μmol/g, $P<0.01$) (Fig. 2D). Similarly, felbamate increased brain glutamate in relation to the effects of lamotrigine alone at doses of 5 mg/kg of lamotrigine (from 7.30 to 8.98 μmol/g, $P<0.01$) and decreased it at doses of 20 mg/kg (from 7.58 to 6.88 μmol/g, $P<0.05$) (Fig. 2E).

## 4. Discussion

This study shows that a low and ineffective dose of lamotrigine increases the protective index of felbamate against seizures induced by 4-aminopyridine by enhancing the felbamate anticonvulsant effect without a significant increase in its neurotoxicity; in contrast, lamotrigine reduces the protective index of felbamate in the pentylenetetrazole model. Conversely, a low and ineffective dose of felbamate increases the protective index of lamotrigine against seizures induced by 4-aminopyridine by enhancing the lamotrigine anticonvulsant effect but not its neurotoxicity. The absence of a significant increase in brain concentrations of each anticonvulsant suggests that a pharmacodynamic interaction underlies these effects.

For the first time, a pharmacodynamic interaction between felbamate and lamotrigine has been demonstrated, adding new evidence of anticonvulsant potentiation between antiepileptic drugs in a preclinical setting. A pharmacodynamic interaction has been described for other associations of these anticonvulsants. Gordon et al. (1993) found a significant potentiation of the anticonvulsant effect of felbamate against the hind limb extension induced by the maximal electroshock test in mice after the addition of nonprotective doses of phenytoin, carbamazepine, valproate or phenobarbitone. Toxicity as determined by the rotarod test was not significantly increased and, therefore, a considerable increase in the therapeutic index of felbamate was observed. This anticonvulsant potentiation could not be accounted for by a pharmacokinetic mechanism. Furthermore, De Sarro et al. (1996) found an increase in the therapeutic index of valproate after adding a non-effective dose of lamotrigine. These investigators observed a potentiation of the valproate anticonvulsant activity against audiogenic seizures without a concomitant increase in neurotoxicity in DBA/2 mice; no pharmacokinetic interactions were observed when they studied the influence of lamotrigine on total plasma levels of valproate. These results regarding a beneficial pharmacodynamic interaction between lamotrigine and valproate in mice support the hypothesis concerning the existence of a beneficial pharmacodynamic interaction between valproate and lamotrigine in humans emerging from clinical studies (Panayiotopoulos et al., 1993; Pisani et al., 1993, 1999; Ferrie et al., 1995; Leach, 1997).

Felbamate did not modify brain concentrations of lamotrigine when it was associated at doses producing an increase in the protective index of lamotrigine in the 4-aminopyridine model. Conversely, a low and ineffective

dose of lamotrigine enhanced the protective index of felbamate in spite of decreasing brain felbamate concentrations. Because the influence on whole brain drug concentrations is the gold standard by which a possible pharmacokinetic interaction in animals is assessed (Levy and Bourgeois, 1997), it is reasonable to discard a pharmacokinetic interaction underlying the anticonvulsant and neurotoxic effects observed in our study.

Our results suggest that the association of a low dose of felbamate with lamotrigine and of a low dose of lamotrigine with felbamate displayed an anticonvulsant potentiation in the 4-aminopyridine model. In contrast, lamotrigine did not increase the anticonvulsant effects of felbamate in the pentylenetetrazole model. Other investigators have tried to relate the effects of some antiepileptic drugs on brain concentrations of epilepsy-related neurotransmitters such as GABA or glutamate to their intimate action mechanism (Leach et al., 1997; Fraser et al., 1999). Fraser et al. (1999) investigated the effects of felbamate on several GABA- and glutamate-related neurochemical parameters in mouse brain and did not find any significant influence on the concentrations of GABA, glutamate and glutamine following both single and multiple doses of felbamate. We have tried for the first time to relate the anticonvulsant interaction between felbamate and lamotrigine to changes in whole brain GABA and glutamate concentrations. The most interesting result in our study was that lamotrigine, an inhibitor of excitatory neurotransmission that reduced brain GABA concentrations in monotherapy, significantly increased GABA concentrations in relation to felbamate alone at doses producing an anticonvulsant potentiation. In contrast, the anticonvulsant enhancement should not be attributed to an antiglutamatergic effect because an increase in brain glutamate was observed both with felbamate in monotherapy and in association with lamotrigine. Certainly, GABA or glutamate changes in the whole brain are not sensitive enough to explain subtle drug effects and it is more suitable to study neurochemical interactions in discrete brain areas and even better at the synaptosomal level. Therefore, studies employing microdissection, microdialysis or neurotransmitter turnover techniques are required. However, the neurochemical analysis in the whole brain performed in our study is arguably applicable to the investigation of the mechanism of action of all antiepileptic drugs and may be a valid approach and an acceptable source of a new hypothesis to investigate (Leach et al., 1997; Fraser et al., 1999).

In this study, the anticonvulsant effect has been tested in two chemical models of convulsion: 4-aminopyridine and pentylenetetrazole. The 4-aminopyridine model has been used in our study instead of the maximal electroshock test. Maximal electroshock test has been widely used to study anticonvulsant synergy between antiepileptic drugs, because it is a well-known model and one of the reference tests used in the screening of new anticonvulsants; it is fast (with a short observation time), and is easy to perform and reproduce (Gordon et al., 1993; Chez et al., 1994; Sofia, 1995; White et al., 1998). 4-Aminopyridine is a potassium channel antagonist; its mechanism of action is partially unknown but it seems to enhance the release of excitatory neurotransmitters. 4-Aminopyridine has been proposed as a substitute for the maximal electroshock test because the overall profile of compounds active against 4-aminopyridine convulsions resembles that against maximal electroshock, and the resultant convulsion resembles that of maximal electroshock. 4-Aminopyridine test is also fast and is easier to perform than maximal electroshock test. The tonic extension of the hind limbs observed in both models is considered representative of seizure spread (Yamaguchi and Rogawski, 1992; Cramer et al., 1994). Our results show that 4-aminopyridine is a suitable model for studying the anticonvulsant interaction between both drugs in mice.

Pentylenetetrazole is a well-known agent extensively used for identifying new anticonvulsant drugs and also to assess the anticonvulsant efficacy of different antiepileptic combinations (Bourgeois, 1988a,b; White et al., 1998). Its mechanism of action is only partially understood, although an inhibition of the chloride conductance by binding to picrotoxin sites of $GABA_A$ receptor complex and a benzodiazepine receptor antagonism have been reported (Sayin et al., 1993). The efficacy against the clonus induced by pentylenetetrazole is considered predictive of the efficacy against generalized absences. However, in spite of the extensive use of the pentylenetetrazole model for screening and research, the validity of the results can hardly be generalized to spontaneous seizures in epileptic patients. In fact, the lack of efficacy of lamotrigine in the pentylenetetrazole model found in our study confirms the results of other investigators (Miller et al., 1986), and it contrasts with the anti-absence effects of lamotrigine observed in humans. Therefore, the influence of felbamate on the anticonvulsant effects of lamotrigine should be analysed in other models in which lamotrigine is active such as photically evoked afterdischarges and photoconvulsive responses (Lamb and Miller, 1985), electrically induced electroencephalographic afterdischarges (Wheatley and Miller, 1989) or the genetic epilepsy-prone rat (Smith et al., 1993). The good relationship between felbamate dose and anticlonus effect found in our study agrees with the results of Swinyard et al. (1986), but contrasts with the poor relationship found by other investigators (Frey and Bartels, 1997).

In summary, we have observed that both the addition of a nonprotective dose of felbamate to increasing single doses of lamotrigine and, conversely, the addition of a nonprotective dose of lamotrigine to increasing single doses of felbamate produced a significant potentiation of the anticonvulsant effect achieved in monotherapy against the hind limb extension produced by 4-aminopyridine in mice without increasing neurotoxicity. Consequently, an increase in the protective index of the association in relation to respective monotherapies was observed. This anticonvulsant potentiation was not explained by a pharmacokinetic interaction. In addition, the anticonvulsant potentiation was

accompanied by an increase of GABA concentration in the whole brain after adding a nonprotective dose of lamotrigine to felbamate. This effect on GABA was unexpected because lamotrigine in monotherapy significantly reduced brain GABA and warrants a further and detailed investigation. Our results add new evidence on a pharmacodynamic interaction between felbamate and lamotrigine and support the need for further experimental studies (in animals and humans) that definitely provide the basis for development of adjunctive therapy between them when monotherapy is ineffective.

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## References

Arzimanoglou, A., Kulak, I., Bidaut-Mazel, C., Baldy-Moulinier, M., 2001. Optimal use of lamotrigine in clinical practice: results of an open multicenter trial in refractory epilepsy. Rev. Neurol. 157, 525–536.

Bourgeois, B.F.D., 1988a. Combination of valproate and ethosuximide: antiepileptic and neurotoxic interaction. J. Pharmacol. Exp. Ther. 247, 1128–1132.

Bourgeois, B.F.D., 1988b. Anticonvulsant potency and neurotoxicity of valproate alone and in combination with carbamazepine or phenobarbital. Clin. Neuropharmacol. 11, 348–359.

Brodie, M.J., Yuen, A.W.C., 105 Study Group, 1997. Lamotrigine substitution study: evidence for synergism with sodium valproate? Epilepsy Res. 26, 423–432.

Chez, M.G., Bourgeois, B.F.D., Pippenger, C.E., Knowles, W.D., 1994. Pharmacodynamic interactions between phenytoin and valproate: individual and combined antiepileptic and neurotoxic actions in mice. Clin. Neuropharmacol. 17, 32–37.

Cilio, M.R., Kartashov, A.I., Vigevano, F., 2001. The long-term use of felbamate in children with severe refractory epilepsy. Epilepsy Res. 47, 1–7.

Colucci, R., Glue, P., Holt, B., Banfield, C., Reidenberg, P., Meenan, J.W., Pai, S., Nomeir, A., Lim, J., Lin, C.-C., Affrime, M.B., 1996. Effect of felbamate on the pharmacokinetics of lamotrigine. J. Clin. Pharmacol. 36, 634–638.

Cramer, C.L., Stagnitto, M.L., Knowles, M.A., Palmer, G.C., 1994. Kainic acid and 4-aminopyridine seizure models in mice: evaluation of efficacy of anti-epileptic agents and calcium antagonists. Life Sci. 54, 271–275.

Cuadrado, A., De las Cuevas, I., Valdizán, E.M., Armijo, J.A., 2002. Synergistic interaction between valproate and lamotrigine against seizures induced by 4-aminopyridine and pentylenetetrazole in mice. Eur. J. Pharmacol. 453, 43–52.

Czuczwar, S.J., Przesmycki, K., 2001. Felbamate, gabapentin and topiramate as adjuvant antiepileptic drugs in experimental models of epilepsy. Pol. J. Pharmacol. 53, 65–68.

Davis, R., Peters, D.H., McTavish, D., 1994. Valproic acid: a reappraisal of its pharmacological properties and clinical efficacy in epilepsy. Drugs 47, 332–372.

De Sarro, G., Nava, F., Aguglia, U., De Sarro, A., 1996. Lamotrigine potentiates the antiseizure activity of some anticonvulsants in DBA/2 mice. Neuropharmacology 35, 153–158.

Dunham, N.W., Miya, T.S., 1957. A note on a simple apparatus for detecting neurological deficit in rats and mice. J. Am. Pharm. Assoc. 46, 208–209.

Fariello, R.G., Varasi, M., Smith, M.C., 1995. Valproic acid: mechanisms of action. In: Levy, R.H., Mattson, R.H., Meldrum, B.S. (Eds.), Antiepileptic Drugs, 4th ed. Raven Press, New York, NY, pp. 581–588.

Ferrie, C.D., Panayiotopoulus, C.P., 1994. Therapeutic interactions of lamotrigine and sodium valproate in intractable myoclonic epilepsy. Seizure 3, 157–159.

Ferrie, C.D., Robinson, R.O., Knott, C., Panayiotopoulos, C.P., 1995. Lamotrigine as an add-on drug in typical absence seizures. Acta Neurol. Scand. 91, 200–202.

Fraser, A.D., MacNeil, W., Isner, A.F., Camfield, P.R., 1995. Lamotrigine analysis in serum by high-performance liquid chromatography. Ther. Drug Monit. 17, 174–178.

Fraser, C.M., Sills, G.J., Forrest, G., Thompson, G.G., Brodie, M.J., 1999. Neurochemical studies with the anticonvulsant felbamate in mouse brain. Pharmacol. Res. 40, 257–261.

French, J., Smith, M., Faught, E., Brown, L., 1999. Practice advisory: the use of felbamate in the treatment of patients with intractable epilepsy. Neurology 52, 1540–1545.

Frey, H.H., Bartels, I., 1997. Felbamate and meprobamate: a comparison of their anticonvulsant properties. Epilepsy Res. 27, 151–164.

Gordon, R., Gels, M., Wichmann, J.K., Diamantis, W., Sofia, R.D., 1993. Interaction of felbamate with several other antiepileptic drugs against seizures induced by maximal electroshock in mice. Epilepsia 34, 367–371.

Lamb, R.J., Miller, A.A., 1985. Effect of lamotrigine and some known anticonvulsant drugs on visually-evoked after-discharge in the conscious rat. Br. J. Pharmacol. 86, 765P (Abstract).

Leach, J.P., 1997. Polypharmacy with anticonvulsants: focus on synergism. CNS Drugs 8, 366–375.

Leach, J.P., Sills, G.J., Butler, E., Forrest, G., Thompson, G.G., Brodie, M.J., 1997. Neurochemical actions of gabapentin in mouse brain. Epilepsy Res. 27, 175–180.

Levy, R.H., Bourgeois, B.F.D., 1997. Drug–drug interactions. In: Engel Jr., J., Pedley, T.A. (Ed.), Epilepsy: A Comprehensive Textbook. Lippincott-Raven, Philadelphia, pp. 1175–1179.

Litchfield, J.T., Wilcoxon, F., 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96, 99–113.

Löscher, W., Fassbender, C.P., Gram, L., Gramer, M., Hörstermann, D., Zahner, B., Stefan, H., 1993. Determination of GABA and vigabatrin in human plasma by a rapid and simple HPLC method: correlation between clinical response to vigabatrin and increase in plasma GABA. Epilepsy Res. 14, 245–255.

Macdonald, R.L., 1999. Cellular actions of antiepileptic drugs. In: Eadie, M.J., Vajda, F.J.E. (Eds.), Antiepileptic Drugs. Pharmacology and Therapeutics Springer-Verlag, Berlin, pp. 123–149.

McCabe, P.H., Michel, N.C., McNew, C.D., 1998. Clinical efficacy of combination therapy with valproic acid and lamotrigine in intractable epilepsy: high success rate with low incidence of rash. Epilepsia 39, 49 (Abstract).

McCabe, P.H., McNew, C.D., Michel, N.C., 2001. Effect of divalproex–lamotrigine combination therapy in frontal lobe seizures. Arch. Neurol. 58, 1264–1268.

Meldrum, B.S., Leach, M., 1994. The mechanism of action of lamotrigine. Rev. Contemp. Pharmacother. 5, 107–114.

Miller, A.A., Wheatley, P., Sawyer, D.A., Baxter, M.G., Roth, B., 1986. Pharmacological studies on lamotrigine, a novel potential antiepileptic drug: I. Anticonvulsant profile in mice and rats. Epilepsia 27, 483–489.

Panayiotopoulos, G.P., Ferrie, C.D., Knott, C., Robinson, R.O., 1993. In-

teraction of lamotrigine with sodium valproate. Lancet 341, 445 (Letter).
Pellock, J.M., 1999. Felbamate. Epilepsia 40 (Suppl. 5), S57–S62.
Pisani, F., Di Perri, R., Perucca, E., Richens, A., 1993. Interaction of lamotrigine with sodium valproate. Lancet 341, 1224 (Letter).
Pisani, F., Oteri, G., Russo, M.F., Di Perri, R., Perucca, E., Richens, A., 1999. The efficacy of valproate–lamotrigine comedication in refractory complex partial seizures: evidence for a pharmacodynamic interaction. Epilepsia 40, 1141–1146.
Privitera, M., Welty, T.E., 1998. Lamotrigine combined with valproate for idiopathic generalized epilepsy. Epilepsia 39, 52 (Abstract).
Rowan, A.J., Meijer, J.W.A., de Beer-Pawlikowski, N., van der Geest, P., Meinardi, H., 1983. Valproate–ethosuximide combination therapy for refractory absence seizures. Arch. Neurol. 40, 797–802.
Sayin, Ü., Cengiz, S., Altug, T., 1993. Vigabatrin as an anticonvulsant against pentylenetetrazole seizures. Pharmacol. Res. 28, 325–331.
Smith, S.E., al-Zubaidy, Z.A., Chapman, A.G., Meldrum, B.S., 1993. Excitatory amino acids, lamotrigine and BW 1003C87 as anticonvulsants in genetically epilepsy-prone rat. Epilepsy Res. 15, 101–111.
Sofia, R.D., 1995. Felbamate: mechanisms of action. In: Levy, R.H., Mattson, R.H., Meldrum, B.S. (Eds.), Antiepileptic Drugs, 4th ed. Raven Press, New York, NY, pp. 791–797.
Swinyard, E.A., Sofia, R.D., Kupferberg, H.J., 1986. Comparative anticonvulsant activity and neurotoxicity of felbamate and four prototype antiepileptic drugs in mice and rats. Epilepsia 27, 27–34.
Turnell, D.C., Cooper, J.D.H., 1982. Rapid assay for amino acids in serum or urine by pre-column derivatization and reversed-phase liquid chromatography. Clin. Chem. 28, 527–531.
Valdizán, E.M., Armijo, J.A., 1992. Effects of single and multiple increasing doses of vigabatrin on brain GABA metabolism and correlation with vigabatrin plasma concentration. Biochem. Pharmacol. 43, 2143–2150.
Waldmeier, P.C., Martin, P., Stocklin, K., Portet, C., Schmutz, M., 1996. Effect of carbamazepine, oxcarbazepine and lamotrigine on the increase in extracellular glutamate elicited by veratridine in rat cortex and striatum. Naunyn-Schmiedeberg's Arch. Pharmacol. 354, 154–162.
Wheatley, P.L., Miller, A.A., 1989. Effects of lamotrigine on electrically induced afterdischarge duration in anaesthetised rat, dog, and marmoset. Epilepsia 30, 34–40.
White, H.S., Wolf, H.H., Woodhead, J.H., Kupferberg, H.J., 1998. The National Institutes of Health Anticonvulsant Drug Development Program: screening for efficacy. In: French, J., Leppik, I., Dichter, M.A. (Eds.), Antiepileptic Drug Development. Advances in Neurology, vol. 76. Lippincott-Raven, Philadelphia, pp. 29–39.
Yamaguchi, S., Rogawski, M.A., 1992. Effects of anticonvulsant drugs on 4-aminopyridine-induced seizures in mice. Epilepsy Res. 11, 9–16.