Effects of three calcium channel antagonists (amlodipine, diltiazem and verapamil) on the protective action of lamotrigine in the mouse maximal electroshock-induced seizure model

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Abstract:
The aim of this study was to assess the effect of three calcium channel antagonists (amlodipine, diltiazem and verapamil) on the anticonvulsant action of lamotrigine (a second generation antiepileptic drug) against maximal electroshock-induced seizures in mice. Results indicated that all three calcium channel antagonists when administered alone [amlodipine (up to 20 mg/kg, ip), diltiazem (up to 10 mg/kg, ip) and verapamil (up to 20 mg/kg, ip)], did not significantly affect the threshold for maximal electroconvulsions in mice. However, amlodipine at a non-protective dose of 20 mg/kg, ip significantly enhanced the anticonvulsant activity of lamotrigine in the maximal electroshock-induced seizure test in mice by reducing its ED₅₀ value from 6.33 to 2.87 mg/kg (p < 0.05). In contrast, amlodipine at lower doses of 5 and 10 mg/kg, ip, diltiazem (at doses up to 10 mg/kg, ip) and verapamil (at doses up to 20 mg/kg, ip) had no significant impact on the antiseizure action of lamotrigine in the maximal electroshock-induced seizure test in mice. In conclusion, one can ascertain that the favorable combination of lamotrigine with amlodipine deserves more attention from a clinical viewpoint because of the enhanced antiseizure action of lamotrigine.

Key words: amlodipine, calcium channel antagonists, diltiazem, lamotrigine, maximal electroshock seizure test, pharmacodynamic interaction, verapamil

Introduction

Overwhelming evidence indicates that calcium ions (Ca²⁺) play an essential role in the pathophysiology of epilepsy. During seizures one can observe a decrease in the extracellular calcium concentrations prior to onset of seizure activity followed by an increase in the intracellular calcium concentrations [14]. Moreover, some calcium channel antagonists reduce the incidence of seizures and possess anticonvulsant proper-
ties in various experimental seizure models [9, 21]. It has been proven that some dihydropyridine derivatives were effective in the maximal electroshock [37], pentylenetetrazole [38], picrotoxin [46], N-methyl-D-aspartic acid [19], pilocarpine [36], amygdala-kindling [48], and sound-induced seizure [11] models in rodents. Interestingly, calcium channel antagonists readily penetrating into the brain potentiated the protective efficacy of some antiepileptic drugs in both, preclinical studies on animals and clinical trials in humans. Several reports revealed beneficial effects of some calcium channel antagonists (i.e., flunarizine, cinnarizine, and nimodipine) as add-on treatment in epileptic patients [2, 10, 40, 41, 43]. Therefore, one can suggest that the use of calcium channel antagonists as add-on treatment in epilepsy has rationally been proven. In preclinical studies, it has been found that diltiazem at a dose of 1.25 mg/kg markedly potentiated the protective action of carbamazepine and phenytoin, but not that of phenobarbital and valproate in the maximal electroshock-induced seizure test [8]. Only, diltiazem at a higher dose of 2.5 mg/kg significantly potentiated the efficacy of all four conventional antiepileptic drugs against maximal electroshock-induced seizures [8]. Similarly, amlodipine at 5 mg/kg significantly enhanced the antiseizure action of carbamazepine, but not that of valproate, phenobarbital and phenytoin in the maximal electroshock-induced seizure test in mice [18]. Amlodipine at a higher dose of 10 mg/kg was required to considerably potentiate the antielectroshock action of carbamazepine, valproate and phenobarbital, but not that of phenytoin in mice [18]. On the contrary, verapamil at up to 10 mg/kg had no effect on the protective action of carbamazepine, phenytoin, phenobarbital, and valproate against maximal electroshock-induced seizures in mice [8].

Generally, it is thought that the blockade of high voltage-activated (L-, N-, P/Q-type) calcium channels is associated with control of partial seizures with or without secondary generalization [14, 21, 37]. There is no doubt that a reduced release of neurotransmitters, including glutamate, is one of consequences of calcium channel blockade [14, 16, 37]. Considering the fact that seizure activity depends on calcium ions [16] and some antiepileptic drugs interfere with calcium ion fluxes [2, 10, 14, 21, 37, 40, 41, 43], we attempted to study the effects of three calcium channel antagonists (amlodipine, diltiazem and verapamil) on the protective activity of lamotrigine in the mouse maximal electroshock-induced seizure model.

Lamotrigine is a second generation antiepileptic drug, licensed as add-on treatment for adults with refractory epilepsy and as monotherapy in newly diagnosed epilepsy (especially, in patients with generalized tonic-clonic seizures and partial convulsions with or without secondary generalization) [4]. The drug blocks the slow-inactivated state of voltage-dependent sodium channels, thereby preventing the presynaptic release of the excitatory neurotransmitter glutamate [7]. Lamotrigine blocks also veratridine-evoked, but not potassium-elicited release of endogenous glutamate [22]. It decreases voltage-gated calcium currents [44], and probably this effect greatly contributes to a decrease in glutamate release.

Generally, it is widely accepted that the maximal electroshock-induced seizure test is an experimental animal model, allowing for the preselection of drugs that are effective in suppression of generalized tonic-clonic seizures, and, to a certain extent, of partial seizures with or without secondary generalization [24]. Thus, it was appropriate to examine the anticonvulsant effects of lamotrigine administered alone and in combination with three calcium channel antagonists in the maximal electroshock-induced seizure test. Moreover, the acute adverse-effect potentials of lamotrigine in combination with amlodipine, diltiazem and verapamil were determined in the chimney test (motor performance), step-through passive avoidance task (long-term memory) and the grip-strength test (muscular strength) in mice. In order to confirm or exclude pharmacokinetic characteristics of interactions between lamotrigine and calcium channel antagonists, total brain lamotrigine concentrations were measured with high-pressure liquid chromatography (HPLC) technique.

Materials and Methods

Animals and experimental conditions

Adult male Swiss mice (weighing 22–26 g) that were kept in colony cages with free access to food and tap water, under standardized housing conditions (natural light-dark cycle, temperature of 23 ± 1°C, relative humidity of 55 ± 5%), were used. After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups comprising...
each 8 mice. Each mouse was used only once and all tests were performed between 08.00 and 15.00 h. Procedures involving animals and their care were conducted in accordance with current European Community and Polish legislation on animal experimentation. Additionally, all efforts were made to minimize animals’ suffering and to use only the number of animals necessary to produce reliable scientific data. The experimental protocols and procedures described in this manuscript were approved by the First Local Ethics Committee in Lublin (License nr. 516/2005/550/2005) and complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Drugs

The following drugs were used in this study: amlodipine (Adamed, Pienkow, Poland), diltiazem (Polfarmex, Kutno, Poland), lamotrigine (Glaxo Wellcome, Kent, UK), and verapamil (Abbott GmbH & Co. KG, Ludwigshafen, Germany). All four drugs were suspended in a 1% aqueous solution of Tween 80 (Sigma, St. Louis, MO, USA) and administered intraperitoneally (ip), in a volume of 5 ml/kg of body weight. Fresh drug solutions were prepared on each day of experimentation and administered as follows: amlodipine was administered 120 min; diltiazem and lamotrigine – 60 min; and verapamil – 30 min, before electroconvulsions. Motor coordination, grip-strength and long-term memory tests, were carried out before brain sampling for the measurement of lamotrigine concentrations. These pretreatment times were based upon information about their biological activity from the literature [8, 17, 18]. The time to the peak of maximum anticonvulsant effects for lamotrigine was used as the reference time in all behavioral tests and pharmacokinetic estimation of brain lamotrigine concentrations.

Maximal electroconvulsions

Electroconvulsions were produced by a current (0.2 s stimulus duration) delivered via ear-clip electrodes by a Rodent Shocker generator (constant-current stimulator Type 221, Hugo Sachs Elektronik, Freiburg, Germany). The criterion for the occurrence of seizure activity was the tonic hind limb extension (i.e. the hind limbs of animals outstretched 180° to the plane of the body axis). In this experiment, two experimental models of maximal electroconvulsions were used:

1) maximal electroshock seizure threshold test
2) maximal electroshock seizure test.

Maximal electroshock seizure threshold test

To evaluate the threshold for maximal electroconvulsions, at least 4 groups of mice, consisting of 8 animals per group, were challenged with electroshocks of various current intensities ranging between 5 and 9 mA, to yield 10–30%, 30–50%, 50–70%, and 70–90% of animals with seizures. Then, a current intensity vs. response curve was constructed, according to a log-probit method by Litchfield and Wilcoxon [23], from which a median current strength (CS50 in mA) was calculated. Each CS50 value represents the current intensity required to induce tonic hindlimb extension in 50% of the mice challenged. Again, after administration of a single dose of each calcium channel antagonist (amlodipine, diltiazem, and verapamil) separately to 4 groups of animals, the mice were subjected to electroconvulsions (each group with a constant current intensity ranging between 5 and 9 mA). The threshold for maximal electroconvulsions was recorded for 3 different doses of amlodipine (5, 10 and 20 mg/kg), diltiazem (2.5, 5, and 10 mg/kg) and verapamil (5, 10 and 20 mg/kg). The experimental procedure has been described in more detail in our earlier papers [25, 26, 30, 32, 33].

Maximal electroshock seizure test

The protective activity of lamotrigine was determined as its median effective dose (ED50 value in mg/kg) against maximal electroshock-induced seizures (0.2 s stimulus duration and fixed current intensity of 25 mA). The animals were administered different drug doses so as to obtain a variable percentage of protection against maximal electroshock seizures, allowing for the construction of a dose-response curve for lamotrigine administered alone, according to Litchfield and Wilcoxon [23]. The ED50 value represents the dose of a drug required to protect half of the animals tested against maximal electroshock seizures. Similarly, the anticonvulsant activity of mixtures of lamotrigine with calcium channel antagonists (amlodipine, diltiazem and verapamil), when these drugs were co-administered with lamotrigine, was evaluated and expressed as ED50, corresponding to the dose of lamotrigine necessary to protect 50% of mice against tonic hindlimb extension in the maximal electroshock sei-
In the present study, lamotrigine was administered at doses ranging between 3 and 9 mg/kg. This experimental procedure has been described in detail in our earlier papers [26, 27, 29, 30, 33].

**Chimney test**

The chimney test of Boissier et al. [3] was used to quantify the adverse effect potential of lamotrigine administered in combination with amlodipine, diltiazem or verapamil on motor performance in mice. In this test, the animals had to climb backwards up a plastic tube (3 cm inner diameter, 25 cm length), and motor performance impairment was indicated by the inability of the mice to climb backward up the transparent tube within 60 s. The adverse effect potential of lamotrigine co-administered with calcium channel antagonists (amlodipine, diltiazem or verapamil) was determined for drugs administered at doses corresponding to their ED$_{50}$ values from the maximal electroshock seizure test. This experimental procedure has been described in detail in our earlier papers [25, 30, 32, 33].

**Grip-strength test**

The effects of combinations of lamotrigine with amlodipine, diltiazem or verapamil at their ED$_{50}$ values from the maximal electroshock seizure test, on muscular strength in mice were quantified by the grip-strength test. The time before the commencement of the grip-strength test (after drug administration) was identical to that for the maximal electroshock seizure test. The grip-strength apparatus (BioSeb, Chaville, France) comprised a wire grid (8 × 8 cm) connected to an isometric force transducer (dynamometer). The mice were lifted by the tails so that their forepaws could grasp the grid. The mice were then gently pulled backward by the tail until the grid was released. The maximal force exerted by the mouse before losing grip was recorded. A mean of 3 measurements for each animal was calculated and subsequently, the mean maximal force of 8 animals per group was determined. The muscular strength in mice was expressed in N (newtons) as the mean ± SD of 8 animals per group. This experimental procedure has been described in detail in our earlier papers [26, 28].

**Light-dark, step-through passive avoidance task**

Each animal was administered amlodipine, diltiazem or verapamil with lamotrigine at doses corresponding to its ED$_{50}$ values from the maximal electroshock seizure test on the first day before training. The time before the commencement of the training session (after drug administration) was identical to that for the maximal electroshock seizure test. Subsequently, animals were placed in an illuminated box (10 × 13 × 15 cm) connected to a larger dark box (25 × 20 × 15) equipped with an electric grid floor. Entrance of animals to the dark box was punished by an adequate electric footshock (0.6 mA for 2 s). The animals that did not enter the dark compartment were excluded from subsequent experimentation. On the following day (24 h later), the pre-trained animals were placed again into the illuminated box and observed for up to 180 s. Mice that avoided the dark compartment for 180 s were considered to remember the task. The time that the mice took to enter the dark box, was recorded and the median latencies (retention times) with 25th and 75th percentiles were calculated. The step-through passive avoidance task gives information about ability to acquire the task (learning) and to recall the task (retrieval). Therefore, it may be regarded as a measure of long-term memory [47]. This experimental procedure has been described in detail in our earlier papers [30, 35].

**Measurement of total brain lamotrigine concentration**

The measurement of total brain concentration of lamotrigine was undertaken at a dose, which corresponded to its ED$_{50}$ value from the maximal electroshock seizure test for the combination of lamotrigine with amlodipine. Mice were killed by decapitation at times chosen to coincide with that scheduled for the maximal electroshock seizure test and the whole brains of mice were removed from skulls, weighed, harvested and homogenized using Abbott buffer (1:2 w/v) in an Ultra-Turrax T8 homogenizer (IKA-Werke, Staufen, Germany). The homogenates were centrifuged at 10,000 × g for 10 min. The supernatant samples (100 µl) were analyzed by HPLC for lamotrigine content. The chromatograph (Laboratorij Pristroje, Praha, Czech Republic) was equipped with a 305 micropump (LCP 3001) and an ultraviolet (UV) detector (HP 1050) with a sensitivity setting of 0.1 absorbance units full scale (AUFS) and a time constant of 0.1 s. The Rheodyne 7125 injector valve with a 100 ml sample loop
was used for sample injection. For HPLC, a stainless steel HP ODS column (200 × 4.6 mm) was used at an ambient temperature of 22°C. The mobile phase was methanol:acetonitrile:citrate buffer (20 mM citric acid/40 mM sodium citrate); 330:90:580 vol/vol/vol (Baker HPLC grade). The mobile phase flow rate was 1 ml/min. Supernatant samples of 200 μl were added to 200 μl of water and 100 μl of methanol : water solution; 1:1. The solutions were evaporated to dryness under a vacuum system and redissolved in 1 ml of tert-buthyl-methyl ether (HPLC, Aldrich) and again evaporated to dryness under a vacuum system. The residue was redissolved in 4 ml of tert-buthyl-methyl ether; samples of 50 μl were then injected into the chromatograph. Lamotrigine concentrations were calculated according to the external standard method using the original Gilson 715 software. The amount of lamotrigine was determined by comparing its peak area with the peak area of the external standard [7-acetyl-5-(4-aminophenyl)-8,9-dihydro-7H-1,3-dioxolo(4,5H)-2,3-benzodiazepine]. Stock solutions of lamotrigine serving as internal standards (0.2 : 0.6 : 1.2 : 2.4 : 4.8 mg/ml) were prepared in mobile phase. They were placed at the beginning and end of each measurement sequence. The wave excitation and emission parameters for the detection of lamotrigine were 270 and 310 nm, respectively. The elution parameter for lamotrigine was 1 ml/min. Total brain concentrations of lamotrigine were expressed in μg/g of wet brain tissue as the means ± SD of 8 separate brain preparations.

Statistical analysis

Both, CS\textsubscript{50} and ED\textsubscript{50} values with their 95% confidence limits were calculated by computer log-probit analysis according to Litchfield and Wilcoxon [23]. Subsequently, the respective 95% confidence limits were transformed into standard errors (SE), as described previously [25, 32]. Statistical analysis of the data from electroconvulsive tests was performed by one-way analysis of variance (ANOVA) followed by the post-hoc Tukey-Kramer test for multiple comparisons [15]. Qualitative variables from the chimney test were compared by the use of the Fisher’s exact probability test, whereas the results obtained in the passive avoidance task were statistically evaluated using Kruskal-Wallis nonparametric ANOVA. The results from the grip-strength test were verified by one-way ANOVA. Total brain lamotrigine concentrations were statistically compared using the unpaired Student’s t-test. Differences among values were considered statistically significant if p < 0.05. All statistical tests were performed using GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA, USA).

Results

Influence of three calcium channel antagonists (amlodipine, diltiazem and verapamil) on the threshold for electroconvulsions

Amlodipine administered systemically (ip) at 120 min before the test at doses of 5, 10 and 20 mg/kg did not affect significantly the threshold for electroconvulsions (Tab. 1). Similarly, diltiazem at doses of 2.5, 5 and 10 mg/kg administered ip, at 60 min before the test, had no significant impact on the threshold for electroconvulsions in mice (Tab. 1). Likewise, the last calcium channel antagonist tested in this study – verapamil – administered alone at 30 min before the threshold for electroconvulsions, at doses of 5, 10 and 20 mg/kg did not affect the threshold for electroconvulsions in mice (Tab. 1).

Effects of amlodipine, diltiazem and verapamil on the protective action of lamotrigine in the mouse maximal electroshock-induced seizure model

Lamotrigine administered alone (ip) produced clearcut anticonvulsant effects against maximal electroshock-induced seizures in mice and its ED\textsubscript{50} value is presented in Table 2. Amlodipine co-administered with lamotrigine enhanced, in a dose-dependent manner, the antielectroshock action of lamotrigine by reducing its ED\textsubscript{50} value in the maximal electroshock-induced seizures. One-way ANOVA followed by the post-hoc Tukey-Kramer test for multiple comparisons revealed that amlodipine at a dose of 20 mg/kg significantly decreased the ED\textsubscript{50} value of lamotrigine from 6.33 to 2.87 mg/kg (p < 0.05; Tab. 2). Amlodipine at lower doses of 5 and 10 mg/kg also reduced the ED\textsubscript{50} value of lamotrigine from 6.33 to 4.72 and 3.76 mg/kg, respectively (Tab. 2). However, in these cases, the differences did not reach statistical analysis when tested with one-way ANOVA. With respect to diltiazem, the drug at doses of 2.5, 5, and 10 mg/kg co-
administered with lamotrigine had no significant impact on the antiseizure action of the latter drug in the maximal electroshock-induced seizure test in mice. It was found that diltiazem at doses of 5 and 10 mg/kg decreased the ED50 value of lamotrigine from 6.33 to 5.19 and 4.00 mg/kg, although, statistical evaluation of the data revealed no significance among the compared ED50 values (Tab. 2). Likewise, verapamil at doses of 5, 10 and 20 mg/kg did not alter significantly the protective action of lamotrigine in the maximal electroshock-induced seizure test in mice, although it reduced the ED50 of lamotrigine from 6.33 to 6.13, 6.03 and 4.37 mg/kg, respectively (Tab. 2).

Effects of lamotrigine in combination with amlodipine, diltiazem and verapamil on motor performance, long-term memory, and muscular strength of animals in the chimney, step-through passive avoidance and grip-strength tests

When lamotrigine was administered in combination with amlodipine, diltiazem and verapamil at doses corresponding to its ED50 from the maximal electroshock seizure test, motor performance as assessed by the chimney test was unaffected (Tab. 3). Furthermore, none of the combinations of lamotrigine with the calcium channel antagonists studied impaired long-term memory as determined in the passive avoidance test, the median retention times being approximately 180 s (Tab. 3). Likewise, lamotrigine combined with amlodipine, diltiazem and verapamil had no significant impact on muscular strength of animals as assessed by the grip-strength test (Tab. 3).

Influence of amlodipine on total brain concentration of lamotrigine

With HPLC technique, the total brain concentration of lamotrigine administered alone (at a dose of 2.9 mg/kg) was $3.780 \pm 0.654 \mu g/g$ of wet brain tissue, and did not differ significantly from that for the combination of lamotrigine (2.9 mg/kg) with amlodipine (20 mg/kg),

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>CS50 (mA)</th>
<th>n</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>6.67 (5.98 – 7.44)</td>
<td>16</td>
<td>0.371</td>
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<tr>
<td>Amlodipine (5)</td>
<td>6.78 (6.07 – 7.57)</td>
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<td>Amlodipine (10)</td>
<td>7.11 (6.30 – 8.04)</td>
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<td>0.443</td>
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<tr>
<td>Amlodipine (20)</td>
<td>8.00 (7.16 – 8.96)</td>
<td>32</td>
<td>0.459</td>
</tr>
<tr>
<td>F (3, 84) = 1.980; p = 0.1233</td>
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<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>CS50 (mA)</th>
<th>n</th>
<th>SE</th>
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<tbody>
<tr>
<td>Vehicle</td>
<td>6.67 (5.98 – 7.44)</td>
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<td>0.371</td>
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<tr>
<td>Diltiazem (2.5)</td>
<td>5.96 (5.11 – 6.94)</td>
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<td>0.465</td>
</tr>
<tr>
<td>Diltiazem (5)</td>
<td>5.70 (4.59 – 7.07)</td>
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<tr>
<td>Diltiazem (10)</td>
<td>5.74 (4.78 – 6.88)</td>
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<tr>
<td>F (3, 84) = 0.5768; p = 0.6319</td>
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<tr>
<th>Treatment (mg/kg)</th>
<th>CS50 (mA)</th>
<th>n</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>6.67 (5.98 – 7.44)</td>
<td>16</td>
<td>0.371</td>
</tr>
<tr>
<td>Verapamil (5)</td>
<td>6.52 (5.54 – 7.67)</td>
<td>24</td>
<td>0.542</td>
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<tr>
<td>Verapamil (10)</td>
<td>6.45 (5.63 – 7.39)</td>
<td>16</td>
<td>0.448</td>
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<tr>
<td>Verapamil (20)</td>
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<td>0.397</td>
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<tr>
<td>F (3, 68) = 0.2124; p = 0.8875</td>
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</table>

Results are presented as median current strengths (CS50 in mA; with 95% confidence limits in parentheses) required to produce tonic hindlimb extension in 50% of animals tested. The CS50 values were calculated using the log-probit method [23], followed by the method transforming 95% confidence limits into SE [25, 32]. Amlodipine was administered ip at 120 min, diltiazem –at 60 min, and verapamil –at 30 min before the threshold test for electroconvulsions. Statistical analysis of data was performed by one-way ANOVA. n – number of animals at those current strengths, whose convulsive effects ranged between 16% and 84% (4 and 6 probals); SE – standard error of CS50 values.

Pharmacological Reports, 2007, 59, 673-692 677
which amounted to 3.984 ± 0.774 μg/g of wet brain tissue (results not shown).

Discussion

In the present study, it was found that amlodipine (up to 20 mg/kg), diltiazem (up to 10 mg/kg) and verapamil (up to 20 mg/kg) did not affect the electroconvulsive threshold in mice and these findings are partially in agreement with those documented earlier [17, 18]. Moreover, amlodipine at a non-protective dose of 20 mg/kg significantly enhanced the antiseizure action of lamotrigine, whereas, diltiazem (up to 10 mg/kg) and verapamil (up to 20 mg/kg) had no effect on the protective activity of lamotrigine in the mouse maximal electroshock-induced seizure model. However, this enhancement appeared at much higher doses than in the case of the conventional antiepileptic drugs. As documented earlier, amlodipine at a dose of 10 mg/kg potentiated the anticonvulsant action of carbamazepine, phenobarbital and valproate, but not that of phenytoin in the maximal electroshock-induced seizures in mice [18]. Similarly, diltiazem at a dose of 1.25 mg/kg enhanced the antiseizure action of carbamazepine and phenytoin, but not that of phenobarbital and valproate in the maximal electroshock-induced seizure test in mice [8]. Only verapamil at doses up to 20 mg/kg did not affect the antielectroshock action of conventional antiepileptic drugs against maximal electroshock-induced seizures in mice [8, 17]. Noteworthy, in the present study, the ED₅₀ values of lamotrigine administered alone and in combination with amlodipine, diltiazem and verapamil were statistically

### Tab. 2. Influence of amlodipine, diltiazem and verapamil on the anticonvulsant activity of lamotrigine in the mouse maximal electroshock seizure model

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>ED₅₀ (mg/kg)</th>
<th>n</th>
<th>SE</th>
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<tbody>
<tr>
<td>Lamotrigine + vehicle</td>
<td>6.33 (4.49 – 8.92)</td>
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<td>1.107</td>
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<td>Lamotrigine + Amlodipine (5)</td>
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<td>Lamotrigine + vehicle</td>
<td>6.33 (4.49 – 8.92)</td>
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<td>1.107</td>
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<tr>
<td>Lamotrigine + Diltiazem (2.5)</td>
<td>6.46 (5.14 – 8.11)</td>
<td>16</td>
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<tr>
<td>Lamotrigine + Diltiazem (5)</td>
<td>5.19 (4.45 – 6.04)</td>
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<td>0.403</td>
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<tr>
<td>Lamotrigine + Diltiazem (10)</td>
<td>4.00 (2.64 – 6.07)</td>
<td>16</td>
<td>0.850</td>
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<tr>
<td>Lamotrigine + vehicle</td>
<td>6.33 (4.49 – 8.92)</td>
<td>24</td>
<td>1.107</td>
</tr>
<tr>
<td>Lamotrigine + Verapamil (5)</td>
<td>6.13 (4.71 – 7.96)</td>
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<td>24</td>
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</tbody>
</table>

Results are presented as median effective doses (ED₅₀ in mg/kg; with 95% confidence limits in parentheses) required to protect 50% of animals tested against maximal electroshock-induced seizures. The ED₅₀ values were calculated by the use of log-probit method [23], followed by the method transforming 95% confidence limits into SE [25, 32]. Amlodipine, diltiazem, verapamil and lamotrigine were suspended in 1% aqueous solution of Tween 80 and administered systemically (ip), as follows: amlodipine at 120 min, diltiazem and lamotrigine at 60 min, and verapamil at 30 min before maximal electroconvulsions. Statistical analysis of data was performed by one-way ANOVA followed by the post-hoc Tukey-Kramer test for multiple comparisons. n – number of animals at those doses, whose anticonvulsant effects ranged between 16% and 84% (4 and 6 probit); SE – standard error of ED₅₀ values; *p < 0.05 vs. the respective control group (lamotrigine + vehicle-treated animals).
analyzed and compared by one-way ANOVA followed by the post-hoc Tukey-Kramer test for multiple comparisons. In contrast, the results presented in the previous studies were compared separately to their control ED$_{50}$ values using log-probit method only [8, 17, 18]. Quite recently, it has been documented that statistical analysis of data obtained from log-probit method should be performed by one-way ANOVA followed by the post-hoc test for multiple comparisons and this method has gained priority over statistical analysis with log-probit method only [27]. The crucial differences between these two statistical methods have been presented and discussed in more detail elsewhere [27]. This is why, in this study, amlodipine was administered at doses up to 20 mg/kg, whereas in that by Kaminski et al. [18], amlodipine was administered at doses up to 10 mg/kg.

To explain the appearance of the favorable interaction between lamotrigine and amlodipine in the maximal electroshock-induced seizure test, one should consider molecular mechanisms of action of both drugs. As regards the anticonvulsant effect of lamotrigine, it has been documented that the drug acts at voltage-dependent sodium channels to decrease the presynaptic release of the excitatory neurotransmitter glutamate [7]. Lamotrigine blocks veratridine-evoked, but not potassium-elicited release of endogenous glutamate [22]. Moreover, lamotrigine decreases voltage-gated calcium currents [44], and probably, this effect contributes also to a decrease in glutamate release. On the other hand, experimental evidence indicates that N-type calcium channels are responsible for glutamate release in the cerebral cortex and hippocampus [45]. It is noteworthy that calcium current through the N-type channel accounted for 20% of total inward calcium current in isolated cortical neurons obtained from epileptic patients [1, 42]. As to amlodipine, the drug belongs to the 1,4-dihydropyridine class of calcium channel antagonists [5], and it blocks N- and P/Q-type calcium channels, showing high affinity for calcium channel antagonists [5], and it blocks N- and P/Q-type calcium channels, showing high affinity for
enhancement of the anticonvulsant activity of lamotrigine in this study. With respect to verapamil (the calcium channel antagonist that hardly crosses blood-brain barrier and penetrates into the brain very poorly [20]), the drug did not affect the protective action of lamotrigine against maximal electroshock-induced seizures in mice. Similarly, diltiazem did not significantly affect the protective action of lamotrigine against maximal electroshock-induced seizures. Noteworthy, diltiazem and verapamil are considered to be L-type calcium channel antagonists, in contrast to amlodipine, which is considered to be the N- and P/Q-type calcium channel antagonist [39]. Thus, one can suggest that amlodipine through the blockade of N- and P/Q-type calcium channels enhances the effects of lamotrigine related to the reduction of glutamate release from neurons, also probably through N-type calcium channel blockade. This hypothesis can readily explain the observed interaction between lamotrigine and amlodipine in the maximal electroshock-induced seizures in mice.

Furthermore, it was found that none of the calcium channel antagonists examined in this study affected acute adverse-effect liability of lamotrigine in animals challenged with the chimney test, passive avoidance task, and grip-strength test. These observations are partially in contrast to the results shown by Kaminski et al. [18], who have found that amlodipine potentiated the impairment of motor coordination of the animals that received carbamazepine, phenytoin, phenobarbital and valproate in the chimney test. The lack of effect of amlodipine on acute adverse-effect liability of lamotrigine, one can explain through more favorable safety and tolerability profiles of lamotrigine in comparison to conventional antiepileptic drugs used in preclinical studies. This is why, in the present study no acute adverse effects produced by lamotrigine were observed after systemic (ip) amlodipine administration.

With respect to the combination of lamotrigine with amlodipine, the latter drug did not alter total brain lamotrigine concentration and thus, the observed interaction was pharmacodynamic in nature. Previously, it has been documented that amlodipine significantly increased free plasma concentration of carbamazepine, but not that of valproate, phenobarbital and phenytoin in mice [18]. It is worth mentioning that pharmacokinetic evaluation of total brain lamotrigine concentrations in this study provided the exact insight into the nature of observed interaction between drugs in the maximal electroshock-induced seizure test. Relatively recently, it has been demonstrated that only total brain concentrations of antiepileptic drugs exactly and precisely characterize pharmacokinetic interactions between drugs, influencing the central nervous system [6, 34]. It is highly likely that some drugs can markedly change total brain concentrations of antiepileptic drugs, having no impact on free (non-protein bound) plasma concentrations and inversely, some agents can considerably alter free plasma concentrations without any significant changes in total brain concentrations of antiepileptic drugs. For instance, it has been reported that 2-phosphonomethyl-pentanedioic acid significantly elevated total brain valproate concentrations, having had no impact on the free plasma concentrations of valproate [31]. Similarly, tiagabine combined with valproate markedly increased total brain concentrations of the latter drug, having had little effect on the free plasma valproate concentrations [31]. In contrast, loreclezole co-administered with valproate significantly increased the free plasma concentrations of valproate, inducing simultaneously no changes in total brain valproate concentrations [32]. This is why, in the present study, total brain concentrations instead of the free plasma lamotrigine concentrations were evaluated with HPLC technique.

**Conclusions**

Amlodipine enhanced the anticonvulsant action of lamotrigine, produced no acute adverse effects when combined with lamotrigine and had no impact on total brain concentrations of lamotrigine in experimental animals. If the results from this study can be extrapolated to the clinical settings, a novel therapeutic option in the management of epilepsy might be created for epileptic patients. Thus, amlodipine deserves more attention from a preclinical point of view, as a potentially favorable drug that could be applied in patients treated with lamotrigine, who additionally required a calcium channel antagonist treatment for other than epilepsy reasons.

**Acknowledgments:**
This study was supported by a grant (KBN 2P05D 306 29) from the State Committee for Scientific Research, Warszawa, Poland. The authors express their thanks to Mr. W. Zgajek (Institute of Agricultural Medicine, Lublin, Poland) for the skillful determination of the brain concentrations of lamotrigine.
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Received: August 12, 2007; in revised form: November 22, 2007.