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Anticonvulsant and Adverse Effects of MK-801, LY 235959, and GYKI 52466 in Combination with Ca^{2+} Channel Inhibitors in Mice

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GASIOR, M., K. BOROWICZ, R. STAROWNIK, Z. KLEINROK AND S. J. CZUCZWAR. *Anticonvulsant and adverse effects of MK-801, LY 235959, and GYKI 52466 in combination with Ca^{2+} channel inhibitors in mice.* PHARMACOL BIOCHEM BEHAV **56**(4) 629–635, 1997.—This study was designed to investigate the influence of the calcium (Ca^{2+}) channel inhibitors nicardipine, nifedipine, and flunarizine on the protective action of MK-801, LY 235959 [*N*-methyl-D-aspartate (NMDA) receptor antagonists], and GYKI 52466 (a non-NMDA receptor antagonist) against electroconvulsions in mice. Unlike nicardipine (15 mg/kg) or flunarizine (10 mg/kg), nifedipine (7.5 and 15 mg/kg) potentiated the protective potency of MK-801 (0.05 mg/kg), as reflected by significant elevation of the convulsive threshold (a CS_{50} value of the current strength in mA producing tonic hind limb extension in 50% of the animals). The protective activity of LY 235959 and GYKI 52466 was reflected by their ED_{50} values in mg/kg, at which the drugs were expected to protect 50% of mice against maximal electroshock-induced tonic extension of the hind limbs. Nicardipine (3.75–15 mg/kg), nifedipine (0.94–15 mg/kg), and flunarizine (2.5–10 mg/kg) in a dose-dependent manner markedly potentiated the antiseizure efficacy of LY 235959. Flunarizine (5 and 10 mg/kg) was the only Ca^{2+} channel inhibitor to enhance the protective action of GYKI 52466 against electroconvulsions. Except with MK-801 + flunarizine (motor performance) or GYKI 52466 + flunarizine (long-term memory), combination of NMDA or non-NMDA receptor antagonists with Ca^{2+} channel inhibitors produced an impairment of motor performance (evaluated in the chimney test) and long-term memory acquisition (measured in the passive avoidance task) as compared with vehicle treatment.

Calcium channel inhibitors MK-801 LY 235959 GYKI 52466 Seizures

OVER 40 years have passed since Hayashi reported on neuronal excitation of ubiquitous glutamate (14). Selective antagonists and agonists of glutamate receptors have been available since the early 1980s, and they have aided in characterization of the role of excitatory amino acids and excitatory amino acid receptors in the pathogenesis of epilepsy (5). Currently, excitatory amino acids are considered critical in seizure models in vivo and in vitro (23). To date, the clinical approval of excitatory amino acid receptor antagonists in the treatment of epilepsy is, unfortunately, limited by their toxicity, reflected in sedation, myorelaxation, and by impairment of learning and memory (20,30). Combination therapy, perhaps, opens new possibilities for some of the excitatory amino acid receptor antagonists in the treatment of epilepsy and other neurological disorders (23). Moreover, investigators have reported that the *N*-methyl-D-aspartate (NMDA) receptor antagonists, in relatively low doses, potentiate the protective action of carbamazepine, diazepam, valproate, phenobarbital, and diphenylhydantoin against electroconvulsions in mice (6,22,27,32). A similar trend was observed when α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate/kainate (AMPA/kainate, also called non-NMDA) receptor antagonists (GYKI 52466 or NBQX) and conventional antiepileptic drugs were combined to protect mice against maximal electroshock (MES)-evoked seizures (3,33). With some combinations, which might be of particular clinical interest, the observed side effects were reduced as compared

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with antiepileptic drugs alone (3,6,22,32,33). A combined treatment with NMDA and non-NMDA receptor antagonists also provided better protection, compared with that yielded by single drugs, against MES and kindling-induced seizures (7,19).

In the present study, we investigated whether calcium (Ca^{2+}) channel inhibitors may affect the anticonvulsant activity of NMDA and non-NMDA antagonists. The question has been raised by numerous observations that Ca^{2+} ions play a pivotal role in both epileptogenesis and excitatory amino acid-mediated events (13,15,16,18,31). We studied the influence of nicardipine, nifedipine, and flunarizine on the protective action of NMDA (MK-801, LY 235959) and non-NMDA (GYKI 52466) receptor antagonists against electroconvulsions in mice. In parallel, these treatments were evaluated for side effects in the chimney test (motor performance) and in the passive-avoidance task (long-term memory acquisition) (2,28).

METHOD

Animals

Experiments were carried out with male Swiss mice weighing 24–30 g. After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups (10–12 per group). Mice were kept in colony cages with free access to tap water and food pellets and were maintained on a natural light/dark cycle. The experiments were performed between 0800 and 1300 h.

Drugs and Administration Regimen

The following NMDA and non-NMDA antagonists were used throughout the study: MK-801 (dizocilpine maleate), LY 235959 [(−)-3R,4aS,6R,8aR-6-(phosphonomethyl) decahydroisoquinoline-3-carboxylic acid] and GYKI 52466 [1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5-2,3-benzodiazepine]. MK- 801 was commercially obtained from RBI (Natick, MA, USA). LY 235959 and GYKI 52466 were generous gifts from Lilly Research Laboratories (Indianapolis, IN, USA) and the Institute for Drug Research (Budapest, Hungary), respectively. MK-801, LY 235959, and GYKI 52466 were dissolved in distilled water and administered 45, 45, and 15 min prior to testing, respectively.

Two dihydropyridine derivatives, nicardipine and nifedipine (both from Sigma, St. Louis, MO, USA), and a diphenylalkylamine, flunarizine (Polfa, Warsaw, Poland), were used as Ca^{2+} channel inhibitors. All Ca^{2+} channel inhibitors were suspended in a 1% solution of Tween 81 (Loba Chemie, Vienna, Austria). Solutions of nicardipine and nifedipine were handled in darkroom conditions. Pretreatment time for Ca^{2+} channel inhibitors was 60 min. Each compound was administered intraperitoneally (IP) in a volume of 10 ml/kg body weight. When drug combinations were used, mice were treated with a Ca^{2+} channel inhibitor first and then with an excitatory amino acid antagonist at the times scheduled as indicated above.

Electroconvulsions

Electroconvulsions were produced by means of an alternating current generator (Hugo Sachs rodent shocker, type 221, March-Hugstetten, FRG), and delivered via ear-clip electrodes. The stimulus duration was 0.2 s. The end point was the tonic extension of the hind limbs. The threshold currents were used to evaluate the protective action of MK-801. MES was not employed, in order to diminish the dosage of this noncompetitive NMDA receptor antagonist. It is evident that when used at doses effective against maximal electroshock, MK-801 exerts a clear-cut excitation due to its central sympathomimetic activity (4). To evaluate the convulsive threshold (CS_{50}), at least four groups of mice were challenged with electrical shocks of various intensities. An intensity–response curve was subsequently calculated on the basis of the percentage of animals showing the end point. Each CS_{50} value represents the current strength (in mA) necessary to produce tonic hind limb extension in 50% of the mice tested. The convulsive threshold was evaluated in drug-free mice and in those injected with Ca^{2+} channel inhibitors and MK-801 alone or in combination. To assess the anticonvulsive action of LY 235959 and GYKI 52466, reflected by respective ED_{50} values (in mg/kg), MES-induced seizures (fixed current intensity of 25 mA) were employed. A drug, at its ED_{50} value, is considered to protect 50% of mice against the MES-induced tonic hind limb extension. To evaluate each ED_{50} value, at least four groups of mice, after receiving progressive doses of a drug, were challenged with MES. A dose–response curve was subsequently calculated on the basis of the percentage of animals protected against the end point. When CS_{50} or ED_{50} values are mentioned throughout the text, the potentiation of the anticonvulsant action of a compound is reflected by an increase in a CS_{50} value and a decrease in an ED_{50} value, respectively.

Chimney Test

The chimney test of Boissier and coworkers was used to evaluate the influence of excitatory amino acid antagonists alone or in combination with the Ca^{2+} channel inhibitors on motor performance (2). Motor impairment was indicated by the inability of the animals to climb backward up the tube (3 cm inner diameter, 25 cm length) within 60 s. Naive mice were tested in advance, and those unable to perform the task correctly were rejected from the experiment. On the following day, the animals were treated with the compounds either alone or in the respective combinations. The test was performed with the pretreatment times scheduled for the convulsive studies. Results were calculated as the percentage of animals failing to perform the test.

Passive-Avoidance Acquisition and Retention Testing

According to Venault et al. (28), the step-through passive-avoidance task may be identified as a measure of long-term memory acquisition (28). We used this test to compare the effects of MK-801, LY 235959, GYKI 52466, nicardipine, nifedipine, and flunarizine alone or in the respective combinations on passive-avoidance acquisition in mice. The animals were injected with the compounds and, as soon as the scheduled time had elapsed, were put into an illuminated box ($10 \times 13 \times 15$ cm) connected to a large dark box ($25 \times 20 \times 15$ cm) with an electric grid floor. Entrance into the dark box was punished by electric footshock (0.6 mA for 2 s; facilitation of acquisition). The testing of the passive-avoidance acquisition was performed 24 h after the facilitation of acquisition. Mice avoiding the dark compartment for over 60 s showed no long-term memory impairment and were regarded as remembering the task. Retention was expressed as a percentage of mice with no memory impairment.

Statistics

Calculation of CS_{50} and ED_{50} values (with 95% confidence limits) and statistical analysis were performed using computerized probit analysis according to the method of Litchfield and Wilcoxon (17). The results obtained in the chimney and passive

avoidance tests were compared statistically by using Fisher's exact probability test. A probability level of 0.05 was accepted as significant.

RESULTS

Effects of Ca^{2+} Channel Inhibitors on the Anticonvulsive Action of MK-801

In our earlier experiments, nicardipine, nifedipine, and flunarizine were demonstrated to increase dose-dependently the convulsive threshold per se. Specifically, nicardipine (20 mg/kg), nifedipine (20 mg/kg), and flunarizine (15 mg/kg) raised the threshold for electroconvulsions from 6.2 mA to 7.6, 7.4, and 7.4 mA (12,13). To avoid an additive effect, based on these results, nicardipine, nifedipine, and flunarizine were administered up to their maximal ineffective doses (15, 15, and 10 mg/kg, respectively) against electroconvulsions when combined with MK-801, LY 235959, and GYKI 52466.

MK-801 (0.05 and 0.1 mg/kg) significantly elevated the convulsive threshold compared with untreated controls. Specifically, the CS_{50} values were 7.3 mA (6.5–8.3) and 10.8 mA (8.9–13.1) vs. 6.3 mA (6.0–6.6). MK-801 at a dose of 0.025 mg/kg did not modify the convulsive threshold.

Co-administration of either nicardipine or flunarizine (15 or 10 mg/kg, respectively) did not significantly alter the convulsive threshold elevated by MK-801 at 0.05 mg/kg (Fig. 1). In contrast, combined treatment with nifedipine (7.5 and 15 mg/kg) and MK-801 (0.05 mg/kg) resulted in marked potentiation of the protective efficacy of MK-801 against electroconvulsions. This was reflected by a significant increase in the CS_{50} value as a result of a parallel leftward shift of the current strength–response curve for this combination, as shown in Fig. 1. The current intensity–response curves evaluated in mice treated with either MK-801 (0.05 mg/kg) alone or in combination with nicardipine (15 mg/kg), nifedipine (15 mg/kg), and flunarizine (10 mg/kg) are presented in the right section of Fig. 1.

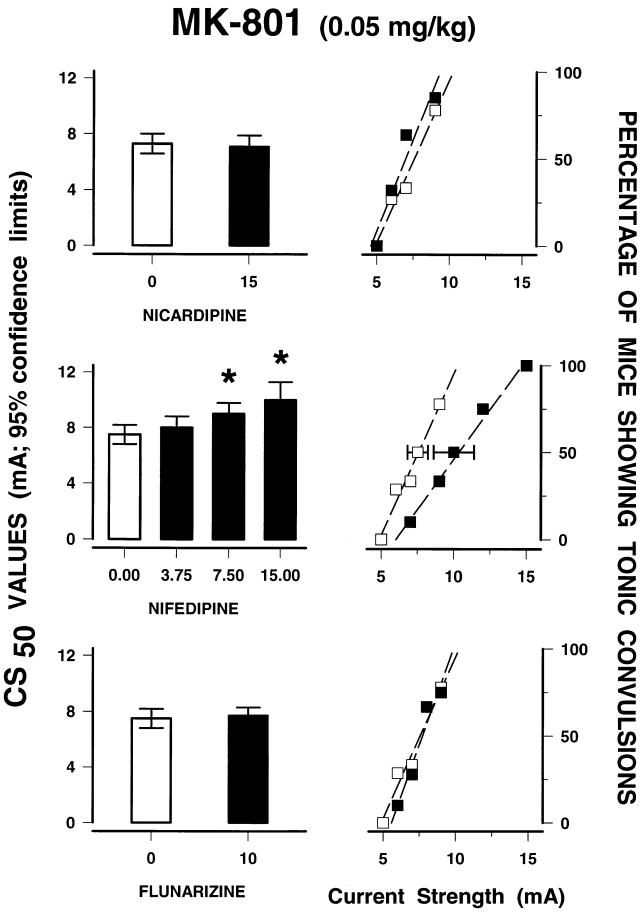

FIG. 1. (Left) Influence of nicardipine (upper section), nifedipine (middle section), and flunarizine (lower section) on the protective activity of MK-801 administered against electroconvulsions at a constant dose of 0.05 mg/kg. Bars represent the convulsive threshold, expressed as CS_{50} value in mA, evaluated in mice pretreated with MK-801 alone (open bars) and in combination with Ca^{2+} channel inhibitors (solid bars) necessary to produce tonic hind limb extension. Doses of Ca^{2+} channel inhibitors are shown below corresponding bars. Vertical error bars represent 95% confidence limits for CS_{50} values. MK-801 was administered 45 min prior to the test. The pretreatment time for Ca^{2+} channel inhibitors was 60 min. Statistical evaluation was carried out according to the method of Litchfield and Wilcoxon (17). *p at least <0.05 was considered to be significantly different from the group treated with MK-801 alone. (Right) Current strength (mA) vs. response (percentage of mice showing tonic convulsions) plots with calculated linear regressions (dashed lines) evaluated in groups treated with MK-801 alone (□) and in combination with nicardipine (15 mg/kg; ■), nifedipine (15 mg/kg; ■), and flunarizine (10 mg/kg; ■), respectively. Each point represents the results obtained from at least 10 mice. Points with horizontal error bars refer to the CS_{50} values with 95% confidence limits, calculated in groups treated with MK-801 alone (□) and MK-801 + nifedipine (■), according to the method of Litchfield and Wilcoxon (17).

Influence of Ca^{2+} Channel Inhibitors on the Protection Offered by LY 235959 and GYKI 52466 Against MES-Induced Seizures

The Ca^{2+} channel inhibitors differently affected the protective action of LY 235959 and GYKI 52466 against MES-induced seizures. The anticonvulsive efficacy of LY 235959 was markedly enhanced, as reflected by a decrease in its ED_{50} values, by nicardipine (3.75–15 mg/kg), nifedipine (0.94–15 mg/kg), and flunarizine (2.5–10 mg/kg) (Fig. 2). In the case of GYKI 52466, its antiseizure efficacy was enhanced only by flunarizine at doses of 5 and 10 mg/kg (Fig. 3). Nicardipine (15 mg/kg) and nifedipine (15 mg/kg) did not affect the protection offered by GYKI 52466 in this test (Fig. 3). Figures 2 and 3 show the ED_{50} values (with 95% confidence limits) and the dose–response curves evaluated for all combinations.

Chimney Test

Nicardipine (15 mg/kg), nifedipine (15 mg/kg), and flunarizine (10 mg/kg) did not significantly affect the motor performance of mice when compared with (saline + vehicle)-treated animals (Table 1). MK-801 (0.05 mg/kg) administered alone or in combination with nicardipine (15 mg/kg), nifedipine (15 mg/kg), and flunarizine (10 mg/kg) did not influence behavior in this test ($p > 0.05$ vs. untreated group). LY 235959, at its ED_{50} dose against MES (1.6 mg/kg), did not produce motor impairment in mice. Co-administration of either nicardipine (15 mg/kg), nifedipine (15 mg/kg), or flunarizine (10 mg/kg) with LY 235959 (at doses equal to those evaluated for the respective combinations) did not significantly decrease the performance of mice when compared with mice pretreated with LY 235959 alone. Regarding GYKI 52466 and the Ca^{2+} channel inhibitors, a similar trend was observed. The combined treatment of nicardipine (15 mg/kg) or nifedipine (15 mg/kg), but not flunarizine (10 mg/kg), with either LY 235959 or GYKI 52466, however,

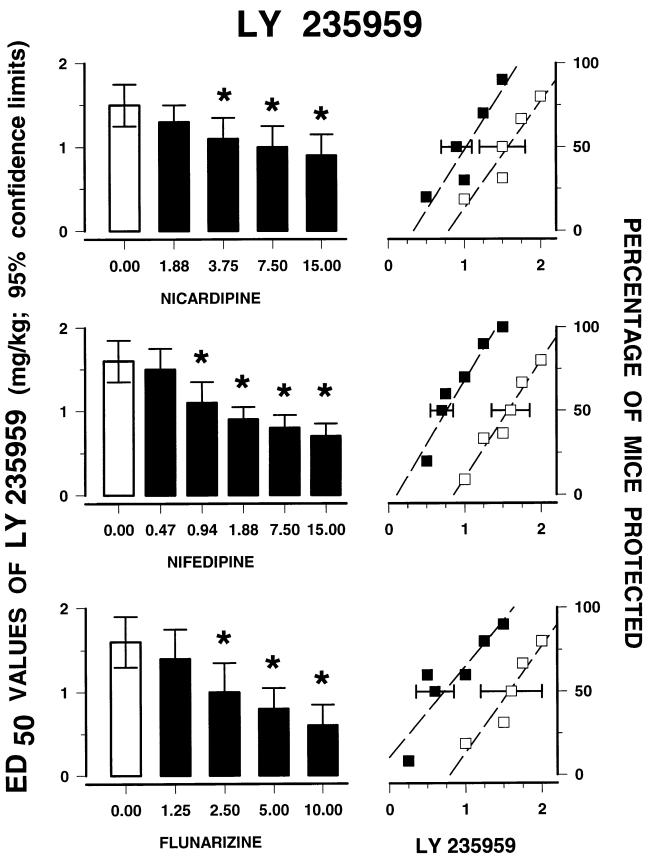

FIG. 2. (Left) Influence of nicardipine (upper section), nifedipine (middle section), and flunarizine (lower section) on the anticonvulsant activity of LY 235959. Bars represent the ED_{50} values of LY 235959 alone (open bars) and in combination with Ca^{2+} channel inhibitors (solid bars) necessary to protect 50% of mice against MES-induced tonic hind limb extension. Doses of Ca^{2+} channel inhibitors are shown below corresponding bars. Vertical error bars represent 95% confidence limits for ED_{50} values. LY 235959 was administered 45 min prior to the test. The pretreatment time for Ca^{2+} channel inhibitors was 60 min. Statistical evaluation was carried out according to the method of Litchfield and Wilcoxon (17). *p at least <0.05 was considered to be significantly different from the group treated with LY 235959 alone. (Right) LY 235959 dose (mg/kg) vs. response (percentage of mice protected) plots with calculated linear regressions (dashed lines) evaluated in groups treated with LY 235959 alone (□) or in combination with nicardipine (15 mg/kg; ■), nifedipine (15 mg/kg; ■), and flunarizine (10 mg/kg; ■), respectively. Each point represents the results obtained from at least 10 mice. The ED_{50} values of LY 235959 with 95% confidence limits were calculated according to the method of Litchfield and Wilcoxon (17) and are shown with horizontal error bars.

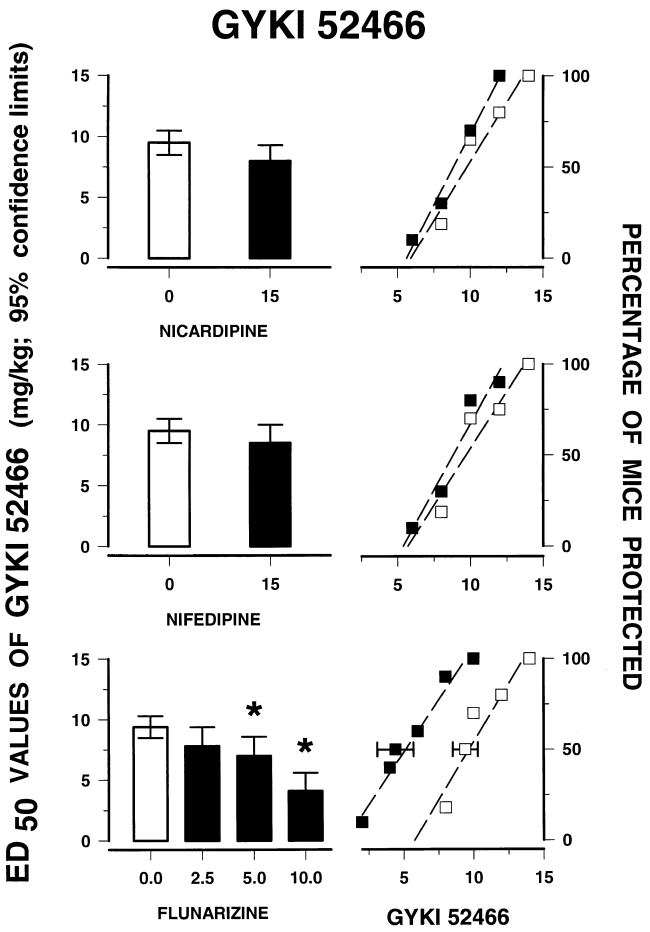

FIG. 3. (Left) Influence of nicardipine (upper section), nifedipine (middle section), and flunarizine (lower section) on the anticonvulsant activity of GYKI 52466. Bars represent the ED_{50} values of GYKI 52466 alone (open bars) and in combination with Ca^{2+} channel inhibitors (solid bars) necessary to protect 50% of mice against MES-induced tonic hind limb extension. Vertical error bars represent 95% confidence limits for ED_{50} values. GYKI 52466 was administered 15 min prior to the test. The pretreatment time for Ca^{2+} channel antagonists was 60 min. Statistical evaluation was carried out according to the method of Litchfield and Wilcoxon (17). *p at least <0.05 was considered to be significantly different from the group treated with GYKI 52466 alone. (Right) GYKI 52466 dose (mg/kg) vs. response (percentage of mice protected) plots with calculated linear regressions (dashed lines) evaluated in groups treated with GYKI 52466 alone (□) or in combination with nicardipine (15 mg/kg; ■), nifedipine (15 mg/kg; ■), and flunarizine (10 mg/kg; ■), respectively. See legend of Fig. 2. for further explanation.

produced significant motor impairment when compared with the (saline + vehicle)-treated group (Table 1).

Dark-Avoidance Task

The task of avoiding the dark compartment was remembered by all of the untreated mice (Table 2). Nicardipine (15 mg/kg), nifedipine (15 mg/kg), and flunarizine (10 mg/kg) caused a significant impairment of long-term memory. A strong memory impairment was observed after administration of MK-801 alone at the dose selected for the convulsive study (0.05 mg/kg). Co-administration of nicardipine (15 mg/kg), nifedipine (15 mg/kg), or flunarizine (10 mg/kg) with MK-801 (0.05 mg/kg) resulted in an impairment of long-term memory comparable to that produced by MK-801 alone (Table 1). Both LY 235959 and GYKI 52466, when administered at their respective ED_{50} doses against MES (1.6 and 9.5 mg/kg, respectively), produced marked memory deficits in 41.7% and 58.4% of animals, respectively. The combined treatment of LY 235959, in doses equal to its ED_{50} values evaluated in combination with nicardipine (15 mg/kg), nifedipine (15 mg/kg), or flunarizine (10 mg/kg), resulted in similar long-term memory deficits, as observed in mice pretreated with LY 235959 alone (Table 2). No significant difference in long-term memory acquisition was observed between

TABLE 1

EFFECT OF MK-801, LY 235959, AND GYKI 52466 ALONE AND IN COMBINATION WITH NICARDIPINE, NIFEDIPINE, AND FLUNARIZINE ON MOTOR PERFORMANCE OF MICE

Treatment	Vehicle	Nicardipine (15)	Nifedipine (15)	Flunarizine (10)
Saline	0	25	25	8.3
MK-801 (0.05)	0	8.3	0	25
LY 235959 (0.6)	8.3	—	—	8.3
LY 235959 (0.7)	8.3	—	33.3*	—
LY 235959 (0.9)	16.6	41.6*	—	—
LY 235959 (1.6)	25	—	—	—
GYKI 52466 (4.4)	8.3	—	—	25
GYKI 52466 (8.5)	16.6	—	41.6*	—
GYKI 52466 (8.9)	25	41.6*	—	—
GYKI 52466 (9.5)	25	—	—	—

Results are expressed as a percentage of animals showing motor impairment in the chimney test (see Method section for the treatment schedule and a description of the test). Doses of compounds in mg/kg are shown in parentheses. *p at least <0.05 vs. (saline + vehicle)-treated group (Fisher's exact probability test). Treatments with LY 235959 and GYKI 52466 alone or combined with Ca^{2+} channel inhibitors provided 50% protection against MES. MK-801 was given in a fixed dose of 0.05 mg/kg, which resulted in significant elevation of the convulsive threshold. Each group consisted of 12 animals.

mice treated with GYKI 52466 alone or in combination with Ca^{2+} channel inhibitors (Table 2).

The combined treatment did not further affect long-term memory as compared with that produced by the tested compounds alone. However, except for a combination of GYKI 52466 (4.4 mg/kg) + flunarizine (10 mg/kg), all other treatments resulted in a substantial impairment of memory acquisition as compared with untreated controls (Table 2).

DISCUSSION

This study demonstrates that the protective action of MK-801, LY 235959, and GYKI 52466 can be differentially modulated by the Ca^{2+} channel inhibitors nicardipine, nifedipine, and flunarizine. The protective action of MK-801 (a noncompetitive antagonist that binds to a specific site within the ion channel of the NMDA receptor complex) was exclusively potentiated by nifedipine in a dose-dependent manner. In contrast, nicardipine (15 mg/kg) and flunarizine (10 mg/kg) did not affect the antiseizure efficacy of MK-801 against electroconvulsions. The protective efficacy of LY 235959, a competitive antagonist of the NMDA receptor, was dose-dependently potentiated by all Ca^{2+} channel inhibitors. The anticonvulsive action against MES of the noncompetitive non-NMDA receptor antagonist GYKI 52466 was markedly enhanced only by flunarizine (5–10 mg/kg). No significant differences in the performance of mice in the chimney test or in the passive avoidance task were observed after administration of MK-801, LY 235959, and GYKI 52466 alone or in combination with Ca^{2+} channel inhibitors. Most combinations, however, produced considerable motor and long-term memory impairment when compared with vehicle-treated controls.

Excitatory amino acid receptors and voltage-operated Ca^{2+}

TABLE 2

EFFECT OF MK-801, LY 235959, AND GYKI 52466 ALONE AND IN COMBINATION WITH NICARDIPINE, NIFEDIPINE, AND FLUNARIZINE ON PERFORMANCE OF A PASSIVE AVOIDANCE TASK BY MICE

Treatment	Vehicle	Nicardipine (15)	Nifedipine (15)	Flunarizine (10)
Saline	100	41.6*	41.6*	66.6*
MK-801 (0.05)	25*	8.3*	8.3*	0*
LY 235959 (0.6)	75	—	—	58.3*
LY 235959 (0.7)	50*	—	41.6*	—
LY 235959 (0.9)	50*	33.3*	—	—
LY 235959 (1.6)	58.3*	—	—	—
GYKI 52466 (4.4)	58.3*	—	—	75
GYKI 52466 (8.5)	33.3*	—	41.6*	—
GYKI 52466 (8.9)	58.3*	33.3*	—	—
GYKI 52466 (9.5)	41.6*	—	—	—

Results are expressed as a percentage of animals showing no long-term memory deficit in the passive avoidance task (see Method section for the treatment schedule and a description of the test). Doses of compounds in mg/kg are shown in parentheses. *p at least <0.05 vs. (saline + vehicle)-treated group. Each group consisted of 12 animals. See also footnote of Table 1.

channels, as mentioned in the introduction, are involved in epileptogenesis. The Ca^{2+} channel inhibitors nifedipine, nimodipine, and flunarizine, but not verapamil, which poorly crosses the blood–brain barrier, show protective activity in various experimental models of epilepsy (1). Moreover, they also considerably potentiate the antiseizure efficacy of conventional antiepileptic drugs against electroconvulsions and pentylenetetrazol-induced seizures (8,9,11). It is likely that potentiation of the protective activity of some excitatory amino acid antagonists resulted from the simultaneous blockade of excitatory events and voltage-dependent Ca^{2+} channels. In addition, the elimination of the NMDA component of glutamate-mediated excitation leads to reduced influx of Ca^{2+} ions through the receptor-operated channels, and the Ca^{2+} channel inhibitors may further diminish this influx via the voltage-dependent channels. The fact, that only some drug combinations were effective seems to speak against nonspecific mechanisms participating in the observed effects.

The exact nature of the interaction between voltage-operated Ca^{2+} channels and excitatory amino acid receptors remains undetermined. NMDA and non-NMDA receptors differ substantially in ion selectivity (16,31). It is known that the NMDA receptor-mediated effects are Ca^{2+} dependent due to the high permeability of the NMDA receptor to Ca^{2+} ions (16,18,31). Non-NMDA receptors, with only a few exceptions, are permeable to monovalent cations (26,31). Ca^{2+} channel inhibitors, therefore, may synergistically increase the protective action of competitive NMDA receptor antagonists against electroconvulsions. A similar effect of Ca^{2+} channel inhibitors was recently reported, when a combined study was performed with two other competitive NMDA receptor antagonists, CGP 40116 and CGP 43487 (12). Further, BAY k-8644 (a dihydropyridine-derivative Ca^{2+} channel agonist) solely impaired the anticonvulsant action of competitive NMDA receptor antagonists (CGP 37849 and D-CPP-ene) and failed to interact with the noncompetitive NMDA (MK-801) and non-NMDA (NBQX and GYKI 52466) receptor antagonists against electroconvulsions in mice (10). These results, taken together, suggest that modulation of Ca^{2+} influx via voltage-dependent Ca^{2+} channels may alter the anticonvulsant potency of competitive NMDA receptor antagonists, and suggest little function for non-NMDA-mediated protection against electroconvulsions. The potentiation by flunarizine of the anticonvulsant action of GYKI 52466 does not seem to be in line with the above-mentioned explanations. However, this seems to be attributed to flunarizine's wide spectrum of pharmacological activity (1,24). Among these actions of flunarizine, which may account for its anticonvulsant properties, are suppression of N- and T-type Ca^{2+} channel conductance, diphenylhydantoin-like effects on sodium channels, and inhibition of adenosine reuptake (1,21,24). Actually, the flunarizine-induced potentiation of the anticonvulsive activity of valproate was not influenced by BAY k-8644 and that of carbamazepine was (13). On the other hand, it was observed that nitrendipine (a dihydropyridine voltage-operated Ca^{2+} channel inhibitor) suppressed NMDA receptor function via a direct interaction with the MK-801 binding site within the NMDA receptor/channel complex in mouse neurons (25). Interestingly, nifedipine, which (unlike nicardipine and flunarizine) potentiated the protective action of MK-801, is a close analogue of nitrendipine. The presynaptic Ca^{2+} channels may also be a site of the dihydropyridine-mediated inhibition of neurotransmitter exocytosis (29). Another possibility to consider, when more than one drug is applied, is pharmacokinetic interaction. Being unable to measure plasma levels of the drugs tested, we cannot entirely rule out possible pharmacokinetic interactions. The fact that the side effects produced by the combined treatment were similar to those exhibited by the individual compounds alone suggests indirectly that pharmacokinetic interactions may not be involved. Previous studies of the influence of Ca^{2+} channel inhibitors on the anticonvulsant potency and plasma levels of classical antiepileptics showed that the pharmacokinetic interaction played a marginal role (8,9,11).

In summary, the present study demonstrates that the anticonvulsant action of some excitatory amino acid antagonists can be dose-dependently enhanced by centrally acting Ca^{2+} channel inhibitors. The combined treatment, however, is not free of side effects. With a few exceptions, combinations of excitatory amino acid antagonists and Ca^{2+} channel inhibitors resulted in an impairment of motor and memory performance as compared with vehicle treatment. The disturbed motor performance was even noted in the absence of a synergism of anticonvulsant activity, as observed in case of GYKI 52466 and dihydropyridines. Nevertheless, further studies are needed to elucidate possible interactions between Ca^{2+} channel inhibitors and excitatory amino acid antagonists. Once characterized, such information could be used to develop new drugs and rationalize polypharmacological approaches in the treatment of epilepsy. It is noteworthy that still up to 25% of epileptic patients (approximately 12.5 million individuals worldwide) remain refractory to therapy with currently available antiepileptic drugs (24).

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