# BIOPHYSICS AND BIOCHEMISTRY

# Effectiveness of Combined Application of Calcium Blockers and Antiepileptic Drugs

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Using the model of electroshock convulsions, we showed that combined administration of blockers of potential-operated (riodipine and nifedipine) or receptor-activated (MK-801) calcium channels with the antiepileptics sodium valproate, phenobarbital, diazepam, ethosuximide, carbamazepine, and Diphenine markedly reduces drug doses and increases therapeutic index of their combinations.

**Key Words:** *antiepileptic preparations; Ca-channel blockers; anticonvulsive and neurotoxic effects; maximum electroshock; complex pathogenetic therapy*

Hyperactivation and epileptization of neurons are associated with $Ca^{2+}$ entry via glutamate N-methyl-D-aspartate activated and potential-operated Ca-channels [8-10]. In the present study, which was performed in the framework of the Pathogenic Therapy of Epilepsy program [1-4], we examined the effectiveness of combined application of blockers of potential-operated 1,4-dihydrodipine-sensitive (riodipine and nifedipine) and receptor-activated (MK-801) Ca-channels and some antiepileptic preparations with different mechanisms of anticonvulsive action.

## MATERIALS AND METHODS

Experiments were carried out on 1140 outbred albino mice weighing 18-24 g. Anticonvulsive activities of the preparations and their combinations were estimated in the maximum electroshock test [4]. Neurotoxicity of the preparations and their combinations were estimated in the rota-rod test [6]. The effectiveness of the preparations and their combinations was expressed as a dose preventing tonic convulsions of hind limbs in 50% mice ($ED_{50}$). This dose and the dose causing toxicity in 50% mice ($TD_{50}$) were determined as described [13] using special software [11]. Effective and toxic doses of combinations were determined by maintaining equal ratios of their doses to their individual $ED_{50}$ and $TD_{50}$. Anticonvulsive and neurotoxic effects of preparations in combinations were analyzed and estimated by isobolographic method [14] with modifications [5] and by calculating fractional index: indices of fractional effective and toxic doses (FED and FTD indices) [7,12]. The effect was regarded as synergistic potentiation if the coefficient was lower than 0.7, as additive synergism when the coefficient varied from 0.7 to 1.3, and as antagonism when the coefficient was higher than 1.3. The prospectiveness of drug combinations was assessed by calculating therapeutic index (TI) as the ratio between their $TD_{50}$ and $ED_{50}$. All preparations were administered *per os* before electroshock so that the peaks of their activities coincided: sodium valproate (Sanofi) 30 min; carbamazepine and diazepam (Relanium, Polfa), ethosuximide (Suxilep, Jenapharm), and MK-801 1 h; riodipine (Foridon) and nifedipine (Sigma) 1.5 h; phenobarbital and Diphenine 3 and

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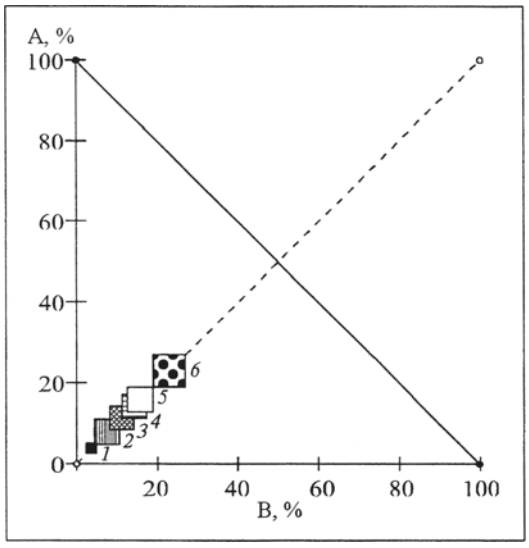


**Fig. 1.** Isobolographic analysis of the effectiveness of riodipine in combination with antiepileptic preparations. Here and in Figs. 2 and 3: ordinate and abscissa: $ED_{50}$ of the preparations designated A and B upon combined administration (individual $ED_{50}$ are taken as 100%). The line connecting $ED_{50}$ of preparations A and B is a theoretical isobole for an additive interaction. Combinations: *1*) riodipine+sodium valproate; *2*) riodipine+diazepam; *3*) riodipine+carbamazepine; *4*) riodipine+Diphenine; *5*) riodipine+ethosuximide; *6*) riodipine+phenobarbital.

4 h, respectively. Sodium valproate, ethosuximide, and MK-801 were dissolved in normal saline; other preparations were dissolved in 5% Tween-80. The total volume of fluid was not higher than 0.2 ml when the drugs were administered individually and 0.4 ml when they were administered in combination. Control mice were given an equal volume of normal saline and/or Tween-80.

## RESULTS

In all cases riodipine and antiepileptic preparations potentiated the effects of each other, as evidenced by the location of the "confidence field:" left to the isobole (Fig. 1). The degree of potentiation depended on the composition of combination (Tables 1 and 2), being the highest in the riodipine—sodium valproate and the lowest in the riodipine—phenobarbital combination.

Potentiation was also confirmed by the FED index: it was smaller than 0.7 in all combinations. Potentiation of anticonvulsive effect of riodipine in combination with antiepileptic preparations was accompanied by increase in their neurotoxicity effect: the doses causing toxic effects decreased. In all combinations the effect of riodipine was not potentiating but additive, since the FTD index varied from 0.7 to 1.3 (Tables 1 and 2).

The highest TI of combinations of riodipine with sodium valproate, Diphenine, and carbamazepine (26.8, 14.4, and 12.4, respectively), which is due to considerable potentiation of anticonvulsive effects and the additive neurotoxic effects of the preparations.

Potentiation of drug effects, occurred in all antiepileptic—nifedipine combinations, as evidenced by isobolograms (Fig. 2) and FED index (Tables 1 and 2). However, the degree of potentiation did not depend on the composition of combination, being practically the same in all cases: $ED_{50}$ of all preparations could be lowered 3.9- to 4.6-fold (Tables 1 and 2). Estimation of neurotoxicity of the preparations by calculating the FTD index showed that their effects were additive, since the index varied from 0.7 to 1.3 (Table 2).

Thus, potentiation of antiepileptic effects and additive neurotoxic effects were observed in all combinations of nifedipine with antiepileptic drugs. Since reduction in $ED_{50}$ of the preparations in combination was comparable to that in $TD_{50}$; TI of the combinations was higher than that of individual preparations or remained virtually unchanged (Tables 1 and 2).

**TABLE 1.** $ED_{50}$, $TD_{50}$, and TI of Anticonvulsive Preparations

| Preparation | $ED_{50}$, mg/kg | $TD_{50}$, mg/kg | TI |
|---|---|---|---|
| Riodipine | 35.1 (27.1-45.6) | 94.0 (81.2-109.0) | 2.7 |
| Nifedipine | 19.6 (13.8-29.0) | 35.0 (25.9-47.1) | 1.8 |
| MK-801 | 0.14 (0.10-0.21) | 0.17 (0.12-0.23) | 1.2 |
| Sodium valproate | 295.7 (271.1-322.5) | 346.4 (305.7-392.6) | 1.2 |
| Diazepam | 6.1 (3.7-10.1) | 5.7 (3.7-8.8) | 0.9 |
| Phenobarbital | 11.1 (8.6-14.2) | 51.3 (40.8-64.6) | 4.6 |
| Ethosuximide | 337.4 (245.9-463.0) | 356.4 (309.9-409.8) | 1.1 |
| Diphenine | 9.6 (7.7-11.9) | 35.3 (26.2-47.5) | 3.7 |
| Carbamazepine | 12.0 (8.1-17.7) | 57.4 (47.9-68.8) | 4.8 |

**Note.** Dispersion is given in parentheses.

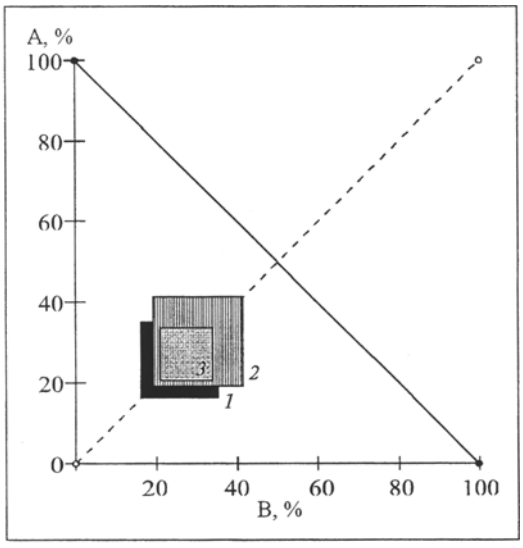


**Fig. 2.** Isobolographic analysis of the effectiveness of nifedipine in combination with antiepileptic preparations. Combination: *1*) nifedipine+phenobarbital; *2*) nifedipine+sodium valproate; *3*) nifedipine+diazepam.

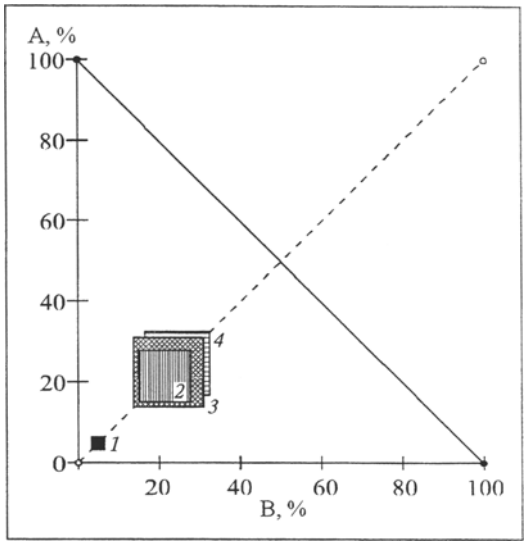


**Fig. 3.** Isobolographic analysis of the effectiveness of MK-801 in combination with antiepileptic preparations. Combination: *1*) MK-801+sodium valproate; *2*) MK-801+diazepam; *3*) MK-801+ethosuximide; *4*) MK-801+phenobarbital.

Synergism of anticonvulsive action was observed after administration of MK-801 with antiepileptic drugs (Fig. 3). Potentiation of effect was observed when MK-801 was administered with sodium valproate: $ED_{50}$ of both preparations could be lowered 26.4-fold (Tables 1 and 2). The degree of potentiation in combinations with diazepam, ethosuximide, and phenobarbital was practically the same: $ED_{50}$ of these preparations could be lowered 5.1-, 5.3-, and 4.2-fold, respectively. The FED index showed that MK-801 potentiates anticonvulsive effects of the antiepileptic drugs. Judging from the FTD index, neurotoxicity of these combinations also increased. Although potentiation of both anticonvulsive and neurotoxic effects was observed when MK-801 was administered with sodium valproate, the TI of this combination increased 8-fold (compared with individual TI) due to greater potentiation of anticonvulsive effect compared with that of neurotoxic effect. Potentiation of anticonvulsive and neurotoxic effects was practically the same when

**TABLE 2.** Changes in Anticonvulsive Activity and Neurotoxicity of Drug Combinations

| Combination | | Decrease in $ED_{50}$ by n times | FED index | FTD index | TI of combination |
|---|---|---|---|---|---|
| **Riodipine+** | | | | | |
| | sodium valproate | 30.3 | 0.07 | 1.05 | 26.8 |
| | diazepam | 15.4 | 0.13 | 0.73 | 7.4 |
| | phenobarbital | 4.8 | 0.43 | 0.70 | 5.7 |
| | ethosuximide | 7.0 | 0.28 | 0.85 | 4.5 |
| | carbamazepine | 10.0 | 0.20 | 0.72 | 12.4 |
| | Diphenine | 7.8 | 0.26 | 1.19 | 14.4 |
| **Nifedipine+** | | | | | |
| | sodium valproate | 3.9 | 0.50 | 0.70 | 2.0 |
| | diazepam | 4.0 | 0.50 | 1.02 | 2.5 |
| | phenobarbital | 4.6 | 0.44 | 0.72 | 4.3 |
| **MK-801+** | | | | | |
| | sodium valproate | 26.4 | 0.08 | 0.62 | 9.6 |
| | diazepam | 5.1 | 0.40 | — | — |
| | phenobarbital | 4.2 | 0.48 | — | — |
| | ethosuximide | 5.3 | 0.38 | 0.45 | 1.3 |

MK-801 was administered with ethosuximide; therefore, TI of this combination was practically the same as that of individual preparations.

Analysis of the effectiveness of the calcium blocker-antiepileptic drug combinations shows that administration of preparations acting on different pathogenetic mechanisms of the epileptic syndrome is prospective.

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