PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

Anticonvulsant Activity of the 1,4-Dihydropyridine Ryodipine and Other Anticonvulsants Administered in Combination

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The study reported here is part of our ongoing research into the development of a novel approach to pharmacological correction of epileptic activity through combined use of drugs that each act on a distinct basic pathogenetic mechanism of epileptogenesis [3-6]. This research is based, on the one hand, on what is currently known about the actions of drugs at the molecular and membrane levels and, on the other, on the systemic principle of combined pathogenetic therapy (CPT) [2]. In this study, CPT of an experimental epileptic syndrome was carried out using binary combinations of drugs that act on two basic pathogenetic mechanisms of epileptogenesis, namely impaired GABA-ergic inhibition and intensified entry of Ca^{2+} and Na^{+} into neurons [9].

In our previous study [6], where sodium valproate, a drug improving GABA metabolism [12], was administered in combination with various blockers of potential-dependent Ca channels, the most effective combination proved to be that of sodium valproate with ryodipine, a derivative of 1,4-dihydropyridine. In this work, therefore, we used combinations of ryodipine with several other anticonvulsants that enhance inhibitory GABA-ergic mechanisms, viz. diazepam, which binds to the GABA-benzodiazepine receptor complex and stimulates GABA effects by increasing the frequency of Cl channel opening [13, 15]; phenobarbital, which prolongs the open state of Cl channels [8, 14]; diphenine, which blocks the potential-dependent Na channels [10]; and ethosuximide and carbamazepine, whose mechanism of antiepileptic action has not been fully elucidated.

MATERIALS AND METHODS

The experiments were conduced on 350 random-bred mice weighing 18-24 g. They were maintained in the vivarium under ordinary conditions and fed a standard diet. The anticonvulsive activity of the drugs and their combinations was assessed by the maximal electroshock test. The electrical current (40 mA for 0.4 sec) was delivered from an ENS-01 electrostimulator (Lvov, Ukraine) through auricular electrodes, as described in detail previously [3]. The drug dose that prevented the occurrence of tonic convulsions of the hind legs in 50% of mice (ED_{50}) was taken as the index of efficacy of the drug after their separate or joint administra-

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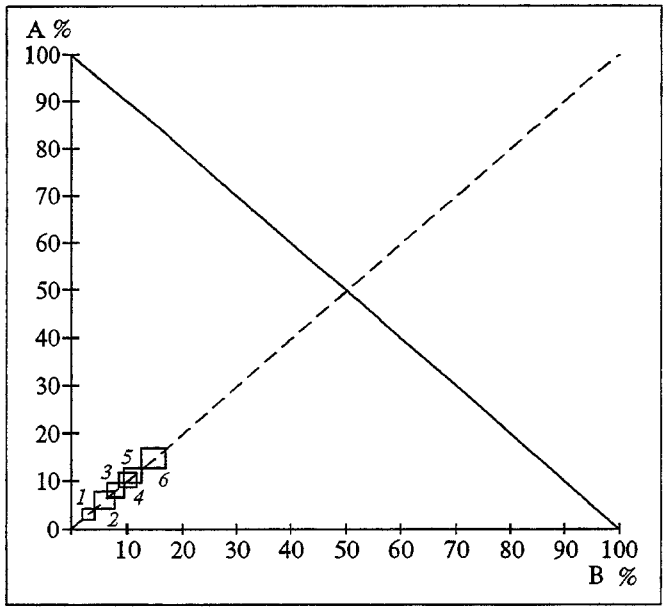

Fig. 1. Isodynamic diagram of anticonvulsant activity shown by combinations of ryodipine with various anticonvulsants. Ordinate and abscissa: percentage ED_{50} values of two drugs (designated A and B) after their combined administration (ED_{50} values of drugs given separately were taken as 100%). The straight line connecting the ED_{50} of drugs A and B is a theoretical isobol for their additive action. The squares are the "confidence fields" for the ED_{50} of the drugs used in the following combinations: *1*) ryodipine + sodium valproate; *2*) ryodipine + diazepam; *3*) ryodipine + carbamazepine; *4*) ryodipine + diphenine; *5*) ryodipine + ethosuximide; *6*) ryodipine + phenobarbital.

tion; the value of ED_{50} was determined in each case by the conventional method of Litchfield and Wilcoxson [11], using computer software. For analysis and subsequent evaluation of the effects from drug combinations, we employed Loewe's isobolographic method in Lisunkin's modification, which involves statistical treatment of the data obtained and the use of a "confidence field" concept [7]. The drugs in the combinations had equal ratios of their doses relative to their ED_{50}. All drugs were administered *per os* at times so selected that the peaks of their activity coincided. Thus, sodium valproate (Sanofi, France) was given 30 min before electroshock; diazepam (Relanium, Polfa) and ethosuximide (Suxilep, Jenapharm) at 60 min; and phenobarbital, diphenine, and ryodipine (Foridon, Latvia) at 3 h, 4 h, and 1.5 h, respectively. Sodium valproate and ethosuximide were dissolved in physiological saline and the remaining drugs, in a 5% Tween-80 solution. The total volume of administered liquid did not exceed 0.2 ml when the drugs were used separately and 0.4 ml when they were used in combination. Control mice received only solvents (physiological saline and/or Tween-80 solution) under the same experimental conditions.

RESULTS

Isobolographic analysis of the efficacy of the various binary drug combinations indicated synergism of the potentiation type for all combinations, as the "confidence fields" lay to the left of the isobol without overlapping it (Fig. 1). The degree of potentiation varied, being greatest with ryodipine+ +sodium valproate and smallest with ryodipine+phenobarbital. The ED_{50} of each drug could be decreased 5-fold for the combination of ryodipine with phenobarbital, 7-fold with ethosuximide, 8-fold with diphenine, 10-fold with carbamazepine, 13-fold with diazepam, and as much as 30-fold with sodium valproate (Table 1 and Fig. 2).

The results of this work, as well those of our other studies using animal models of focal penicillin-induced and generalized corasole-induced epileptic activity and of electroconvulsive shock [3-6], indicate that a CPT with combinations of drugs that act upon the basic pathogenetic mechanisms of epileptogenesis can be effective in suppressing this activity. Relying on the principle of CPT enables the dose of each drug to be substantially reduced without diminishing their antiepileptic activity - an advantage that is of particular importance in the clinical practice of treating epileptic patients with polymorphic syndromes [1].

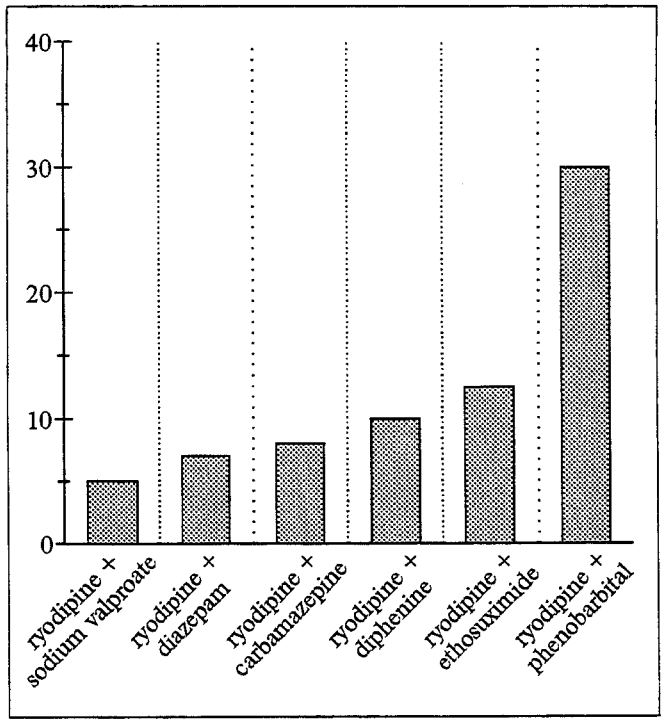

Fig. 2. Potentiation of antiepileptic effects resulting from combined use of ryodipine with another anticonvulsant. Figures at the vertical line show the number of times the ED_{50} of the drugs concerned could be decreased in the respective combinations relative to their ED_{50} when they are administered singly.

TABLE 1. ED_{50} Values of Anticonvulsant Drugs and Ryodipine Administered Separately or in Combination

Combination	ED_{50} of drugs given separetely, mg/kg	ED_{50} of drugs given in combination, mg/kg	ED_{50} of drugs given in combination as % of values for separate administration
Sodium valproate	295.7 (271.1–322.5)	9.7 (6.9–13.5)	3.3
+ ryodipine	35.1 (27.1–45.6)	1.15 (0.82–1.60)	(2.3–4.6)
Diazepam	6.1 (3.7–10.1)	0.46 (0.29–0.72)	6.7
+ ryodipine	35.1 (27.1–45.6)	2.7 (1.7–4.1)	(4.4–10.1)
Carbamazepine	12.0 (8.1–17.7)	1.17 (0.90–1.53)	10.0
+ ryodipine	35.1 (27.1–45.6)	3.4 (2.6–4.5)	(7.7–13.0)
Diphenine	9.6 (7.7–11.9)	1.22 (0.99–1.51)	12.7
+ ryodipine	35.1 (27.1–45.6)	4.5 (3.6–5.5)	(10.5–15.6)
Ethosuximide	337.4 (245.9–463.0)	48.4 (39.4–59.4)	14.3
+ ryodipine	35.1 (27.1–45.6)	5.0 (4.1–6.2)	(11.8–17.4)
Phenobarbital	11.1 (8.6–14.2)	2.3 (1.9–2.8)	20.7
+ ryodipine	35.1 (27.1–45.6)	7.3 (6.1–8.7)	(17.3–24.8)

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