Anticonvulsive Activity of Sodium Valproate and Some Calcium Antagonists Used in Combination in Mice

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UDC 616.853-02:615.31:546.41].015.23]-092.9-07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 3, pp. 231-233, March, 1993. Original article submitted November 2, 1992.

Key Words: *sodium valproate, calcium antagonists; maximal electroshock; combined pathogenetic therapy*

Our previous studies [2,3] on the development of combined pathogenetic therapy (CPT) for epilepsy [1] showed that combined use of sodium valproate, which enhances GABA-ergic processes, and riodipine, which is a calcium antagonist from the 1,4-dihydropyridine group, results in a stronger antiepileptic effect than does either of these drugs when used alone. This effect was discovered on animal models of focal penicillin-induced and generalized corazol (pentylenetetrazol)-induced epileptic activity (EA) [2,3]. The present experiments were a continuation of the above-mentioned studies on CPT in epilepsy through concurrent activation of the processes that weaken the development of EA. To this end, sodium valproate was administered together with various calcium antagonists, namely nifedipine, riodipine, or IOS-1.1212 from the 1,4-dihydropyridine group, verapamil from the phenylalkylamine group, or diltiazem from the benzothiazepine group.

MATERIALS AND METHODS

The experiments were conducted on 500 noninbred male mice weighing 18-24 g. The animals were kept in the vivarium under ordinary conditions and fed a standard diet. The anticonvulsive activity of the drugs and their combinations was assessed by a maximal electroshock test. The current (40 mA for 0.4 sec) was delivered from an electrostimulator (ENS-01, Lvov) via two auricular electrodes in the form of light "alligator" clips with flat silver-plated jaws. The clips were fixed to the auricles in such a manner as to ensure the maximal possible area of contact with the skin. The dose that prevented the occurrence of convulsions in 50% of the animals (ED_{50}) was taken as the index of activity of the drugs after their separate or joint administration. The value of ED_{50} was determined in each particular case by the conventional method of Litchfield and Wilcoxson [13] using computer software [12]. For the analysis and subsequent evaluation of the effects from drug combinations, Loewe's graphic method [14] in Lisunkin's modification [4] was employed. The drugs in the combinations had equal ratios of the doses relative to their respective ED_{50} values. All drugs were administered *per os* at times so selected that the peaks of their activity coincided: thus, sodium valproate (Sanofi, France) was given at 30 min before the electroshock; verapamil (finoptin, Orion, Finland) at 60 min; and nifedipine (Sigma, USA), riodipine (foridon, Latvia), IOS-1.1212 (a drug synthesized at the Institute of Organic Synthesis of the Latvian Academy of Sciences), and diltiazem (Sigma, Italy) at 90 min. The calcium antagonists (with the exception of diltiazem) were dissolved in a 3-5% Tween-80 solu-

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0007-4888/93/0003-0237$12.50

tion. 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

Anticonvulsive activity, as assessed by the maximal electroshock test, was found to be possessed only by the 1,4-dihydropyridines nifedipine, riodipine, and IOS-1.1212 (Table 1). Verapamil and diltiazem were ineffective under the experimental conditions used. It has been shown, however, that diltiazem (in doses of 1.5 and 5 mg/kg intraperitoneally), but not verapamil, increases the threshold of electroshock-induced convulsions, and in further experiments we therefore used diltiazem at 5 mg/kg. The failure of verapamil to display anticonvulsive activity as assessed by that test appears to be due not only to its poor penetration via the blood-brain barrier [8], since this drug did not affect audiogenic convulsions in mice when administered intraventricularly either [7].

The effects from the administration of drugs in binary combinations, evaluated by performing an isobolographic analysis, showed that sodium valproate and any of the calcium antagonists acted synergistically (potentiated each other's effects): the "confidence field" occurred to the left of the isobol and did not overlap it (Fig. 1). In the case of the sodium valproate and nifedipine combination, the ED_{50} of each of these drugs could be decreased threefold. A similar result was obtained with the combination sodium valproate and IOS-1.1212. The greatest potentiation was noted with sodium valproate + riodipine: the ED_{50} of each could be decreased 30-fold (Table 1).

An isobolographic analysis of the combination of two 1,4-dihydropyridines - riodipine and IOS-1.1212

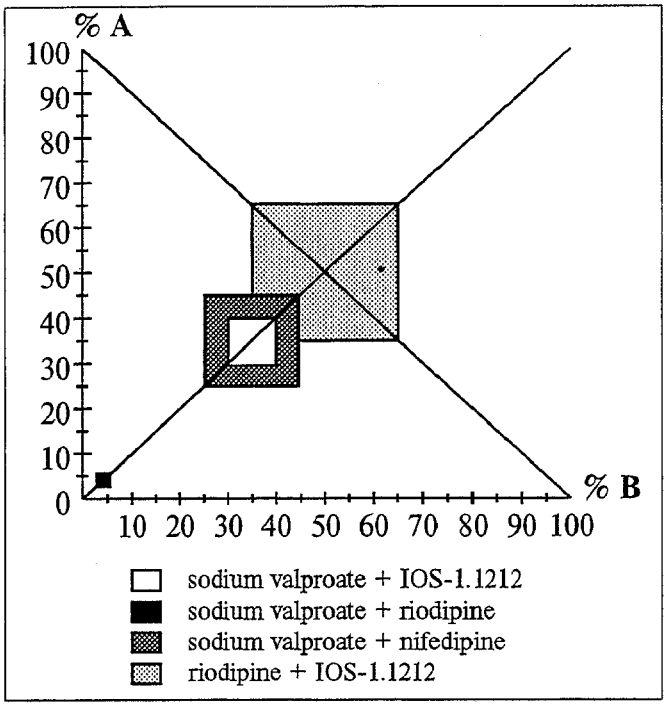

Fig. 1. Isobolographic analysis of effects from binary combinations of drugs. Ordinate and abscissa: percentage ED_{50} values of two drugs (designated A and B) after their combined administration (ED_{50} values of drugs when given alone were taken as 100%). The straight line connecting the ED_{50} of drugs A and B is a theoretical isobol for their additive action.

- indicated an additive effect, since the "confidence field" overlapped the isobol (Fig. 1): the ED_{50} of each drug decreased twofold (Table 1).

The combined use of two calcium antagonists from different groups, nifedipine (1,4-dihydropyridine) and diltiazem (benzothiazepine), also resulted in their synergistic action of the potentiation type: the ED_{50} for nifedipine could be decreased 12-fold (Table 1).

Calcium antagonists acting on the outer part of the calcium channel (flunarizine, dihydropyridines) are known to exert a more powerful effect than those acting on its inner part (diltiazem, verapamil) or on

TABLE 1. ED_{50} Values of Sodium Valproate and Some Calcium Antagonists Administered in Combination

Combination	ED_{50} of drugs given separately, mg/kg	ED_{50} of drugs given in combination, mg/kg	ED_{50} of drugs given in combination as % of ED_{50} for separate administration
1. Sodium valproate + nifedipine	295.7 (271.1–322.5) 19.6 (13.8–29.0)	106.8 (81.7–134.7) 7.1 (5.6–8.9)	35.8 (28.9–44.4)
2. Sodium valproate + riodipine	295.7 (271.1–322.5) 35.1 (27.1–45.6)	9.7 (6.9–13.5) 1.2 (0.8–1.6)	3.3 (2.3–4.6)
3. Sodium valproate + 10S–1.1212	295.7 (271.1–322.5) 80.7 (73.5–88.6)	108.8 (93.7–126.2) 29.7 (25.6–34.4)	38.4 (33.0–44.8)
4. Riodipine + 10S–1.1212	35.1 (27.1–45.6) 80.7 (73.5–88.6)	15.8 (10.8–23.2) 36.3 (24.9–53.2)	45.0 (30.8–65.9)
5. Nifedipine + diltiazem	19.6 (13.8–29.0) –	1.6 (1.0–2.2) –	8.0 (5.7–11.3)

intracellular membranes (NA 1004) [9-11]. Riodipine and IOS-1.1212 act on the same parts of the calcium channel and so produce only an additive effect when administered in combination. In contrast, nifedipine and diltiazem act on different parts of the channel [9-11] and hence a potentiating effect is observed from their combined administration. Possibly, the potentiating effect is also associated with the ability of diltiazem to raise the nifedipine concentration in the plasma by inhibiting the oxidative enzymes metabolizing nifedipine [15], and to increase the binding of dihydropyridines to their receptor [5].

The reported results thus indicate that combined use of sodium valproate and a calcium antagonist from the 1,4-dihydropyridine group leads to their synergistic action of the potentiation type. These two drugs act on different initial components of the epileptogenic process, which explains the potentiation of their effects observed upon their joint administration. The potentiation results, on the one hand, from enhancement of the inhibitory GABA-ergic mechanisms (the effect of valproate) and, on the other, from inhibition of a proepileptic mechanism such as neuronal hyperactivity associated with the entry of Ca^{2+} (the effect of calcium antagonists).

In conclusion, the results of this and previous studies suggest the desirability of using a combined pathogenetic therapy with a combination of antiepileptic drugs acting on different basic pathogenetic mechanisms of the epileptic syndrome.

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