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Effect of Sodium Valproate, Phenobarbital, and Diazepam Administered in Combination on Convulsions Induced by Electroshock in Mice

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UDC 615.8-009.24-092.9-02:615.869]-085

Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 115, № 3, pp. 238-240, March, 1993. Original article submitted November 21, 1992.

Key Words: *sodium valproate; diazepam; phenobarbital; maximal electroshock; combined pathogenetic therapy*

A prerequisite for the genesis of epileptic activity in the brain is deficiency of inhibitory, in particular GABA-ergic, mechanisms [2]. GABA-ergic inhibition results from activation of the $GABA_A$-receptor complex consisting of subunits that bind GABA, benzodiazepines, and barbiturates [10]. These subunits mutually potentiate each other, and this increases the activity of the whole complex and thus leads to an increased chloride current, which causes hyperpolarization inhibition of the neuron [3,9,12].

The present study was undertaken to examine the efficacy of the combined use of two drugs that act on the above-mentioned subunits, namely diazepam (which stimulates GABA activity by increasing the frequency with which the chloride channels open [9,13]) and phenobarbital (which acts by prolonging the open state of these channels [6,11]), as well as of sodium valproate, which enhances the inhibitory GABA-ergic mechanisms by raising the GABA levels in the brain [8].

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MATERIALS AND METHODS

The experiments were conducted on 350 noninbred mice weighing 18-24 g. The animals were kept in the vivarium under ordinary conditions and fed a standard diet. Anticonvulsive activity of the drugs and their combinations was assessed by the maximal electroshock test. The current (40 mA for 0.4 sec) was delivered from an electrostimulator (ENS-01, Lvov) through auricular electrodes (the electrostimulation procedure is described elsewhere [4]). 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 using computer software. For the analysis and subsequent evaluation of the effects from

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TABLE 1. Changes in ED_{50} Values of Sodium Valproate, Diazepam, and Phenobarbital Resulting from their Combined Administration

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	295.7 (271.1–322.5)	41.6 (34.1–53.7)	14.1
Diazepam	6.1 (3.7–10.1)	0.86 (0.67–1.1)	(11.5–18.2)
2. Sodium valproate	295.7 (271.1–322.5)	24.2 (15.5–36.2)	8.2
Phenobarbital	11.1 (8.6–14.2)	0.91 (0.58–1.41)	(5.3–12.3)
3. Phenobarbital	11.1 (8.6–14.2)	0.77 (0.55–1.15)	7.0
Diazepam	6.1 (3.7–10.1)	0.42 (0.31–0.63)	(5.1–10.4)
4. Sodium valproate	295.7 (271.1–322.5)	11.3 (7.3–17.6)	3.8
Phenobarbital	11.1 (8.6–14.2)	0.42 (0.27–0.66)	(2.5–6.0)
Diazepam	6.1 (3.7–10.1)	0.23 (0.15–0.36)	

drug combinations, Loewe's graphic method in Lisunkin's modification was used, with statistical processing of the results and introduction of the concept of the "confidence field" [5]. The drugs in the combination had equal ratios of their doses relative to the respective ED_{50} values. The drugs were administered *per os* at times so selected that the peaks of their activity coincided: sodium valproate (Sanofi, France) was given 30 min before electroshock, while diazepam and phenobarbital, dissolved in Tween-80, were given 60 and 120 min, respectively, beforehand. The 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.

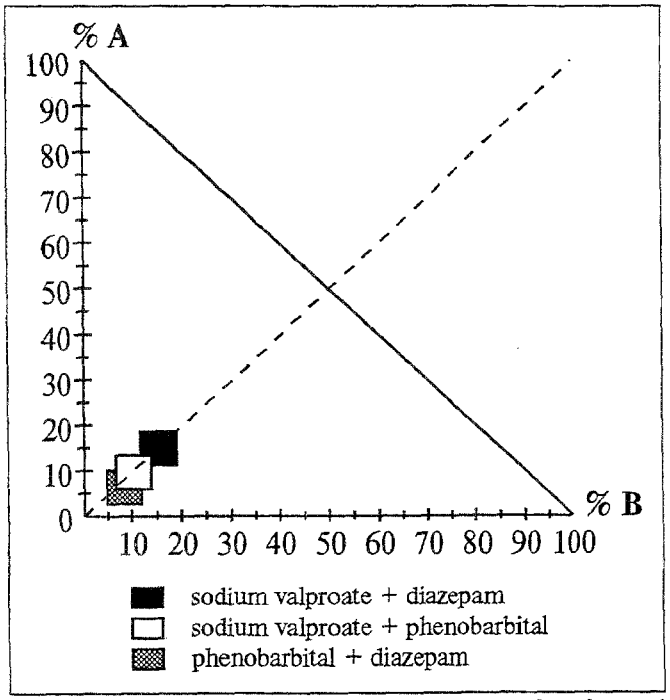

Fig. 1. Isobolographic analysis of effects from combined use of drugs. Ordinate and abscissa, percentage ED_{50} values of two drugs (designated a and b) after their combined administration (ED_{50} values of drugs given separately taken as 100%). The straight line connecting ED_{50} of drugs A and B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

RESULTS

Isobolographic analysis indicated that synergistic effects of the potentiation type occurred with all three binary combinations of the drugs (the "confidence fields" lay to the left of the isobol - Fig. 1), but that the degree of potentiation varied, being smallest with the sodium valproate + diazepam combination: the ED_{50} of each of these drugs in the combination could be decreased sevenfold (Table 1). With sodium valproate + phenobarbital, ED_{50} could be decreased 12-fold and with phenobarbital + diazepam, 14-fold (Table 1).

The greatest potentiation was observed in the case of a ternary combination of sodium valproate, phenobarbital, and diazepam: in this case the ED_{50} of each drug in the combination could be decreased by as many as 26 times (Table 1).

The results indicate that combined use of drugs acting on different mechanisms of GABA inhibitory control leads to a considerable potentiation of the final effect. There is evidence [1] for a potentiating action of certain other anticonvulsants and tranquilizers that also exert their influence via the GABA-ergic mechanisms. The results of this and a previous [4] study permit the conclusion that a combined pathogenetic drug therapy in epilepsy may ensure potentiation of the effects of each of the drugs used in substantially reduced doses.

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BIOCHEMISTRY AND BYOPHYSICS

Effect of Proteoglycans on Erythrocytes in the Circulating Blood

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UDC 616.155:1−02.547.995.1].−07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 3, pp. 240−242, March, 1993
Original article submitted September 18, 1992

Key Words: *proteoglycans, erythrocytes, aggregation, blood, circulation*

Among the various biological functions of proteoglycans (PG) in the animal organism, the capacity of these biopolymers to ensure spatial exclusion and cell concentration in a definite volume, preventing their dispersion, is of great importance for the organism [3,6,10,12]. The best studied in this respect are hyaluronic acid (HA), protein-chondroitin-keratansulfate (PCKS), and PG aggregates represented by natural complexes of HA, PCKS, and binding protein [4-6]. These are the very PG used in the modern treatment of various diseases [6,7].

In this connection it is important to consider the correlation between the therapeutic effect of the above mentioned biopolymers and their activity as factors of spatial exclusion. One of the approaches to this problem could be a study of the effect of HA and PCKS introduced into the blood flow on the erythrocyte (Er) spatial exclusion, i.e., aggregation. This report presents the results of such a study. The need for such studies is further dictated by the fact that they make it possible to compare the results of model experiments on the mechanism of PG-mediated cell spatial exclusion [4,7] with the data obtained at the level of the organism.

MATERIALS AND METHODS

Sodium salts of HA and PCKS were used in this study. Highly purified HA preparations of high polymerization were derived from human umbilical cord [2]. PCKS of similar parameters was obtained from bovine tracheal cartilage [1]. The data of analyses of

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(Presented by I. B. Zbarskii, Member of the Russian Academy of Medical Sciences)

0007−4888/93/0003−0248$12.50