Antiseizure and Neurotoxic Effects of Various Combinations of Phenobarbital, Diazepam, Sodium Valproate and 1,4-Dihydropyridine Riodipin

M. N. Karpova, G. N. Kryzhanovskii, and I. Yu. Abrosimov

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The use of different anticonvulsants as 2-, 3-, or 4-member combinations considerably potentiates their antiseizure effects and, though to a lesser degree, increases their neurotoxicity. In most of these combinations the therapeutic index is considerably higher than in the case of individual preparations.

Key Words: *complex pathogenetic therapy; maximum electroshock; antiseizure preparations; 1,4-dihydropyridine riodipin*

Impaired GABAergic inhibition and increased Ca^{2+} entry into the neuron play an important role in epileptogenesis [9]. In this study, which was undertaken in the framework of the development of a complex pathogenetic therapy [1-5], we attempted to assess the efficacy of combined application of preparations stimulating GABAergic inhibition (sodium valproate, phenobarbital, and diazepam) and 1,4-dihydropyridine riodipin, which blocks Ca^{2+} entry into the neuron. We also tried to find out whether the neurotoxicity of these combinations exceeds the neurotoxicity of individual preparations and how the therapeutic index (TI) of preparations changes when they are used individually and in combination.

MATERIALS AND METHODS

Experiments were performed on 600 outbred mice weighing 18-24 g. The animals were kept in a standard vivarium and received a standard diet. The antiseizure activity of the drugs and their combinations consisting of 2, 3, or 4 preparations was assessed in the maximum electroshock test. Current (40 mA, 0.4 sec) was applied via ear electrodes using an ENS-01 electrostimulator (Lvov) [5]. Neurotoxicity of the preparations was assessed in the rotor-rod test [8]. In preliminary tests the animals were checked for their ability to hold for 10 min onto a rod 2.5 cm in diameter rotating at a speed of 6 rpm. The mice that did not pass this test were not included in the study. Two falls of a mouse from the rod during the test period was the criterion for considering the preparation or combination to possess neurotoxic activity. The dose preventing the development of tonic seizures of the hind limbs in 50% of the animals (ED_{50}) was chosen as the parameter reflecting the efficacy of the preparation or combination. The dose causing a toxic effect in 50% of the animals (TD_{50}) and ED_{50} were calculated by the method of Litchfield and Wilcoxon using computer software [10]. When the preparations were applied in combination, ED_{50} and TD_{50} were determined keeping an equal ratio of their doses relative to ED_{50} that had been found for each preparation. The efficacy of the combination was assessed by calculating the

Institute of General Pathology and Pathological Physiology, Russian Academy of Medical Sciences, Moscow

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TD_{50}/ED_{50} ratio for the preparations and their combinations. All the preparations were administered *per os* in such a way that their maximum effects would coincide: sodium valproate (SV, Sanofi) 30 min, diazepam (DP, Relanium, Polfa) 60 min, phenobarbital (PB, pharmacopeic production) 3 h, and riodipin (RD, Foridon, Latvia) 1.5 h before electroshock. Sodium valproate was administered in normal saline; the other drugs were administered in Tween-80. The total amount of liquid was not more than 0.2 ml for individual and 0.4 ml for combined application. Control animals were given the same volumes of normal saline and/or Tween-80 under the same conditions.

RESULTS

In all cases combined application of two preparations potentiated their antiseizure effects (Table 1). The degree of potentiation depended on the composition of the combination. The highest potentiation was observed in the SV-RD combination: ED_{50} of each preparation could be decreased 30-fold. According to their antiseizure efficacy, the preparations were ranked as follows:

SV+RD>DP+RD>PB+DP>SV+PB>SV+DP>PB+RD.

Potentiation of the antiseizure activity of two simultaneously administered preparations was accompanied by an increase in their neurotoxic activity: the doses producing toxic effects were smaller. For example, TD_{50} for SV and RD in combination decreased 1.3- and 3.3-fold, respectively. Since upon combined application the decrease in ED_{50} of the preparations was much greater than the decrease in TD_{50}, TI rose in four of the six tested combinations. The SV+RD combination proved to be the most effective: a 30-fold decrease in ED_{50} of these combined preparations was accompanied by a considerable increase in their TI (10-fold for SV and 22-fold for RD). According to TI, the combinations were ranked as follows:

SV+RD>DP+RD>PB+RD>PB+DP>SV+PB>SV+DP.

The antiseizure effect was potentiated when the preparations were used in a 3-member combination

TABLE 1. Antiseizure Efficacy, Toxicity, and TI of Preparations Applied Individually or as 2-Member Combinations

No. of combination	Composition of combination	ED_{50} (upper line) and TD_{50} (lower line) of preparation, mg/kg		TI of preparation	TI of combination	Decrease in ED_{50}, *n*-fold
		applied individually	applied in combination			
1	SV	295.7 (271.1–322.5) 346.4 (305.7–409.8)	9.7 (6.9–13.5) 260.2 (185.8–364.5)	1.2	26.8	30.5
	RD	35.1 (27.1–45.6) 94.0 (81.2–109.0)	1.15 (0.82–1.60) 28.4 (20.8–38.8)	2.7		
2	PB	11.1 (8.6–14.2) 51.3 (40.8–64.6)	2.4 (1.9–3.1) 12.9 (8.4–19.8)	4.6	5.4	4.6
	RD	35.1 (27.1–45.6) 94.0 (81.2–109.0)	7.7 (6.0–9.7) 41.5 (27.7–63.4)	2.7		
3	DP	6.1 (3.7–10.1) 5.7 (3.7–8.8)	0.41 (0.27–0.62) 3.1 (2.3–4.1)	0.9	7.5	14.9
	RD	35.1 (27.1–45.6) 94.0 (81.2–109.0)	2.3 (1.5–3.6) 17.1 (12.8–23.0)	2.7		
4	SV	295.7 (271.1–322.5) 346.4 (305.7–409.8)	24.2 (15.5–36.2) 82.5 (57.5–118.6)	1.2	3.4	12.2
	PB	11.1 (8.6–14.2) 51.3 (40.8–64.6)	0.91 (0.58–1.41) 3.1 (2.1–4.4)	4.6		
5	SV	295.7 (271.1–322.5) 346.4 (305.7–409.8)	41.6 (34.1–53.7) 135.3 (102.0–179.3)	1.2	3.2	7.1
	DP	6.1 (3.7–10.1) 5.7 (3.7–8.8)	0.86 (0.67–1.10) 2.8 (2.1–3.7)	0.9		
6	PB	11.1 (8.6–14.2) 51.3 (40.8–64.6)	0.77 (0.56–1.15) 3.4 (2.2–5.1)	4.6	4.4	14.4
	DP	6.1 (3.7–10.1) 5.7 (3.7–8.8)	0.42 (0.31–0.63) 1.8 (1.2–2.8)	0.9		

Note. Here and in Table 2 confidence limits are given in parentheses.

TABLE 2. Antiseizure Efficacy, Toxicity, and TI of Preparations Applied Individually and as 3- or 4-Member Combinations

No. of combination	Composition of combination	ED_{50} (upper line) and TD_{50} (lower line) of preparation, mg/kg		TI of preparation	TI of combination	Decrease in ED_{50}, n-fold
		applied individually	applied in combination			
1	PB	11.1 (8.6-14.2) 51.3 (40.8-64.6)	0.30 (0.19-0.47) 3.1 (2.3-4.1)	4.6		
	DP	6.1 (3.7-10.1) 5.7 (3.7-8.8)	0.17 (0.11-0.26) 1.7 (1.3-2.2)	0.9	10.2	36.6
	RD	35.1 (27.1-45.6) 94.0 (81.2-109.0)	0.96 (0.61-1.49) 9.8 (7.4-13.0)	2.7		
2	PB	11.1 (8.6-14.2) 51.3 (40.8-64.6)	1.33 (0.95-1.87) 6.3 (4.5-8.8)	4.6		
	SV	295.7 (271.1-322.5) 346.4 (305.7-409.8)	35.6 (25.4-49.9) 142.0 (108.1-186.7)	1.2	4.0	8.3
	RD	35.1 (27.1-45.6) 94.0 (81.2-109.0)	4.2 (3.0-5.9) 19.8 (14.1-27.8)	2.7		
3	DP	6.1 (3.7-10.1) 5.7 (3.7-8.8)	0.37 (0.24-0.56) 1.9 (1.4-2.5)	0.9		
	SV	295.7 (271.1-322.5) 346.4 (305.7-409.8)	18.0 (11.9-27.4) 90.7 (66.3-124.1)	1.2	5.0	16.4
	RD	35.1 (27.1-45.6) 94.0 (81.2-109.0)	2.1 (1.4-3.3) 10.6 (7.7-14.5)	2.7		
4	SV	295.7 (271.1-322.5) 346.4 (305.7-409.8)	11.3 (7.3-17.6) 115.3 (64.5-205.8)	1.2		
	PB	11.1 (8.6-14.2) 51.3 (40.8-64.6)	0.42 (0.27-0.66) 4.3 (2.4-7.7)	4.6	10.3	26.2
	DP	6.1 (3.7-10.1) 5.7 (3.7-8.8)	0.23 (0.15-0.36) 2.4 (1.3-4.2)	0.9		
5	SV	295.7 (271.1-322.5) 346.4 (305.7-409.8)	9.3 (5.6-15.7) 47.3 (33.6-66.7)	1.2		
	PB	11.1 (8.6-14.2) 51.3 (40.8-64.6)	0.35 (0.21-0.59) 1.8 (1.3-2.5)	4.6		
	DP	6.1 (3.7-10.1) 5.7 (3.7-8.8)	0.19 (0.11-0.32) 0.96 (0.67-1.37)	0.9	5.1	31.8
	RD	35.1 (27.1-45.6) 94.0 (81.2-109.0)	1.11 (0.66-1.86) 5.6 (4.0-8.0)	2.7		

(Table 2). According to the antiseizure activity, these combinations were ranked as follows:

PB+DP+RD>SV+PB+DP>DP+SV+RD>PB+SV+RD.

According to the TI value the order was as follows:

SV+PB+DP>PB+DP+RD>DP+SV+RD>PB+SV+RD.

In 3 of the 4 tested combinations TI was higher than for individual preparations.

Combined application of four preparations also resulted in the potentiation of their antiseizure effects: ED_{50} of each preparation could be decreased 32-fold (Table 2). At the same time, ED_{50} of such a combination did not differ considerably from the most effective combinations consisting of two or three preparations. However, since the toxicity of each preparation in a 4-member combination was considerably higher than their toxicity in 2- and 3-member combinations, there was no substantial increase in the TI of the 4-member combination, compared, for example, with the TI of PB (Tables 1 and 2).

Thus, our results indicate that combined application of preparations affecting different mechanisms of epileptogenesis is well-reasoned. These preparations potentiate the GABAergic inhibition (DP interacting with the $GABA_A$-benzodiazepine receptor complex and increasing the probability of the Cl channel being open [12,14], PB prolonging the period of the Cl channel being open [6,13], and SV altering the GABA metabolism [7,11]); combined application of these drugs potentiates their effects and inhibit the hyperactivation of neurons associated with Ca^{2+} entry (RD).

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REFERENCES

1. G. N. Kryzhanovskii, M. N. Karpova, E. M. Abramova, and I. Yu. Abrosimov, *Byull. Eksp. Biol. Med.*, **114**, № 10, 376-378 (1992).
2. G. N. Kryzhyzhanovskii, M. N. Karpova, I. Yu. Abrosimov, and E. M. Abramova, *Ibid.*, 369-370.
3. G. N. Kryzhanovskii, M. N. Karpova, and I. Yu. Abrosimov, *Ibid.*, **115**, № 3, 231-233 (1993).
4. G. N. Kryzhanovskii, M. N. Karpova, and I. Yu. Abrosimov, *Ibid.*, 238-240.
5. G. N. Kryzhanovskii, M. N. Karpova, and I. Yu. Abrosimov, *Ibid.*, **116**, № 7, 15-17 (1993).
6. N. Akaike, K. Hattori, N. Inomata, *et al.*, *J. Physiol. (Lond.)*, **360**, 367-386 (1985).
7. F. Baldino and H. M. Geller, *J. Pharmacol. Exp. Ther.*, **217**, 445-450 (1981).
8. N. W. Dunbam and T. S Miya, *J. Amer. Pharmacol.*, **46**, 208-209 (1957).
9. U. Heinemann, C. L. Zhang, Y. Dreier, *et al.*, *Path. Physiol.*, **4**, 37-43 (1992).
10. Kam Pui Fung, *Comput. Biol. Med.*, **19**, 131-135 (1989).
11. W. Loscher, in: *Antiepileptic Drugs*, Eds. H. -H. Frey and D. Janz, Berlin (1985), pp. 507-536.
12. R. L. McDonald, M. G. Weddle, and R. A. Gross, in: *Transmission and Anxiety*, Eds. G. Biggio and E. Costa, New York (1986), pp. 67-78.
13. R. W. Olsen and A. Snowman, *J. Neurosci.*, **2**, 1812-1823 (1982).
14. R. E. Study and J. L. Barker, *JAMA*, **247**, 2147-2151 (1982).

Characterization of Cardiac Function in Hypertensive Rats of the NISAG Strain (an ECG Study)

G. S. Yakobson, D. G. Sakharov, and A. L. Markel'

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Analysis of ECGs recorded in 3 standard leads, 3 augmented limb leads, and 3 chest leads revealed typical signs of left ventricular hypertrophy with a relative deterioration of coronary blood supply in NISAG rats, a new strain with hereditary arterial hypertension. These signs are considered to be characteristic of an established arterial hypertension and may be taken as evidence that the NISAG strain can serve as an adequate animal model of human hypertensive disease.

Key Words: *hereditary arterial hypertension; heart; electrocardiography*

A new rat strain (NISAG), which is an animal model of stress-sensitive arterial hypertension, has been developed by selective breeding at the Institute of Cytology and Genetics in the Siberian Division of the Russian Academy of Sciences [3,5]. Behavioral characteristics [4] and functions of the neurochemical [1,2,6] and neuroendocrine [8] regulatory systems in this strain have now been examined.

The purpose of the present work was to evaluate cardiac function in the NISAG strain through a detailed ECG study, which is necessary for characterizing the hypertensive status of the new strain.

Institute of Physiology, Siberian Division of the Russian Academy of Medical Sciences, Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences, Novosibirsk. (Presented by V. A. Trufakin, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Six-month-old male rats of two strains were used: 60 NISAG (hypertensive) rats and 60 Wistar (normotensive) rats. Cardiac function in these strains was evaluated electrocardiographically. For this, the animals were anesthetized with ether (etherrausch), placed into a screened box in the supine position, and connected to a Mingograf-34 cardiograph (Sweden) by means of thin needle electrodes. These were placed subcutaneously in all four limbs and in the chest at the level of the

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