ANTICONVULSANT ACTION OF N-DIPROPYL ACETATE IN CONJUNCTION WITH BENZODIAZEPINES, PHENOBARBITAL, AND PHENYTOIN

M. Yu. Kosoi

UDC 615.213.015.21:615.214.24

n-Dipropyl acetate (sodium valproate, Depakine, DPA) is an effective anticonvulsant whose experimental and clinical pharmacology has been studied now for more than 25 years. Various workers [10, 12] have shown that DPA increased the brain concentration of GABA, the inhibitory mediator of the CNS.

In this investigation the combined action of the calcium salt of DPA, synthesized in the nationalized Arzneimittelwerk organization (Dresden, East Germany), with benzodiazepine derivatives (phenazepam and diazepam) and also with phenobarbital and phenytoin, which are widely used in clinical practice for the treatment of epilepsy, was studied.

EXPERIMENTAL METHOD

Tests were carried out on noninbred male albino mice weighing 18-22 g. Tests of maximal electric shock and antagonism with subcutaneously injected metrazol [1], prognostic tests relative to the therapeutic activity of the drugs in patients with grand mal and petit mal, respectively [4], and also the test of antagonism with thiosemicarbazide, a substance causing a fall in the brain GABA level [3], were used. All anticonvulsants were injected intraperitoneally in suspension with Tween-80. The results were subjected to statistical analysis [6].

EXPERIMENTAL RESULTS

According to the maximal electric shock test a combination of DPA, in a constant subthreshold dose (ED_3 = 150 mg/kg) with benzodiazepines, given in different doses, enhanced their anticonvulsant activity, as reflected in a decrease in ED_{50} of phenazepam by 2.7 times and of diazepam by 3.4 times (Fig. 1A). Similarly, benzodiazepines in ineffective subthreshold doses (2.5 mg/kg) combined with DPA in various doses, reduced the doses of the latter required to obtain an anticonvulsant effect (Fig. 1B).

In the antagonism with metrazol test addition of DPA in a subthreshold dose (ED_3 = 100 mg/kg) to the benzodiazepines reduced their ED_{50} by 2.8 times (Fig. 2A); conversely, benzodiazepines in a subthreshold dose of 0.05 mg/kg reduced ED_{50} of DPA. Under these circumstances as regards the degree of potentiation of the anticonvulsant activity of DPA phenazepam was twice as effective as diazepam (Fig. 2B).

In the antagonism with thiosemicarbazide test DPA in a subthreshold dose (ED_3 = 100 mg/kg), combined with phenazepam in different doses, led to a marked decrease in the effective anticonvulsant doses, reflected in an appreciable shift to the left of the dose–effect curve and by a two-thirds decrease in ED_{50} of phenazepam. When phenazepam, in a constant subthreshold dose (ED_1 = 0.0025 mg/kg), was combined with DPA, potentiation of the anticonsulvant activity of the latter also was observed, with a statistically significant decrease in its ED_{50} by 1.4 times.

It has been suggested that the mechanism of GABA accumulation in the brain under the influence of DPA is linked with a change in the balance between the GABA-synthesizing enzyme (glutamate decarboxylase) and GABA-catabolizing enzymes (GABA transaminase and succinic semialdehyde dehydrogenase) in favor of inhibition of the latter [7, 9, 12, 14].

KEY WORDS: convulsive states; GABA; n-dipropyl acetate; anticonvulsants (phenazepam, diazepam, phenobarbital, phenytoin); combined action.

Laboratory of Pharmacology of the Nervous System, Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 4, pp. 458-460, April, 1981. Original article submitted October 20, 1980.

 0007-4888/81/9104-0496$07.50 © 1981 Plenum Publishing Corporation

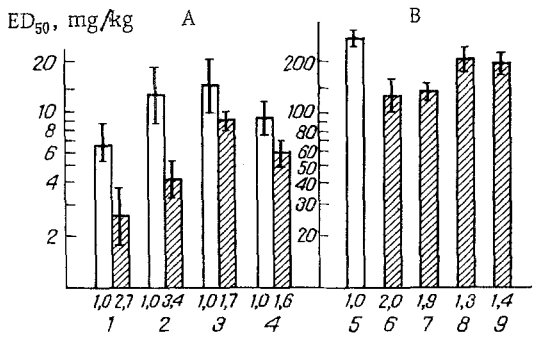

Fig. 1

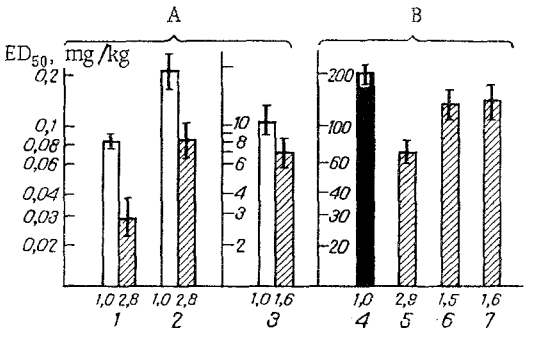

Fig. 2

Fig. 1. Combined action of DPA with anticonvulsants in maximal electric shock test on mice. Ordinate, ED_{50} (in mg/kg on logarithmic scale). A) Combination of DPA in subthreshold dose (ED_3) with anticonsulvants in various doses: 1) phenazepam + DPA; 2) diazepam + DPA; 3) phenobarbital + DPA; 4) phenytoin + DPA; B) combination of anticonvulsants in subthreshold doses (ED_3) with DPA in various doses; 5) DPA; 6) DPA + phenazepam; 7) DPA + diazepam; 8) DPA + phenobarbital; 9) DPA + phenytoin. Unshaded columns — only one substance; shaded columns — combination of two substances. Numbers below columns represent degree of change in effective dose (ED_{50}).

Fig. 2. Combined action of DPA with anticonvulsants in antagonism with metrazol test on mice. Legend as to Fig. 1.

The anticonvulsant properties of the benzodiazepines also are linked with the GABA system [2, 15]. In particular, it has been suggested on the basis of investigations with diazepan [2] that this drug increases the sensitivity of postsynaptic GABA-ergic receptors. It has also been shown [10] that diazepam, in large doses, depresses succinic semialdehyde dehydrogenase activity by 40% or more. Benzodiazepin derivatives thus evidently not only act at the mediator—receptor level, but they can also directly influence biochemical conversions of GABA, by increasing the concentration of succinic semialdehyde; this, in turn, inhibits GABA transamination and leads to an increase in the GABA concentration in the CNS.

Analysis of data in the literature and the results of the present observations indicate that the high anticonvulsant activity of combinations of DPA and benzodiazepine on different models of epileptic fits is evidently attributable mainly to the synergic coupled effect of these substances on the GABA system.

The second stage in the work was to study the anticonvulsant action of DPA in combination with phenobarbital or phenytoin. In experiments to study prevention of tonic extension in mice during maximal electric shock DPA, in a constant subthreshold dose, reduced ED_{50} both of phenobarbital (by 1.7 times) and of phenytoin (by 1.6 times) (Fig. 1A). Conversely the classical anticonvulsants, in ineffective subthreshold doses, also reduced ED_{50} of DPA statistically significantly (Fig. 1B).

In experiments to study the effect of the test substances on convulsions induced by metrazol, marked potentiation was obtained with a combination of DPA and phenobarbital. For instance, injection of DPA in a constant subthreshold dose, in combination with phenobarbital in various doses and vice versa, reduced the dose of the drug required to protect against clonic convulsions in half of the animals by 1.6 times, in whichever way the substances were combined (Fig. 2). The results of these investigations thus demonstrated marked reciprocal potentiation of the anticonvulsant activity of the substances used.

Comparison of these data with results obtained by a combination of DPA with phenazepam and diazepam shows that the potentiating effect of DPA, in a constant subthreshold dose, on the anticonvulsant activity of benzodiazepines, phenobarbital, and phenytoin is stronger than when the substances were given in the reverse order. Moreover, in whichever way the substances were combined, combinations of DPA with benzodiazepines proved more effective than combinations of DPA with phenobarbital or phenytoin. The fact that combined administration of DPA with phenobarbital or phenytoin proved to be moderately effective even when the drugs were given only once can be explained, just as in the case with benzodiazepines, evidently by the synergic action of the combined drugs on those inhibitory structures of the CNS for which GABA is the mediator. This suggestion is supported by data showing that barbiturates can directly

activate GABA-receptors and inhibit the effects of excitatory neurotransmitters — glutamate and aspartate [8], whereas phenytoin, by raising the calcium level in presynaptic endings in the cerebral cortex [11], accelerates GABA uptake and establishes equilibrium between the excitatory action of glutamine and the inhibitory action of GABA, thus restricting the generalization of convulsions [13]. DPA is known [5] not only to increase the GABA concentration in the brain, but also to reduce the aspartate concentration.

All drugs with anticonvulsant action studied in this investigation thus exert their anticonvulsant effects to some degree or other indirectly through the GABA system, by acting synergically on different stages of the inhibitory function of the GABA-ergic system, as is evidently confirmed by the marked reciprocal potentiation of the anticonvulsant activity of the drugs when used in combination.

LITERATURE CITED

1. Yu. I. Vikhlyaev, T. A. Klygul', A. V. Bogatskii, et al., in: Physiologically Active Substances [in Russian], No. 3, Kiev (1971), p. 265.
2. S. N. Kozhechkin and R. U. Ostrovskaya, Byull. Eksp. Biol. Med., No. 12, 1448 (1977).
3. C. F. Baxter and E. Roberts, Proc. Soc. Exp. Biol. (New York), 104, 426 (1960).
4. E. H. Jenney and C. C. Pfeiffer, Ann. New York Acad. Sci., 64, 679 (1956).
5. K. Kukino and T. Geguchi, Chem. Pharm. Bull., 25, 2257 (1977).
6. J. Litchfield and F. Wilcoxon, J. Pharmacol. Exp. Ther., 96, 99 (1949).
7. B. S. Meldrum, Lancet, 2, 304 (1978).
8. R. A. Nicoll, cited by W. E. Haefely, Agents Actions, 7, 353 (1977).
9. R. M. Pinder, R. N. Brogden, T. M. Speight, et al., Drugs, 13, 81 (1977).
10. M. C. Sawaya, R. W. Horton, and B. S. Meldrum, Epilepsia, 16, 649 (1975).
11. R. S. Sohn and I. A. Ferrendelli, Arch. Neurol. (Chicago), 33, 626 (1976).
12. J. W. Van der Laan, T. de Boer, and J. Bruinvels, in: Proceedings at the 2nd Meeting of the European Society of Neurochemistry, Weinheim (1978), p. 625
13. J. Weinberger, W. J. Niclas, and S. Berl. Neurology (Minneapolis), 26, 162 (1976).
14. S. R. Whittle and A. J. Turner, J. Neurochem., 31, 1453 (1978).
15. V. V. Zakusov, R. U. Ostrovskaya, V. V. Markowitch, et al., Arch. Int. Pharmacodyn. Ther., 214, 188 (1975).