# TRANQUILIZING EFFECT OF n-DIPROPYL ACETATE AND OTHER GABA-ERGIC DRUGS IN A CONFLICT SITUATION

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Reports have recently been published that GABA-ergic drugs which are structural analogs of GABA, such as fenibut ($\beta$-phenyl-$\gamma$-aminobutyric acid), pyracetam, and baclofen, possess tranquilizing properties [8, 11, 13]. These facts, and also proof of the participation of a GABA-ergic component in the mechanism of action of the benzodiazepine tranquilizers [10, 15], suggested that anxiolytic properties may be a feature of other GABA-ergic drugs, including those inducing an increased GABA concentration in the brain. This group is known to include n-dipropyl acetate (n-DPA, Depakine) and aminohydroxyacetic acid (AHAA) [12, 14].

The object of this investigation was to compare n-DPA, AHAA, and structural analogs of GABA – pyracetam and pantogam – under conditions of a conflict situation, a method widely used for the detection and evaluation of the tranquilizing effect of new compounds [2, 9].

## EXPERIMENTAL METHOD

To stimulate a conflict situation the instrument designed at the Institute of Pharmacology, Academy of Medical Sciences of the USSR [6] was used. For two days the rats were deprived of fluids, and during the next three days each animal was placed in an experimental chamber for 10 min in order to develop the habit of taking water from a feeding bowl. On the 6th day the experimental conditions were modified so that at the time of taking water the animal received an electric shock (80 V), with the result that a conflict situation developed. During the experimental session lasting 20 min the following parameters were recorded automatically: the number of times water was taken from the feeding bowl, the number of approaches to the feeding bowl, total motor activity. Experiments were carried out on 70 noninbred male rats weighing 180-200 g. Substances increasing the GABA concentration in brain tissue were used: AHAA (from "Sigma") and n-DPA (Depakine, "Labaz"), and also structural analogs of GABA – pyracetam (S. Ordzhonikidze All-Union Pharmaceutical Chemical Research Institute) and pantogam (calcium homopantothenate, All-Union Vitamin Research Institute). Substances used for analysis included bicuculline ("Sigma"), which blocks GABA receptors, and the analeptics metrazol and caffeine. n-DPA (200 mg/kg), pyracetam (1450 mg/kg), and pantogam (500 mg/kg) were injected

TABLE 1. Effect of GABA-ergic Drugs on Behavior of Animals in a Conflict Situation (M ± m)

| Drug | No. of visits to water bowl | Number of visits to feeding bowl | Motor activity |
|---|---|---|---|
| Control | 2,1±0,4 | 4,3±1,2 | 23,0±4,5 |
| AHAA | 3,5±0,4* | 4,1±0,8 | 22,4±8,6 |
| Pantogam | 3,1±0,5 | 4,6±1,6 | 34,1±8,0* |
| Pyracetam | 3,8±0,9* | 5,8±0,7* | 20,0±2,4 |
| n-DPA | 23,5±6,6† | 12,3±2,3* | 18,8±5,6 |

*P < 0.05.
†P < 0.01.

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intraperitoneally 30 min before, and AHAA (20 mg/kg) 60 min before the experiment. Bicuculline (2 mg/kg), metrazol (25 mg/kg), and caffeine (25 mg/kg) were injected subcutaneously, the first 5 min, the rest 15 min before the beginning of the experiment. In a separate series of experiments the effects of the same drugs were studied on drinking motivation by measuring the quantity of fluid drunk by the animals in the course of 60 min. The results were subjected to statistical analysis by the Student–Fisher test.

## EXPERIMENTAL RESULTS

The main indicator of the tranquilizing effect, the number of times water was taken from the feeding bowl, showed a statistically significant increase under the influence of AHAA, pyracetam, and n-DPA (Table 1). Pantogam caused a small increase, not statistically significant (at the $P = 0.05$ level), in this parameter. When pyracetam and n-DPA were used, the number of visits to the feeding bowl also increased, whereas in the experiments with pantogam, there was a significant increase in motor activity of the rats in the conflict situation.

Although the increase in the times water was taken after administration of AHAA and pyracetam was significant, it was insufficiently conclusive to allow the conclusion to be drawn that these substances have a definite tranquilizing effect. The increase in motor activity of the animals under the influence of pantogam without any parallel change in the other indices could point to the presence of a stimulating component in the spectrum of action of the drug, as has been noted for tranquilizers [3, 5].

It was n-DPA which had the strongest effect on the number of times water was taken by animals in a conflict situation. As Fig. 1 shows, this index was increased elevenfold, 30 min after injection of n-DPA in a dose of 200 mg/kg, which can be regarded as the characteristic effect of tranquilizers. It is important to note that diazepam possesses this same property to a comparable degree, when tested by the same method [7].

To test the hypothesis that the tranquilizing effect of n-DPA is connected with accumulation of GABA in the brain and potentiation of its postsynaptic action (n-DPA in a dose of 200 mg/kg, according to the observation of Godin et al. [12], causes an increase in the brain GABA level of 30-40%), experiments were carried out with bicuculline, an antagonist of GABA receptors. As Fig. 2 shows, in a dose of 2 mg/kg, bicuculline completely prevented the tranquilizing effect of n-DPA, in agreement with the results obtained in the experiments with diazepam [7]. Metrazol, and, to a lesser degree, caffeine, had a similar action to that of bicuculline. Metrazol is known to have the property of abolishing or weakening the tranquilizing effect of benzodiazepine derivatives – diazepam and phenazepam [4]. Evidence in support of the possible presence of a GABA-ergic

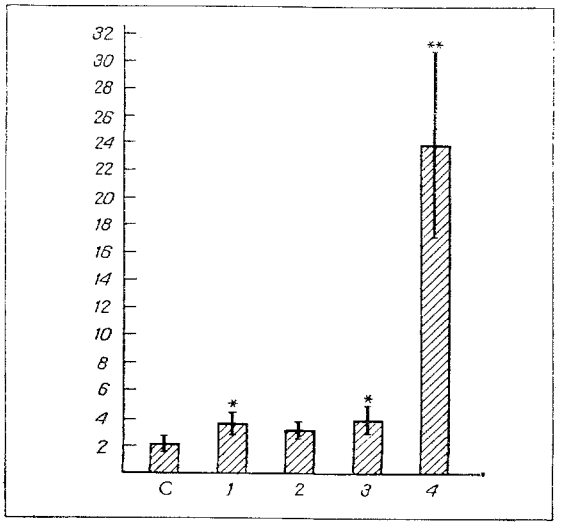


Fig. 1. Effect of GABA-ergic drugs on the principal indicator of the tranquilizing effect (number of times of taking water). Abscissa: C) control, 1) AHAA, 2) pantogam, 3) pyracetam 4) n-DPA; ordinate, number of times water taken from feeding bowl. 1, 3) $P < 0.05$; $P < 0.01$.

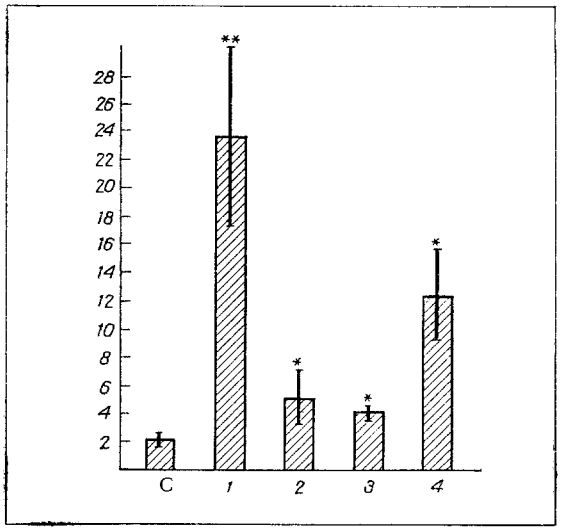


Fig. 2. Antagonism of bicuculline, metrazol, and caffeine with tranquilizing effect of n-DPA. Abscissa: C) control, 1) n-DPA, 2) n-DPA plus bicuculline, 3) n-DPA plus metrazol, 4) n-DPA plus caffeine; ordinate: number of times of taking water from feeding bowl. 1) $P < 0.01$; 2, 3, 4) $P < 0.05$.

component in the mechanism of action of metrazol is also given by electrophysiological studies of antagonism between metrazol, on the one hand, and diazepam and GABA on the other hand, with respect to their effect on the inhibitory phase of the recovery cycle of the intercortical response [15]. As regards caffeine, considering the marked ability of this substance to stimulate cortical processes, it can be tentatively suggested that the partial antagonism with n-DPA observed in the present experiments is realized with the participation of other mechanisms, unconnected with the GABA system.

Special experiments in which the quantity of water drunk by the animals was recorded showed that AHAA and pyracetam reduced the consumption of fluid, whereas n-DPA and pantogam did not change it. This may partly explain the apparently contradictory fact that AHAA, a substance with a similar effect to n-DPA on the brain GABA concentration [14], has a weak tranquilizing effect.

It can be concluded from the results obtained as a whole that n-DPA can be regarded as a tranquilizer whose effect is evidently realized with the participation of a GABA-ergic component.

Considering the similarity between the effect of n-DPA in a conflict situation and the analogous action of substances of the diazepam type, it can be suggested that n-DPA may be used as a tranquilizer under clinical conditions, for we know that the action of the benzodiazepine tranquilizers, as revealed by experimental tests, correlates well with their clinical efficacy [1].

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# EFFECT OF NICOTINAMIDE ON EPILEPTIC ACTIVITY IN THE CEREBRAL CORTEX

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It has been shown [14] that nicotinamide is an endogenous agent or ligand which can bind specifically with benzodiazepine receptors found in the brain [13, 17] and give rise to a benzodiazepine-like effect, as reflected in various indices and, in particular, it can influence certain forms of activity of the spinal reflex apparatus [14]. Nicotinamide is known to prevent reactions catalyzed by glutamate decarboxylase, i.e., to cause insufficiency of GABA synthesis [14].

In the investigation described below it was shown that nicotinamide can suppress electrical activity both in the single focus and in complexes of epileptic foci evoked in the cerebral cortex. The use of an epileptic complex to study the effect of nicotinamide is of special interest, for the complex is a model of a pathological epileptic system arising from foci with comparatively low initial activity under the influence of a powerful determinant focus, which intensifies excitation in other foci, synchronizing their activity, and unites them into a single complex, determining the character of its activity [3, 4]. In such a system, its separate parts (foci) are functionally unequal and they behave differently toward inhibitory procedures [4-6].

## EXPERIMENTAL METHOD

Acute experiments were carried out on 12 cats. Under ether anesthesia the skin and subcutaneous cellular tissues were divided by a midline incision running from the nasal bones to the occiput. The eyeball was drained. The cranial bones and orbit were trephined to give wide access to different parts of the frontal and temporal cortex, after which administration of ether was discontinued. The animal was immobilized (with 0.5-1 mg/kg diplacin) and artifically ventilated. Scattered foci of epileptic activity were created by application of filter paper (2 $mm^2$) soaked in 0.1-0.5% strychnine solution. These foci were formed in different parts of the coronary, anterior and posterior sigmoid, and ectosylvian gyri. A focus of powerful epileptic activity was created in the orbital or coronary gyri by application of a 1-3% solution or a crystal of strychnine. Biopotentials were derived by a monopolar electrode, the reference electrode was fixed in the nasal bones, and cotton threads soaked in Ringer's solution were used as active electrodes. Potentials were recorded on a 4-EEG-3 ink-writing electroencephalograph. A 5% solution of nicotinamide was injected intravenously in a dose of 50-100 mg/kg, but in some experiments larger doses (500-800 mg/kg) were used.

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