Effects of Phenazepam in Ultralow Doses on Bioelectric Activity of the Brain and Behavior of Rats in Various Models of Anxiety

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Phenazepam in ultralow doses produced an anxiolytic effect on male outbred albino rats in the conflict situation and elevated plus-maze models and inhibited θ-activity in EEG, which is typical of tranquilizers. As differentiated from standard doses, phenazepam in this concentration did not affect other frequency bands. Our results suggest that phenazepam in ultralow doses acts as the anxioselective tranquilizer.

Key Words: *tranquilizers; phenazepam; ultralow doses; EEG; anxiolytic activity*

Benzodiazepine tranquilizers produce various side effects. The search for new methods of pharmacotherapy of patients with neurosis-like and borderline disorders that would increase anxioselectivity of therapeutics is of considerable importance. Phenazepam in ultralow doses (10^{-10} mol/kg) exhibits potent anxiolytic activity on the model of conflict situation without producing side effects [5]. Here we studied electrophysiological characteristics and behavior of animals in various models of anxiety after treatment with phenazepam in low doses.

MATERIALS AND METHODS

Experiments were performed on male outbred albino rats weighing 200-220 g. EEG was recorded in freely moving animals using a Neurograph 18-channel electroencephalograph (O.T.E. Biomedica) [6]. Amplifiers were adjusted to standard recording conditions (time constant 0.3 sec, frequency band 32 Hz). Bioelectrical signals were processed using BrainSys software (Hard Soft). The effects of phenazepam on EEG power spectrum in the frontal cortex and hippocampus at 0-32 Hz were evaluated.

After adaptation of rats to experimental conditions baseline EEG was recorded for 5 min. Phenazepam in a dose of 10^{-10} mol/kg was administered through a gastric tube. EEG was recorded and processed every 15 min over the first hour and then every hour. Control animals received placebo. The effects of phenazepam in ultralow and standard doses were compared.

Anxiolytic activity of phenazepam was determined in the conflict situation [3,13] and elevated plus-maze test [9]. The number of punished drinkings (conflict situation) and the latency of the first entry into open arms, number of entries, and time spent in open arms (plus-maze) were recorded. Emotionality of rats in the elevated plus-maze was determined by the number of urinations and boluses.

The effect of phenazepam on animal behavioral was evaluated by total locomotor activity, interaction with cagemates, and reactions to handling and new environmental conditions.

RESULTS

Background EEG in rats was mainly presented by low-frequency θ- and δ-bands and to a lesser extent by high-frequency fluctuations (Fig. 1, *a*). The θ-band included all frequencies (4-7 Hz). Phenazepam in a dose of 10^{-10} mol/kg produced changes in EEG θ-band typical of tranquilizers: increase in power spectrum and a shift toward lower frequencies (4-6 Hz). It should be emphasized that other EEG bands (slow δ-waves and fast α- and β-waves) remained unaffected. This selectivity is typical of low but not standard doses of phenazepam. Phenazepam in a dose of 1 mg/kg not only modified the θ-band, but also enhanced slow δ-activity and high-frequency β-activity (Fig. 1, *b*) [6]. Other benzodiazepine derivatives produce similar changes in EEG [10,11].

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TABLE 1. Effects of Phenazepam in Ultralow and Standard Doses on the Rat Behavior in the Plus-Maze Test

Parameter	Control	Phenazepam	
		10^{-10} mol/kg	1 mg/kg
Latency of entry into open arms, sec	49.6±11.3	6.2±1.3*	53.1±12.5
Number of entries into open arms	2.1±0.7	8.3±1.9**	1.4±0.7
Time spent in open arms, sec	12.7±2.4	51.8±10.1*	18.3±3.6

Note. *p<0.001 and **p<0.05 compared to the control.

The efficiency of phenazepam in various models of anxiety depended on the dose of this preparation. In the conflict test phenazepam was effective in ultralow and standard doses, which is consistent with published data [5]. Phenazepam in ultralow and standard doses increased the number of punished drinking from 17.4±4.1 to 36.1±7.8 (p<0.05) and 127.3±4.1 (p<0.001), respectively.

Thus, in the conflict test we revealed only quantitative differences in the effects of ultralow and standard doses of phenazepam. However, in the elevated plus-maze test phenazepam in ultralow and standard doses produced qualitatively different effects. In ultralow doses this preparation produced an anxiolytic effect: increased the number of entries and the time spent in open arms and decreased the latency of entry into open arms (Table 1). As for general behavior, the rats receiving phenazepam in ultralow doses displayed normal reaction to handling and did not demonstrate aggression against cagemates. Reduced emotionality manifested also in decreased number of boluses and urinations during exploration of the maze.

The effects of phenazepam in a dose of 10 mg/kg were different. The animals rapidly entered closed arms and stayed there motionlessly throughout the observation period. This behavior was probably determined by the sedative effect of this dose of phenazepam [7].

Therefore, the effects of phenazepam depend on its dose. This preparation in ultralow doses produces a selective effect on the θ-band of EEG. In standard concentrations phenazepam changes not only θ-activity, but also δ- and β-frequency bands. In ultralow doses this tranquilizer produces an anxiolytic effect in the elevated plus-maze and exhibits moderate activity in the conflict test. In standard doses phenazepam produces a potent anticonflict effect in the conflict test, but is ineffective in the plus-maze test.

The selective effect of phenazepam on EEG θ-activity is associated with anxioselectivity of tranquilizers [1]. However, stimulation of electrical activity in other bands (*e.g.*, β-activity) is related to the side effect of tranquilizers [2,10-12]. On the other hand high activity in the conflict test is typical of potent tranquilizers, which are effective at both high and low intensities of punishing current [4] and usually produce side effects. Mild anxiolytics are less effective in the conflict test and their effect develops with decreasing current strength [4]. These compounds are

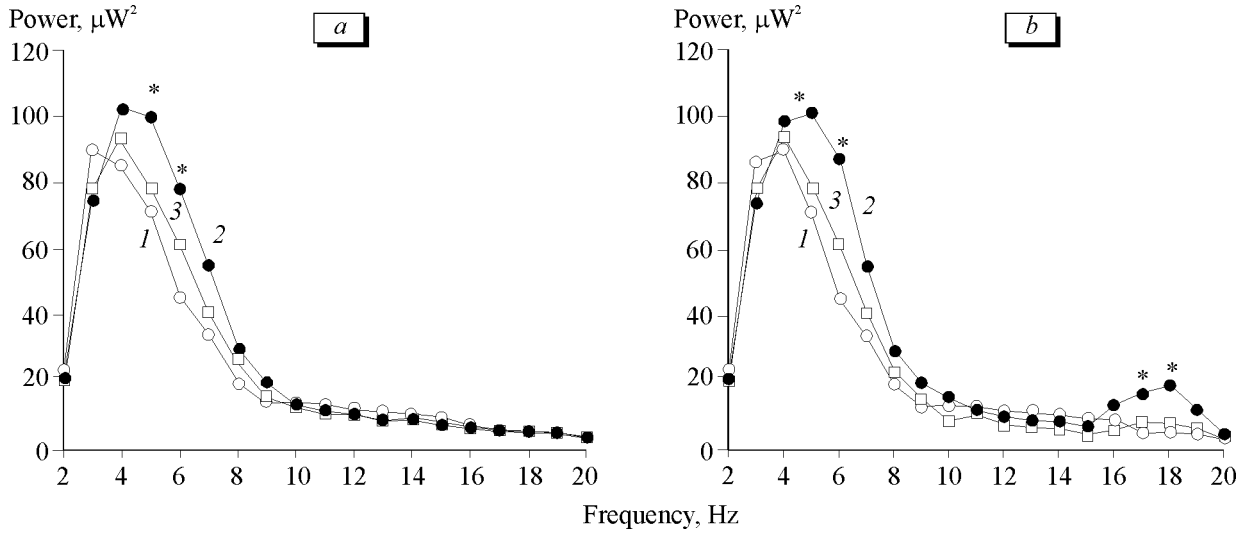

Fig. 1. Power spectra of electrical activity in rat hippocampus before (*1*) and 90 (*2*) and 180 min (*3*) after administration of phenazepam in ultralow (10^{-10} mol/kg, *a*) and standard doses (1 mg/kg, *b*). *p<0.05 compared to the control.

usually efficient in the plus-maze test [8,14,15] and produce less pronounced (or do not produce) side effects typical of potent benzodiazepine tranquilizers [14,15]. Previous studies showed that phenazepam in ultralow doses acts as the anxioselective agent in the conflict test. The preparation is effective at low punishing current (0.5 mA), but loses its activity with increasing the current strength to 1 mA [5]. In these doses the tranquilizer produces no side effects, but increases animal activity in the plus-maze test and produces changes in EEG typical of anxioselective compounds.

Our results indicate that phenazepam in standard doses acts as a potent tranquilizer: exhibits high efficiency in the conflict situation, being less effective in the plus-maze test, and modulates θ-, δ-, and β-activity in EEG. In ultralow doses the effects of phenazepam are similar to those of anxioselective tranquilizers. The preparation is efficient in the plus-maze test, possesses moderate anticonflict activity, and produces specific changes in the θ-band of EEG.

REFERENCES

1. N. N. Bogdanov, *Zh. Vyssh. Nervn. Deyat*., **44**, No. 3, 403-413 (1994).
2. S. V. Krapivin and R. K. Khafiz'yanova, *Byull. Eksp. Biol. Med*., **113**, No. 6, 567-570 (1992).
3. G. M. Molodavkin and T. A. Voronina, *Eksp. Klin. Farmakol*., **58**, No. 2, 54-56 (1995).
4. G. M. Molodavkin and T. A. Voronina, *Byull. Eksp. Biol. Med*., **121**, No. 1, 63-66 (1996).
5. G. M. Molodavkin, E. B. Burlakova, L. I. Chernyavskaya, *et al*., *Ibid*., **121**, No. 2, 164-166 (1996).
6. L. N. Nerobkova and T. A. Voronina, *Ibid*., **56**, No. 8, 62-64 (1983).
7. *Phenazepam* [in Russian], Kiev (1982).
8. D. Benjamin, H. Lal, and L. R. Meyerson, *Life Sci*., **47**, 195-203 (1990).
9. S. E. File, P. S. Mabbutt, and P. K. Hitchcott, *Psychopharmacology*, **102**, 98-101 (1990).
10. J. W. Mandema, L. N. Sansom, M. C. Dios-Vieitez, *et al*., *J. Pharmacol. Exp. Ther.*, **257**, 472-478 (1991).
11. M. Massotti, L. Mele, and C. De Luca, *Pharmacol. Biochem. Behav*., **35**, 933-936 (1990).
12. A. H. Tang, S. R. Franklin, C. S. Himes, and P. M. Ho, *J. Pharmacol. Exp. Ther.*, **259**, 248-254 (1991).
13. J. Vogel, B. Beer, and E. Clody, *Psychopharmacologia*, **21**, 1-7 (1971).
14. C. Wolfman, H. Viola, A. Paladini, *et al*., *Pharmacol. Biochem. Behav*., **47**, 1-4 (1994).
15. H. Yasumatsu, Y. Morimoto, Y. Yamamoto, *et al*., *Br. J. Pharmacol*., **111**, 1170-1178 (1994).