Effect of Superlow Doses of Phenazepam on the EEG and Behavior of Rats in Different Models of Anxiety

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Phenazepam administered in superlow doses exerts an anxiolytic effect in albino male rats under conditions of plus-maze and conflict test and induces typical inhibition of θ-activity in the EEG. Contrary to the effect of usual doses, other frequency intervals remain practically unchanged. Phenazepam in superlow doses acts as an anxioselective tranquilizer.

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

Since benzodiazepines usually have various side effects, new approaches to pharmacotherapy of neurosis-like and borderline-neurotic states are directed toward anxioselectivity of the treatment. It has been previously demonstrated that phenazepam in low doses (up to 10^{-10} mol/kg) exerts a pronounced anxiolytic effect under conditions of a conflict situation without inducing adverse effects [3].

Our goal was to evaluate electrophysiological effects of low doses of phenazepam and their relationship with rat behavior in different models of anxiety.

MATERIALS AND METHODS

Experiments were carried out on random-bred albino male rats with initial body weight of 200-220 g.

The EEG was recorded under conditions of free behavior [6] using a Neurograph 18-channel electroencephalograph (O.T.E. Biomedica) with amplifiers set at a standard mode: 0.3 sec time constant and 32 Hz upper frequency band, and analyzed with BrainSys software. The effect of phenazepam on the EEG power spectra (0-32 Hz) in the frontal cortex and hippocampus was evaluated.

After initial habituation, the background EEG was recorded for 5 min. Phenazepam (10^{-10} mol/kg) was administered intragastrally through a tube; EEG was recorded and analyzed every 15 min during the first hour and then every hour. Control animals received placebo according to the same scheme. The effects of phenazepam administered in superlow and usual doses were compared.

Anxiolytic activity of phenazepam was assessed in the standard conflict test [5,13] and in elevated plus-maze test [9].

The conflict situation test is based on a conflict between drinking motivation and painful electrical stimulation in water-deprived animals. A considerably increased number of punished drinkings in the experimental group in comparison with the control served as a measure of the effect.

The elevated plus-maze test is based on a natural fear of open space and heights. The maze consists of 4 perpendicular arms (0.5 m length and 10 cm width), two opposite arms are closed and two other are open. The animal is placed into the center, and its behavior (latency of the first entry into the open arm and the number of entries and the time spent in open arms) is recorded during a 3-min period.

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Emotionality of rats during their stay in the maze was assessed by the number of boluses and urinations.

The effect of phenazepam on general behavior (motor activity, contacts with other animals in the cage, reaction to handling and to new environment) was also assessed.

RESULTS

Experiments showed that in the background EEG the low-frequency activity (θ and δ rhythms) predominates, while the high-frequency rhythms are less pronounced (Fig. 1). In the θ band (4-7 Hz) all frequencies are presented. Phenazepam in a dose of 10^{-10} mol/kg induces tranquilizer-specific changes in the θ band: it increased power density and a shift to lower frequency (4-6 Hz), while lower (δ) and higher (α and β) frequency bands remained practically unaffected. This selectivity is typical of low but not of conventional doses of phenazepam. Indeed, in doses of 1-5 mg/kg phenazepam induces changes in the θ band, which are accompanied by an increase in low- (δ) and high-frequency (β) activities [6]. Similar changes are induced by other benzodiazepines [10,11].

Behavioral experiments showed that the effect of phenazepam in different models of anxiety is dose-dependent.

In support of our previous observations [3], both usual and superlow doses of phenazepam increased the number of punished drinkings in the conflict test (Table 1), the effect of usual doses was much more pronounced.

Unlike the conflict test, where only quantitative difference between the effects of different doses of the drug was noted, the effect of these doses on the behavior in elevated plus-maze was qualitatively different. In superlow doses the drug exerted a marked anxiolytic effect, which manifested itself in increased number of entries, prolonged the time spent in the open arms, and shortened transfer latency (Table 2). Some changes were also noted in animal behavior: no aggression to handling and toward other animals in the cage were noted. The lowered emotionality was confirmed by reduced number of boluses and urinations during the test.

Another behavioral pattern was observed in rats treated with phenazepam in usual doses (1-10 mg/kg): they hid into closed arms with a short latency and spent there all the time motionless, which is presumably due to sedative effect of these doses of the drug [7].

Thus, the spectrum of pharmacological activity of phenazepam is dose-dependent. In superlow doses this drug has a selective effect on the θ band of

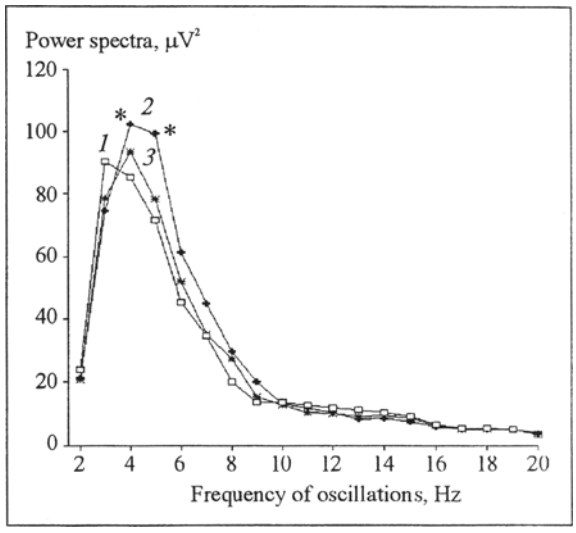

Fig. 1. Power spectra of electrical activity in rat hippocampus before (*1*) and 90 (*2*) and 180 min (*3*) after administration of phenazepam in a dose of 10^{-10} mol/kg. *$p<0.05$ compared with the control.

EEG, whereas in conventional doses it, apart from modulation of the θ band, induces changes in the δ and β bands. Moreover, in superlow doses the drug exerts an anxiolytic effect in the elevated plus-maze test and exhibits moderate activity in the conflict test, while in conventional doses it exhibits a potent anticonflict effect and is practically ineffective in the maze test. On the one hand, the selective effect of tranquilizers on θ activity of EEG can be considered as a marker of their anxioselectivity [1], whereas their stimulating effect on other EEG bands (in particular, the β band) are usually related to their side effects [2,10-12]. On the other hand, high activity in a conflict situation is usually characteristic of potent tranquilizers, which are effective in both weak and strong punishing current [4]. These preparations usually have adverse effects. Less potent anxiolytics are less effective in a conflict situation and exhibit their activity only in weak electrical stimulation [4]. These agents are usually effective in the plus-maze test [8,14,15], and side effects typical of potent benzodiazepines with these drugs are less pronounced or absent [14,15]. Previous studies showed [3] that in a conflict situation phenazepam administered in super-

TABLE 1. Effect of Phenazepam on Rat Behavior in the Conflict Test

Treatment	Dose, mol/kg	Number of punished reactions
Control	—	17.4±4.1
Phenazepam	10^{-10}	36.1±7.8*
Phenazepam	2.4×10^{-6} (1 mg/kg)	127.3±19.9**

Note. Here and in Table 2: *$p<0.05$, **$p<0.001$ compared with the control.

TABLE 2. Effect of Phenazepam Administered in Superlow and Usual Doses on Rat Behavior in the Plus-Maze Test

Parameter	Control	Phenazepam, 10^{-10} mol/kg	Phenazepam, 1 mg/kg
Latency of transition 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

low doses acts similarly to anxioselective drugs: it is effective in weak punishing current (0.5 mA) and ineffective in stronger current (1 mA). In these doses the tranquilizer has no side effects. Phenazepam in superlow doses stimulates animal behavior in the plus-maze test and induces EEG changes typical of anxioselective drugs.

Thus, our findings suggest that in usual doses phenazepam acts as a potent tranquilizer: it is highly effective in the conflict test and ineffective in the plus-maze test and induces changes in both the θ activity and δ and β activities of EEG. When administered in superlow doses, phenazepam is similar to anxioselective tranquilizers: it is effective in the plus-maze test and induces specific changes in the θ band of EEG.

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