Pharmacological Activity of Phenazepam and Flunitrazepam in Ultralow Doses

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Experiments on male outbred albino rats showed that benzodiazepine tranquilizers phenazepam and flunitrazepam in ultralow doses (10^{-9}-10^{-15} mol/kg) produced an anxiolytic effect in the conflict situation test. This effect was not accompanied by myorelaxing and sedative side effects typical of standard doses of tranquilizers.

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

Tranquilizers (primarily benzodiazepine compounds) in high doses are widely used in experimental studies (0.1-10.0 mg/kg or 2.4×10^{-7}-2.4×10^{-5} mol/kg) and outpatient treatment (0.5-5.0 mg/kg or 1.2×10^{-6}-1.2×10^{-5} mol/kg). Benzodiazepine tranquilizers possess anxiolytic activity, but often produce undesirable myorelaxing and hypnotic effects. Therefore, these preparations can not be used for the therapy of individuals, whose professional activity requires concentration and good coordination. Tranquilizers are expensive, which also limits their wide use.

Here we studied whether administration of benzodiazepine tranquilizers phenazepam and flunitrazepam in ultralow doses can reduce their undesirable effects.

MATERIALS AND METHODS

Experiments were performed on male outbred albino rats weighing 180-220 g. The anxiolytic effect of preparations was studied in the conflict test based on the conflict between drinking motivation and painful electrical stimulation [3,6,10].

Each experimental series was performed for 3 days. After 24-h water deprivation (day 1) the animals were trained to take water from a drinking bowl. To this end the animals were placed in experimental chamber for 5 min. The animals explored the chambers, found the bowls, and drank. After training the rats were randomly divided into groups. On day 3 the test preparation were injected intraperitoneally 40 min before behavioral experiments.

In series I we determined the ultralow dose of tranquilizers producing the maximum effect. To this end, control rats (n=10) received 0.9% NaCl (0.25 ml/100 g) and experimental groups received phenazepam or flunitrazepam (Rogipnol) suspended in Tween 80 to concentrations of 2.4×10^{-5} mol/kg (10 mg/kg), 10^{-9}, 10^{-10}, 10^{-11}, 10^{-13}, and 10^{-15} mol/kg. Ten rats received daytime tranquilizer medazepam in a dose of 10 mg/kg. For evaluation of the anxiolytic effect of the test preparations the rats were placed into experimental chambers for 10 min, and 10 sec after the first drink direct electric current (0.5 or 1 mA) was applied to a drinking bowl, so that each attempt to drink was punished. The increase in punished responding in the experimental group compared to the control served as the measure of the anxiolytic effect. Horizontal and vertical locomotor activities were visually evaluated.

In series II the anticonflict effect of the test preparation was evaluated at different current strengths. Control rats (n=10) received 0.9% NaCl (0.25 ml/100 g) and experimental groups received phenazepam or flunitrazepam (Rogipnol) suspended in Tween 80 to concentrations of 2.4×10^{-5} mol/kg (10 mg/kg) and 10^{-10} mol/kg. Ten rats received daytime tranquilizer medazepam in a dose of 10 mg/kg. The anxiolytic effect of the test preparations was evaluated as in series I but the current strength was 1 mA. In addition, the myorelaxing effect of preparations was estimated by the rotarod test (2 min at 0.5 and 3 rpm).

The data were processed statistically. The means and standard deviations were calculated (p<0.05) and significance of differences was evaluated using Mann—Whitney U test.

RESULTS

Phenazepam and flunitrazepam in doses of 10^{-15}-10^{-9} mol/kg produced atranquilizing effect on animals

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under conditions of conflict situation. The dependence of punished responding on the dose of preparations was described by a bell-shaped curve. The maximum effective dose was 10^{-10} mol/kg (Fig. 1). It should be emphasized that the anticonflict effect of phenazepam and flunitrazepam in ultralow doses surpassed that of the daytime tranquilizer medazepam, but was less pronounced than the effect produced by their standard doses (Fig. 2).

It was interesting to evaluate the effects of tranquilizers on locomotor activity of rats in the anticonflict test. In control animals both horizontal and vertical activities were markedly suppressed during the experiment compared to the training period. In rats receiving phenazepam in a dose of 10^{-10} mol/kg this inhibition of locomotor activity was less pronounced. Phenazepam in a dose of 10 mg/kg significantly suppressed locomotor activity. Flunitrazepam produced the same effect. These results indicate that phenazepam and flunitrazepam in a dose of 10^{-10} mol/kg produced pronounced tranquilizing effects without inhibiting behavioral activity.

Tranquilizers in ultralow doses increased punished responding, which can be explained by their nonspecific activating effect. We studied the effect of stimulator caffeine on this behavioral parameter. The preparation in a dose of 20 mg/kg markedly increased locomotor activity of animals, but in parallel decreased punished responding (compared to the control). Therefore, caffeine produced proconflict and anxiogenic effect.

Increasing current strength to 1 mA abolished not only the anticonflict effect of phenazepam and flunitrazepam in ultralow doses, but also the influence of medazepam. However, the effects of phenazepam and flunitrazepam in standard doses decreased insignificantly (Fig. 2).

Phenazepam and flunitrazepam in low doses produced no myorelaxing effect and did not disturb the rotarod performance. Phenazepam and flunitrazepam in standard doses, as well as the daytime tranquilizer medazepam caused pronounced muscular relaxation: ED_{50} for phenazepam, flunitrazepam, and medazepam inthis test were 2.48 (1.65-3.72), 2.8 (1.65-3.95), and 16.7 mg/kg (9.27-30.06), respectively.

Thus, phenazepam and flunitrazepam in ultralow and standard doses possess different pharmacological activity. In standard doses these preparations produce not only anxiolytic, but also sedative and myorelaxing effects [2,5].

Previous studies showed that the effect of benzodiazepine tranquilizers is realized via their interaction with GABA-benzodiazepine receptors [8]. The efficiency of tranquilizers in clinical [9] and experimental studies [7] correlates with their affinity. Stress is accompanied by changes in the GABA-benzodiazepine receptor complex [11]. Because of high affinity of phenazepam and flunitrazepam for the receptors, the effects of these drugs manifest at ultralow doses during moderate stress produced by application of low-intensity current (0.5 mA) to the drinking bowl [4]. In can be hypothesized that more intensive stimulation (1 mA) induces more pronounced changes in the ligand-receptor interaction, which abolished the effects produced by tranquilizers in low doses. Under these conditions activity of phenazepam and flunitrazepam in standard doses decreases, but does not disappear due to high affinity of preparations.

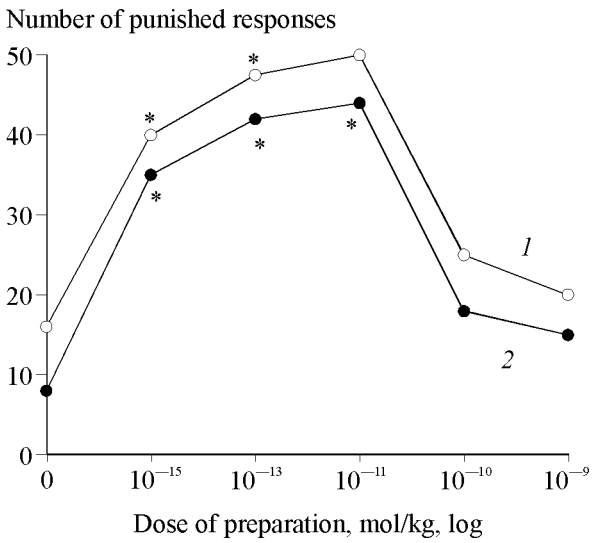

Fig. 1. Effects of phenazepam (*1*) and flunitrazepam (*2*) on punished responding in the conflict test. *p<0.05 compared to the control (dose "0").

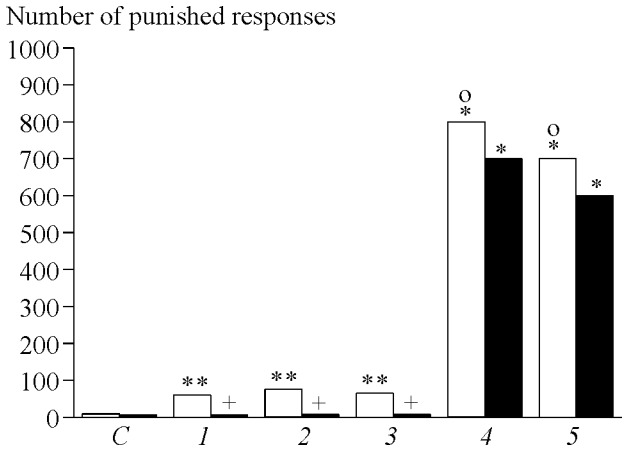

Fig. 2. Anticonflict effect of medazepam, phenazepam, and flunitrazepam in ultralow and standard doses during application of low (0.5 mA, light bars) and intense current (1 mA, dark bars) to a drinking bowl. *p<0.001 and **p<0.05 compared to the control; ^{+}p<0.05 compared to low current; ^{o}compared to medazepam, phenazepam, and flunitrazepam in ultralow doses. Medazepam (10 mg/kg, *1*), phenazepam in doses of 10^{-10} mol/kg (*2*) and 10 mg/kg (*4*), and flunitrazepam in doses of 10^{-10} mol/kg (*3*) and 10 mg/kg (*5*).

The mechanisms underlying the effect of bioactive substances in ultralow doses are extensively studied. However, there is no general opinion regarding the common principles of action of biological active substances in ultralow doses. It can be hypothesized that their effects are mediated by putative receptors characterized by receptor-ligand dissociation constant far below 10^{-12} mol. However, this assumption cannot be experimentally verified due to technical difficulties.

Our results indicate that benzodiazepine tranquilizers phenazepam and flunitrazepam in ultralow doses produce an anxiolytic effect in the conflict test and do not exhibit myorelaxing and sedative activity.

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