PHARMACOLOGY AND TOXICOLOGY

Pharmacological Analysis of the Activity of Phenazepam and Flunitrazepam Administered in Superlow Doses

G. M. Molodavkin, E. B. Burlakova, L. I. Chernyavskaya, T. A. Voronina, N. I. Khorseva, and S. B. Seredenin

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Benzodiazepine tranquilizers, phenazepam and flunitrazepam, administered to random-bred albino male rats in superlow doses (10^{-9}-10^{-15} mol/kg), are shown to exert an anxiolytic effect in the conflict test. In contrast to the case with the usual doses, the above effect is not accompanied by marked myorelaxant or sedative effects.

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

The use of tranquilizers, primarily of the benzodiazepine family, is currently on the rise. They are used in rather high doses (0.1-10 mg/kg or 2.4×10^{-7}-2.4×10^{-5} mol/kg in experiments and 0.5-5 mg or 1.2×10^{-6}-$1.2\times\times10^{-5}$ mol/kg in clinical practice and for outpatient treatment. However, apart from their pronounced anxiolytic action, the known benzodiazepines usually have adverse side effects (myorelaxant and soporific), limiting the use of these preparations. In addition, the tranquilizers are expensive, which also restricts their wide usage.

Our goal was to explore the possibility of administering the benzodiazepines phenazepam (PZ) and flunitrazepam (FNZ) in superlow doses in order to minimize the adverse effect of these drugs.

MATERIALS AND METHODS

The experiments were carried out on random-bred albino male rats weighing 180-220 g. The anxiolytic effect of the preparations was studied using the conflict situation test based on a conflict between drinking motivation and painful electrical stimulation [2,4,8]. A reliably increased number of punished drinkings in the experimental groups in comparison with the control

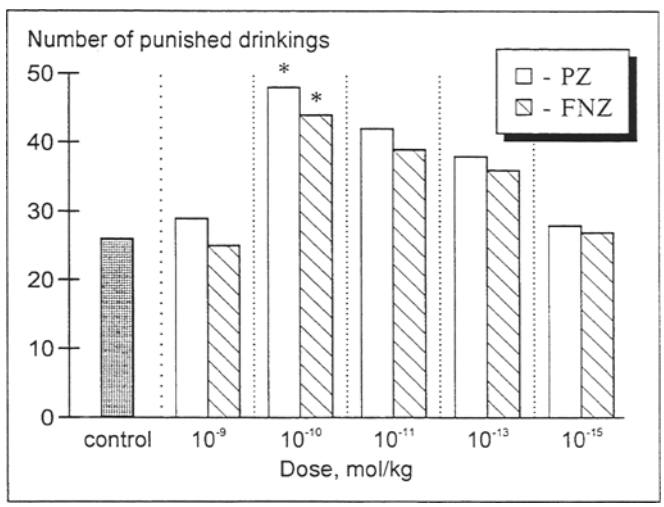

Fig. 1. Effect of phenazepam (PZ) and flunitrazepam (FNZ) on the number of punished drinkings in the conflict test. *$p<0.05$.

Institute of Chemical Physics, Russian Academy of Sciences, Research Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow

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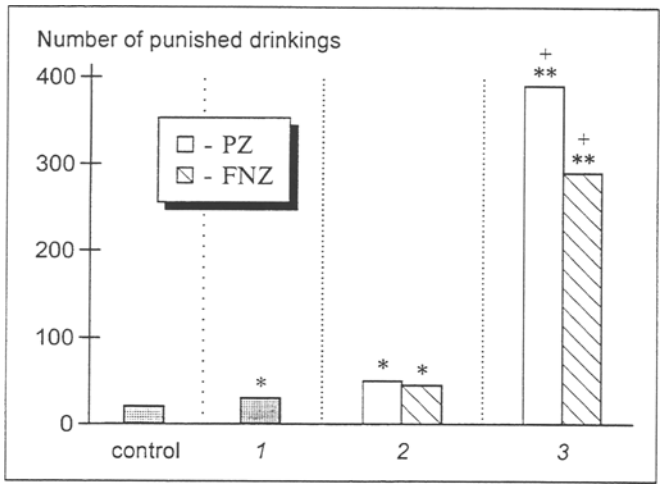

Fig. 2. Effect of medazepam and superlow and usual doses of phenazepam (PZ) and flunitrazepam (FNZ) on the number of punished drinkings in the conflict test. *1*) medazepam, 10 mg/kg, *2*) potent tranquilizers, 10^{-10} mol/kg; *3*) potent tranquilizers, 10 mg/kg (2.4×10^{-5} mol/kg). Here and in Fig. 3: $^{*}p<0.05$, $^{**}p<0.001$ in comparison with the control, $^{+}p<0.001$ in comparison with the effect of a superlow dose.

served as a measure of the effect. In addition to the number of punished responses, we also evaluated the motor activity of rats during conditioning and experiments. To this end, the floor of the experimental chamber was divided into 4 squares and horizontal activity (the number of crossed squares), vertical activity (number of upright postures), and the number of grooming acts (scratching) were visually evaluated. All animals were trained and then randomly divided into 3 groups. On day 3 of the experiment the rats were injected with isotonic NaCl (group 1, control), PZ suspended in Tween-80 (group 2), or FNZ (Rogipnol, group 3) 40 min before being placed in the

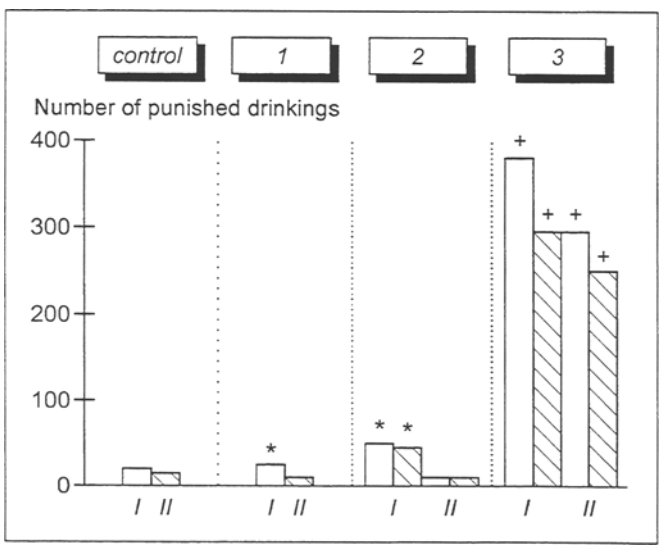

Fig. 3. Effect of strength of current delivered to water dish on anticonflict effect of medazepam, phenazepam (PZ), and flunitrazepam (FNZ). *I*) weak current, *II*) strong current; *1*) medazepam, 10 mg/kg, *2*) PZ and FNZ, 10^{-10} mol/kg; *3*) PZ and FNZ, 10 mg/kg.

experimental chamber. The myorelaxant effect of the preparations was evaluated by changes in the ability of rats to stay on a rotating rod for 2 min at 0.5 and 3 rpm rotation rates. The effects of superlow doses of the tranquilizers were compared with those of the usual doses and with the effect of the daytime tranquilizer medazepam. All preparations were injected intraperitoneally.

The data were processed using standard statistical software.

RESULTS

Our experiments demonstrated that PZ and FNZ in doses ranging from 10^{-15} to 10^{-9} mol/kg had a pronounced tranquilizing effect in the conflict test at a 0.5 mA current. The dose-response curve depicting the number of punished drinkings was bell-shaped, the maximally effective dose being 10^{-10} mol/kg. This dose corresponded to an approximately 2-fold increase in punished drinkings in comparison with the control (Fig. 1). It should also be noted that the effect of superlow doses of PZ and FNZ surpassed that of the daytime tranquilizer medazepam, but was inferior to the effect of the usual doses of these preparations (0.25-10 mg/kg) (Fig. 2).

In considering the spectrum of pharmacological activity of the tranquilizers, special attention was paid to studying their effect on motor activity in the conflict test. In the control animals both types of activity were shown to be suppressed during the experimental session in comparison with the conditioning (training) period. This suppression was less pronounced after the injection of PZ in a dose of 10^{-10} mol/kg. Injection of 10 mg/kg PZ considerably suppressed locomotion during the experimental session (Table 1). Similar effects were observed with FNZ. Thus, PZ and FNZ in a dose of 10^{-10} mol/kg exhibit a marked tranquilizing effect without causing a concomitant suppression of behavior.

When the current delivered to the water dish and floor of the experimental chamber was increased to 1 mA, the anticonflict effect of superlow doses of tranquilizers as well as the effect of the daytime tranquilizer medazepam disappeared, whereas the effect of PZ and FNZ diminished and remained at a lower level (Fig. 3).

In low doses PZ and FNZ were shown to have no myorelaxant effect, as is evidenced from the fact that the ability to cling to the rotating rod was completely preserved. However, when injected in the usual doses, PZ and FNZ, like medazepam induced pronounced myorelaxation, the ED_{50} in this test was 2.48 (1.65-3.72) mg/kg for PZ, 2.8 (1.65-3.95) mg/kg for FNZ, and 16.7 (9.27-30.06) mg/kg for medazepam.

TABLE 1. Effect of Phenazepam (PZ) on Motor Activity of Rats in the Conflict Test

Type of activity	Control		PZ, 10^{-10} mol/kg		PZ, 10 mg/kg (2.4×10^{-5} mol/kg)	
	conditioning	conflict	conditioning	conflict	conditioning	conflict
Horizontal	17.9±1.7	8.2±1.72*	18.95±1.19	12.9±1.28*	21.4±3.6	7.9±1.4**
Vertical	21.5±3.2	8.3±1.94**	17.26±1.4	11.27±1.31*	18.4±1.9	3.1±1.2**
Grooming acts	4.0±0.61	2.5±0.45*	3.0±0.34	2.27±0.28	4.5±0.9	1.1±0.6**

Note. *$p<0.05$, **$p<0.01$ in comparison with this parameter during conditioning.

Benzodiazepine tranquilizers are known to exert their effect by interacting with the GABA-benzodiazepine receptor complexes [6], their efficiency both in the clinical setting [7] and under experimental conditions [5] correlating with their ability to interact with the receptors. Stress is also known to modify this complex [9]. Due to the high affinity of PZ and FNZ [3] for the receptor, superlow doses of the preparations produce an effect in weak stress modeled by the delivery of a weak current (0.5 mA) to the water dishes. It may be assumed that a stronger stress factor (1 mA current) will produce more pronounced changes in the conditions of ligand-receptor interaction, which will abolish the effect of superlow doses of the tranquilizers. Under these conditions the usual doses of PZ and FNZ preserve their effect, albeit at a lower level, due to high receptor affinity.

At present, the mechanism of action of superlow doses of biologically active substances is being widely discussed [1], but no consensus has been reached. The existence of some receptors with a dissociation constant lying far below 10^{-12} M may be hypothesized. However, difficulties in the experimental measurement of such values hampers verification of this hypothesis.

Thus, the benzodiazepines phenazepam and flunitrazepam in superlow doses exhibit an anxiolytic effect in a conflict situation and this effect is not accompanied by myorelaxant or sedative effects.

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