Study of Bipathic Effect of Phenazepam

O. I. Epstein, T. A. Voronina, G. M. Molodavkin, M. V. Belopol'skaya, I. A. Kheyfets, J. L. Dugina, and S. A. Sergeeva

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 144, No. 10, pp. 417-419, October, 2007
Original article submitted June 29, 2007

Phenazepam in ultralow doses significantly potentiated anxiolytic and anticonvulsant effects of therapeutic doses of the same drug both after preliminary or simultaneous administration, which attests to bipathic action of phenazepam. The combination of ultralow and therapeutic doses of phenazepam prevented the development of its specific myorelaxant and sedative side effects.

Key Words: *phenazepam; ultralow doses; bipathic effect*

The benzodiazepine-like tranquilizers possess a number of pronounced side effects limiting their use. This stimulates the search for novel preparations exhibiting pronounced anxiolytic activity not accompanied by side effects. Phenazepam applied in ultralow doses (10^{-14}-10^{-9} M) demonstrated a pronounced anxiolytic action not accompanied by myorelaxant and sedative side effects characteristic of benzodiazepine tranquilizers [2,3,8].

It was shown that ultralow doses of phenazepam modify the effect of the same drug administered in usual therapeutic doses; this phenomenon was termed as bipathic effect [8]. Phenazepam is widely used in clinical practice as a potent anxiolytic and anticonvulsant preparation, although administration of the drug in high doses or during long time is fraught with side effects [5-7]. Based on bipathic phenomenon, one can hypothesize that combination of therapeutic and ultralow doses of phenazepam (ULDP) produces a bipathic effect modifying the action of phenazepam by moderating its side effects, but preserving the anxiolytic and anticonvulsant effects.

Our aim was to examine the anxiolytic, sedative, myorelaxant, and anticonvulsant effects of phenazepam related to its bipathic use.

Materia Medica Holding Research-and-Production Company, Moscow. ***Address for correspondence:*** belopolskayamv@materiamedica.ru. M. V. Belopol'skaya

MATERIALS AND METHODS

The experiments were carried out on random-bred male albino rats weighing 230-250 g. The rats were randomized into 6 groups (10 animals per group). Controls received distilled water. Rats of experimental group 1 received phenazepam in a dose of 1 mg/kg (0.5 mg/kg in the study of anticonvulsant action) 20 min before testing. Rats of experimental groups 2 and 3 received ULDP (a mixture of homeopathic dilutions C12+C30+C200 corresponding to 10^{-27} M, 2.5 ml per 1 kg body weight) 20 and 30 min before testing, respectively. In group 4 rats, ULDP (2.5 ml/kg) and phenazepam (1 mg/kg) were administered simultaneously 20 min before testing. Group 5 rats received ULDP (2.5 ml/kg) 10 min before phenazepam (1 mg/kg, delayed administration), and the testing was started 20 min after phenazepam administration.

For evaluation of the anxiolytic effect of the drugs, we used Vogel conflict test based on conflict between drinking motivation and painful electrical shock [1,4,9,10]. The number of punished water licks was recorded. The myorelaxant effect of the drug was examined in rotarod test and the sedative effect was evaluated by changes in explorative activity in the open field test. The anticonvulsant activity was tested in rats with corazol-induced seizures [5-7]. To this end, corazol was

0007-4888/07/1444-0536

TABLE 1. Anxiolytic Effect of Phenazepam (1 mg/kg), ULDP, and Their Combination Assessed by Vogel Conflict Test in Rats (*M*±*m*)

Group	Time between administration of the drugs and testing (min)	Number of punished water licks electrical current strenght (mA)	
		0.25	0.5
Control	20	177.75±43.02	28.00±16.14
Phenazepam, 1 mg/kg	20	415.67±113.96*	211.00±136.72*
ULDP	20	260.67±38.21	No data
ULDP	30	358.33±60.75*	19.60±10.74
ULDP+phenazepam (1 mg/kg), simultaneously	20	1279.33±82.28**	60.67±21.38
ULDP before phenazepam (1 mg/kg)	20	1022±46.36**	68.20±24.67

Note. *p<0.05, **p<0.01 compared to the control.

injected subcutaneously in a dose of 100 mg/kg. The effects of the examined drugs on the severity of seizures, mortality, and latency to the first seizure attack were assessed.

RESULTS

In the conflict test (0.25 mA current), phenazepam (1 mg/kg) produced a characteristic anticonflict effect manifested in a significant increase in the number of punished licks, which attested to the anxiolytic effect of the drug (Table 1). ULDP administered 20 min before the experiment also produced an anticonflict action, which agreed with previous data [1]. When the interval between the injection and testing was prolonged from 20 to 30 min, the effect of ULDP became more pronounced: the number of punished drinks significantly surpassed the control, but was lower than after administration of phenazepam in therapeutic doses. Potentiation of the anxiolytic action of phenazepam was observed during simultaneous administration of phenazepam (1 mg/kg) and ULDP. Similar potentiation was observed in the experiment with delayed (by 10 min) administration of phenazepam in therapeutic doses.

ULDP applied 20 min before conflict test with electrical current of 0.5 mA produced no significant anticonflict action under any administration regimen in contrast to therapeutic dose of phenazepam (1 mg/kg), which exerted a pronounced effect. In experiments with combined administration of drugs (simultaneous or with an interval), a clear-cut anxiolytic effect was observed, which manifested in 1.6-1.7-fold increased number of punished water licks in comparison with the control. However, combined administration of ULDP and phenazepam resulted in a decrease of anxiolytic potency of the latter (1 mg/kg) suggesting "antagonistic" action of ULDP on the effect of phenazepam in therapeutic dose.

Phenazepam in the therapeutic dose of 1 mg/kg produced marked myorelaxation: 60% animals fell in the rotarod test and fall latency was 56.8±12.7 sec, while no falls were observed in the control group. In contrast to the therapeutic dose of phenazepam, ULDP produced no myorelaxation. ULDP injected 10 min before therapeutic dose decreased

TABLE 2. Effect of ULDP on Sedative Action of Phenazepam (1 mg/kg) in Open Field Test in Rats (*M*±*m*)

Index	Control	Phenazepam, 1 mg/kg	ULDP	ULDP before phenazepam	ULDP+ phenazepam, simultaneously
Horizontal activity	16.00±4.66	7.1±3.2*	26.33±3.20*+	11.67±10.32	37.33±5.79*+
Vertical activity	10.00±3.29	3.0±2.5*	16.00±4.19+	3.0±1.3*	13.00±3.55+
Explorative activity	4.67±1.53	1.33±1.31*	8.00±3.17+	2.67±0.58	3.00±1.73
Number of grooming acts	3.00±1.07	2.33±0.58	4.35±2.65	0±0	0.67±0.58
Fecal boluses	7.67±0.58	1.67±1.53*	2.00±1.73*	0.33±0.28	4.00±1.05
Number of entries into the center	0±0	0±0	1.67±0.58	0±0	1.33±0.53

Note. *p<0.05 compared to the control; +p<0.05 compared to phenazepam.

TABLE 3. Effect of Therapeutic Phenazepam Dose (0.5 mg/kg), ULDP, and Their Combination on Corazol-Induced Convulsions in Random-Bred Albino Rats (*M*±*m*)

Index	Corazol	Corazol+ phenazepam	Corazol+ ULDP	Corazol+ULDP (applied 10 min before phenazepam)	Corazol+ULDP+ phenazepam (simulta-neously)
Latency (h:min:sec)					
clonic convulsions	0:11:20± 0:03:13	0:22:20± 0:05:42*	0:09:40± 0:00:35	0:37:20± 0:06:48*+	0:32:00± 0:10:26*
tonic convulsions	0:16:40± 0:04:56	—	0:13:20± 0:00:34	—	0:33:00± 0:09:24*
Percent of rats with tonic convulsions	100	0	100	0	40
Mortality, %	80	0	100	0	20

Note. *$p<0.05$ compared to corazol; +$p<0.05$ compared to therapeutic dose of phenazepam.

the myorelaxant effect of the latter: number of falls decreased to 30% and their latency increased to 82.2±15.9 sec. After simultaneous administration ULDP and therapeutic dose, the myorelaxant effect was preserved.

Phenazepam in the therapeutic dose of 1 mg/kg produced a pronounced sedative effect manifested in a significant decrease in exploratory and motor activity in the open field test. ULDP increased open field activity; the rats visited the center of the field more frequently (Table 2).

Simultaneous administration of ULDP and phenazepam potentiated the activating effect of ULDP: vertical and horizontal activities increased, and visits to the center of the open field were preserved.

The study of the anticonvulsant action of the drugs showed that therapeutic dose of phenazepam (0.5 mg/kg) exerted a characteristic anticonvulsant effect manifested in prolongation of preconvulsion latency, moderation of their severity, and decrease in mortality (Table 3).

Individual administration of ULDP did not affect corazol-induced convulsions. Combined administration of ULDP with phenazepam (0.5 mg/kg) modified the anticonvulsant effect of the latter. The effect of ULDP given before phenazepam manifested in significant prolongation of the latency of clonic and tonic convulsions, which became less severe. The number of fatal outcomes markedly decreased. Simultaneous use of the therapeutic doses of phenazepam and ULDP significantly increased the anticonvulsant effect of the former, which manifested in lengthening of convulsion latency, decrease in the percent of rats with convulsions, and decrease in animal mortality.

Thus, this study showed that anxiolytic and anticonvulsant effects of phenazepam are significantly potentiated when it is applied in combination with ULDP (irrespective whether ULDP was administered before or simultaneously with phenazepam), which attested to bipathic effect of the drug. Moreover, the combination of ULDP with therapeutic dose of phenazepam moderated myorelaxant and sedative side effects of the drug.

REFERENCES

1. T. A. Voronina and G. M. Molodavkin, *Byull. Eksp. Biol. Med.*, **121**, No. 1, 63-66 (1996).
2. T. A. Voronina and G. M. Molodavkin, L. I. Chernyavskaya, *et al*., *Ibid.*, **124**, No. 9, 308-310 (1997).
3. G. M. Molodavkin, E. B. Burlakova, L. I. Chernyavskaya, *et al., Ibid*, **121**, No. 2, 63-66 (1996).
4. G. M. Molodavkin and T. A. Voronina, *Eksp. Klin. Farmakol.,* **58**, No. 2, 54-56 (1995).
5. S. B. Seredenin, T. A. Voronina, G. G. Neznamov, *et al*., *Phenazepam: 25 Years in Medical Practice* [in Russian], Moscow (2007).
6. *Pharmacology of Modern Anticonvulsant Preparations* [in Russian], St. Petersburg (1994), pp. 3-30.
7. *Characterization of Pharmacological Profile of Phenazepam. Research Methods* [in Russian], Kiev (1982), pp. 145-150.
8. O. I. Epstein, *Byull. Eksp. Biol. Med.,* Suppl. 4, 8-14 (2002).
9. S. Liljequist and J. A. Engel, *Pharmacol. Biochem. Behav.,* **21**, No. 4, 521-525 (1984).
10. R. Vogel, B. Beer, and D. E. Clody, *Psychopharmacologia*, **21**, No. 1, 1-7 (1971).