Experimental and clinical *pharmacology2010 Vol. 73 No. 11*
C. 10 - 14

GENDER DIFFERENCES IN THE EFFECTS OF PHENOTROPIL AND ITS STRUCTURAL ANALOGUE - COMPOUND RGPU-95 ON ANXIETY-DEPRESSIVE BEHAVIOUR OF ANIMALS

И. N. Tyurenkov1, **B. B.** Bagmetova1, **A. B.** Shishkina1, **B. M.** Berestovitskaya2, O. **S.** Basilieva2, **E. S.** Ostrogliadov2

Phenotropil and its structural analogue - compound RGPU-95 to a greater extent reduce the severity of anxiety-depressive behaviour in male rats than in females. In terms of expression of anxiolytic action, the compound RGPU-95 is statistically significantly superior to phenotropil, but inferior to diazepam; in terms of antidepressant activity it is comparable to melipramine and superior to phenotropil.

Keywords: gender differences, phenotropil, anxiolytic action, anti-depressive action

INTRODUCTION

One of the most productive ways to search for psychotropic substances is to modify the structure of already available drugs, allowing to increase the specificity of action, reduce toxicity, increase efficacy and bioavailability. Pheno-tropil (N-carbomail-methyl-4-phenyl-2-pyrrolidone), a GABA derivative, is an effective nootropic drug; it also has weak anxiolytic, antidepressant [1] and other properties [3], but it can cause psychomotor agitation, agitation, sleep disturbance [4], i.e. side-effects unacceptable in the treatment of anxiety and depression.

As a result of a targeted search for substances with N-carbomail-methyl-4-para-chloro-phenyl-2-pyrrolidone with the laboratory code RGPU-95 (Fig. 1) was found to have anxiolytic and antidepressant effects in a number of compounds close in structure to phenotropil.

This paper analyses comparative data on the effects of phenotropil and RGPU-95 on anxiety-depressive behaviour in male and female rats.

RESEARCH METHODS

The study was performed on 50 female and 50 male Wistar rats weighing 250 - 280 g, kept in standard vivarium conditions with natural light and dark regime, at air temperature 20 - 21 °C and with free access to water and food. The animals were obtained from the Federal State Unitary Enterprise "Rappolovo Laboratory Animal Nursery" of the Russian Academy of Medical Sciences (Leningrad Region). The animals were kept in accordance with rules of laboratory practice for preclinical research in the Russian Federation (GOST Z 51000.3-96 and 51000.4-96) and Order of the Ministry of Health of the Russian Federation No. 267 from 19.06.2003 г. "On Approval of the Laboratory Practice Regulations (GLP) in compliance with the International Guidelines of the European Convention for the Protection of Vertebrate Animals Used in Experimental Research (1997).

The effect of phenotropil and the compound RGPU-95 on spontaneous individual behaviour of animals was carried out in the "open field" test [7]. To study the anxiolytic properties of the investigated substances, classical models of anxiety were used - the "elevated cruciform maze" test and the method of conflict situation, Vogel variant, in combination with 48-hour pre-deprivation by drinking in conditions of free access to dry food [4]. The antidepressant activity of the compound RGPU-95 was studied using a model of stress-induced depression in animals in the Porsolt test of unavoidable forced swimming [6, 7].

Compound RGPU-95 and phenotropil were administered to animals in equimolar concentration in doses of 1/10 of the molecular weight: RGPU-95 - 25 mg/kg, phenotropil - 22 mg/kg. Both substances were synthesised at the Department of Organic Chemistry of the Herzen Russian State Pedagogical University (St. Petersburg). Diazepam - 1 mg/kg (Simplex pharma Pvt. Ltd., India), melipramine - 15 mg/kg (Egis Pharmaceuticals, Hungary) were also used as positive control agents. Since the investigated substance is not completely water soluble, in order to create a uniform suspension, it and the phenotro-pil substance were diluted in 2% starch mucus. Control animals received a similar solution of starch mucus in an equivalent volume. Administration of the compound, positive control preparations and starchy mucus solution to control animals

[1] Department of Pharmacology and Biopharmacy, Faculty of Pharmacy (Head - Prof. I. H. Tyurenkov) Volgograd State Medical University, 400131, Volgograd, 1a Pashykh Bortsov Square.

[2] Department of Organic Chemistry (Head - Prof. V. M. Berestovitskaya) Russian State Pedagogical University, 48, Moika River Embankment, St. Petersburg, 191186.

was administered once orally 60 min before the tests.

The obtained results were subjected to statistical processing using non-parametric Mann-Whitney U-test, Kruskal-Wallis rank one-factor analysis, Dana criterion for multiple-

The statistical comparison, chi-square test. Statistically significant were considered effects at $p < 0,05$.

RESULTS AND DISCUSSION

In the "open field" test, females of the control group had higher indices of locomotor and exploratory behaviour than males, as well as the number of acts of short-term grooming (lasting less than 5c), characterising the state of emotional discomfort and tension (Fig. 2 *a*, *b*, *d*). These data indicate higher anxiety in females compared to males. The compound RGPU-95 had no significant effect on locomotor and orientation-research-ing behaviour.

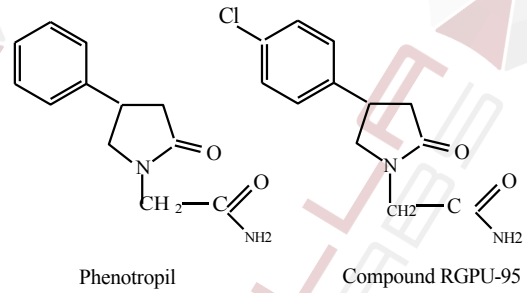

Figure 1. Structural formula of RGPU-95.

In contrast, phenotropil statistically significantly increased both locomotor and orientation-seeking activity in male and female rats under conditions of stress of "situation novelty". Phenotropil, on the contrary, statistically significantly increased both locomotor and orientation-exploratory activity in male and female rats.

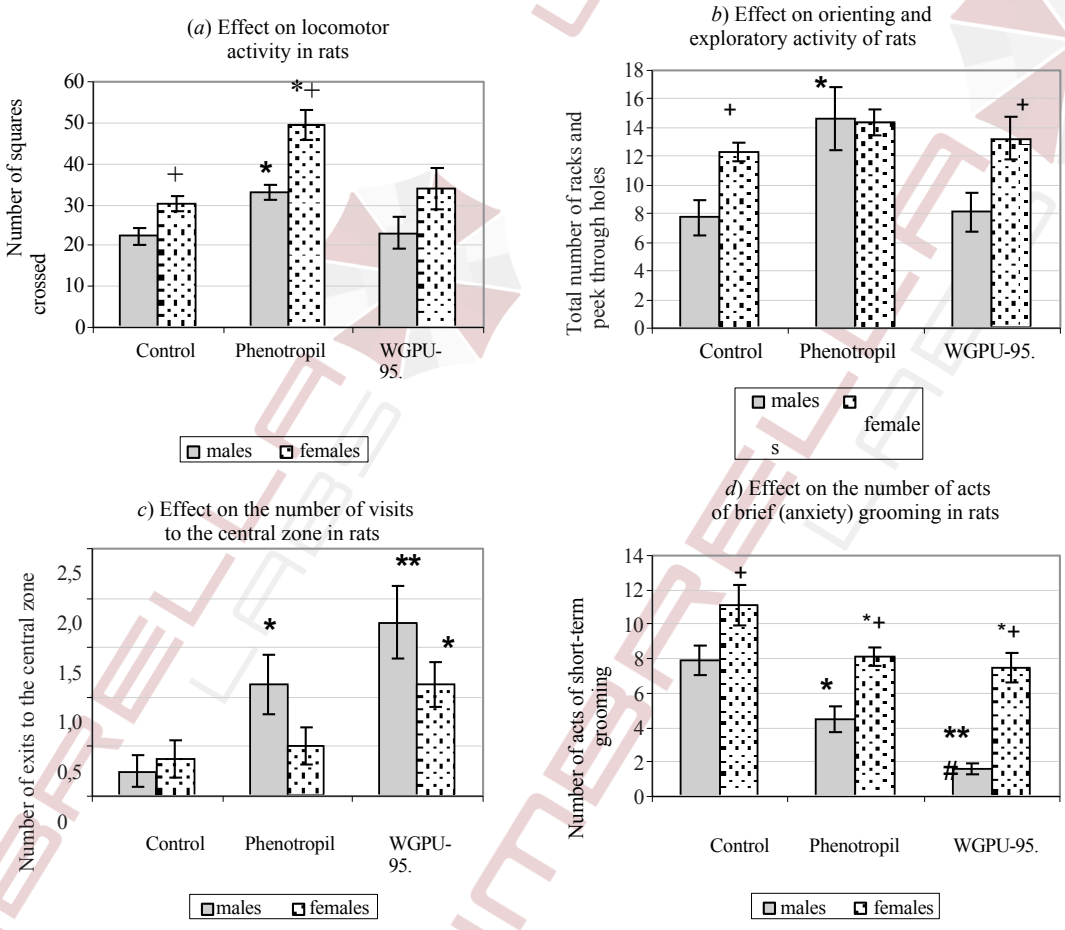

Fig. 2. Results of studying the effect of substance RGPU-95 and phenotropil on the behaviour of male and female rats in the "open field" test (*a* - *d*).

Here and in Figs. 3 and 4: Differences are significant when comparing: * - $p < 0.05$; ** - $p < 0.01$ - with the control group of animals;$^{\#}$ - $p < 0.05$;$^{\#\#}$ - $p < 0.01$ - with the group of animals receiving phenotropil;$^{+}$ - $p < 0.05$;$^{++}$ - $p < 0.01$ - with the corresponding group of males (Cruz-Kahl-Wallis rank one-factor analysis, Dunn's criterion for multiple comparisons).

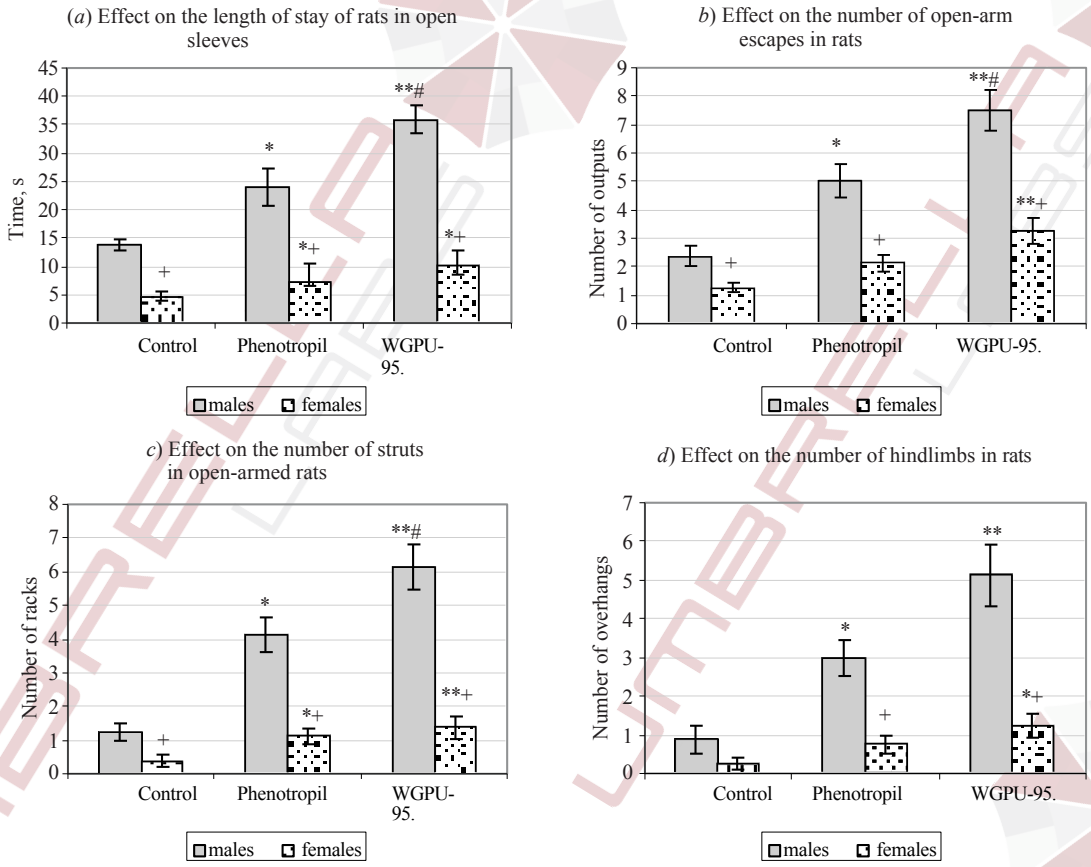

Fig. 3. The results of studying the effect of the substance RGPU-95 and phenotropil on the behaviour of male and female rats in the test "elevated cruciform maze" (*a* - *d*).

The designations are the same as in Fig. 2.

In addition, phenotropil increased orientation activity in males and locomotor activity in females. In addition, phenotropil increased the number of exits to the central zone (Fig. 2 *c*) and decreased the number of acts of short-term "anxious" grooming mainly in males.

Effect of RGMU-95, phenotropil and diazepam on the behaviour o f iris in a Vogel ionfluid situation (*M* ± *m*)

Group		Latent first approach period to the drinker, with	Quantity of punishable water withdrawals
Control	males	39,4 ± 1,7	5,6 ± 1,1
	females	61,6 ± 2,6^{+}	2,6 ± 0,5^{+}
Phenotropil	males	33,4 ± 2,5	9,2 ± 0,7*
	females	34,8 ± 4,0*	3,8 ± 0,6^{+}
WGPU-95.	males	19,2 ± 1,3$^{**\#}$	14,6 ± 1,2$^{**\#}$
	females	11,4 ± 1,0$^{**\#+}$	5,0 ± 0,7^{+}
Diazepam	males	10,6 ± 1,4$^{**\#}$	20,6 ± 2,3$^{**\#}$
	females	9,6 ± 1,1$^{**\#}$	9,0 ± 0,7$^{**\#+}$

Note. Differences are significant when comparing: * - $p < 0.05$; ** - $p < 0.01$ - with the control group of animals; # - $p < 0.05$; ## - $p < 0.01$ - with the group of animals receiving phenotropil; + - $p < 0.05$; ++ - $p < 0.01$ - with the corresponding group of males (Kruskal-Wallis single factor rank analysis, Dunn's criterion for multiple comparisons).

In the "elevated cruciform maze" test in the control group, females had a higher level of anxiety compared to males. They statistically significantly spent less time in the open arms of the maze and entered them less (Fig. 2 *a*, *b*). Females had practically no standing in the open arms and no hanging. In this test, the substance RGPU-95 and phenotropil increased in animals of both sexes (to a greater extent in males) the number of stands and exits to open arms of the maze,
the total time spent in them, the number of hang-ups from open sleeves - that is, they contributed to overcoming the natural fear of heights and brightly lit unfamiliar space. The anxiolytic effect of the compound RGPU-95 was significantly greater in male males than in phenotropil (Fig. 3).

Under Vogel conflict conditions, based on a clash of food and defensive behaviour, females were also more susceptible to anxiety: they stayed away from the drinker longer than males and made fewer punishable attempts to quench the drinker.
thirst. The substance RGPU-95, the benzodiazepine anxiolytic diazepam and phenotropil decreased the expression of anxious behaviour in animals and had an anti-conflict anti-anxiety effect in this model: it decreased in animals of both sexes.

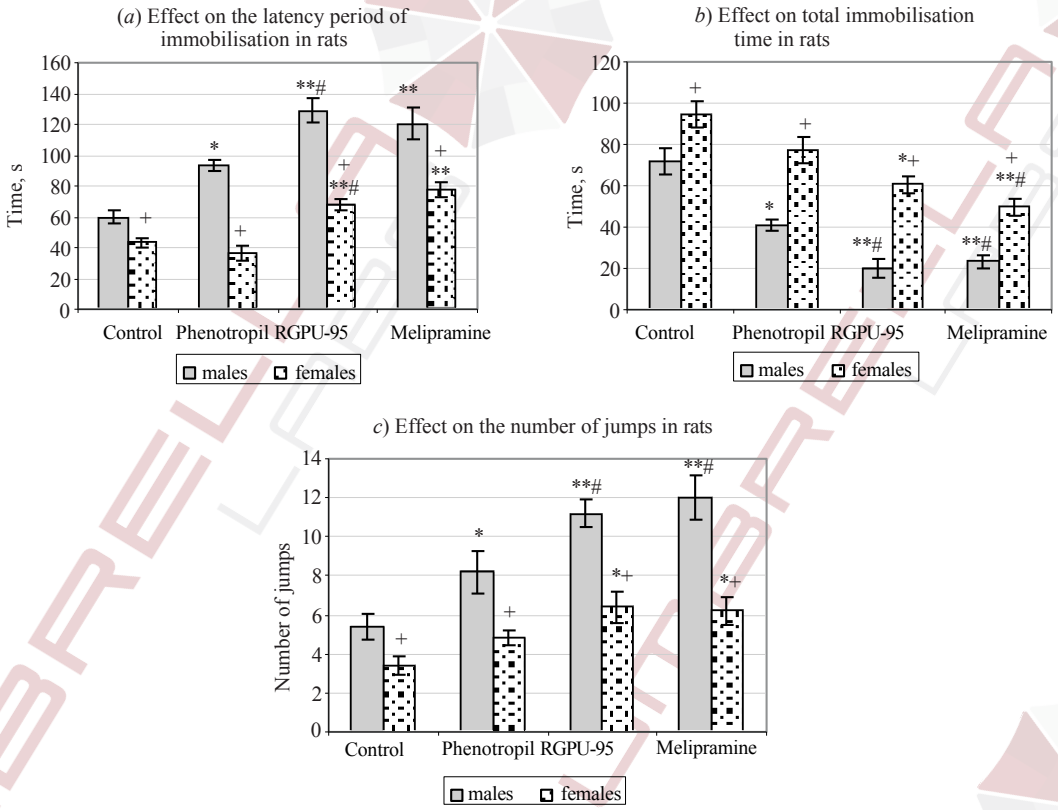

Fig. 4. Results of studying the effect of substance RGPU-95, phenotropil and melipramine on the behaviour of male and female rats in the Porsolt test of unavoidable forced swimming (*a* - *c*).

The designations are the same as in Fig. 2.

the latency period of the first punishment approach to the drink and increased the number of such approaches (tab-faces). The substance RGPU-95 and both comparison drugs had a significantly stronger effect on the latency of the punishable approach to the drinker in females, but to a significantly greater extent increased the number of punishable approaches to satisfy drinking motivation in males (the main index of this technique), which also indicates a greater expression of their anxiolytic effect in male animals, initially less prone to anxiety. In terms of the expression of anxiolytic effect in this test, the RGPU-95 compound was significantly superior to pheno-tropil, but inferior to diazepam.

Analysis of the results obtained in the Porsolt test of unavoidable forced swimming showed that female control animals exhibited greater expression of depressive behaviour - they had a longer total duration of immobilisation (despair behaviour characteristic of depression), a shorter latency period of immobilisation (depression developed faster), and a lower number of jumps (active behaviour of avoiding a stressful situation) than males (Fig. 4 *a-c*). The above differences were statistically significant and confirmed again.

The current opinion about the high predisposition to anxiety and depression in female individuals [2, 5].

The compound RGPU-95 and the comparison drugs phenotropil and melipramine caused a statistically significant increase in the latency period of immobilisation and a decrease in the total immobilisation time both in males and, to a lesser extent, in females, indicating their antidepressant activity. In terms of influence on the majority of indicators of this test the substance RGPU-95 was statistically superior to phenotropil and practically not inferior to melipramine.

Thus, in the model of stress-induced depression in the Porsolt unavoidable swimming test, the compound RGPU-95, as well as the comparison drugs melipramine and, to a lesser extent, phenotropil showed antidepressant activity with predominant efficacy in male rats. The antidepressant effect of RGPU-95 was statistically significantly more pronounced than that of phenotropil and was not inferior to that of melipramine.

CONCLUSIONS

1. A new structural analogue of phenotropil, the compound RGPU-95, reduces the severity of anxiety.

It has anxiolytic and antidepressant properties both in male rats, which are more resistant to anxiety and depression, and, to a lesser extent, in females, which are highly predisposed to anxiety-depressive behaviour.

2. In terms of the severity of anxiolytic action the compound RGPU-95 statistically significantly exceeds phenotropil, but is inferior to diazepam; in terms of antidepressant activity it is comparable to melipramine and superior to phenotropil. The investigated substance, as well as the comparison drugs, are characterised by greater efficacy when administered to male than to female rats.

LITERATURE

1. B. I. Akhapkina, R. V. Akhapkin, Y. A. Alexandrovsky, A. S. Avedisova, T. A. Voronina, V. V. Nesteruk, Russian Patent 2232578 (2003), 7 A61K31 / 4152, A61P25 / 24 (2004).
2. Э. B. Arushanyan, *Expert. u kltsn. pharmacol.*, **70**(1), 63 - 71 (2007).
3. Y. B. Belousov, M. A. Mukhina, *Kachestvennaya klentskaya praktstka*, No. 3 (2005).
4. T. Y. Kulikova, *Phenotropol: Expert and Clinical Pharmacology and Practical Application*, Moscow (2007), pp. 72 - 77.
5. Э. A. Manvelyan, *Milk Dissemination of the Effects of Pharmaceuticals*, SGU, Stavropol (2008).
6. *Manual on expert (preclinical) review of new pharmacological substances*, R. U. Khabriev (ed.), Moscow (2005).
7. R. D. Porsolt, M. Le Pinchon, and M. Jalfre. *Nature,* **266**, 730 - 732, (1977).

Received 16.03.10

GENDER DIFFERENCES IN ACTION FENOTROPIL AND ITS STRUCTURAL ANALOGUE - COMPOUND RGPU-95 ON ANXIETY-DEPRESSIVE BEHAVIOUR ANIMALS

I. N. Tyurenkov[1] , V. V. Bagmetova[1] , A. V. Shishkina[1] ; V. V. M. Berestovitskay[2] , O. S. Vasileva[2] , E. S. Ostroglaydov.

[1] Volgograd State Medical University, pl. Pavshikh Bortsov 1a, Volgograd, 400131, Russia

[2] Russian State Pedagogical University, 191186, St. Petersburg, Russia

Fenotropil and its structural analog - compound RGPU-95 to a greater extent reduce the severity of anxious and depressive behaviour in male rats than in fema- les. On expression of the anxiolytic compound RGPU-95 significantly exceeds Fenotropil, but inferior to Diazepam; of antidepressant activity - comparable to Melipramin and exceeds Fenotropil.

Key words: Gender differences, Fenotropil, anxiolytic, antidepressant.