Effect of a Prolyl Endopeptidase Inhibitor Benzyloxycarbonyl-Alanyl-Proline on the Development of Experimental Depressive Syndrome in Rats

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The effects of a competitive prolyl endopeptidase inhibitor benzyloxycarbonyl-alanyl-proline were studied in rats with experimental dopamine deficiency-dependent depressive syndrome due to systemic administration of a proneurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine for 14 days. The inhibitor was injected intraperitoneally 30 min before treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (2nd week of the study). This substance contributed to rapid disappearance of depressive symptoms during the recovery of behavioral activity. Our results indicate that benzyloxycarbonyl-alanyl-proline has the antidepressant properties.

Key Words: *experimental depressive syndrome; 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; prolyl endopeptidase inhibitor; benzyloxycarbonyl-alanyl-proline; rats*

High incidence of anxiety-depression disorders [5] necessitates the development of new pathogenetic methods for their correction. According to current concepts of neurobiological mechanisms for emotional-and-behavioral disturbances, the symptoms of depression and anxiety are associated with function of some proline-containing neuropeptides (*e.g.*, neuropeptide Y and substance P) [11]. These molecules are hydrolyzed by enzymes that belong to a class of serine proline-specific peptidases. They include prolyl endopeptidase (PEP, EC 3.4.21.26) and dipeptidyl peptidase IV (DP-IV, EC 3.4.14.5) [14].

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Clinical studies revealed that the activity of these enzymes in blood plasma and serum is modified in patients with anxiety-depression disorders [12]. We showed for the first time that the activity of PEP and DP-IV in target structures of the central dopaminergic brain system increases in rats with experimental dopamine deficiency-dependent depressive syndrome [3]. Our experiments demonstrated that a noncompetitive synthetic inhibitor of PEP, benzyloxycarbonyl-methionyl-2(S)-cyanopyrrolidine (Z-Met-Prd-N) has the antidepressant activity [8]. Dipeptide inhibitors of PEP (Z-AA-Pro-OH, where AA are the residues of amino acids Gly, Ala, Ile, and Pro), including benzyloxycarbonyl-alanyl-proline (Z-Ala-Pro-OH), prevent the symptoms of depressiveness in mice on the model of stress-induced reactive depression (Porsolt forced swimming test) [1].

Here we studied the effect of a synthetic competitive inhibitor of PEP, benzyloxycarbonyl-ala-

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nyl-proline (INH), on the development of experimental 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced (MPTP) depressive syndrome in rats.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 400-450 g. The animals were housed in individual cages and had free access to water and food (dry feed). Depressive state in rats was induced by systemic administration of a proneurotoxin MPTP. This compound has a specific effect on dopaminergic neurons. MPTP was synthesized at the Institute of Pharmacology (Russian Academy of Medical Sciences). MPTP was injected intraperitoneally in a daily dose of 20 mg/kg for 14 days [4]. Control rats were administered with physiological saline (PS). Some animals of the treatment and control groups received intraperitoneal injections of INH (3 mg/kg) 30 min before administration of MPTP or PS (2nd week of the study). INH was synthesized at the V. V. Zakusov Institute of Pharmacology (Russian Academy of Medical Sciences). The inhibition constant for PEP from subcortical structures and brain cortex was 20 and 90 mmol/liter, respectively (substrate Z-Ala-Pro-7-amino-4-coumarylamide) [1]. The INH+MPTP and INH+PS groups consisted of 7 and 8 rats, respectively. The remaining animals received PS and were divided into the groups of PS+MPTP (n=8) and PS+PS (n=8). The preparations were administered in a dose of 1 ml per kg body weight. No between-group differences were found in the basal locomotor activity, degree of anxiety, and body weight. The animals were also examined over 2 weeks after withdrawal of test preparations (recovery of behavioral activity).

The severity of MPTP-induced depressive syndrome was evaluated from the following symptoms of experimental depressive syndrome: hedonic disturbances in the sucrose preference test (decrease in preference); behavioral despair (increase in the immobility time) and biorhythmic disorders (increase in the depression index (DI), ratio of short immobility periods (up to 6 sec) to the total number of episodes of intensive swimming) in the forced swimming test; and reduction of drinking (decrease in fluid intake) and food motivation (decrease in body weight as an indirect symptom) [4]. The overall severity of depression was expressed in points [2]. Low value of the total score (0-1 point) reflected the absence of behavioral depression. The degree of anxiety was determined by a special scale for anxious-and-phobic symptoms [7]. Locomotor activity was studied in the open-field test for 3 min [2].

The results were analyzed by Statistica 6.0 software. The correspondence of an empirical data distribution to a normal distribution was evaluated by Kolmogorov—Smirnov test. In a normal distribution, the mean values of several independent samples were compared by one-way analysis of variance (ANOVA, F test). A mean-variance analysis involved the Duncan test. The nonparametric one-way analysis of variance by Kruskal—Wallis (H test) was applied in a non-normal distribution. The nonparametric Mann—Whitney U test was used for post-hoc analysis. The significance of intragroup differences was determined by means of repeated measures ANOVA (F test, post-hoc analysis by Duncan test) and paired nonparametric Wilcoxon test. The significance level was 5%.

RESULTS

Chronic administration of the proneurotoxin to rats of the PS+MPTP group was followed by a decrease in the preference for 10% sucrose over water on day 4 of treatment. Daily fluid intake was reduced on day 2 of MPTP injection. These parameters remained low over the 2nd week of the study (subsequent treatment with PS and MPTP; $F_{(29,\ 174)}$= 3.025, p=0.000), as well as for 2 weeks after withdrawal of test preparations ($F_{(29,\ 174)}$=3.268, p=0.000; Fig. 1, *a*, *b*). A significant decrease in body weigh was revealed over 2 weeks of proneurotoxin treatment. Body weigh returned to normal after drug withdrawal ($F_{(4,\ 28)}$=5.209, p=0.003; Fig. 2). The immobility time and DI in the forced swimming test did not differ by the end of 2-week treatment with MPTP and 2 weeks after drug withdrawal (p>0.05, Wilcoxon test; Fig. 3, *a*, *b*). The overall severity of behavioral depression decreased after cessation of treatment. The degree of depression during MPTP injection and 2 weeks after drug withdrawal was 3.8±0.8 and 2.3±0.8 points, respectively (p<0.05, Wilcoxon test).

One-week administration of the proneurotoxin to rats of the INH+MPTP group was followed by a decrease in daily fluid intake and reduction of sucrose preference (similarly to the PS+MPTP group). These parameters remained low over the 2nd week of subsequent treatment with INH and MPTP ($F_{(29,\ 174)}$= 5.376, p=0.000; and $F_{(29,\ 174)}$=6.069, p=0.000, respectively). On day 4 after drug withdrawal, all parameters did not differ from the baseline (Fig. 1, *a*, *b*). Body weight of animals decreased during injection of MPTP, but returned to normal after combined treatment with INH and MPTP. As differentiated from the PS+MPTP group, body weight of rats increased and exceeded the baseline after

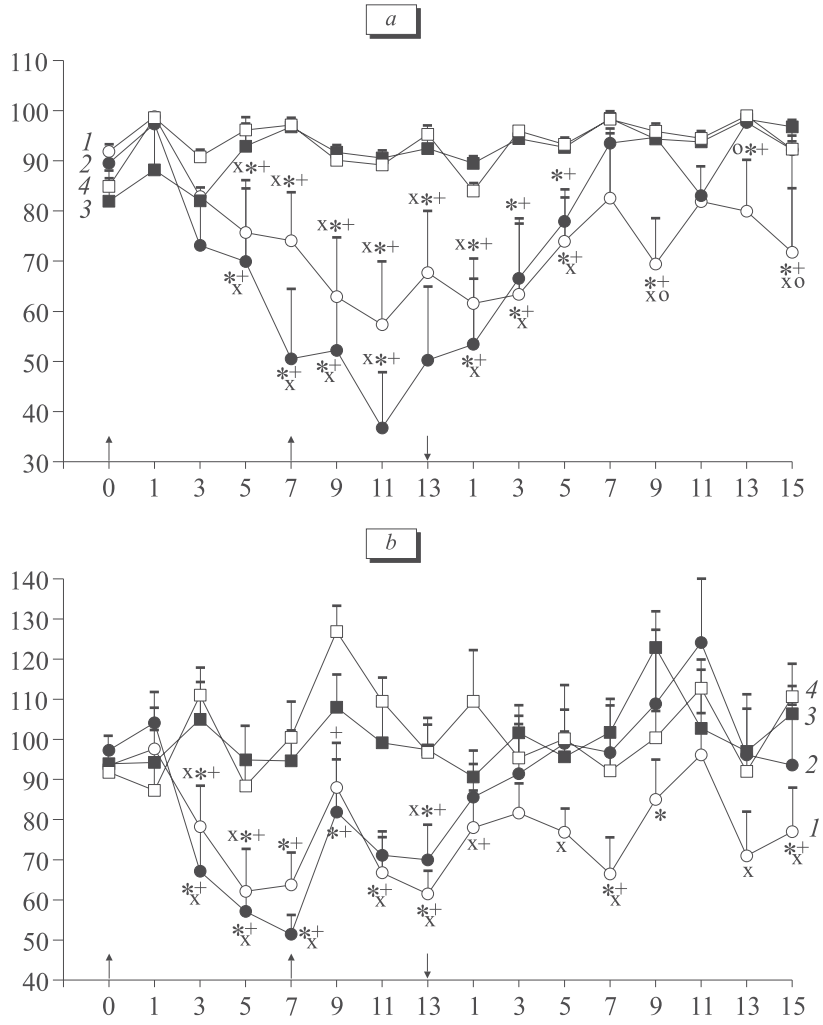

Fig. 1. Preference for 10% sucrose solution over water (*a*) and daily fluid intake (*b*) in rats with experimental depressive syndrome. Here and in Figs. 2 and 3: PS+MPTP group (*1*), INH+MPTP group (*2*), PS+PS group (*3*), and INH+PS group (*4*). Abscissa, days; 0, baseline value (before drug treatment); 1st arrow, start of treatment with MPTP or PS; 2nd arrow, start of treatment with an inhibitor or PS; 3rd arrow, cessation of treatment. Here and in Fig. 2: post-hoc analysis by Duncan test, $p<0.05$ compared to the ^{x}baseline value. Mann—Whitney test: *differences between *1-2* and *3* on the same day; $^{+}$differences between *1-2* and *4* on the same day; ^{o}differences between *1* and *2* on the same day.

drug withdrawal ($F_{(4, 24)}=16.524$, $p=0.000$; Fig. 2). The immobility time and rhythmic DI after drug withdrawal were lower than those during MPTP injection ($p<0.05$, Wilcoxon test; Fig. 3, *a*, *b*). The score of behavioral depression after drug withdrawal was lower that that observed by the end of MPTP treatment (0.7±0.3 and 3.3±0.6 points, respectively; $p<0.05$, Wilcoxon test).

Behavioral depression was not found in animals of the PS+PS and INH+PS groups. The volume of fluid intake and preference for sucrose in some periods of the study exceeded the baseline value ($F_{(29, 203)}=18.335$ and $F_{(29, 203)}=3.077$; and $F_{(29, 203)}=3.550$ and $F_{(29, 203)}=3.220$, respectively; $p=0.000$; Fig. 1, *a*, *b*). The increase in body weight was revealed during administration of test preparations and after drug withdrawal ($F_{(4, 28)}=27.196$, $p=0.000$ for the PS+PS group; and $F_{(4, 28)}=21.333$, $p=0.000$ for the INH+PS group; Fig. 2). The immobility time and rhythmic DI in these rats decreased after drug withdrawal (as compared to the period of MPTP treatment; $p<0.05$, Wilcoxon test; Fig. 3, *a*, *b*). The overall score of depression in animals of the PS+PS and INH+PS groups remained low during administration of test preparations (0.6±0.3 and 0.3±0.3, respectively) and after drug withdrawal (0.01±0.1 and 0.0± 0.0, respectively). These data illustrate the absence of behavioral depression in specimens of both groups.

An intergroup comparative study showed that the degree of sucrose preference in rats of the PS+ MPTP and INH+MPTP groups is lower than in animals of the PS+PS and INH+PS groups on days 4-

14 of drug treatment. This parameters was lowest on day 4 ($H_{(3,30)}$=8.097, p=0.044), but highest on day 10 ($H_{(3,30)}$=20.393, p=0.000; Fig. 1, *a*). After drug withdrawal, sucrose preference in rats of the PS+MPTP group remained lower than in animals of the PS+PS, INH+PS, and INH+MPTP groups ($H_{(3,30)}$= 12.794, p=0.005; last day of the study). No differences were revealed between the INH+MPTP and control groups on day 6 after drug withdrawal.

Daily fluid intake during proneurotoxin administration in rats of the PS+MPTP and INH+MPTP groups was lower compared to the PS+PS and INH+PS groups. This parameters was lowest on day 3 ($H_{(3,30)}$=9.825, p=0.020), but highest on day 7 ($H_{(3,30)}$=17.771, p=0.000; Fig. 1, *b*). Drug withdrawal was accompanied by an increase in the overall volume of fluid intake in animals of the INH+ MPTP group, which did not differ from the control value (day 3 after cessation of treatment). After drug withdrawal, daily fluid intake in rats of the PS+MPTP group was lower than in specimens of the PS+PS and INH+PS groups ($H_{(3,30)}$=10.098, p=0.018; last day of the study).

Body weight in INH-receiving animals increased only by the end of the study (similarly to specimens of the PS+PS and INH+PS groups; $F_{(3,27)}$= 5.801, p=0.003; Fig. 2).

An intergroup comparative study showed that the immobility time and DI in animals of the PS+ MPTP and INH+MPTP groups do not exceed the corresponding parameters in specimens of the PS+ PS and INH+PS groups ($H_{(3,31)}$=2.801, p=0.423; $H_{(3,31)}$=4.185, p=0.242; $H_{(3,31)}$=5.244, p=0.155; and $H_{(3,31)}$=3.281, p=0.350, respectively; Fig. 3, *a*, *b*).

No differences were found in the overall severity of depression in rats of the PS+MPTP and INH+ MPTP groups during treatment with test preparations. The mean values were shown above. However, the degree of depression in these animals was higher than in specimens of the PS+PS and INH+PS groups ($H_{(3,31)}$=16.574, p=0.001; p<0.05, Mann—Whitney test). After drug withdrawal (recovery of behavioral activity) the overall score of depression in rats of the PS+MPTP group significantly differed from than in specimens of other groups ($H_{(3,31)}$= 13.675, p=0.003; p<0.05, Mann—Whitney test).

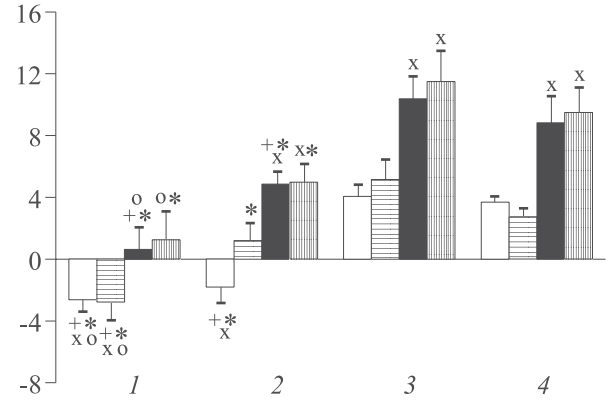

Fig. 2. Body weight of rats during administration of test preparations (light bars, 1st week of the study; horizontal shading, 2nd week of the study) and behavioral recovery after cessation of treatment (dark bars, 3rd week of the study; vertical shading, 4th week of the study). Abscissa: changes in body weight (percent of the initial value).

In groups of PS+MPTP, INH+MPTP, PS+PS, and INH+PS, baseline anxiety levels (6.6±1.2, 6.4± 1.0, 5.6±0.9, and 6.7±1.2 points, respectively) and locomotor activity (51.2±4.3, 43.3±5.9, 50.1±3.9, and 50.7±8.4 squares, respectively) remained practically unchanged in all periods of the study.

Hence, administration of MPTP to rats is followed by the development of experimental dopamine deficiency-dependent depressive syndrome.

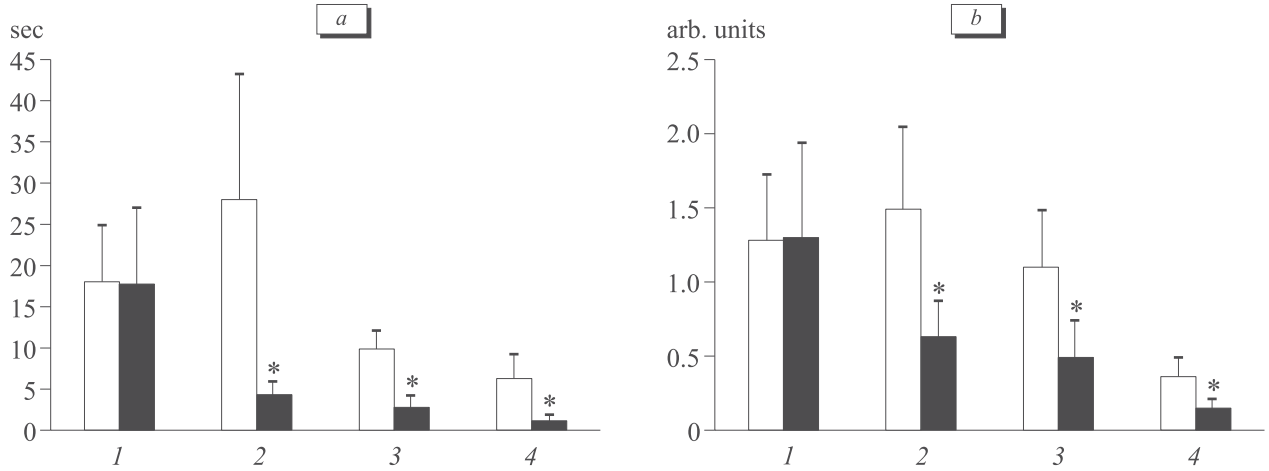

Fig. 3. Immobility time (*a*) and rhythmic DI (*b*) of rats in the forced swimming test. Light bars, administration of preparations; dark bars, 2 weeks after drug withdrawal. *p<0.05 compared to the baseline value (Wilcoxon test).

These data are consistent with the results of our previous studies [2]. Subchronic treatment with INH before administration of MPTP (days 8-14) had no effect on the majority of symptoms of experimental depressive syndrome. At the stage of behavioral recovery (after cessation of treatment), a PEP inhibitor Z-Ala-Pro-OH contributed to the disappearance of depressive symptoms in rats with experimental depressive syndrome.

Our findings confirm the fact that a PEP inhibitor Z-Ala-Pro-OH has the antidepressant activity. Similar results were obtained in experiments on the model of behavioral despair in the forced swimming test [1]. Dysfunction of the central monoaminergic system, including a decrease in the activity of catecholaminergic structures [3], has an important role in the pathogenesis of psychoaffective disorders [9]. *In vivo* experiments showed that PEP inhibitors improve functional activity of the brain dopaminergic system [6]. PEP inhibitors have a modulatory effect on the content of substrates for this enzyme (*i.e.*, neuropeptides that mediate the development of depression and anxiety) [10]. These compounds produce a direct or indirect effect on the activity of brain monoaminergic systems [15].

Our previous experiments showed that the activities of PEP and DP-IV increase in brain structures of rats with experimental MPTP-induced depressive syndrome [3]. We conclude that proline-specific peptidases are involved in the central mechanisms for depressive states. Therefore, PEP inhibitors hold much promise for the combination pathogenetic therapy of depressive disorders.

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