Effects of Prolylendopeptidase Inhibitor Benzyloxycarbonyl-Methionyl-2(S)-Cyanopyrrolidine on Experimental Depressive Syndrome Development in Rats

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Model of experimental depressive syndrome in rats induced by repeated systemic injection of proneurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine showed that chronic injection of prolylendopeptidase inhibitor benzyloxycarbonyl-methionyl-2(S)-cyanopyrrolidine 30 min before pro-neurotoxin injection prevents the development of a number of depressive syndrome symptoms such as behavioral despair and biorhythmic disorders in forced swimming test, precludes the increase in anxiety-phobic level, prevents reduction of relative thymus mass. These results indicate that benzyloxycarbonyl-methionyl-2(S)-cyanopyrrolidine possesses antidepressant, anxiolytic, and/antistress properties.

Key Words: *experimental depressive syndrome; 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; prolylendopeptidase inhibitor; benzyloxycarbonyl-methionyl-2(S)-cyanopyrrolidine; rats*

Recent clinical observations and experimental data suggest the involvement of peptidergic systems in anxiety-depressive disorder development [8]. Prolyl-specific endopeptidase, serine peptidohydrolase, cleaving neuropeptide substrates, mediating the development of depressive and anxiety states, may play an essential role in the pathogenesis of these disorders [10]. Patients with major depression and anxiety symptoms have altered serum levels of prolylendopeptidase (PEP; EC 3.4.21.26) and dipeptidylpeptidase IV (DPP-IV; EC 3.4.14.5) [11,12]. Our studies showed that the development of experimental 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine(MPTP)-induced dopamine-deficient depressive syndrome in rats is associated with increased PEP and DPP-IV activities in the target structures of mesocortical and nigro-striatal central dopaminergic brain systems (striatum, frontal cortex, nucleus accumbens, and hypothalamus) [3]. Experiment using the model of acute stress-induced reactive depression (Porsolt test) revealed, that dipeptide PEP inhibitors with typical structure Z-AA-Pro-OH (where Z is benzyloxycarbonyl and AA are Gly, Ala, Ile, and Pro residues) exerted antidepressant action on animals, reducing the duration of floating in the forced swimming test [2]. PEP inhibitor benzyloxycarbonyl-methionyl-2(S)-cyanopyrrolidine (INH) was found to possess anxiolytic properties in experimental anxiety models (plus — maze and conflict tests) [1].

The aim of this work was evaluation of INH (artificial non-competitive PEP inhibitor) action on the development of experimental MPTP-induced

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depressive syndrome, anxiety level, and state of stress-marker organs in rats.

MATERIALS AND METHODS

Experiments were carried out on 47 male Wistar rats, weighting 320-450 g, which were caged individually during the study. During the experiment, the animals received only pellefed food and water *ad libitum*. Depressive state was induced by systemic injection of proneurotoxin MPTP, which is specific for dopaminergic neurons (synthesized in Institute of Pharmacology, Russian Academy of Medical Sciences, 20 mg/kg intraperitoneally daily for 14 days) [5]. Rats from control groups were injected with saline solution (Sal) according to the same schedule. Some animals from experimental and control groups received INH (synthesized in Institute of Pharmacology Russian Academy of Medical Sciences, 1 mg/kg intraperitoneally; groups INH+MPTP, n=8, and INH+Sal, n=8) 30 min before MPTP or saline injection, others received Sal (groups Sal+MPTP, n=12, and Sal+Sal, n=12). Compounds were administered in a volume of 1 ml/kg body weight. Rats receiving no preparations served as intact controls (INT, n=7). The groups did not differ by motor activity, anxiety level, and body weight.

The severity of MPTP-induced depressive syndrome was evaluated by the development of depression-like state symptoms: hedonic disorders (decrease in the preference of 10% sucrose solution consumption, by the development of "behavioral despair" and appearance of biorhythmic disorders (prolongation of floating and increase in rhythmic depression index (DI), which is determined as a ratio of the number of short floating episodes (<6 sec) to total number of active swimming episodes in the forced swimming test), by the decrease vital motivation level (decrease in orientation behavior, water consumption and body weight as indirect measure of reduced nutritional motivation) [4,5]. Anxiety level was assessed using specific scale for anxiety-phobic state assessment [7]. Motor activity was measured in the open field test for 3 min. 24 h after MPTP injection, when animals behavior was markedly depressive, the rats were decapitated and relative weight of stress-competent organs, adrenals and thymus, was evaluated.

Results were analyzed using Statistica 6.0 software. Correspondence of empirical data distribution to normal law was examined using Kolmogorov—Smirnov's test: in case of correspondence, the mean values of several independent samples were compared using one-way ANOVA with subequent comparison of dispersion complex mean values by Newman—Keuls test; in case of disconformity, the nonparametric one-way ANOVA test was used, and in this case post-hoc analysis was performed using nonpaired nonparametric Mann—Whitney U test. Within-group changes were evaluated using ANOVA for repeated measurements (post-hoc analysis using Duncan's test) and paired nonparametric Wilcoxon's test. The significance level was set at 5%.

RESULTS

At the end of 2-week proneurotoxin injection period, in rats from Sal+MPTP group, floating duration in the forced swimming test and depression index exceeded the corresponding values in all other groups (H (3, N=40)=8.090, p=0.044; H (3, N=40)= 8.547, p=0.036, respectively; results of post-hoc analysis are presented at Fig. 1). This data suggests the development of behavioral despair state and biorhythmic disorders of swimming behavior, *i.e.* the presence of depression-like behavior signs. Values of these parameters in rats from INH+MPTP group did not differ from control values.

Daily water consumption was deceased in comparison with baseline values in rats from groups Sal+MPTP ($F(14,154)$=4.036, p=0.000) and INH+ MPTP ($F(14,98)$=4.990; p=0.000) starting from day 5 of preparations injection (Fig. 2, *a*). Daily fluid consumption did not change in rats from Sal+Sal group ($F(14,140)$=1.695, p>0.05), and increased in INH+Sal group ($F(14,84)$=2.187, p=0.015). In rats from Sal+MPTP and INH+MPTP groups, daily fluid consumption was reduced in comparison with animals from Sal+Sal and INH+ Sal groups from day 3 to day 14 of treatment (minimal $F(3,36)$=3.220 was observed on day 6; p<0.050; maximum on day 14: $F(3,36)$=12.203, p<0.000). Cumulative volume of consumed fluid in Sal+MPTP and INH+MPTP groups was similar. Cumulative volume of consumed fluid in INH+Sal group was maximum and sometimes significantly surpassed that in Sal+Sal group (Fig. 2).

Preference of 10% sucrose decreased with time in animals from Sal+MPTP ($F(14,154)$=2.533; p=0.003) and INH+MPTP ($F(14,98)$= 3.161; p=0.000) groups in comparison with baseline values starting from day 8 of treatment (Fig. 2, *b*), while in Sal+Sal and INH+Sal groups statistically significant differences did not appear ($F(14,140)$=1.380; $F(14,84)$=1.712; for both p>0.05). Intergroup comparison revealed that on days 13 and 14 of treatment preference of sucrose consumption in Sal+MPTP and INH+MPTP groups was lower than in Sal+Sal and INH+Sal groups ($F(3,36)$=4.164 and $F(3,36)$=4.639, respectively; p<0.01 for both). By preference of sucrose

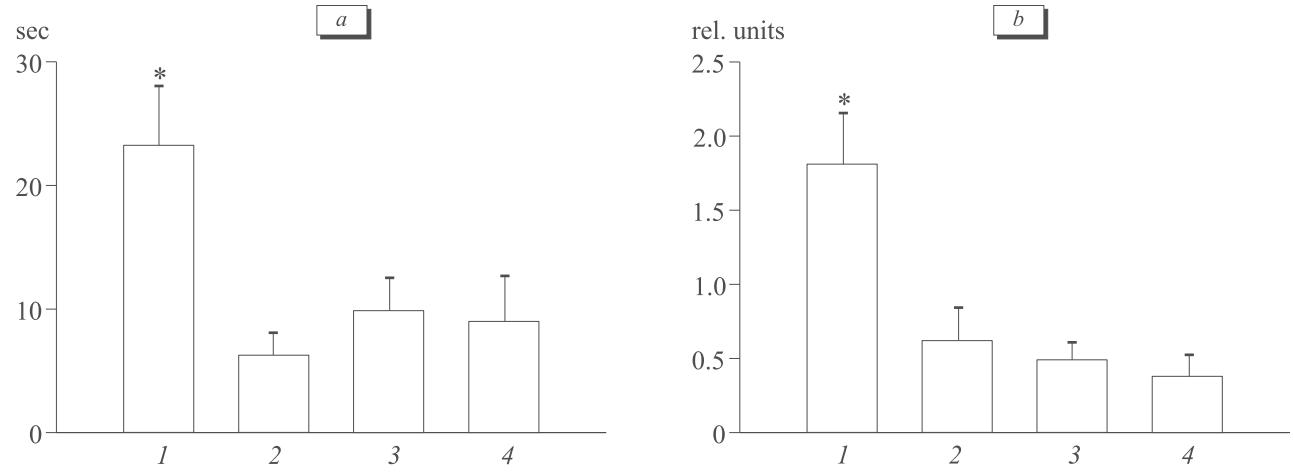

Fig. 1. Duration of immobilization (*a*) and rhythmologic depression index (*b*) in the forced swimming test in rats at the stage of marked depressive behavior. *1*) Sal+MPTP, *2)* Sal+Sal, *3)* INH+MPTP, *4)* INH+Sal. **p*<0.01 compared to other groups (Mann—Whitney test).

solution consumption Sal+ MPTP and INH+MPTP groups did not differ signifcantly.

In INT, Sal+Sal, and INH+Sal groups, body weight increased in comparison with baseline values, and in Sal+MPTP and INH+MPTP groups it decreased (at the baseline 397.9±21.4; 414.4±11.4; 433.8± 21.0; 419.8±11.5; 403.8±14.1 g, respectively, and 418.0±20.6; 431.9±11.0; 453.1±12.6; 405.9±10.0, and 382.1±10.1 g after 2 weeks of treatment; $p<0.05$).

Initial anxiety levels were similar in all groups: 5.1±1.8 (INT), 5.4±0.7 (Sal+MPTP), 4.6±0.6 (Sal+Sal), 6.1±0.5 (INH+MPTP), and 6.0±0.9 (INH+Sal) points. Anxiety level significantly increased in animals with preliminary Sal injection (Sal+MPTP and Sal+ Sal) in comparison with baseline values ($p<0.05$). No significant changes in anxiety level were noted in intact animals and rats, preliminary receiving inhibitor (INH+MPTP and INH+Sal groups, Fig. 3). Motor activity in groups remained unchanged.

At the stage of marked depression, relative weight of the adrenal in Sal+MPTP and INH+MPTP groups increased in comparison with that in INT, Sal+ Sal, INH+Sal groups ($F(4,38)=6.754$; $p=0.000$; results of post-hoc analysis are shown in Table 1). Relative thymus mass in Sal+MPTP group was lower than in other groups ($F(4,38)=8.401$; $p=0.000$; Table 1). Relative weight of the thymus in INH+ MPTP group did not differ from that in INT, Sal+ Sal, INH+ Sal groups and exceeded the value in Sal+ MPTP group.

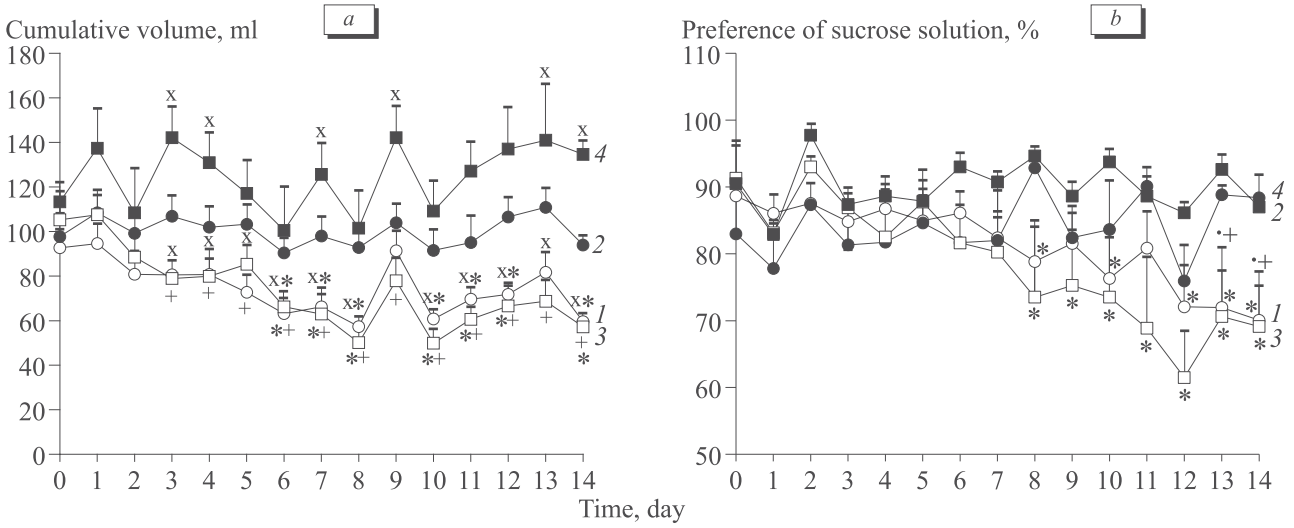

Fig. 2. Dynamics of daily fluid consumption (*a*) and preference of 10% sucrose solution (*b*) in rats with experimental depressive syndrome. *1*) Sal+MPTP, *2*) Sal+Sal, *3*) INH+MPTP, *4* — INH+Sal. Post-hoc analysis using Duncan's test: $p<0.05$ compared to: *baseline values, ^{x}2nd group, $^{+}$4th group.

Thus, MPTP injection was associated with development of experimental dopamine-deficient depressive syndrome (model of chronic depression-like state), which agrees with our previous data [4]. Chronic INH injections before proneurotoxin MPTP injection prevented the development of such symptoms of depressive syndrome as behavioral despair and biorhythmic disorders of swimming behavior, but did not affect the appearance of other symptoms of depressive syndrome. These results indicate that INH possesses antidepressant activity, which corresponds to the data on the presence of antidepressant properties in another type of PEP inhibitors, Z-AA-Pro-OH, in Prosolt's forced swimming test (classical model of acute reactive depression) [2]. These results suggest that antidepressant activity is a common feature of different types of PEP inhibitors, and prove the hypothesis on the involvement of prolyl-specific peptidases into central mechanisms of depression development [3]. On the basis of data obtained in this and previous studies [2,3], the increase in PEP activity in brain structures might be regarded as one of the mechanisms of depressive disorders development.

INH prevented the increase in anxiety level in experimental animals, which could be associated with injection procedure by itself. These data confirm the presence of anxiolytic properties in the studied PEP inhibitor.

An essential role in the pathogenesis of depressive disorders is played by disturbances in central monoaminergic systems activity [9], in particular, the decrease in catecholaminergic systems activity [4]. *In vivo* experiments showed that PEP inhibitors of Z-AA-Pro-OH type can intensify functional activity of brain dopaminergic system [6]. Probably, INH possesses the same property, what underlies its antidepressant action in the model of dopamine-deficient MPTP-induced depressive syndrome. PEP inhibitors through reducing enzyme activity may alter the level of enzyme substrates, neuropeptides, mediating depression and anxiety states [10], which in turn, directly or indirectly affect activity of brain monoaminergic systems [14].

Chronic stress and associated disorders, in particular, depression, are accompanied by an increase in the weight of the adrenals and decrease in thymus weight [13, 15]. Our study revealed an increase in relative adrenals weight and decrease in relative thymus weight in rats with MPTP-induced depressive syndrome, what serves as a new evidence for validity of this model of depression-like state. Repeated INH injections prevented changes in relative weight of one of studied organs (thymus), what suggests that INH possesses antistress properties.

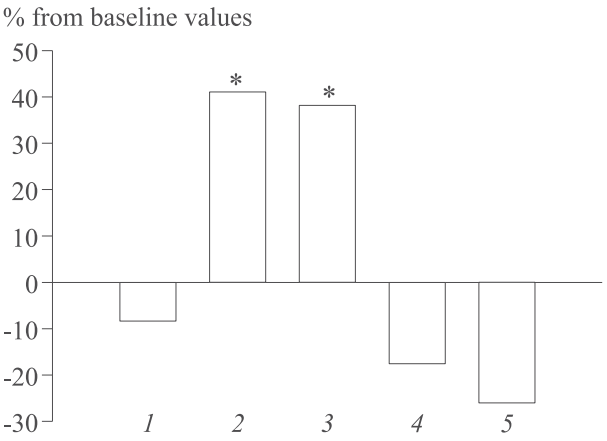

Fig. 3. Changes in anxiety level at the stage of marked depressive behavior (% from baseline values). *1*) INT; *2*) Sal+MPTP; *3*) Sal+Sal; *4*) INH+MPTP; *5*) INH+SAL. *$p<0.05$ compared to baseline level (paired Wilcoxon test).

TABLE 1. Changes in Relative Adrenal Weight of the Glands and Thymus Mass in Rats with Experimental Depressive Syndrome

Group	Adrenals, g	Thymus, g
Intact	0.14±0.01	1.08±0.04
Sal+MPTP	0.17±0.01*x+	0.61±0.09*x+
Sal+Sal	0.14±0.01	0.91±0.05
INH+MPTP	0.17±0.01*x+	0.89±0.07
INH+Sal	0.13±0.01	1.05±0.06

Note. $p<0.01$ in comparison with *intact animals; xwith Sal+Sal group; +with INH+Sal group (Newman—Keuls test).

We found slight increase in daily fluid consumption under conditions of repeated INH injections. Mechanisms underlying this action require further investigations.

The data obtained suggest that INH possesses antidepressant, anxiolytic and antistress properties and that inclusion of INH in complex pathogenetic treatment of depressive disorders will be promising.

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