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Effect of Prolyl Endopeptidase Inhibitor Benzyloxycarbonyl-Methionyl-2(S)-Cyanopyrrolidine on Activity of Proline-Specific Peptidases in Brain Structures of Rats with Experimental MPTP-Induced Depressive Syndrome

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A noncompetitive synthetic inhibitor of prolyl endopeptidase benzyloxycarbonyl-methionyl-2(S)-cyanopyrrolidine (1.0 mg/kg intraperitoneally for 2 weeks) prevented the increase in activity of prolyl endopeptidase in the frontal cortex, striatum, and hypothalamus and activation of dipeptidyl peptidase IV in the frontal cortex of rats with experimental dopamine deficiency-dependent depressive syndrome caused by administration of proneurotoxin MPTP (2 weeks). Our results suggest that the antidepressive effect of prolyl endopeptidase inhibitor is at least partly related to prevention of enzyme activation in the frontal cortex. The antistress effect of this substance can be associated with prevention of enzyme activation in the hypothalamus.

Key Words: *MPTP-induced depressive syndrome; prolyl endopeptidase inhibitor benzyloxycarbonyl-methionyl-2(S)-cyanopyrrolidine; prolyl endopeptidase; dipeptidyl peptidase IV; rat brain structures*

Proline-specific peptidases belong to a relatively small group of exo- and endopeptidases that cleave bonds formed by proline residue that can act as a specific regulatory signal for peptide prevention from degradation with wide-specificity enzymes [13]. A large body of evidence indicates that these peptidases are involved in the regulation of affective disorders. Activities of prolyl endopeptidases (PEP, EC 3.4.21.26) and dipeptidyl peptidase IV (DP-IV, EC 3.4.14.5) in the serum and plasma are modified in patients with mental disorders [10,15]. Experiments on rats showed that DP-IV deficiency caused by mutation in the enzyme gene is associated with less pronounced affective behavior and stress sensitivity of animals [11].

Our studies showed that administration of a synthetic DP-IV inhibitor, methionyl-2(S)-cyanopyrrolidine, to rats during the early postnatal period causes the development of a depression-like state and increase in activities of PEP and DP-IV in brain structures, which play a role in affective behavior [4,8]. A noncompetitive PEP inhibitor benzyloxycarbonyl-methionyl-2(S)-cyanopyrrolidine abolished depressive symptoms and had a normalizing effect on peptidase activities in brain structures on this experimental model [3,8]. The development of experimental depression in rats (MPTP-induced depressive syndrome)

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was accompanied by an increase in PEP and DP-IV activities in the target structures of the mesolimbic and nigrostriatal dopaminergic systems of the brain [2]. Synthetic PEP inhibitors of different structure and various mechanisms for action had the antidepressive effect on this experimental model [6,7,9].

Here we studied the effect of a PEP inhibitor benzyloxycarbonyl-methionyl-2(S)-cyanopyrrolidine on activities of proteolytic enzymes PEP and DP-IV in brain structures of rats with experimental MPTP-induced depressive syndrome.

MATERIALS AND METHODS

Experiments were performed on 42 male Wistar rats weighing 320-450 g. They were housed in cages (5-7 specimens per cage) and had free access to water and food. All procedures and experiments were conducted according to the Rules of Studies with Experimental Animals (Ministry of Health of the USSR, order No. 755 of 12.08.1977) and Rules of Laboratory Practice in Russian Federation (Ministry of Health of Russian Federation, order No. 267 of 19.06.2003).

The depression-like state in rats was induced by systemic administration of proneurotoxin MPTP (Institute of Pharmacology, Russian Academy of Medical Sciences), which has a specific effect on dopaminergic neurons [6]. MPTP was injected intraperitoneally in a daily dose of 20 mg/kg for 14 days. Control rats were administered with physiological saline (PS) by the same scheme. Some animals of the treatment and control groups received intraperitoneal injections of a noncompetitive PEP inhibitor benzyloxycarbonyl-methionyl-2(S)-cyanopyrrolidine (INH, 1.0 mg/kg; Institute of Pharmacology, Russian Academy of Medical Sciences) 30 min before administration of MPTP or PS. The inhibition constant for PEP from subcortical structures and brain cortex was 4.4 and 1.4 nmol/liter, respectively (substrate Z-Ala-Pro-7-amino-4-coumarylamide) [1]. Each of the groups (INH+MPTP and INH+PS) consisted of 8 rats. Other animals received PS and were divided into the groups of PS+MPTP (n=9) and PS+PS (n=10). The preparations were administered in a dose of 1 ml per kg body weight. Intact rats (n=7) received no injections.

The methods and results of studying the severity of depressive symptoms, anxiety, and degree of stress in rats were described previously [6]. The animals were divided into groups so that no between-group differences existed in locomotor activity, degree of anxiety, and body weight.

All animals of the treatment and control groups were decapitated 1 day after the last administration of test preparations. The frontal cortex, hypothalamus, and striatum were isolated on ice under visual control. Brain structures from intact rats were isolated similarly. Activities of proline-specific peptidases in brain tissues were estimated fluorometrically from the release of 7-amino-4-methylcoumarin during hydrolysis of synthetic fluorogenic substrates Z-Ala-Pro-7-amino-4-coumarylamide (for PEP) and glycyl-proline 4-methylcoumarin-7-amide (for DP-IV). Substrate hydrolysis was recorded on a LS-5B spectrofluorometer (380/460 nm; Perkin-Elmer) after 30-min incubation. Peptidase activity was expressed in nmol/mg protein/min. Protein content was measured spectrophotometrically by the method of Bradford with Coomassie blue G-250 using a KFK-2-UKhL 4.2 photocolorimeter (light filter 590 nm). Human serum albumin (Sigma) served as the standard.

The results were analyzed by Statistica 6.0 software. The empirical data did not conform to a normal distribution (as shown by Kolmogorov–Smirnov test). Hence, between-group differences were evaluated by the nonparametric Kruskal–Wallis one-way analysis of variance (ANOVA) followed by a multiple comparison test (Mann–Whitney). The significance level in testing the null hypothesis was 0.05. The data are presented as $M \pm SEM$ (*SEM*, standard error of the mean).

RESULTS

Table 1 shows the summary data on depression-like behavior, degree of anxiety, and stress reactivity of experimental rats (described previously [6]). Subchronic administration of INH before treatment with proneurotoxin MPTP prevented the development of some depressive symptoms (*e.g.*, behavioral despair and change in the rhythmic structure of swimming behavior), but had no effect on other symptoms of depressive syndrome (anhedonia and reduction of drinking and feeding motivation). Under these conditions, INH prevented an increase in the degree of anxious phobia and decrease in the relative weights of the thymus in rats. These data indicate that INH possesses the antidepressive, anxiolytic, and antistress properties.

At the stage of severe behavioral depression, PEP activity in the frontal cortex of the brain, hypothalamus, and striatum of PS+MPTP group rats was higher than inother groups (Table 2). DP-IV activity in the frontal cortex of these rats was higher than in other specimens (Table 3). INH prevented an increase in PEP and DP-IV activities in brain structures.

The results of our previous experiments were reproduced in the present study. We found that PEP activity is elevated in the frontal cortex and striatum, while DP-IV activity increases only in the frontal cortex at the peak of MPTP-induced behavioral depression [2]. Studies on the model of depression-like behavior (stress-induced behavioral despair in the forced

TABLE 1. Emotional and Motivational Behavior of Rats with MPTP-Induced Depressive Syndrome

Test	Group			
	PS+MPTP (*n*=9)	PS+PS (*n*=10)	INH+MPTP (*n*=8)	INH+PS (*n*=8)
	Anxiety			
Complex multiparametric method for integral estimation of anxious and phobic states in rats	+	+	=	=
	Depressiveness			
Forced swimming (behavioral despair, rhythmic disorders)	+	=	=	=
Preference/consumption of sucrose (anhedonia)	+	=	+	=
Fluid consumption (decreased drinking motivation)	+	=	+	=
Body weight loss (reduced feeding motivation)	+	=	+	=
	Stress reaction			
Increase in the relative weight of the adrenal glands (compared to intact animals)	+	=	+	=
Decrease in the relative weight of the thymus (compared to intact animals)	+	=	=	=

Note. +, significant increase in anxiety/depressiveness; =, no statistically significant differences from the control.

swimming test) showed that PEP activity is increased in the frontal cortex and striatum. In contrast, DP-IV activity was elevated in the striatum, but not in the frontal cortex (similarly to the results of experiments with MPTP-induced depressive syndrome) [1]. These data suggest that the involvement of PEP and DP-IV in the central mechanisms of depressive states of different etiology depends on the structure of the brain. The heterogeneity of depressions due to various neurochemical patterns [14] includes different changes of proline-specific peptidase activities (PEP and DP-IV) in brain structures that play a role in the pathogenesis and manifestation of depressive states.

This assumption is confirmed by the results of studying the effect of a PEP inhibitor benzyloxycarbonyl-methionyl-2(S)-cyanopyrrolidine on various experimental models of psychoemotional disorders. This inhibitor exhibits antidepressant activity on the model of MPTP-induced depressive syndrome and prevented the increase in activities of both peptidases in brain structures (as shown in the present work). It should be emphasized that the disappearance of depressive symptoms in animals on this experimental model after MPTP withdrawal was accompanied by a decrease in peptidase activities in the frontal cortex and striatum to the control level [2]. Our studies on the model of behavioral despair showed that administration of benzyloxycarbonyl-methionyl-2(S)-cyanopyrrolidine in the dose suppressing depression-like behavior prevents the increase in peptidase activities in brain structures [1].

Interestingly, experiments on rats with anxious depression due to postnatal administration of a DP-IV inhibitor showed that the appearance of behavioral symptoms of anxiety and/or depression at various stages of the study is accompanied by an increase in the activities of both proline-specific peptidases in the frontal cortex, hypothalamus, and striatum [5]. The results of our study suggest that an increase in peptidase activities in these structures is not associated with anxiety symptoms. We revealed that the level of anxiety increases in rats of the treatment and control groups (Table 1), while the elevation of peptidase activities is observed only animals with the symptoms of behavioral depression. Published data indicate that peptidase activities in the hypothalamus increase under stress conditions [12]. It can be suggested that the elevation of PEP activity in the hypothalamus of rats with MPTP-induced depressive syndrome reflects increased sensitivity of these animals to a stress procedure, which is related to the daily administration of test preparations for 2 weeks (twice per day) [7]. These changes are probably associated with the common pathogenetic mechanisms of depression and stress response (*e.g.*, change in functional activity of the HPA axis) [14]. The animals with anxious depression and high peptidase activities in the hypothalamus are probably highly sensitive to stress factors. Benzyloxycarbonyl-methionyl-2(S)-cyanopyrrolidine

TABLE 2. PEP Activity in Brain Structures of Rats with MPTP-Induced Depressive Syndrome after Preventive Intraperitoneal Injection of PEP Inhibitor Benzyloxycarbonyl-Methionyl-2(S)-Cyanopyrrolidine

Group	PEP activity, nmol/mg protein/min		
	frontal cortex	striatum	hypothalamus
Intact (*n*=7)	0.087±0.004	0.083±0.002	0.092±0.004
PS+MPTP (*n*=9)	0.119±0.003^{+x*o}	0.124±0.002^{+x*o}	0.105±0.003^{+x*o}
PS+PS (*n*=10)	0.093±0.004	0.091±0.002	0.088±0.002
INH+MPTP (*n*=8)	0.084±0.004	0.091±0.006	0.085±0.005
INH+PS (*n*=8)	0.085±0.003	0.088±0.003	0.093±0.002

Note. Kruskal–Wallis ANOVA. Frontal cortex: H(4,N=42)=22.501 (p<0.001); striatum: H(4,N=42)=22.635 (p<0.001); hypothalamus: H(4,N=42)=16.606 (p=0.002). Here and in Table 3: p<0.05: ^{x}compared to the intact group; $^{+}$compared to the PS+PS group; *compared to the INH+PS group; ^{o}compared to the INH+MPTP group (Mann–Whitney test).

TABLE 3. DP-IV Activity in Brain Structures of Rats with MPTP-Induced Depressive Syndrome after Preventive Intraperitoneal Injection of a PEP Inhibitor Benzyloxycarbonyl-Methionyl-2(S)-Cyanopyrrolidine

Group	DP-IV activity, nmol/mg protein/min		
	frontal cortex	striatum	hypothalamus
Intact (*n*=7)	0.049±0.003	0.051±0.003	0.051±0.004
PS+MPTP (*n*=9)	0.066±0.002^{+x*o}	0.053±0.001	0.054±0.004
PS+PS (*n*=10)	0.050±0.002	0.044±0.003	0.048±0.002
INH+MPTP (*n*=8)	0.052±0.002	0.053±0.002	0.055±0.002
INH+PS (*n*=8)	0.050±0.005	0.043±0.003	0.046±0.005

Note. Kruskal–Wallis ANOVA. Frontal cortex: H(4,N=42)=17.773 (p=0.001).

prevented changes in the weight of the thymus (Table 1) and increase in PEP activity in the hypothalamus on the model of MPTP-induced depressive syndrome. These effects probably underlie one of the mechanisms for antistress activity of benzyloxycarbonyl-methionyl-2(S)-cyanopyrrolidine.

The results of this study confirm our previous hypothesis [8] that the frontal cortex, striatum, and hypothalamus are the components of a complex pathological system for depression-like states.

REFERENCES

1. N. A. Krupina, N. G. Bogdanova, N. N. Khlebnikova, *et al*., *Bull. Exp. Biol. Med*., **154**, No. 5, 606-609 (2013).
2. N. A. Krupina, N. N. Zolotov, N. G. Bogdanova, *et al*., *Ibid*., **142**, No. 5, 554-556 (2006).
3. N. A. Krupina, E. Yu. Kushnareva, N. N. Khlebnikova, *et al*., *Ibid*., **147**, No. 3, 285-290 (2009).
4. N. A. Krupina, E. Yu. Kushnareva, N. N. Khlebnikova, *et al*., *Zh. Vyssh. Nervn. Deyat.*, **59**, No. 3, 349-361 (2009).
5. E. Yu. Kushnareva, N. A. Krupina, N. N. Khlebnikova, *et al*., *Bull. Exp. Biol. Med*., **151**, No. 6, 675-679 (2011).
6. N. N. Khlebnikova, N. A. Krupina, N. G. Bogdanova, *et al*., *Bull. Exp. Biol. Med.*, **147**, No. 1, 26-30 (2009).
7. N. N. Khlebnikova, N. A. Krupina, N. G. Bogdanova, *et al*., *Bull. Exp. Biol. Med*., **155**, No. 2, 190-193 (2013).
8. N. N. Khlebnikova, N. A. Krupina, E. Yu. Kushnareva, *et al*., *Bull. Exp. Biol. Med*, **152**, No. 4, 409-412 (2012).
9. N. N. Khlebnikova, N. A. Krupina, I. N. Orlova, *et al*., *Bull. Exp. Biol. Med*, **147**, No. 3, 291-295 (2009).
10. G. Breen, A. J. Harwood, K. Gregory, *et al*., *Bipolar Disord*., 6, No. 2, 156-161 (2004).
11. N. Frerker, K. Raber, F. Bode, *et al*., *Clin. Chem. Lab. Med*., 47, No. 3, 275-287 (2009).
12. J. Idanpaan-Heikkila, P. Rauhala, R. K. Tuominen, *et al*., *Pharmacol. Toxicol.*, 78, No. 3, 129-135 (1996).
13. L. Klimaviciusa, R. K. Jain, K. Jaako, *et al*., *J. Neurosci. Methods*, 204, No. 1, 104-110 (2012).
14. R. Lam and H. Mok, *Depression*, New York (2008), pp. 104-110.
15. M. Maes, Bonaccorso, V. Marino, *et al*., *Mol. Psychiatry*, **6**, No. 4, 475-480 (2001).