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Effect of Imipramine and Prolyl Endopeptidase Inhibitor Benzyloxycarbonyl-Methionyl-2(S)-Cyanopyrrolidine on Activity of Proline-Specific Peptidases in the Brain of Rats with Experimental Anxious-Depressive Syndrome

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Activities of prolyl endopeptidase and dipeptidyl peptidase IV in the frontal cortex, hypothalamus, nucleus accumbens, striatum, and hippocampus were measured in rats with the experimental anxious-depressive syndrome induced by treatment with a dipeptidyl peptidase IV inhibitor during the early postnatal period (days 5-18). Prolyl endopeptidase activity was elevated in the frontal cortex, hypothalamus, and nucleus accumbens. Increased activity of dipeptidyl peptidase IV was observed in the hypothalamus and striatum. Norepinephrine/serotonin reuptake inhibitor, imipramine, and noncompetitive prolyl endopeptidase inhibitor, benzyloxycarbonyl-methionyl-2(S)-cyanopyrrolidine, were shown to abolish depression-like behavior of animals in the forced swimming test. These compounds had a normalizing effect on activities of prolyl endopeptidase and dipeptidyl peptidase IV in brain structures of rats.

Key Words: *dipeptidyl peptidase IV; prolyl endopeptidase; depression; rats; brain structures*

The results of clinical observations and experimental studies indicate that proline-specific peptidases belonging to a group of serine proteases play a role in the pathophysiological mechanisms of affective disorders. Strong clinical evidence exists that variations in activities of prolyl endopeptidase (EC 3.4.21.26, PEP) and dipeptidyl peptidase IV (EC 3.4.14.5, DP-IV) in the serum and blood plasma are associated with the severity of depressive symptoms and anxiety [10,11]. Experiments on adult rats with dopamine deficiency-dependent depressive syndrome induced by treatment with proneurotoxin MPTP showed that the development of depression-like behavior is accompanied by an increase in activities of PEP and DP-IV in brain structures, which play a role in the emotional-and-motivational behavior (frontal cortex and striatum) [1].

Our studies showed that systemic subchronic administration of a DP-IV inhibitor methionyl-2(S)-cyanopyrrolidine during the early postnatal period is followed by persistent behavioral disorders in adult animals (anxious-depressive state) [2]. Various stages of these disorders were characterized by an increase in activities of PEP and DP-IV in brain structures, which mediate emotional-and-motivational behavior

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[5]. Classical antidepressant imipramine (IMI) and noncompetitive PEP inhibitor benzyloxycarbonyl-methionyl-2(S)-cyanopyrrolidine (INH) were shown to relieve the symptoms of depression-like behavior in rats with the MPTP-induced depressive syndrome, as well as in animals with a DP-IV inhibitor-induced anxious-depressive state [2,3,6].

Here we studied the effects of IMI and INH on activities of proline-specific peptidases PEP and DP-IV in the brain of rats after treatment with a DP-IV inhibitor during early ontogeny.

MATERIALS AND METHODS

Experiments were performed on 48 male Wistar rats (bred in a vivarium of the Institute of General Pathology and Pathophysiology). The animals were maintained in a vivarium under standard conditions and natural light-dark regimen. They were housed in cages (5-7 specimens per cage) and had free access to water and food (except for the testing of fluid intake and sucrose preference/intake). All procedures and experiments were conducted according to the Rules of Laboratory Practice in Russian Federation (Ministry of Health of Russian Federation, order No. 267 of 19.06.2003).

The animals (50% specimens) of the treatment groups were treated with a noncompetitive DP-IV inhibitor methionyl-2(S)-cyanopyrrolidine (Gly-Pro-7-amino-4-coumarylamide inhibition constant 2.7 nmol/liter, synthesized at the V. V. Zakusov Institute of Pharmacology; 1.0 mg/kg intraperitoneally, 0.1 ml per 10 g body weight) during the early postnatal period (days 5-18). The remaining rats (50% specimens) of the control groups received physiological saline (PS). Each of the treatment and control groups included the rat pups of 3-4 litters.

The effect of antidepressant compounds on proline-specific peptidase activity in the brain was studied in rats with experimental anxious-depressive syndrome. For this purpose, we used animals demonstrating relief of depressive symptoms after treatment with IMI and INH. Behavioral experiments were described previously [2,4]. The symptoms of depression in 2-month-old rats were evaluated from the development of behavioral despair and biorhythmic disturbances in the forced swimming test. Three pairs of groups were formed after behavioral testing. In each pair, the severity of depressive symptoms in treated animals was much greater than in control specimens. Tricyclic antidepressant IMI (EGIS) was administered in a daily dose of 10 mg/kg to animals from the first pair of groups for 10 days (IMI-control, n=7; IMI-treatment, n=6). The second pair of groups was treated with a noncompetitive INH (Z-Ala-Pro-7-amino-4-coumaryl-amide inhibition constant 2.0 nmol/liter, synthesized at the V. V. Zakusov Institute of Pharmacology) in a dose of 2 mg/kg (INH-control, n=8; INH-treatment, n=7). PS was injected to rats from the third pair of groups (PS-control, n=8; PS-treatment, n=7). The substances were injected intraperitoneally (1 ml/kg).

The rats were decapitated after behavioral testing. The frontal cortex, hypothalamus, striatum, nucleus accumbens, and hippocampus were isolated. Tris-HCl buffer (0.02 M, pH 8.0) containing 1 mM EDTA and 0.1 mM dithiothreitol (1:19 ratio) was added to the weighted tissue sample. The tissue was subjected to ultrasonic treatment (22 kHz). Activities of proline-specific peptidases PEP and DP-IV in the homogenate were measured fluorometrically (Mannisto and Tuomainen, 1994) [11]. To prepare the sample, 1.9 ml Tris-HCl buffer and 0.03 ml Z-Ala-Pro-MCA/Gly-Pro-MCA in DMSO (substrate concentration 1 mg/ml) were added to 0.01 ml enzyme preparation. Incubation was performed at 37°C for 60 min. The reaction was stopped by addition of 1 ml 20% acetic acid to the incubation mixture. Substrate hydrolysis was studied on a LS-5B spectrofluorometer (Perkin-Elmer). Protein content was measured spectrophotometrically by the method of Bradford with Coomassie blue G-250 (Serva). The amount of the enzyme that caused the release of 1 μM product over 1-min incubation was taken as one unit of enzyme activity. Specific activity was expressed in mU per 1 mg enzyme preparation.

The results were analyzed by Statistica 7.0 software. The empirical data did not conform to a normal distribution (as shown by Kolmogorov–Smirnov test). Hence, several independent samples were compared using the Kruskal–Wallis one-way analysis of variance. Post-hoc analysis involved the Mann–Whitney U test. The data are presented as $M \pm SEM$. The significance level was 5%.

RESULTS

PEP activity in the frontal cortex, hypothalamus, and nucleus accumbens of rats from the PS-treatment group was higher than in specimens of the PS-control group (Table 1). PEP activity in the striatum tended to increase in animals of the PS-treatment group (p=0.070). PEP activity in the frontal cortex of rats from the INH-treatment group was higher than in specimens of the INH-control group. Enzyme activity in the nucleus accumbens of treated animals was slightly higher than in control specimens (p=0.065). PEP activity in rats of the IMI-treatment group did not differ from the control.

Statistically significant differences in PEP activity in animals of the control groups were observed only in the hypothalamus [H (2, N=26)=6.229, p=0.044]. PEP

activity in rats of the INH-control group was lower than in specimens of the PS-control group. PEP activity in the frontal cortex and nucleus accumbens of animals from the INH-control was slightly lower than in specimens of the PS-control group (p=0.071 and p=0.103, respectively).

Comparison of treatment groups revealed significant differences in PEP activity in the frontal cortex [H (2, N=23)=0.012, p=0.049], hypothalamus [H (2, N=23)=9.354, p=0.009], and nucleus accumbens [H (2, N=25)=6.900, p=0.032]. PEP activity in brain structures of animals from the IMI-treatment and INH-treatment groups was much lower than in specimens of the PS-treatment group.

DP-IV activity in the hypothalamus and striatum of rats from the PS-treatment group was higher than in specimens of the PS-control group. Enzyme activity tended to increase in the striatum (p=0.059; Table 2). DP-IV activity in brain structures of animals from the IMI-treatment and INH-treatment groups did not differ from the control.

DP-IV activity in brain structures of rats from the IMI-control and INH-control groups not differ from that in specimens of the PS-control group. A comparison of animals from the treatment groups revealed significant differences in DP-IV activity in the hypothalamus [H (2, N=25)=8.560, p=0.014] and striatum [H (2, N=23)=6.366, p=0.042]. Enzyme activity in the hypothalamus of rats from the IMI-treatment group was lower than in specimens of the PS-treatment group. Similar results were obtained for DP-IV activity in the striatum of animals from the IMI-treatment and INH-treatment groups.

These data are consistent with the results of our previous experiments. Peptidase activity in rats was studied in the dynamics of an anxious-depressive state induced by postnatal treatment with a DP-IV inhibitor [5]. Comparison of these data shows that the symptoms of behavioral depression and, probably, high anxiety in adolescent and adult rats are accompanied by an increase in activities of PEP (in the frontal cortex and hypothalamus) and DP-IV (in the hypothalamus). The present study demonstrates that adult rats with the symptoms of behavioral depression are characterized by high activities of PEP and DP-IV in the striatum. Previous experiments on this model revealed that an increase in peptidase activities in the striatum is observed in adult rats with the symptoms of anxiety. These structures mediate the emotional-and-motivational behavior [7].

The results of experiments on the models of MPTP-induced depressive syndrome and DP-IV inhibitor-induced anxious-depressive state (postnatal treatment with a DP-IV inhibitor) suggest that an abnormal increase in activities of PEP and DP-IV in the frontal cortex, hypothalamus, and striatum serves as the general mechanism for affective disorders of different etiology.

Increased activities of PEP and DP-IV were observed only in the striatum of adult rats. Our results suggest the existence of general ontogenetic mechanisms for the involvement of these structures (according to an increase in activity of proline-specific peptidases) in the formation of a pathological system of anxious-depressive state. The system probably includes not only these structures of the brain, but also the nucleus accumbens. PEP activity was elevated in the nucleus accumbens of adult rats. Strong evidence exists that this nucleus plays an important role in the positive reinforcement system and motivational behavior [15].

This study was performed with the following antidepressants of various mechanisms of action: imipra-

TABLE 1. PEP Activity in Brain Structures of Rats from the Treatment and Control Groups (nmol/mg protein/min)

Group	Brain structure				
	frontal cortex	hypothalamus	nucleus accumbens	striatum	hippocampus
PS-control	0.088±0.017	0.097±0.017	0.045±0.009	0.062±0.012	0.094±0.017
PS-treatment	0.196±0.035*	0.221±0.046*	0.108±0.022*	0.132±0.035	0.086±0.018
IMI-control	0.067±0.011	0.077±0.015	0.041±0.008	0.061±0.014	0.084±0.013
IMI-treatment	0.099±0.009^{+}	0.063±0.07^{+}	0.038±0.017^{+}	0.075±0.016	0.082±0.011
INH-control	0.049±0.010	0.047±0.006^{+}	0.027±0.007	0.054±0.005	0.110±0.009
INH-treatment	0.089±0.015*$^{+}$	0.080±0.026^{+}	0.051±0.010^{+}	0.064±0.016	0.124±0.032

Note. Here and in Table 2: Mann–Whitney test: *p<0.05 compared to the control group; ^{+}p<0.05 compared to the corresponding parameter in PS-receiving rats.

TABLE 2. DP-IV Activity in Brain Structures of Rats from the Treatment and Control Groups (nmol/mg protein/min)

Group	Brain structure				
	frontal cortex	hypothalamus	nucleus accumbens	striatum	hippocampus
PS-control	0.036±0.006	0.037±0.006	0.017±0.003	0.026±0.004	0.036±0.005
PS-treatment	0.035±0.007	0.084±0.015*	0.041±0.008	0.077±0.017*	0.031±0.005
IMI-control	0.033±0.005	0.036±0.003	0.016±0.002	0.023±0.004	0.040±0.004
IMI-treatment	0.046±0.007	0.032±0.002+	0.019±0.005	0.035±0.005+	0.039±0.005
INH-control	0.048±0.004	0.036±0.004	0.025±0.004	0.039±0.006	0.043±0.005
INH-treatment	0.040±0.010	0.066±0.014	0.027±0.004	0.034±0.006+	0.039±0.008

mine (norepinephrine/serotonin reuptake inhibitor) and INH. A PEP inhibitor was potent in decreasing activity of PEP, but not of DP-IV in control rats (Tables 1 and 2). These data illustrate specificity of the test inhibitor. Both products with antidepressant properties had a normalizing effect on increased activity of peptidases in rats of the treatment groups. However, PEP activity in the frontal cortex after treatment with a PEP inhibitor remained above the control level. These variations in enzyme activity probably reflect a specific role of frontal cortex dysfunction in the functioning of a pathological system of anxious-depressive state (at various stages, including the latent period with no clinical symptoms).

There are new data on the cellular and molecular mechanisms for action of antidepressant compounds (*e.g.*, modulation of gene expression, synaptic plasticity, and neurogenesis) [13]. The antidepressant effect of chronic treatment with imipramine is probably mediated by the regulatory influence of this agent on activity of serine proteases in the prefrontal cortex [9]. A specific inhibitor of PEP had a normalizing effect on activity of both peptidases in brain structures of treated rats. Unidirectional changes in enzyme activity are probably associated with the induction of various regulatory mechanisms, including the interaction of enzyme substrates [8] and variations in functional activity of the second-messenger system under the influence of a PEP inhibitor. These changes result in the increased expression of genes for both enzymes [14].

We demonstrated that normalization of the animal behavior after treatment with antidepressant compounds is accompanied by restoration of proline-specific peptidase activity in brain structures, which play a role in emotional-and-motivational states. These data support the hypothesis that the increase in activities of PEP and DP-IV serves as one of the pathogenetic mechanisms for the development of affective disorders.

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