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Activities of Prolyl Endopeptidase and Dipeptidyl Peptidase IV in Brain Structures of Rats with Dopamine Deficiency-Dependent MPTP-Induced Depressive Syndrome

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The development of MPTP-induced depressive syndrome in rats was accompanied by activation of prolyl endopeptidase and dipeptidyl peptidase IV in the brain frontal cortex. Prolyl endopeptidase activity in the striatum also increased under these conditions. Our results indicate that proline-specific peptidases in the target structures of the brain dopaminergic system are involved in the pathogenesis of dopamine deficiency-dependent depressive states.

Key Words: *proline endopeptidase; dipeptidyl peptidase IV; brain; MPTP-induced depressive syndrome; rats*

The pathogenesis of depressive disorders is poorly understood. It was hypothesized that this process involves various systems of the organism [13]. The role of central monoaminergic systems was described elsewhere [7]. Much attention was given to the peptidergic mechanisms [14,15]. The interaction between these mechanisms was evaluated [6,9].

Plasma activities of proline-specific peptidases, prolyl endopeptidase (PEP) and dipeptidyl peptidase IV (DP-IV), are changed in patients with symptoms of major depression [10,11]. Previous experiments showed that PEP inhibitors have antidepressant properties [1] and can modulate functional activity of the brain dopaminergic system [5], involved in the development of depressive states [2]. Published data show that proline-specific peptidases play a role in the pathophysiological mechanisms of depressions. However, the contribution of these enzymes into the development of depressive disorders remains unclear. Measurement of peptidase activities in CNS structures during depressive states can help to solve this problem.

We previously developed a model of experimental dopamine deficiency-dependent MPTP-induced depressive syndrome in rats [2,4]. Our results and published data show that target structures of the brain dopaminergic system, specifically frontal cortex (mesocortical system), striatum (nigrostriatal system), and nucleus accumbens (mesolimbic system) are probably involved in the development of experimental depressive states [2]. This work was designed to search experimental evidence that PEP and DP-IV in the striatum, frontal

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cortex, nucleus accumbens, and hypothalamus play a role in the development of experimental MPTP-induced depressive syndrome in rats.

MATERIALS AND METHODS

Experiments were performed on 47 male Wistar rats weighing 320-450 g. The animals were maintained in individual cages. Two series of experiments were conducted by the same scheme. Depressive state was induced by systemic intraperitoneal injection of MPTP, proneurotoxin specifically damaging dopaminergic neurons (Institute of Pharmacology, Russian Academy of Medical Sciences), in a daily dose of 20 mg/kg for 14 days. Controls received physiological saline (PS). The severity of MPTP-induced depressive syndrome was evaluated by the decrease in motivation activity (integral score) [3]. The animals were tested for hedonic dysfunction (decrease in preference of 10% sucrose to water), behavioral despair (modification of swimming behavior in the forced swimming test), reduction of water consumption, and decrease in body weight. The total score was low (0-1 point) in animals without behavioral depression. The rats fed dry food and had free access to water.

Some animals were decapitated 1 day after the last injection of MPTP or PS. Other rats were killed on day 15 after withdrawal of drugs. Activities of PEP and DP-IV in brain tissues were estimated by hydrolysis of synthetic fluorogenic substrates carbobenzoxy-alanyl-proline 4-methylcoumarin-7-amide and glycyl-proline 4-methylcoumarin-7-amide, respectively [12]. Peptidase activity was expressed in nmol products released per mg protein over 1 min. Similar results were obtained in both series and therefore were pooled for calculation.

Statistical treatment of data was performed by means of STATISTICA 6.0 software with parametric one-way analysis of variance (ANOVA) and nonparametric one-way analysis of variance (Kruskal—Wallis test). A multiple comparison procedure for mean values in series of the variance complex involved Newman—Keuls test and Mann—Whitney test, respectively.

RESULTS

Two-week administration of MPTP was followed by the development of a depressive state in rats (behavioral depression, score 5.25±0.77). The behavior of animals returned to normal 2 weeks after

TABLE 1. PEP Activity in Brain Structures of Rats with Experimental Depressive Syndrome (nmol/mg/min, $M \pm m$)

Group, period		Frontal cortex	Striatum	Hypothalamus	Nucleus accumbens
Intact (untreated)		0.034±0.006 (n=14)	0.042±0.010 (n=13)	0.062±0.015 (n=14)	0.032±0.006 (n=13)
MPTP	2 weeks after the start of administration	0.041±0.004* (n=16)	0.112±0.031**$^{+xx}$ (n=16)	0.035±0.004^{x} (n=16)	0.028±0.004 (n=15)
	2 weeks after withdrawal	0.030±0.004 (n=16)	0.032±0.004 (n=16)	0.034±0.007^{x} (n=16)	0.025±0.003 (n=16)
PS	2 weeks after the start of administration	0.021±0.002 (n=17)	0.025±0.003 (n=17)	0.012±0.002^{xxx} (n=17)	0.020±0.002 (n=17)
	2 weeks after withdrawal	0.022±0.003 (n=16)	0.024±0.005 (n=16)	0.028±0.007^{x} (n=16)	0.035±0.007 (n=16)

Note. Frontal cortex, $F(4.74)=5.03$ (p=0.001); striatum, $F(4.73)=6.23$ (p=0.000); hypothalamus, $F(4.74)=5.29$ (p=0.001). Newman—Keuls test: *p<0.01 and **p<0.001 compared to PS in the same period; ^{+}p<0.01 compared to the same group in the next period; ^{x}p<0.05, ^{xx}p<0.01, and ^{xxx}p<0.001 compared to intact animals.

TABLE 2. DP-IV Activity in Brain Structures of Rats with Experimental Depressive Syndrome (nmol/mg/min, $M \pm m$)

Group, period		Frontal cortex	Striatum	Hypothalamus	Nucleus accumbens
Intact (untreated)		0.010±0.001 (n=14)	0.011±0.002 (n=13)	0.009±0.001 (n=14)	0.005±0.000 (n=13)
MPTP	2 weeks after the start of administration	0.017±0.003*$^{+x}$ (n=16)	0.007±0.001 (n=16)	0.008±0.001 (n=16)	0.009±0.001^{xx} (n=15)
	2 weeks after withdrawal	0.010±0.001 (n=16)	0.007±0.001 (n=16)	0.010±0.001 (n=16)	0.007±0.001 (n=16)
PS	2 weeks after the start of administration	0.008±0.001 (n=17)	0.011±0.002 (n=17)	0.008±0.001 (n=17)	0.008±0.001^{+x} (n=17)
	2 weeks after withdrawal	0.011±0.001 (n=16)	0.008±0.001 (n=16)	0.006±0.000 (n=16)	0.005±0.001 (n=16)

Note. Frontal cortex, $F(4.74)=5.00$ (p=0.001); nucleus accumbens, $F(4.72)=5.78$ (p=0.000). Newman—Keuls test: *p<0.001 compared to PS in the same period; ^{+}p<0.05 compared to the same group in the next period; ^{x}p<0.05 and ^{xx}p<0.01 compared to intact animals.

MPTP withdrawal (score 1.38±0.43). Signs of behavioral depression were not revealed in animals of the PS group (2-week treatment with PS, score 0.35±0.12; 2 weeks after PS withdrawal, score 0.38±0.18). The severity of depression in rats during MPTP administration exceeded not only the control level, but also that observed 2 weeks after withdrawal of MPTP or PS ($H(3, N=65)=36.6467$, $p<0.0000$, Kruskal—Wallis test; $p<0.05$, Mann—Whitney test). The severity of behavioral depression remained high 2 weeks after withdrawal of the drugs ($p=0.05$).

By the end of 2-week treatment, activities of PEP and DP-IV in the frontal cortex increased in rats with severe behavioral depression. Striatal PEP activity also exceeded the control level (Tables 1 and 2). Behavioral characteristics of animals returned to normal 2 weeks after withdrawal of drugs, which was accompanied by the recovery of peptidase activity in the frontal cortex and striatum. DP-IV activity in the nucleus accumbens of rats receiving MPTP or PS for 2 weeks was higher than in intact animals (Table 2). Hypothalamic PEP activity in treated rats was lower than in intact animals during both stages of the study (Table 1).

Our results indicate that PEP activity in the hypothalamus and DP-IV activity in the nucleus accumbens undergo similar changes in rats receiving MPTP and PS. The observed changes are nonspecific for depressive states and reflect the reaction of peptidases in these structures to stress stimulation (repeated administration of drugs). This assumption is confirmed by published data on variations in hypothalamic peptidase activities in stress situation after morphine withdrawal results [8]. In our experiments, changes observed in the hypothalamus were more persistent than those in the nucleus accumbens.

PEP activity in target structures of 2 central dopaminergic systems (mesocortical and nigrostriatal systems) in rats of the MPTP group exceeded that in PS-treated animals. MPTP-receiving rats had high activity of DP-IV in the target structure of only one system (mesocortical system). Therefore, these peptidases play different roles in the central mechanisms of the depressive state. Our results hold much promise for the development of new approaches to complex pathogenetic therapy of depressive disorders with peptidase-modulating drugs.

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