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Activities of Proline-Specific Peptidases in Brain Structures of Rats with Experimental Anxiety-Depressive State Caused by Administration of Dipeptidyl Peptidase IV Inhibitor in the Early Postnatal Period

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We studied the dynamics of activity of dipeptidyl peptidase IV (DP-IV) and prolyl endopeptidase (PEP) in the frontal cortex, hypothalamus, striatum, nucleus accumbens, and hippocampus of rats with experimental anxiety-depression state induced by administration of methionyl-2(*s*)-cyano-pyrrolidine, an inhibitor of DPP-IV, in the early postnatal period. In 1-month-old experimental males, PEP and DP-IV activities increased in the frontal cortex and hypothalamus, while in 1-month-old experimental females PEP activity increased in the hippocampus and DP-IV activity increased in all studied brain structures. At the age of 3 months, increased PEP activity in the hypothalamus and nucleus accumbens was detected in males and decreased DP-IV activity in the nucleus accumbens and decreased PEP activity in the hippocampus were detected in females. At the age of 7 months, PEP activity increased in the frontal cortex and striatum and DP-IV activity increased in all studied brain structures in males; in 7-months-old females, activity of both enzymes increased in the striatum.

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

Numerous clinical observations and experimental data suggest the involvement of some neuropeptides in the development of emotional and behavioral disorders [4]. Neuropeptides mediating the development of depression and anxiety, *e.g.* neuropeptide Y, substance P, thyroliberin, oxytocin, vasopressin [9,14], contain a proline residue and serve as substrates for proline-specific proteinases, *e.g.* dipeptidyl peptidase-IV (DP-IV; EC 3.4.14.5) and prolyl endopeptidase (PEP; EC 3.4.14.5). The contribution of these enzymes into the development of anxiety-depression disorders is confirmed by clinical observations [11]. Our experimental studies demonstrated increased PEP and DP-IV activities in various brain structures of rats with experimental dopamine-deficient depressive syndrome [1]. Administration of PEP inhibitors normalized the behavior of rats with experimental depressive states [5].

F344 rats carrying mutation in DP-IV gene manifesting in deficit of this enzyme demonstrate reduced stress-reactivity in different behavioral tests [10]. DP-

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IV gene-knockout mice are also characterized by reduced stress-reactivity and decreased depression-like behavior and anxiety against the background of hyperactive phenotype [8]. Our previous studies showed that subchronic postnatal administration of methionyl-2(S)-cyano-pyrrolidine (Met-Prd-N), an inhibitor of DP-IV, led to sustained behavioral disturbances similar to anxiety-depression-like state in adolescent and adult rats [3].

Here we studied activity of prolin-specific peptidases PEP and DP-IV in various brain structures in rats receiving Met-Prd-N in the early postnatal period.

MATERIALS AND METHODS

The work was performed on albino Wistar male and female rats (n=91) from the vivarium of Institute of General Pathology and Pathophysiology Russian Academy of Medical Sciences. The animals were kept under standard vivarium conditions in groups of 5-7 rats, separated by sex, with the natural light-dark regimen and free access to food and water, except that during periods of testing for fluid intake and sucrose preference/consumption.

All procedures and animal experiments were carried out in accordance with "Rules of Laboratory Practice in the Russian Federation" approved by the Order of the Public Health Ministry No. 267, 19.06.2003.

Two experimental series were performed (I series, n=33; II series, n=58). In each series, half of the animals (experimental group) in the early postnatal period (days 5-18 inclusive) were injected with synthetic non-competitive DP-IV inhibitor type Met-Prd-N (synthesized at V. V. Zakusov Institute of Pharmacology, Russian Academy of Medical Sciences, the inhibition constant for the substrate Gly-Pro-7-amino-4-cumaril-amide is 2.7 nmol/liter) in a dose of 1.0 mg/kg intraperitoneally in a volume of 0.1 ml per 10 g body weight. Freshly prepared Met-Prd-N solution was used. The substance was dissolved by adding 1-2 drops Tween-80 and brought to the required volume with saline. The second half of the rats (control group) was injected with saline in the same way.

At the age of 1 month, the pups were separated from the mother. Sex-matched experimental and control groups included similar number of pups from 3-4 litters. The results and methods of testing for anxiety and depression of rats at the age of 1 month (II series), 3 months (series II), and 7 months (series I) were described in detail previously [3]. Upon behavioral testing, the animals were decapitated; the frontal cortex and subcortical structures (hypothalamus, striatum, nucleus accumbens, and hippocampus) were promptly isolated under visual control on ice.

Activity of proline-specific peptidases in the isolated brain structures was measured fluorometrically by hydrolysis of synthetic fluorogenic substrates 4-methyl-coumarin-7-amide carbobenzoxyalaninproline (for PEP) and 4-methyl-coumarin-7-amide glycilproline (for DP-IV). Hydrolysis was recorded after 30-min incubation at 37°C on a LS-5B spectrofluorometer (Perkin-Elmer) at 380/460 nm [13]. Protein content was measured spectrophotometrically by the method of Bradford with Coomassie blue G-250.

Statistical analysis was performed using unpaired nonparametric Mann–Whitney U test, because the data did not fit the normal distribution according the Kolmogorov–Smirnov test. The accepted significance level was 5%.

RESULTS

In 1-month-old males of the experimental group, activities of PEP and DP-IV in the frontal cortex and hypothalamus were increased in comparison with the control (Table 1). At the age of 3 months, increased activity of PEP in the hypothalamus and nucleus accumbens was detected (Table 2); at the age of 7 months, increased activity of PEP in the frontal cortex and striatum and increased activity of DP-IV in all studied brain structures were demonstrated (Table 3).

In 1-month-old experimental females, PEP activity in the hippocampus and DP-IV activity in all studied brain structures were increased in comparison with the control (Table 1); at the age of 3 months, reduced DP-IV activity in the nucleus accumbens and reduced PEP activity in hippocampus were found (Table 2); at the age of 7 months, increased activity of both enzymes in the striatum was revealed (Table. 3).

In control groups, activity of DP-IV in all studied brain structures in males was higher than in females at the age of 1 month, PEP activity in males was higher than in females only in the hippocampus (Table 1). At the age of 3 months, no significant differences in the levels of peptidases in brain structures of males and females were detected. At the age of 7 months, activity of DP-IV in the frontal cortex, striatum, and hippocampus, in contrast, was higher in females, while PEP activity was similar in all brain structures (Table 3).

Behavioral experiments showed that 1-month-old males are characterized by increased anxiety (elevated plus maze behavior and increased latency of exit from the center in the open field test) and depression-like behavior (by the development of anhedonia signs in sucrose consumption/preference test and reduced drinking motivation). Adolescent experimental females demonstrated less pronounced signs of depression (only transient anhedonia) in comparison with males and no signs of anxiety [3]. Analysing behavioral changes and the data on peptidase activities in brain structures we assumed that PEP and DP-IV in the frontal cortex and hypothalamus (and hippocampal PEP in

TABLE 1. Activity of PEP and DP-IV (nM×min×mg^{-1} protein) in Brain Structures of Experimental and Control Rats at the Age of 1 Month (*M*±*m*)

Gender	Group	Frontal cortex		Hypothalamus		Nucleus accumbens		Striatum		Hippocampus	
		PEP	DP-4	PEP	DP-4	PEP	DP-4	PEP	DP-4	PEP	DP-4
Males	Control (*n*=6)	0.040± 0.007	0.009± 0.001	0.024± 0.001	0.010± 0.002	0.029± 0.007	0.011± 0.002	0.035± 0.007	0.009± 0.001	0.032± 0.003	0.011± 0.002
	Experiment (*n*=8)	0.068± 0.008*	0.023± 0.002**	0.046± 0.006**	0.018± 0.004*	0.021± 0.004	0.007± 0.001	0.022± 0.004	0.007± 0.001	0.031± 0.002	0.011± 0.001
Females	Control (*n*=8)	0.023± 0.003	0.004± 0.001^{++}	0.016± 0.003	0.003± 0.000^{++}	0.021± 0.004	0.002± 0.001^{+}	0.025± 0.026	0.004± 0.001^{++}	0.016± 0.002^{++}	0.004± 0.001^{++}
	Experiment (*n*=7)	0.027± 0.004	0.008± 0.001*	0.031± 0.012	0.010± 0.002*	0.027± 0.004	0.008± 0.002**	0.026± 0.004	0.009± 0.001**	0.045± 0.011***	0.013± 0.004**

Note. Here and in Tables 2 and 3: *p<0.05, **p<0.01, ***p<0.001 in comparison with the control; ^{+}p<0.05, ^{++}p<0.01 in comparison with males.

TABLE 2. Activity of PEP and DP-IV (nM×min×mg^{-1} protein) in Brain Structures of Experimental and Control Rats at the Age of 3 Months (*M*±*m*)

Gender	Group	Frontal cortex		Hypothalamus		Nucleus accumbens		Striatum		Hippocampus	
		PEP	DP-4	PEP	DP-4	PEP	DP-4	PEP	DP-4	PEP	DP-4
Males	Control (*n*=7)	0.015± 0.004	0.005± 0.002	0.013± 0.004	0.004± 0.002	0.013± 0.005	0.003± 0.002	0.028± 0.012	0.009± 0.004	0.031± 0.009	0.007± 0.003
	Experiment (*n*=9)	0.024± 0.011	0.004± 0.002	0.036± 0.007*	0.016± 0.006	0.031± 0.006*	0.011± 0.004	0.023± 0.006	0.005± 0.002	0.037± 0.009	0.014± 0.005
Females	Control (*n*=7)	0.033± 0.011	0.014± 0.004	0.032± 0.010^{+}	0.007± 0.002	0.020± 0.004	0.002± 0.000	0.022± 0.006	0.005± 0.003	0.041± 0.009	0.010± 0.002
	Experiment (*n*=6)	0.041± 0.014	0.014± 0.004	0.029± 0.010	0.009± 0.004	0.014± 0.003	0.000± 0.000*	0.036± 0.009	0.008± 0.003	0.015± 0.006*	0.012± 0.004

TABLE 3. Activity of PEP and DP-IV (nM×min×mg^{-1} protein) in Brain Structures of Experimental and Control Rats at the Age of 7 Months (*M*±*m*)

Gender	Group	Frontal cortex		Hypothalamus		Nucleus accumbens		Striatum		Hippocampus	
		PEP	DP-4	PEP	DP-4	PEP	DP-4	PEP	DP-4	PEP	DP-4
Males	Control (*n*=8)	0.012± 0.003	0.002± 0.002	0.010± 0.003	0.001± 0.001	0.015± 0.004	0.002± 0.001	0.009± 0.002	0.001± 0.000	0.015± 0.004	0.002± 0.001
	Experiment (*n*=12)	0.022± 0.002**	0.005± 0.001**	0.015± 0.003	0.005± 0.001**	0.021± 0.002	0.004± 0.001**	0.016± 0.003*	0.004± 0.001***	0.035± 0.012	0.005± 0.001***
Females	Control (*n*=7)	0.024± 0.005	0.005± 0.001^{+}	0.012± 0.003	0.003± 0.000	0.013± 0.003	0.003± 0.001	0.013± 0.002	0.002± 0.000^{++}	0.023± 0.005	0.004± 0.000^{++}
	Experiment (*n*=6)	0.017± 0.003	0.003± 0.001	0.012± 0.002	0.004± 0.001	0.020± 0.003	0.004± 0.001	0.020± 0.002*	0.005± 0.001**	0.019± 0.003	0.005± 0.000

females) were involved into the formation of mixed anxiety-depressive states at the early stage.

In 3-month-old rats of both sexes, no signs of increased anxiety and depression were revealed in this series [3], but some changes in peptidase activity were observed. For comparative analysis of behavioral and biochemical changes it is important to keep in mind that anxiety and depression were detected in these animals at the age of 1 and 2 months. It can be assumed that experimental anxiety-depressive states develop via the formation of a complex multistructural pathological system [2] manifesting in a number of clinical symptoms at different stages, including latent periods.

In 7-month-old males, only signs of increased anxiety were revealed (by the scale for evaluating anxiety-phobic states in rats). On the contrary, in 7-month-old females only symptoms of depression were found ("behavioral despair" in the forced swimming test and signs of anhedonia) [3]. Experimental males in this series demonstrated increased anxiety at the age of 1 and 2 months and depressive-like behavior at the age of 2 and 3 months. Experimental females in this series exhibited signs of anxiety at the age of 1 and 3 months. Activity of both enzymes in striatum was increased in these animals at the age of 7 months. The striatum seems to play an important role in the genesis and manifestation of mixed anxiety-depression. This assumption is consistent with the concept on a special role of striatal structures in the pathophysiological machinery of experimental dopamine deficiency-dependent depressive syndrome in rats [2].

Different dynamics of DP-IV and PEP activities in brain structures of control and experimental male and female rats during the ontogeny appears to be associated with the effects of steroid hormones [15].

It cannot be excluded that increased activity of DP-IV in all studied brain structures (in experimental females at the age of 1 month and experimental males at the age of 7 months) accompanied by signs of anxiety and depression in animal behavior can be an adaptive response to prolonged enzyme inhibition in neonatal rats asynchronously developing in males and females. Activation of monoamine oxidase in rat brain due to active immunization against complexes of monoamine oxidase inhibitor with a carrier protein was accompanied by the development of depression and anxiety [7]. Our findings suggest that increased activity of DP-IV can be a pathogenetic mechanism of affective disorders. The dynamics of DP-IV activity in rat brain during administration of the enzyme inhibitor and the mechanisms mediating changes in PEP activity under conditions of modulation of DP-IV activity in this model remain unclear.

In patients with major depressive disorder, neurobiological abnormalities were revealed in several zones

of the prefrontal cortex, ventral striatum including the nucleus accumbens, hippocampus, anterior cingulate cortex, and amygdala [12]. We did not study biochemical changes in the cingulate cortex and amygdala, but the other listed structures were involved in the course of experimental anxiety-depressive disorder.

In our study, DP-IV inhibitor was administered to rats from the 1st to the 3rd week of development, which corresponds to the period of maturation of this enzyme system [6]. These data suggest that modulation of DP-IV activity with Met-Prd-N in the early ontogeny leads to the development of plastic changes in CNS manifesting at the biochemical and behavioral levels in increased proline-specific peptidase activity and in the development of anxiety and depressive states, respectively. The results of the study allow us to consider the increase in DP-IV and PEP activities in rat brain as pathogenetic mechanism of affective disorder development.

REFERENCES

1. N. A. Krupina, N. N. Zolotov, N. G. Bogdanova, *et al*., *Byull. Eksp. Biol. Med.,* **142**, No. 11, 497-500 (2006).
2. N. A. Krupina and G. N. Kryzhanovskii, *Zh. Nevrol. Psikhiatr*., No. 4, 42-47 (2003).
3. N. A. Krupina, E. Y. Kushnaryova, N. N. Khlebnikova, *et al*., *Zh. Vyssh. Nervn. Deyat.,* **59**, No. 3, 349-361 (2009).
4. N. A. Krupina and N. N. Khlebnikova, *Uspekhi Fiziol. Nauk*, **40**, No. 2, 3-26 (2010).
5. N. N. Khlebnikova, N. A. Krupina, N. G. Bogdanova, *et al*., *Byull. Eksp. Biol. Med.,* **147**, No. 1, 27-31 (2009).
6. P. Brust, A. Bech, R. Kretzschmar, R. Bergmann, *Peptides*, **15**, No. 6, 1085-1088 (1994).
7. R. A. Danilova, T. A. Moskvityna, M. F. Obukhova, *et al*., *Neurochem. Res*., **24**, No. 9, 1147-1151 (1999).
8. M. El Yacoubi, J. M. Vaugeois, D. Marguet, *et al*., *Behav. Brain. Res*., **171**, No. 2, 279-285 (2006).
9. J. A. García-Horsman, P. T. Männistö, and J. I. Venäläinen, *Neuropeptides*, **41**, No. 1, 1-24 (2007).
10. T. Karl, T. Hoffmann, R. Pabst, and S. von Horsten, *Pharmacol. Biochem. Behav.,* **75**, No. 4, 869-879 (2003).
11. M. Maes and S. Bonaccorso, *Acta Psychiatr. Scand*., **109**, No. 2, 126-131 (2004).
12. V. Maletic, M. Robinson, T. Oakes, *et al*., *Int. J. Clin. Pract*., **61**, No. 12, 2030-2040 (2007).
13. P. T. Mannisto, P. Tuomainen, O. Kutepova, *et al*., *Pharmacol. Biochem. Behav*., **49**, No. 1, 33-40 (1994).
14. R. Mentlein, *Regul. Pept*., **85**, No. 1, 9-24. (1999).
15. P. Palanza, *Neurosci Biobehav Rev*., **25**, No. 3, 219-233 (2001).