GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Behavioral Changes in Rats Induced by a Dipeptidyl Peptidase IV Inhibitor Methionyl-2(S)-Cyanopyrrolidine: Experimental Model of Anxiety-Depression Disorder

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Administration of a synthetic dipeptidyl peptidase IV inhibitor methionyl-2(S)-cyanopyrrolidine (1 mg/kg) to rats during the early postnatal period was followed by the development of behavioral changes in young and adult animals. The degree of anxiety in the elevated plus maze increased in treated rats at the age of 1-2 months. Depressive behavior in the forced swimming test was typical of animals aging 2-3 months. Diazepam reduced the severity of anxiety in treated rats. Melipramine had a normalizing effect on swimming behavior. A novel prolyl endopeptidase inhibitor benzyloxycarbonyl-methionyl-2(S)-cyanopyrrolidine had the antidepressant properties.

Key Words: *dipeptidyl peptidase IV inhibitor methionyl-2(S)-cyanopyrrolidine; anxiety; depression; model; rats*

The increased anxiety and depressive disorders are associated with dysfunction of various systems in the organism, including the neuroanatomical, neurotransmitter, neuroendocrine, neuroimmune, neuropeptide, and neurotrophic systems [11,12]. Published data suggest that depression and anxiety are related to dysregulation of intracellular signal transduction (*e.g.*, system of secondary messengers) [7, 15]. However, the cause of an abnormally increased anxiety and depression remains unknown.

Previous studies showed that neuropeptides have a role in the development of anxiety and depressive disorders [9,11]. Little is known about the system for degradation of these substances. Clinical observations revealed that the activities of proline-specific peptidases prolyl endopeptidase (PEP) and dipeptidyl peptidase IV (DP-IV) in blood plasma and serum increase in patients with anxiety and depressive disorders [10]. The substrates for these peptidases include peptides that mediate the development of anxiety and depression. They include thyroliberin, substance P, cholecystokinin, neuropeptide Y, oxytocin, and vasopressin [8,13].

We showed for the first time that the activities of PEP and DP-IV in brain structures increase in rats with experimental dopamine deficiency-dependent depressive syndrome [1]. Moreover, a PEP inhibitor benzyloxycarbonyl-methionyl-2(S)-cyanopyrrolidine (Z-Met-Prd-N) had the antidepressant properties under these conditions [4]. A change in the development of central nervous structures in animals during early ontogeny may affect the emotional-and-motivational state of adult specimens [6].

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Here we studied the emotional-and-behavioral state of rats after treatment with a synthetic irreversible noncompetitive inhibitor of DP-IV (methionyl-2(S)-cyanopyrrolidine, INH) in the early postnatal period.

MATERIALS AND METHODS

Experiments were performed on 103 male Wistar rats. The animals were housed in cages (5-7 specimens per cage) under the natural light/dark cycle and had free access to food and water. The study was conducted in 3 repetitions to evaluate the reproducibility of behavioral changes. In each series, some animals (treatment group) received intraperitoneal injections of DP-IV INH on days 5-19 after birth. This substance was synthesized at the V. V. Zakusov Institute of Pharmacology (Russian Academy of Medical Sciences). The inhibition constant was 2.7 nmol/liter (substrate Gly-Pro-7-amino-4-coumarylamide). DP-IV INH was administered in a dose of 1.0 mg/kg (0.1 ml per 10 g body weight). A freshly prepared solution of DP-IV INH was used. The substance was dissolved by addition of Tween 80 (1-2 drops) and diluted to a required volume with physiological saline. The remaining animals (control group) received physiological saline (PS). Each group of animals consisted of rat pups from 3-4 litters. The behavior of rats was studied in series I (1, 2, 3, and 7 months of age; n=21), II (1, 2, and 3 months of age; n=30), III (1, 2, and 3 months of age; n=52). All manipulations and experiments were performed in accordance with the Rules of Laboratory Practice of the Russian Federation (Order of the Russian Ministry of Health, No. 267 of 19.06.2003).

Locomotor and exploratory activity of animals was evaluated from the total number of crossed squares and vertical rearing postures in the open-field test over 3 min [2].

The degree of anxiety was determined in the elevated plus maze. The maze was located at a distance of 80 cm from the floor level. Two open arms (without the wall; 36×10 cm) and two closed arms (surrounded by the wall, open ends; 37×10 cm) were perpendicular to each other. The central area was 10×10 cm. The central zone of the maze was illuminated with 39 lx. Before the study, each animal was placed in a new environment (brightly illuminated small cage) for 6 min to increase the behavioral activity [14]. The behavior of rats in the maze was studied for 5 min. The following parameters were recorded: number of entries into (crossing of the boundaries of the arm by four limbs) and time spent in the open and closed arms of the maze; number of overhanging postures in the open and closed arms of the maze; number of overhanging postures in the central zone (not studied in series I); risk-assessment behavior (looking out (at least up to the interaural line) from the open ends of the closed arms and immediate return); and preference for the open arms (ratio of the number of entries into the open arms to the total number of entries into the open and closed arms). Seven-month-old animals were not tested in the maze. In series II, some rats of the treatment and control groups (1 month of age) received intraperitoneal injection of a benzodiazepine tranquilizer diazepam (0.5 mg/kg, RUSAN FARMA LTD) on day 21 after the first test. These specimens were repeatedly tested in the maze 30 min after injection.

The symptoms of behavioral depression were studied in the Porsolt forced swimming test with modification [2]. The temperature of water in a reservoir was 25-26°C. The time of active swimming, passive swimming, and immobility (absence of swimming movements) and number of episodes of active swimming and immobility (up to 6 sec) were recorded to calculate the rhythmic index of depression.

In series III, the effect of test compounds were studied on 3 pairs of groups that consisted of 2-month-old rats. The immobility time and depression index in rats of the treatment subgroup were much higher compared to those in animals of the control subgroup. The first-pair animals received a tricyclic antidepressant melipramine (EGIS) in a daily dose of 10 mg/kg for 10 days (n=7, control group; n=6, treatment group). A PEP inhibitor Z-Met-Prd-N in a dose of 2 mg/kg was administered to the second-pair animals (n=8, control group; n=7, treatment group). Z-Met-Prd-N was synthesized at the V. V. Zakusov Institute of Pharmacology (Russian Academy of Medical Sciences). The inhibition constant was 2.0 nmol/liter (substrate Z-Ala-Pro-7-amino-4-coumarylamide). The third-pair animals were treated with PS (n=8, control group; n=7, treatment group). All compounds were injected intraperitoneally (1 ml per kg body weight). The swimming behavior was repeatedly studied 1 day after the last treatment with test compounds.

The results were analyzed by nonparametric Mann—Whitney test and Wilcoxon test (Statistica 6.0 software). Symptom frequency was estimated by Fischer's exact test. The significance level was 5%.

RESULTS

One-month-old males of the treatment group (Fig. 1) were characterized by a decrease in the number of entries into the open arms (series II and III), time

spent in the open arms (series II and III), and incidence of overhanging postures in the open arms (series II). These animals exhibited an increase in the time spent in the closed arms (series II and III), number of overhanging postures in the closed arms (series II), and frequency of risk-assessment behavior (series I and II). Moreover, we revealed a decreased number of overhanging postures in the central zone (series II; 3.4±0.7 for the control group, 1.3±0.5 for the treatment group) and reduced preference for the open arms (series III; 0.3±0.0 for the control group, 0.1±0.0 for the treatment group; $p<0.05$, Mann—Whitney test). Control males of this age demonstrated a greater number of entries into the open arms as compared to specimens of the treatment group (80.4 and 57.4%, respectively; $p<0.05$, Fischer's exact test).

Two-month-old males of the treatment groups exhibited a decrease in the number of overhanging postures in the closed arms (series III) and increase in the frequency of risk-assessment behavior (series II and III). At the age of 3 months, no significant differences were revealed between control and treated animals.

Injection of diazepam was followed by a decrease in the frequency of risk-assessment behavior in rats of the treatment group. The time spent in the open arms and number of overhanging postures in the open arms, closed arms, and central zone of the maze tended to increase under these conditions ($p<0.14$). Control animals were characterized by a decrease in the number of entries into the open arms (no changes in the time spent in the open arms and preference for the open arms) and increase in the incidence of overhanging postures in the closed arms (compared to the baseline value, Fig. 2). The observed changes in control rats probably reflect a modulatory effect of diazepam on locomotor acti-

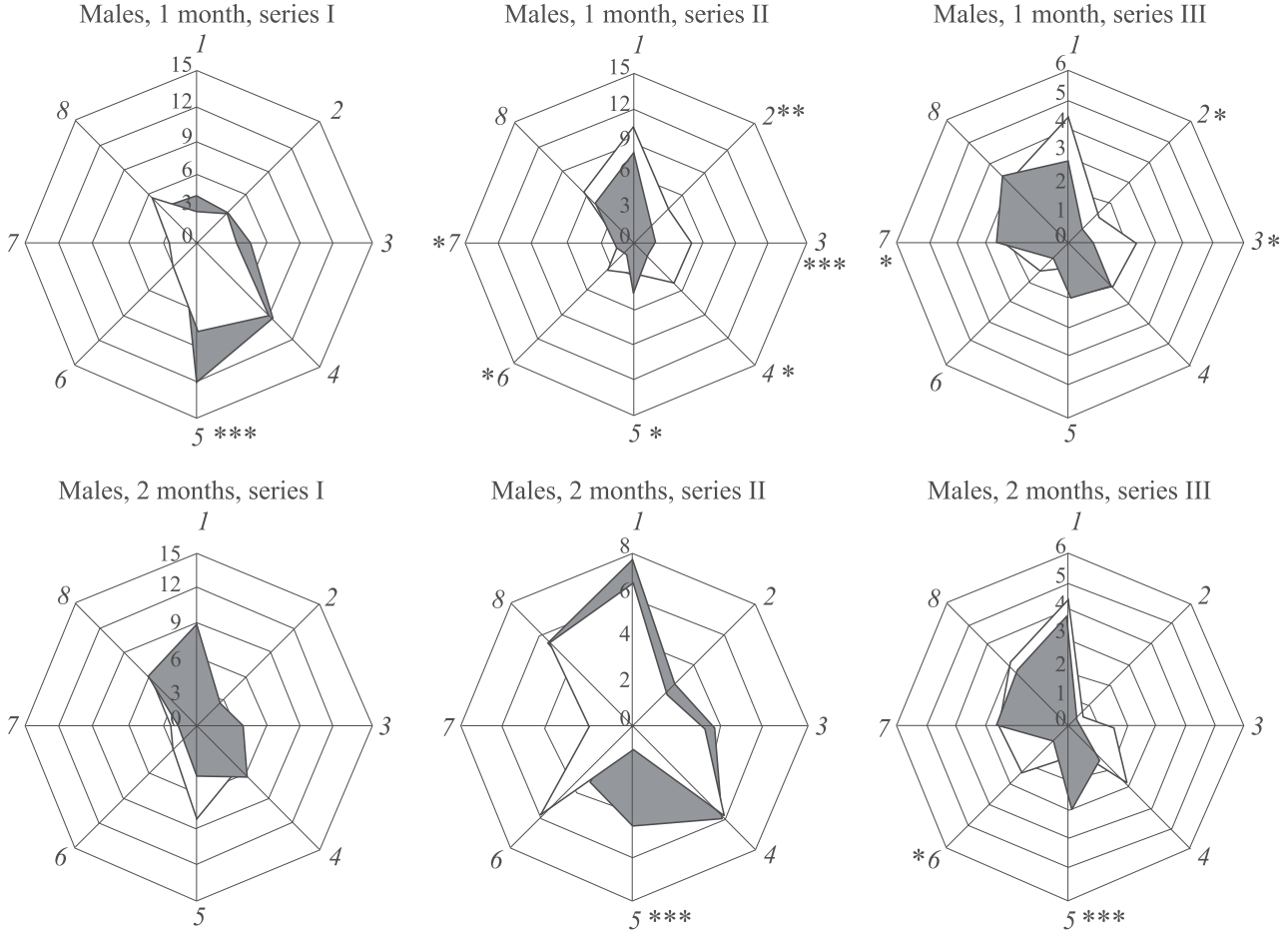

Fig. 1. Behavioral activity of treated rats (administration of DP-IV INH during the early postnatal period) and control animals in the elevated plus maze. Time spent in the central zone of the maze (sec×10, *1*); number of entries into the open arms (*2*); time spent in the open arms (sec×10, *3*); number of overhanging postures in the open arms (*4*); incidence of risk-assessment behavior (*5*); number of overhanging postures in the closed arms (*6*); time spent in the closed arms (sec×100, *7*); and number of entries into the closed arms (*8*). Light area, control group; dark area, treatment group. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ compared to the control group in the same period (Mann—Whitney test).

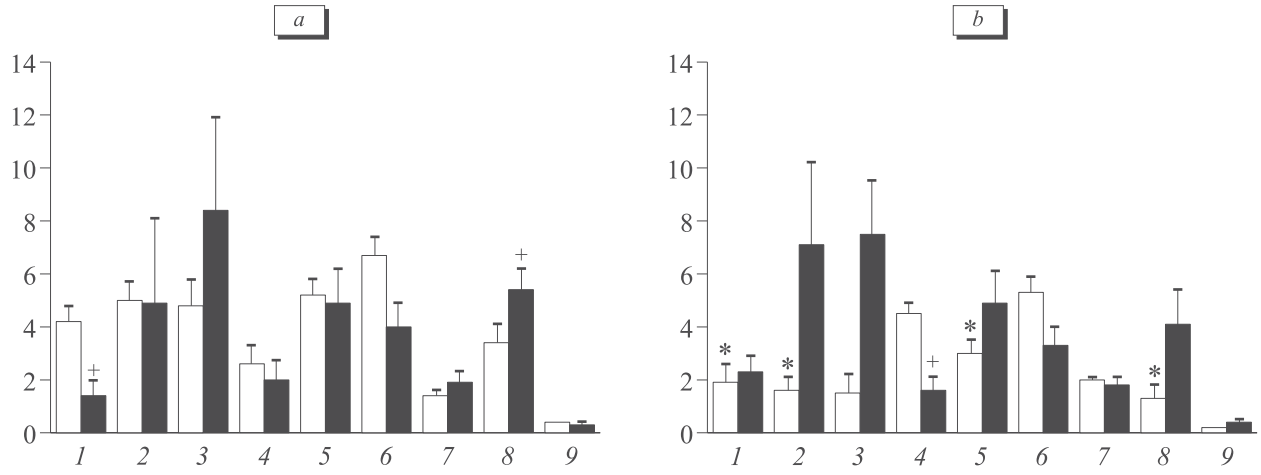

Fig. 2. Effect of diazepam (0.5 mg/kg, acute administration) on the behavior of treated rats (administration of DP-IV INH during the early postnatal period, *a*) and control animals (*b*) in the elevated plus maze. Number of entries into the open arms (*1*); time spent in the open arms (sec×10, *2*); number of overhanging postures in the open arms (*3*); incidence of risk-assessment behavior (*4*); number of overhanging postures in the central zone of the maze (*5*); number of entries into the closed arms (*6*); time spent in the closed arms (sec×100, *7*); number of overhanging postures in the closed arms (*8*); and preference for the open arms (*9*). Light bars, control (*a*) and treatment groups (*b*) before administration of diazepam; dark bars, after administration of diazepam. $p<0.05$: *compared to the control group in the same period; +compared to the same group before administration of diazepam (paired Wilcoxon test).

vity of animals. Before treatment with diazepam, animals of the treatment group exhibited a smaller number of entries into the open arms, little time spent in the open arms, and low incidence of overhanging postures in the central zone and closed arms (as compared to control specimens; Fig. 2). Behavioral parameters of control and treated rats did not differ after administration of diazepam.

Locomotor and exploratory activity of treated rats in the open-fled test remained unchanged during all periods of the study (various series).

No behavioral changes of 1-month-old rats from the treatment group were found in the forced swimming test. The immobility time and depression index of treated animals aging 2 months were shown to increase in various series (Table 1, Fig. 3). At the age of 3 months, an increase in the immobility time and depression index of treated specimens was revealed in series I. No changes were found in 7-month-old rats.

Subchronic administration of PS was not followed by a statistically significant increase in the immobility time and depression index of 2-month-old rats from the treatment group (Fig. 3). By contrast, melipramine and PEP inhibitor Z-Met-Prd-N decreased the immobility time and depression index in treated animals.

These changes reflect an increase in the degree of anxiety of treated rats aging 1-2 months (study in the elevated plus maze). The increased anxiety

TABLE 1. Immobility Time and Rhythmic Index of Depression in Treated (Administration of DP-IV INH) and Control Rats (Administration of the Solvent for DP-IV INH) in the Forced Swimming Test

Group	Immobility, sec		Depression index	
	Series I	Series II	Series I	Series II
Control, 1 month	14.3±6.9 (*n*=9)	13.1±6.0 (*n*=13)	0.6±0.2 (*n*=9)	0.5±0.1 (*n*=13)
Treatment, 1 month	14.5±4.5 (*n*=12)	19.4±5.8 (*n*=17)	1.1±0.3 (*n*=12)	1.5±0.3 (*n*=17)
Control, 2 months	6.8±1.3 (*n*=8)	2.6±1.2 (*n*=7)	0.4±0.1 (*n*=8)	0.1±0.0 (*n*=7)
Treatment, 2 months	47.8±7.8** (*n*=12)	34.9±11.8* (*n*=9)	2.2±0.4** (*n*=12)	1.8±0.5* (*n*=9)
Control, 3 months	3.0±2.2 (*n*=8)	4.1±1.6 (*n*=7)	0.2±0.1 (*n*=8)	0.2±0.1 (*n*=7)
Treatment, 3 months	31.5±6.5** (*n*=12)	5.2±2.2 (*n*=9)	1.4±0.3* (*n*=12)	0.5±0.2 (*n*=9)
Control, 7 months	0.6±0.4 (*n*=8)	Not measured	0.1±0.1 (*n*=8)	Not measured
Treatment, 7 months	10.9±3.8 (*n*=12)	Not measured	1.1±0.5 (*n*=12)	Not measured

Note. *$p<0.01$ and **$p<0.001$ compared to the control group in the same period (Mann—Whitney test).

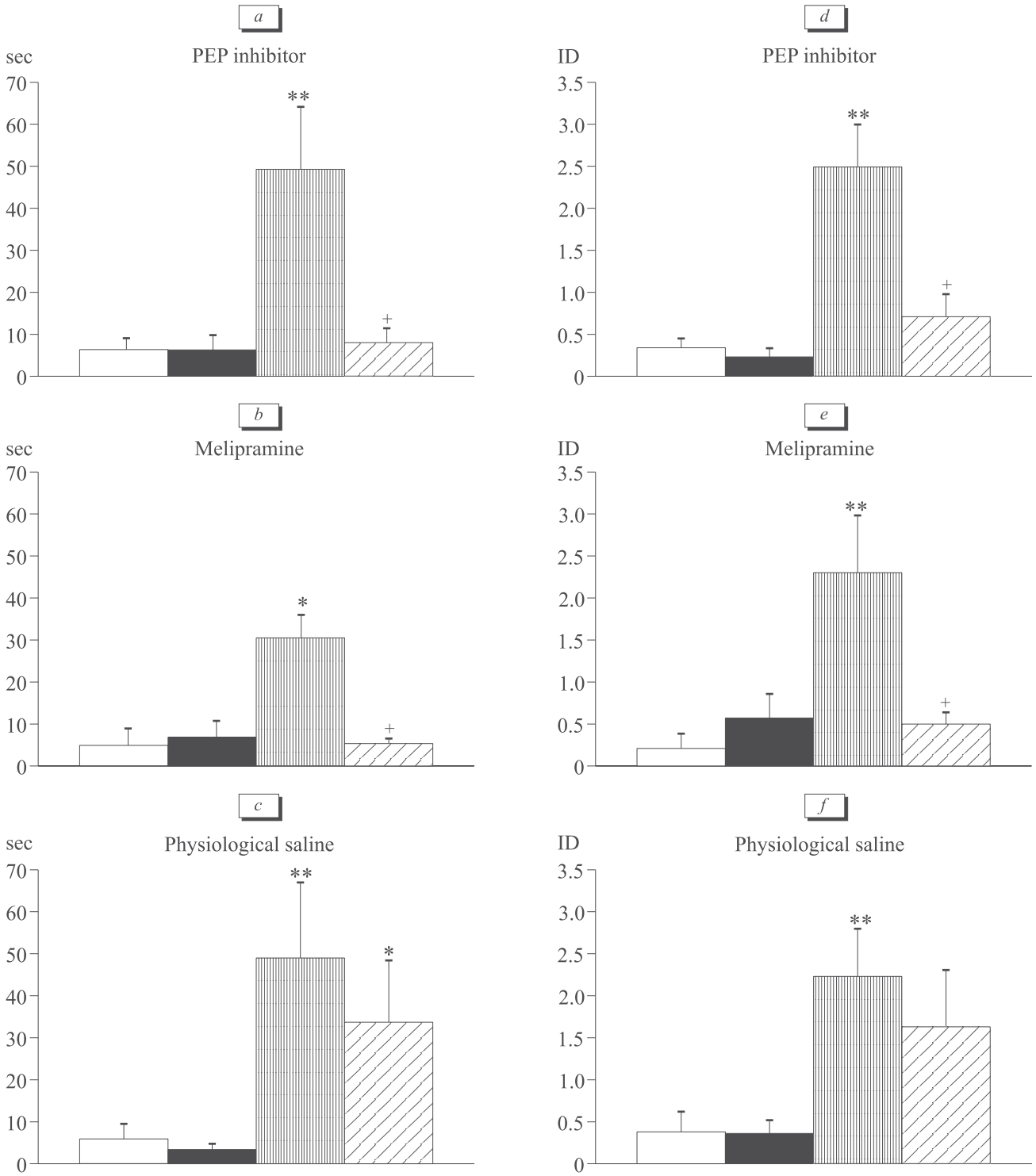

Fig. 3. Effects of melipramine (10 mg/kg) and PEP inhibitor Z-Met-Prd-N (2 mg/kg, subchronic administration) on the behavior of treated rats (administration of DP-IV INH during the early postnatal period) and control animals in the forced swimming test. (*a-c*) Immobility time and (*d-f*) rhythmic index of depression (ID). Light bars, control group before administration of test preparation; dark bars, control group after administration of test preparation; vertical shading, treatment group before administration of test preparation; horizontal shading, treatment group after administration of test preparation. *$p<0.05$ and **$p<0.01$ compared to the control group in the same period (non-paired Mann—Whitney test); $^{+}p<0.05$ compared to the same group before administration of test preparation (paired Wilcoxon test).

(failure to execute some tasks in the maze, chronological study) and normal locomotor activity of rats indicate that the observed changes do not reflect the reduction of movements. The degree of anxiety in the maze was evaluated from the indexes not common for this test (overhanging postures in

the central zone and closed arms; and risk-assessment behavior). Diazepam abolished the differences between general and additional indexes of anxiety in control and treated animals. Moreover, this substance suppressed the risk-assessment behavior (treatment group) and increased the number of overhanging postures in the closed arms (control group). Our findings illustrate the increased anxiety of treated rats. Therefore, an increase in risk-assessment behavior and decrease in the number of overhang postures in the closed arms and central zone may serve as reliable criteria of animal's anxiety.

A study in the forced swimming test showed that treated rats aging 2 and 3 months are characterized by behavioral despair and biorhythmic disorders, which results from the state of depression. The development of behavioral depression in animals was confirmed by high efficacy of melipramine in reducing the symptoms of this disorder. The efficacy of a PEP inhibitor Z-Met-Prd-N in reducing the symptoms of behavioral depression in rats compares well with that of melipramine. However, the dose of Z-Met-Prd-N was 5-fold lower than that of melipramine. These data are consistent with the results of our previous experiments. We showed that a PEP inhibitor Z-Met-Prd-N has the antidepressant properties during MPTP-induced depression [4]. It may be suggested that PEP plays a role in the development of depressive state in rats due to administration of a DP-IV inhibitor during the early postnatal period.

In clinical practice the symptoms of anxiety and depression serve as diagnostic criteria for several diseases, including mixed anxiety-depression disorder. It is impossible to evaluate the dominant affect under these conditions [3]. The symptoms of anxiety often precede the appearance of depressive signs. Experimental and clinical observations indicate that emotional-and-behavioral disturbances in rats due to postnatal treatment with INH are similar to anxiety-depression disorders in humans. The experimental anxiety-depression state may serve as a new model of emotional-and-behavioral disturbances in rats with anxiety and depressive disorders. This model complies with the principle of phenomenological and, partially, pharmacological isomorphism for a clinical prototype of mixed anxiety-depression disorder.

DP-IV INH was administered to rats from the 1st to the 3rd week of postnatal development, which coincides with the maturation of this enzyme system during ontogeny [5]. Our results confirm the notion that various diseases in adults are formed during early ontogeny. In the present study, anxiety and depression were induced by modulation of DP-IV activity during early ontogeny. Therefore, that DP-IV is involved in the development of anxiety-depression disorder. The mechanisms for this involvement require further investigations.

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