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Anxiolytic and Antidepressant Effects of Divaza and Brizantin

N. N. Yakovleva, T. A. Voronina, N. I. Suslov*, I. A. Ertuzun, G. M. Molodavkin, V. I. Poseva, V. V. Andreeva, Yu. L. Dugina, M. A. Putilovskii, and O. I. Epshtein

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The anxiolytic and antidepressant activities of complex preparations divaza and brizantin containing antibodies to brain-specific protein S100 were estimated using Vogel conflict test and Nomura forced swimming test. Course treatment (5 days) of brizantin in a dose of 2.5 ml/kg and divaza in a dose of 7.5 ml/kg significantly increased punished drinking in the Vogel conflict test in comparison with the control. Both drugs also improved general emotional behavior during training prior to the test procedure. Brizantin and divaza in a dose of 7.5 ml/kg increased the number of wheel revolutions in the Nomura forced swimming test in comparison with the control; the effect of divaza was more pronounced. High correlation coefficients between the number of wheel revolutions during the first and second 5-min sessions are also indicative of antidepressant action of divaza and brizantin.

Key Words: *divaza; brizantin; S100; anxiolytic effect; antidepressant effect*

At present, neurotic disorders are one of the major medical and social problem. Neuroses and neurosis-like states are usually treated with tricyclic antidepressants and benzodiazepine anxiolytics, as well as selective serotonin reuptake inhibitors. These pharmacological groups, however, produce a number of side effects. Benzodiazepine anxiolytics exert sedative and muscle relaxant action, which leads to impaired attention, sleepiness, coordination disorders and injuries; antidepressants can impair cognitive functions; treatment with selective serotonin reuptake inhibitors is associated with the risk of maniacal states, suicide, and sexual dysfunction [9,10,12-14].

Brizantin and divaza are complex drugs based on release-active antibody forms [6]. Divaza (as part of complex therapy) was designed to restore brain integrative activity in a wide range of organic CNS disorders, including neurodegenerative, cerebrovascular (and ischemic) diseases, neuroinfections, and traumatic brain injuries. Brizantin is used in treatment of alcohol and tobacco dependence. Both drugs contain release-active form of anti-S100 antibody with a wide range of psychotropic and neuroprotective activities [6,7,11,15] and produce no side effects typical of other anxiolytics [2].

The aim of this study was to evaluate potential anxiolytic and antidepressant activity of divaza and brizantin using standard methods and to compare them with benzodiazepine anxiolytic diazepam and tricyclic antidepressant amitriptyline.

MATERIALS AND METHODS

Divaza was used in the form of aqueous dilutions of a combination of release-active forms of anti-S100 antibodies and antibodies to endothelial NO-synthase. Brizantin was also used in the form of aqueous dilutions of a combination of release-active forms of antibodies to protein S100 and antibodies to type 1 cannabinoid receptor. The doses of dilutions were expressed in ml/kg body weight. Divaza, brizantin, and distilled water were provided by Materia Medica Holding in

Materia Medica Holding, Moscow; *E. D. Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk, Russia.
Address for correspondence: guryanovann@materiamedica.ru.
N. N. Yakovleva

coded form. Diazepam (Gedeon Richter) and amitriptyline (vials for injection, 20 mg/2 ml, Moscow Endocrine Plant) were used as the reference drugs.

The study was carried out at the E. D. Goldberg Research Institute of Pharmacology and Regenerative Medicine on white mongrel male rats (n=140) weighing 264.87±57.62 g and aged 2.5 months at the beginning of the study. The animals were obtained from the nursery of E. D. Goldberg Research Institute of Pharmacology and Regenerative Medicine. The animals were kept in accordance with the rules of good laboratory practice (GLP) and with regulations adopted in Russian Federation. The rats delivered from the nursery were quarantined for 7 days.

The study was conducted in accordance with the Manual on Experimental (Preclinical) Study of New Pharmacological Substances [1,3]. To study the anxiolytic effect of drugs in the Vogel conflict test, the rats (a total of 6 groups, 10 rats per each) received divaza, brizantin, and distilled water through a gastric tube in volumes of 2.5 and 7.5 ml/kg once a day for 5 days. Diazepam (2 mg/kg once a day for 5 days, n=10) was used as the reference drug. The last dose of the drugs was administered 60 min prior to the final test (72 h after the start of drinking deprivation). The Vogel conflict is modeled by collision of drinking and defense motivations (every water lick is punished by electrical pain stimulation) using the previously described operant chamber [4]. The test was conducted for 3 days. On day 1, the animals were completely deprived of water. On day 2, they were placed in the experimental chamber and learned to drink water from the drinker fitted on the wall of experimental chamber for 5 min without punishment. On day 3, the animals were again placed into the chamber, but in 10 sec after the first lick, electric current of 0.25 mA was delivered to the drinker and electrode floor in such a way that each lick was punished. The number of punished licks was counted over 10 min. Anxiolytic activity of the test substances was evaluated by the increase in punished drinking in comparison with the control.

The antidepressant effect of the drugs was evaluated in the Nomura forced swimming test. To this end, the rats intragastrically received divaza, brizantin, and distilled water in doses of 2.5 and 7.5 ml/kg once a day (6 groups, 10 rats per each) for 5 days. Amitriptyline (10 mg/kg, once a day for 5 days, n=10) was used as the reference drug. The study was conducted according to Nomura protocol in modification of G. M. Molodavkin [5,8]. Experimental tank (64×30×42 cm) divided into 4 equal compartments with freely rotating wheels (15 cm in diameter) was filled with water (t=25°C) up to the middle of the wheels. The measure of depressive condition in rats was the number of wheel revolutions while they tried to get out of the container: the lower was the number of revolutions, the more pronounced was depression. The number of revolutions was recorded over 10 min using a magnetic sensor. In order to find whether the drug belongs to a certain neurotropic group, the number of wheel revolutions over the first 5 min of the test and the second 5-min interval were counted separately, and the coefficient of correlation between the number of revolutions during the first and second 5-min intervals was calculated. Significant increase in the number of wheel revolutions and in the correlation coefficient in comparison with the control was used as measure of the antidepressant action of the drug [5,8].

Statistical data processing included calculation of mean number (M) of punished licks or wheel revolutions and their standard deviations (SD) for each group. Significance of changes in these parameters in comparison with the control was assessed using the Student's t test. The correlation coefficient was calculated using Microsoft Excel software.

RESULTS

In the Vogel conflict test, the control rats receiving 2.5 and 7.5 ml/kg distilled water made 57.40±15.24 and 58.20±11.87 punished licks, respectively (Table 1); they were characterized by high emotionality and left many fecal boluses (Table 2). Diazepam in a dose of 2 mg/kg produced a pronounced anxiolytic effect in the conflict test: the number of licks was by 3.2 times higher than in the control (p<0.05; Table 1). Moreover, diazepam-treated rats showed calm behavior: they allowed handling and left no fecal boluses during training in the apparatus (Table 2). Thus, in the Vogel conflict test diazepam in a dose of 2 ml/kg showed a pronounced anxiolytic effect.

TABLE 1. Effect of Brizantin, Divaza, and Diazepam on the Number of Punished Water Licks in the Vogel Conflict Test (M±SD)

Group		Number of licks
Control (distilled water)	2.5 ml/kg	57.40±5.24
	7.5 ml/kg	58.20±1.87
Diazepam	2 mg/kg	186.40±5.51*
Brizantin	2.5 ml/kg	121.1±41.69*+
	7.5 ml/kg	65.2±21.56+
Divaza	2.5 ml/kg	58.00±20.28+
	7.5 ml/kg	144.80±21.53*+

Note. p<0.05 in comparison with *corresponding control, +diazepam.

TABLE 2. Effect of Brizantin, Divaza, and Diazepam on the Number of Fecal Boluses during Training in the Vogel Conflict Test (*M*±*SD*)

Group		Number of fecal boluses
Control (distilled water)	2.5 ml/kg	2.80±1.20
	7.5 ml/kg	1.80±1.90
Diazepam	2 mg/kg	0±0
Brizantin	2.5 ml/kg	0.20±0.42
	7.5 ml/kg	0.10±0.32
Divaza	2.5 ml/kg	0.20±0.42
	7.5 ml/kg	0.40±0.70

In rats receiving 2.5 ml/kg brizantin, the number of punished water licks increased by 2.1 times in comparison with the controls animals ($p<0.05$) despite painful stimulation (Table 1). Animals also demonstrated more adequate behavior, the number of fecal boluses was lower (Table 2). Administration of brizantin in a dose of 7.5 mg/kg was followed by an insignificant increase in the number of water licks in comparison with the control (by 12%; Table 1), but the number of fecal boluses was lower, similar to the group treated with 2.5 ml/kg brizantin (Table 2). Thus, brizantin in a dose of 2.5 ml/kg (but not 7.5 ml/kg) produced an anxiolytic effect in the Vogel conflict test. Effect of brizantin in the dose of 2.5 ml/kg was somewhat less pronounced than that of diazepam in a dose of 2 mg/kg.

In the group of rats treated with 2.5 ml/kg divaza, the number of punished licks did not differ from the corresponding control (Table 1). Rats in this group were emotional, struggled in arms, left many fecal boluses (Table 2). A similar pattern was observed in the control group.

When divaza was administered in a dose of 7.5 ml/kg, the number of punished licks in the conflict test increased by 2.5 times in comparison with the control ($p<0.05$), thus, the effect of divaza was only slightly inferior to the action of diazepam in a dose of 2 mg/kg. Animals in this group were more adequate, easily contacted the experimenter, did not struggle in arms, and practically did not leave fecal boluses during training (Table 2). Thus, in the Vogel conflict test, divaza in the dose of 7.5 ml/kg (but not 2.5 ml/kg) showed pronounced anxiolytic effect that was slightly inferior to that of diazepam (2 mg/kg).

In the Nomura forced swimming test, amitriptyline in a dose of 10 mg/kg produced a pronounced antidepressant effect manifested in significantly increased number of wheel revolutions in comparison with the control (2.3-fold, $p<0.05$; Table 3). The antidepressant effect of amitriptyline was also confirmed by high correlation coefficient (-0.83).

After administration of 2.5 ml/kg brizantin, the number of wheel revolutions did not increase in comparison with the control and the correlation coefficient was low (Table 3), which is indicative of the absence of antidepressant action of the drug. Administration of 7.5 ml/kg brizantin produced an increase in the number of wheel revolutions by 42.2% ($p>0.05$), when in comparison with the corresponding control, and an increase in correlation coefficient (Table 3), which is indicative of the antidepressant effect of the drug. Thus, brizantin in the dose of 2.5 ml/kg exhibited no antidepressant action, but in the dose of 7.5 ml/kg produced this effect.

In the group of rats treated with 2.5 ml/kg divaza, the number of wheel revolutions did not differ from the control, although the correlation coefficient in this group was significantly higher (Table 3). When divaza was administered in a dose of 7.5 ml/kg, the number of wheel revolutions was by 77.7% ($p<0.05$) higher than in the corresponding control, and the correlation coefficient was 0.94. Thus, this dose of divaza was not inferior to 10 mg/kg amitriptyline in the strength of antidepressant effect (Table 3).

Thus, course administration (5 days) of brizantin (2.5 ml/kg) and divaza (7.5 ml/kg) significantly increased the number of punished water licks in the Vogel conflict test, which is indicative of their anxiolytic action. The strength of anxiolytic effect of brizantin (2.5 ml/kg) and divaza (7.5 ml/kg) was inferior to that of diazepam (2 mg/kg). In addition, improvement in general emotional behavior during training prior to test was observed under the influence of brizantin and divaza. Brizantin and divaza in doses of

TABLE 3. Effect of Brizantin, Divaza, and Diazepam on Rat Behavior in Nomura Forced Swimming Test (*M*±*SD*)

Group		Number of revolutions	Correlation coefficient
Control (distilled water)	2.5 ml/kg	42.40±2.77	0.22
	7.5 ml/kg	72.10±27.30	0.5
Amitriptyline	10 mg/kg	131.30±24.87*	0.83
Brizantin	2.5 ml/kg	36.70±6.02^{+}	0.03
	7.5 ml/kg	102.50±49.72	0.77
Divaza	2.5 ml/kg	43.70±26.18^{+}	0.76
	7.5 ml/kg	128.10±47.34*	0.94

Note. $p<0.05$ in comparison with *corresponding control, $^{+}$amitriptyline.

7.5 ml/kg also produced antidepressant effects in the Nomura test. The action of the drugs manifested in increased number of wheel revolutions, the effect of divaza being more pronounced. Antidepressant action of brizantin and divaza was also confirmed by high values of correlation coefficients between the number of wheel revolutions during the first and second 5-min intervals. Antidepressant effect of 7.5 mg/kg divaza was not inferior to that of amitriptyline.

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