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Research Article

Influence of Diazepino[1,2-a]benzimidazole derivative (DAB-19) on behavioral aspects of animals

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Abstract

Introduction: Diazepino[1,2-a]benzimidazole derivatives showed anxiolytic (EPM, L/D box, Vogel test), antidepressant (Tail Suspension test, Porsolt test), anticonvulsant (Pentylenetetrazole-Induced seizures) and analgesic (Tail-Flick, Hot Plate) actions, which were described earlier (Spasov et al. 2020). 11-(4-tert-butylbenzyl)-2,3,4,5-tetrahydro[1,3] diazepino[1,2-a]benzimidazole hydrobromide (compound DAB-19) has evident anxiolytic, antidepressant and anticonvulsant effects. In the present study, compound DAB-19 was screened for its influence on animals` behavior patterns, such as aggression, obsessive-compulsive behavior, emotional lability, and unsociability.

Materials and methods: 112 outbred white animals (rats and mice) were used. Compound DAB-19 (2.34 mg/kg, p.o.) and diazepam (2 mg/kg, p.o.) were injected to the treatment groups. Using an Open Field (OF) test, we evaluated a spontaneous motor activity, a search activity of mice and a degree of their emotionality. During a Marble Burying (MB) test, we monitored anxiolytic and anticompulsive effects of DAB-19. Using a Resident-intruder (RI) test, we evaluated a degree of aggression in the experimental animals, properties of their social interaction, as well as defensive and individual behavior.

Results and discussion: Compound DAB-19 has positive influence on the search activity, mood stabilizing activity and antiaggressive actions. Administration of both DAB-19 (2.34 mg/kg, p.o.) and diazepam (2 mg/kg, p.o.) reduced anxiety-like behavior in OP, RI and MB tests, as indicated by a significantly increased number of entries to the center of OP; a decreased number of marbles buried in MB and reduced aggressive behavior in RI. It was stated that using DAB-19 leads to a decrease in affective reactions of the animals – aggression, obsessive-compulsive behavior, neurotic condition and emotional instability.

Conclusions: The compound (DAB-19) produces anxiolytic-like effects, compared with those of diazepam, in various anxiety paradigms.

Keywords

anxiolytic; DAB-19; diazepam; combined structures; marble burying (MB); resident-intruder (RI); open field (OF).

Introduction

Nowadays anxiety disorders are among wide-spread diseases (Bahi et al. 2019). Some of the most significant ones for clinical practice are general anxiety disorder, post-traumatic stress disorder, panic disorder (Carpenter et al. 2018; Freire et al. 2020), obsessive-compulsive disorder, social anxiety disorders, and mixed anxiety-depressive disorder. The attributes of clinical picture of patients with those pathologies are a gradual decrease in communicative and cognitive skills (Nechita et al. 2018), as well as disturbance of motivation, motor and vegetative spheres. It leads to problems in the professional sphere, which can be a factor of both economic instability (Ziebold et al. 2019) and interpersonal relationships which can lead to inferiority complex (Zethraeus et al. 1962). Most often anxiety disorders are accompanied by behavior instability, unmotivated aggression, hyperactivity, puerility or, on the contrary, asociality (Craske et al. 2016).

At present, there are a lot of medicine agents for treating anxiety disorders, but all of these have serious side effects (Ziebold et al. 2019). Classic tranquilizers – derivatives of benzodiazepine (diazepam, alprazolam) – after long-term usage can cause mental and physical dependence, as well as addictive behavior, muscle relaxation, and sedation. A number of scientific information sources (Ait-Daoud et al. 2018; Manz et al. 2018) describe cases of significant withdrawal syndrome after interruption of the treatment, when anxiety was increasing in the patients after they learned about a decrease in thetherapeutical dose of the agent (Soyka 2017). Daily sedatives (tofisopam, medazepam, gidazepam) have milder side effects than classic tranquilizers. However these drugs can cause psychomotor agitation, irritation, aggression, sleeping disorder, and confusional state. A derivative of mercaptobenzimidazole – fabomotizole – decreases behavioral components of psychopathological conditions, but has a delayed start of the main effect (Uyanaev et al. 2006).

Following the above, nowadays it is vital to search and study drugs with a pronounced anxiolytic effect and positively affecting the majority of psychoemotional components as well as having a minimum of side effects.

In a previous study, we found a compound (DAB-19) – diazepino[1,2-a]benzimidazole, a derivative having in the core structure 2 privileged subclasses – diazepine and benzimidazole, showing a high tranquilizing action. It was shown that the compound demonstrated a pronounced anxiolytic effect not only comparable with a classic tranquilizer – diazepam, but exceeding it in a number of tests (Spasov et al. 2018).

The aim of the present study was to evaluate the influence of (DAB-19) on some components of animal behavior – aggression, communicative skills and obsessive-compulsive behavior.

Materials and methods

Experimental animals

The present research work was performed using 48 outbred male mice (18–22 g), 32 outbred male rats-residents (230–250 g) and 32 male rat-intruders (160–180 g), obtained from Rappolovo Nursery, Leningrad region, Russia. The animals were maintained in standard laboratory conditions (temperature (22 ± 2)°C and room humidity (60 ± 10)%), with a 12:12 h light/dark cycle. Standard diet and filtered water were provided ad libitum. The experimental procedures on the animals were carried out in accordance with the Local Ethics Committee of Volgograd State Medical University, Volgograd, Russia (Protocol No. IRB 00005839 IORG 0004900 (OHRP)).

Drugs and treatment

Diazepam was procured from Organica J.S.C., Novokuznetsk, Russia. The tested molecule DAB-19 was synthesized at the Research Institute of Physical and Organic Chemistry of the Southern Federal University, Rostov-on-Don, Russia and selected from a series of diazepino-[1,2-α]-benzimidazole derivatives due to its pronounced anxiolytic and antidepressant actions. Chemistry and synthesis of (DAB-19) were reported in our earlier publications (Anisimova et al. 2007). Diazepam and compound DAB-19 were freshly prepared in distilled water and administered per oral (p.o.) in a constant volume of 10 mL/kg.

Experimental design

The dose of (DAB-19) was equimolar to diazepam (2 mg/kg), in which it demonstrates a pronounced anti-anxiety effect without muscle relaxation and sedation (Spasov et al. 2018). Forty-eight outbred mice (n = 8/group) were randomly divided into six experimental groups. Group I consisted of control mice receiving vehicle (distilled water); group II was a group treated with the comparison drug diazepam (2 mg/kg), and group III received (DAB-19) (2.34 mg/kg). Besides sixty-four outbred rats (n = 8/group) were randomly divided into eight experimental groups. Group I consisted of control rats; group II received distilled water; group III was a group of diazepam (2 mg/kg), and group IV received (DAB-19) (2.34 mg/kg). The rodents were orally administered with (DAB-19) or diazepam or vehicle with an atraumatic metal gastric catheter 30 min before behavioral tests.

Behavioral tests

Open Field test

The apparatus consisted of a plastic arena (40×40×30 cm) with the floor divided with black parallel lines into 16

squares (15×15 cm). At intersection of lines, there were holes (1×1 cm), which made it possible to evaluate the search activity of the experimental mice (da Silva et al. 2018). The apparatus was illuminated with a 100 W bulb suspended at the height of 100 cm above the base of the arena. At the beginning of the test, the mice were placed individually at the center of the square arena. The ambulation scores (number of squares crossed), rearing number (upright standings on hind legs) and search activity (number of holes explored), as well as the number of short and long grooming acts were recorded for a 5 min period (Seibenhener et al. 2015). Open Field test can also be used for non-specific evaluation of muscle relaxation effects of compounds and to characterize the general rodent`s emotional state (rearings, sedation, a long grooming act as an indicator of comfortable condition, boluses) in addition to behavioral activity. After each individual test session, the apparatus was cleaned with 70% ethanol.

Marble Burying test

According to Egashira et al. (2013), a plastic cage (350×250×200 mm) was filled approximately 5 cm deep with sawdust. Twenty-four glass marbles (15 mm in diameter) were evenly spaced on the sawdust surface. The container was covered with a metal grid. During the testing phase, each mouse was placed in the corner of the cage individually and was allowed to explore it for 30 min (Taylor et al. 2017). At the end of the test, the mouse was removed from the cage, and the number of marbles buried to at least two-thirds of their depth was counted.

Resident-intruder test

An intruder male was introduced to the resident's cage, and then the main behavioral patterns (individual, aggressive, communicative and protective behavior) were registered. Individual (not associated with partner) behavior implied autogrooming, rearing number, resting state, on-site movement, and freezing (Koolhaas et al. 2013). Bites, attacks, aggressive poses, side poses were considered as aggressive behavior. Communicative behavior included following the partner, sniffing their bodies and genitals, grooming the partner, creeping towards and onto the partner. Avoiding the intruder, defense poses, submission pose on the back were considered as protective behavior (Wei et al. 2014). These parameters were assessed by the number of the registered forms (each act or pose of an experimental animal) of behavior within 5 minutes of monitoring.

Statistical analysis

Data were analyzed using GraphPad Prism v.5.0 (GraphPad Software, La Jolla, CA, USA). All the results are expressed as mean±standard error of mean (SEM). The significance of differences between the groups were analyzed using Kruskal-Wallis test followed by post-hoc Dunn's test. A value of $p \leq 0.05$ was considered as statistically significant.

Results and discussion

Based on the results of the study of behavioral activity in the Open Field test, it was found that the animals treated with (DAB-19) actively moved throughout the area of the experimental apparatus, explored its segments and holes, and more often went out into the central area (Table 1). It was shown that the number of crossed squares (locomotor activity) under the influence of DAB-19 1.6 times ($p \leq 0.05$) exceeds that number for the control group and corresponds to the indicator of the diazepam group. The number of rearings of the animals influenced by DAB-19 was 6.6 times more than that in the control group and 2.6 times more than in diazepam group ($p \leq 0.05$). The search activity of the animals treated with DAB-19, 1.9 times exceeded the control group activity and 1.7 times exceeded the diazepam group activity. Entries to the installation center by this group of animals were performed 4.1 times statistically more often than in the control; however, they were twice as few as diazepam ($p \leq 0.05$). The number of short-term self-grooming acts of the animals influenced by DAB-19 was 3.3 times ($p \leq 0.05$) less pronounced compared to the control and corresponded to the level of the diazepam group. The received data correlated to the indicators of long-term self-grooming: a 6.3-time increase in this parameter was recorded for the animals that received distilled water ($p \leq 0.05$). It is at the level of the diazepam group ($p \leq 0.05$), which indicates the leveling of anxiety behavior in the DAB-19 group. The amount of fecal boli in the DAB-19 group was 2.2 times ($p \leq 0.05$) less than that in the control group, which proves lack of pronounced emotionality.

Table 1. Influence of Compound DAB-19 (2.34 mg/kg, p.o.) and Diazepam (2 mg/kg, p.o.) on Behavior of Mice in the Open Field Test (M ± SEM).

Parameter		Control group	Diazepam (2 mg/kg)	DAB-19 (2.34 mg/kg)
Locomotor activity		55.3 ± 3.61	92.5 ± 6.91*	93.0 ± 3.41*
Rearings		3.2 ± 0.61	8.4 ± 1.32*	21.0 ± 2.00*
Search activity		3.2 ± 0.61	3.6 ± 0.50	6.0 ± 0.61*
Number of entries to the center of arena		0.8 ± 0.31	7.0 ± 1.21*	3.3 ± 0.42*
Self-grooming	Short-term	7.5 ± 0.92	2.7 ± 0.42*	2.3 ± 0.49*
	Long-term	0.8 ± 0.40	4.5 ± 0.85*	5.0 ± 0.73*
Fecal boli		2.2 ± 0.31	1.3 ± 0.33	1.0 ± 0.45

Note: * – differences are significant compared to control ($p \leq 0.05$, Kruskal-Wallis test, Dunn`s post hoc test).

In the Marble Burying test, after DAB-19 administration, compulsive marble burying was 6.9 times lower (1.7 ± 0.70) than in the control group (11.8 ± 2.60), which

Table 2. Influence of Compound DAB-19 (2.34 mg/kg, p.o.) and Diazepam (2 mg/kg, p.o.) on Behavior of Rats in the Resident-intruder Test(M ± SEM).

Group	Individual behavior, points	Aggressive behavior, points	Communicative behavior, points	Defensive behavior, points
Control group	9.2 ± 1.54	17.3 ± 3.77	16.0 ± 1.17	2.4 ± 0.65
Control (vehicle)	6.0 ± 2.67	17.6 ± 3.68	11.4 ± 1.12*	5.6 ± 1.17*
Diazepam (2 mg/kg)	5.1 ± 1.03*	9.5 ± 2.18*	10.5 ± 2.17*	2.4 ± 1.42
DAB-19 (2.34 mg/kg)	7.8 ± 1.18	1.5 ± 0.67*	12.8 ± 2.14	0.0 ± 0.01*

Note: * –differences are significant compared to control ($p \leq 0.05$, Kruskal-Wallis test, Dunn`s post hoc test).

was the same level with diazepam group (2.1 ± 1.24), $p \leq 0.05$. The animals in the group of DAB-19 moved actively in the apparatus, but unlike the control group made fewer attempts in marble burying. Smooth-faced layer of sawdust in the cage and the absence of pits indicate that.

In the Resident-intruder test, a significant change in the number of acts of protective and communicative behavior in the positive control group compared to the intact one was registered, which may be associated with the consequences of animal stress from p.o. administration of the solvent (Table 2). Aggressive behavior reduced ($p \leq 0.05$) after administration of diazepam, which is a characteristic feature of the benzodiazepine drugs. Protective behavior after administration of DAB-19 was practically not observed. However, the indicator of aggressive behavior not only more than 10 times decreased compared to the intact control group, but 6 times exceeded that in the diazepam group ($p \leq 0.05$). Important changes in individual and communicative behavior patterns after using DAB-19 were not recorded.

Among the most effective classic tranquilizers – derivatives of benzodiazepine used in the treatment of anxiety pathologies, diazepam stands out. However, it has side effects on the behavioral component – fatigue and lack of emotions or, on the contrary, acute agitation, anxiety, hallucinations and fits of rage (Talarek et al. 2018). The group of "daytime" sedatives has milder unwanted actions, but even their use can result in increased irritability, psychomotor agitation, confusion (tofisopam), sedation, drowsiness, fatigue and a slow-down in reactivity (medazepam).

The derivative of diazepino[1,2-a]benzimidazole–DAB-19 – does not tend to inhibit the spontaneous locomotor activity of animals, including ambulation scores and rearings number in the Open Field test. As it was observed in previous studies, DAB-19 was confirmed to lack muscle relaxation (Ziebold et al. 2019). High spontaneous activity is probably associated with an increased search activity of mice. It can indicate either pronounced emotionality in the animals, which is not confirmed by the number of boluses as one of the observed parameters, or a positive effect on the cognitive aspect of the experimental rodents` behavior, which is supported by a distinct anxiolytic effect of DAB-19 in previous study (Spasov et al. 2018).The suppression of natural fear of open spaces allows animals to show interest in exploring new territories. The anxiolytic effect of DAB-19 is also confirmed by a high number of entries to the central lit area, rarely recorded short-term self-groomings (which is known to indicate the nervous state of the animal) and a large number of acts of prolonged groomings (which is consistent with the results of screening and in-depth studies of the anti-anxiety activity of the compound) (Spasov et al. 2018; Spasov et al. 2020).

The anxiolytic pattern of action is associated with anti-compulsive effect of DAB-19 shown in the Marble Burying test. The number of acts of compulsive burying was significantly reduced relative to the reference groups. The obtained data of DAB-19 and diazepam do not result from the development of a muscle relaxant effect, which is evident from the data of the previously conducted tests: Elevated Plus Maze (parameter of the total number of moves between the arms), Actometer and Open Field (Spasov et al. 2018). According to the previously obtained data, tofisopam (2 mg/kg) was shown to have an anti-neurotic effect at the level of DAB-19 (2.34 mg/kg) – 1.2 ± 0.49 marbles on average (Skripka et al. 2018). So, DAB-19 in the Marble Burying test demonstrated an anti-compulsive effect slightly exceeding diazepam and not connected with muscle relaxation in the studied dose, which broadens the understanding of the clinical potential of the new diazepino[1,2-a]benzimidazole derivative.

The Resident-intruder test is employed to quantify the intraspecific aggression between male rats. One of the most important components of behavioral activity is social interaction to determine the structure of which the Resident-intruder test was used. For high spontaneous activity of animals in the Open Field test, aggressive genesis was not confirmed since this type of behavior in the group DAB-19 was 10 times reduced compared to control values, which distinguishes its spectrum of action from that of tofisopam. Given the correspondence between the parameters of the individual and communicative behavior of the DAB-19 group to the control group values, a decrease in the aggressive component is associated with the leveling of the protective pattern of rats in the Resident-intruder test. In addition, the lack of influence on individual and communicative behavior may indicate the absence of sedative effect in the studied compound (DAB-19).

The therapeutic correction of affective mental disorders often has some difficulties associated with the biochemical individuality of development mechanisms of these pathologies (Beesdo et al. 2009; Byrd et al. 2014; Cooney et al. 2017). Compound DAB-19 is a derivative of diazepino[1,2-a]benzimidazole, so it has fragments of the two privileged subclasses in its core structure: diazepine and benzimidazole. Perhaps, a combination of chemical compounds with a proven tranquilizing effect

(Spasov et al. 2018), carried out through various mechanisms, is manifested in DAB-19 in the multi-targeting of its action, which results in the reduction or leveling of a number of undesirable behavioral components.

Conclusion

As a result of the study, it was found that a diazepino[1,2-a]benzimidazole derivative, compound DAB-19, at the dose of 2.34 mg/kg, which has previously proven anxiolytic and antidepressant activities, changes various pathological behavioral reactions towards normalization. Thus, theoretically, it can be a promising compound for the relief of a number of psychopathological conditions manifested in pronounced aggression (gambling, alcohol or mental addiction), predominance of compulsive behavior (obsessive-compulsive disorder, addictive behavior) or increased unsociability (anxiety disorder, neurosis). The obtained data indicate the presence of normotymic and anti-aggressive action of DAB-19, as well as its positive effect on research activity.

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Conflict of interests

The authors declare no conflict of interests.

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