Anxiolytic and Anxiogenic Effects of Diazepam in Male Mice with Different Experience of Aggression

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 133, No. 4, pp. 429-433, April, 2002
Original article submitted December 14, 2001

Effects of diazepam (0.5 mg/kg intraperitoneally) on aggressive and anxious behavior were studied on male C57Bl/6J mice with different experience of aggression. Diazepam reduced aggression in animals with previous 3- and 20-day experience of aggression, but the plus-maze test revealed an anxiogenic effect of this drug in the former group and anxiolytic effect in the latter group. It was hypothesized that previous aggression experience modified animal sensitivity to diazepam. The effect of the drug depends on psychoemotional status of experimental animals, and this status differs in male mice with short- and long-term experience of aggression.

Key Words: *aggressive behavior; anxiety; anxiolytics; diazepam*

Neuroleptics, anxiolytic drugs and sedatives are used for correction aggressive behavior and aggressiveness of different etiology. The antiaggressive effects of buspirone, an agonist of serotonin receptors (5-HT_{1A}) were previously reported [7,8]; this agent was effective in the therapy of children aggressiveness [4] and aggressiveness caused by organic brain pathology in adult patients [13]. Antiaggressive effects of buspirone were demonstrated experimentally on mice and rats in different models of aggression: aggression induced by social isolation [14] or apomorphine [11], in the resident-intruder model [1], and under conditions provoking maternal aggression [5].

Patients with personality disorders [8] and subjects capable of aggression and violence [2] demonstrate reduced sensitivity to buspirone. Our experiments showed that buspirone was absolutely ineffective in animals with long-term experience of aggression, but reduced aggression in male mice with short-term experience showing in this case an anxiogenic effect, which could be regarded as a negative effect of the drug.

The search for methods of adequate drug correction of manifest aggression is a very important social and clinical problem. We investigated the antiaggressive and anxiolytic effects of diazepam in animals with different aggressive experience in daily intermale confrontations and compared its effects with the effects of buspirone under similar conditions.

MATERIALS AND METHODS

Experiments were carried out on adult (2.5-3-month-old) male C57Bl/6J mice weighing 21-24 g. The animals were kept under standard vivarium conditions (12-h day/night regimen) with free access to water and food (standard granulated fodder). After separation from mothers (at the age of 1 month) the males of each litter were kept together before the experiment.

Males with different experience of aggression in daily agonistic confrontations were selected using the model of sensory contact [9]. Aggressive behavior in these mice was formed after repeated experience of social victories. We used male mice on days 3 (T3 aggressors) and 20 (T20 aggressors) of agonistic confrontations.

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0007-4888/02/1334-0372$27.00

During 5-min testing in an elevated plus-maze (EPM) [12] the following parameters were recorded: number of entries and time spent in open arms, central platform, and closed arms. Additional parameters such as exploratory head dipping from closed arms and the number of transitions between closed arms were also assessed.

For evaluation of aggressive and antagonistic behavior the separating wall was removed and the following forms of behavior were recorded in aggressive males for 10 min: attacks (bites and persecution of the partner), aggressive grooming (long-term biting of the snout, back, and nape of the conquered animal; the victim demonstrates obedient posture during these attacks and freezes under the aggressor), threats (frightening the partner by tail striking against cage floor and walls), throwing around partner's bedding (stirring and tearing of the bedding, nest, and toilet site of the conquered male at his territory), self-grooming (washing and scratching in some cases reflect shifted activity), rotation (rapid sharp turn by 180°). If the animal demonstrated no attack or aggressive grooming, the latency for this form of behavior was taken as 600 sec (duration of the test) and other parameters were taken as zero. The total duration of attacks, aggressive grooming, and throwing of opponent's bedding was used as the index of hostile (harmful for the partner) behavior.

The dose of diazepam not affecting EPM behavior of intact animals was selected in preliminary experiments. The optimal dose was 0.5 mg/kg. One day before testing standard opponents (previously kept in groups) were caged with T3 and T20 aggressors behind the wall. These opponents more intensely stimulated aggressive behavior in aggressive males in comparison with animals with a history of social defeat. On the next day 2.5 h after intraperitoneal injection of diazepam or vehicle (water) aggressor behavior was tested in 10-min antagonistic confrontation. Animal behavior during confrontations was videotaped and the tapes were then processed. In other experiments the effect of diazepam on EPM behavior of T3 and T20 aggressors was studied.

All animals were used only once. Experimental groups consisted of 10-15 animals. The diazepam-treated and control groups and T3 and T20 groups were compared using nonparametric Mann—Whitney *U* test.

RESULTS

The behavior of animals with short- and long-term experience of aggressions in agonistic confrontations was different (Table 1). The number (U=34; p<0.03) and total duration (U=27.5; p<0.02) of attacks was decreased and the time of throwing opponent's bedding was increased (U=36; p<0.05) in T20 aggressors compared to T3 aggressors. Direct attacks demonstrated 100% T3 aggressors and 92% T20 aggressors. Hostile behavior evaluated by the number of attacks, aggressive grooming, and bedding throwing was less pronounced in T20 aggressors (U=30; p<0.02). Rotation was recorded in only T20 aggressors.

Diazepam reduced the number of attacks in T3 aggressors (U=19.5; $p\leq$0.02), duration of self-grooming (U=22; p<0.02), total duration of hostile behavior (U=27; p<0.05) and prolonged the latency of the first attack (U=25.5; p<0.04) compared to T3 aggressors injected with vehicle. The percentage of animals demonstrating threats (tail strikes) decreased from 60% to 36.3% after injection of diazepam.

In T20 aggressors diazepam decreased the total (U=59.5; p<0.05) and mean (U=56; p<0.04) durations of attacks, mean time of throwing opponent's bedding (U=61; p<0.05) and increased latency of the first attack (U=48; p<0.02). Diazepam injection stimulated aggressive grooming: the total (U=68; p<0.05) and mean (U=67; p<0.05) time increased, while its latency decreased (U=65; p<0.04) in comparison with animals injected with vehicle. The total duration of hostile behavior also decreased after injection of diazepam (U=52; p<0.02). The percentage of animals demonstrating direct attacks and tail strikes slightly decreased after diazepam injection (from 92.9 to 66.7 and from 50 to 33.3%, respectively) and the percentage of animals demonstrating aggressive grooming and rotations increased from 14.3 to 46.7 and from 14.2 to 60%, respectively.

The behavior of T3 and T20 aggressors in EPM was virtually the same (Table 2), except head-deeping (5.4±1.1 in T3 and 2.6±0.7 in T20; U=24; p<0.02).

The drug modulated open arm behavior of T3 aggressors: the number of entries (U=9; p<0.001) and the time spent in open arms (U=1; p<0.001) decreased compared to those in control males receiving vehicle (Table 2). The number of closed arm entries increased in the diazepam group (U=10; p<0.001). Other parameters did not differ from the control (Table 2).

In T20 aggressors diazepam increased the number of open arm entries (U=23; p<0.05), the time spent in open arms (U=21; p<0.03) and central platform (U=22; p<0.04) and decreased the number of closed arm entries (U=22; p<0.04) and the time spent in closed arms (U=18.5; p<0.02) in comparison with animals receiving vehicle. Other parameters remained unaffected (Table 2).

In our experiments, similarly to previous studies carried out in humans [3,8] and animals [5,15], diazepam reduced aggression. However our experiments showed specific effects of diazepam on animals with different experience of aggression. The total duration

of hostile behavior was longer in T3 aggressors compared to T20 aggressors. This difference in the number and duration of intensive attacks was most demonstrative. Diazepam produced an antiaggressive effect (which manifested in decreased duration of hostile behavior) in both groups, but in animals with longer experience of aggression this effect was more pronounced. The number of attacks decreased 2-fold and the latency of the first attack was longer in T20 aggressors. This was paralleled by an increase in the number of animals demonstrating aggressive grooming, a ritual behavior demonstrating aggressiveness and superiority over the opponent. The latency of the first manifestation decreased and the duration of demonstration of this form of behavior was prolonged in T20 animals. However the mechanisms underlying the antiaggressive effect of diazepam differ in T3 and T20 aggressors. In T20 aggressors diazepam produced a pronounced anxiolytic effect: it prolonged the time spent in the open arms increased the number of open arm entries, and decreased both these parameters for closed arms. It can be hypothesized that reduced ag-

TABLE 1. Effect of Diazepam on Behavior of Animals with Different Experience of Aggression in Antagonistic Confrontation ($M \pm m$)

Parameter	T3		T20	
	water (n=10)	diazepam (n=11)	water (n=14)	diazepam (n=15)
Attacks				
number of reactions	13.4±2.2	7.6±1.4**	7.1±1.4^{++}	5.1±1.4
duration, sec				
total	185.9±23.4	133.4±20.3	100.7±21.8^{+}	46.9±11.9**
mean	17.4±4.2	18.2±2.2	15.1±2.8	7.1±1.6**
latent	45.9±10.7	99.6±19.1**	106.1±42.4	323.4±63.3**
percentage of animals	100	100	92.9	66.7
Aggressive grooming				
number of reactions	0.1±0.1	0	0.4±0.2	1.1±0.4
duration, sec				
total	7.5±7.5	0	1.9±1.3	12.9±5.2**
mean	7.5±7.5	0	0.8±0.5	8.1±4.7**
latent	582.5±17.5	600±0	569.3±24.9	402.5±57.6**
percentage of animals	10	0	14.3	46.7
Throwing around of partners bedding				
number of reactions	5.6±1.1	7.5±1.2	8.6±1.2	6.2±0.8
duration, sec				
total	29.3±7.1	35.9±7.5	57.1±8.1^{++}	36.9±5.5
mean	4.8±0.7	5.3±1.0	6.7±0.5	5.9±0.4**
percentage of animals	100	100	100	100
Total duration of hostile behavior, sec	222.7±18.1	169.3±17.0**	159.6±23.3^{++}	96.6±9.2**
Autogrooming				
number of reactions	4.5±0.7	2.7±0.6	3.7±0.4	3.1±0.5
duration, sec				
total	31.0±4.7	15.8±3.6**	23.9±5.5	17.7±3.7
mean	8.3±1.5	5.2±1.3	6.6±1.3	6.0±1.1
percentage of animals	100	100	100	100
Threats				
percentage of animals	60	36.3	50	33.3
Rotations				
percentage of animals	0	9	14.2	60

Note. Here and in Table 2: *p<0.001, **p<0.05 compared to water; ^{+}p<0.01, ^{++}p<0.05 compared to T3 aggressors, Mann—Whitney U test.

TABLE 2. Effect of Diazepam on EPM Behavior of Animals with Different Experience of Aggression (*M*±*m*, *n*=10-15)

Parameter	T3		T20	
	water	diazepam	water	diazepam
Total number of entries-exits	34.6±3.0	27.4±1.9	25.8±3.9	30.8±3.1
Number of entries, %				
open arms	12.2±1.3	4.3±1.0*	7.4±1.9	14.5±2.2**
central platform	49.9±0.2	50.0±0.6	49.8±0.3	50.0±0.0
closed arms	37.9±1.3	45.7±1.3*	42.7±2.0	35.5±2.2**
Number of transitions	7.5±1.3	5.7±1.0	4.9±0.9	5.5±1.1
Time spent in, %				
open arms	7.3±1.0	1.2±0.3*	5.6±2.0	14.3±3.1**
center	20.5±2.2	20.3±1.5	20.3±1.9	24.6±1.1**
closed arms	72.2±2.6	78.5±1.6	74.2±3.3	61.1±2.8**
Peeping	4.3±1.2	3.8±0.8	5.3±0.7	5.7±0.2
Head dipping	5.4±1.1	3.8±0.8	2.6±0.7	5.7±1.4

gressiveness of males with long-term aggressive experience is a result of the anxiolytic effect of the drug. The sedative effect can be excluded, because the chosen dose did not modify parameters of motor activity (total number of entries and exits, number of entries from one closed arm to anther, and exploratory head-dipping). On the other hand, antiaggressive effect of diazepam in T3 aggressors was associated with anxiety and fear. EPM test revealed a pronounced anxiogenic effect of diazepam: the preparation shortened the time spent in open arms and number of open arm entries, and had no effects on other parameters. In intact animals injection of diazepam in this dose did not modulate EPM behavior, and therefore, aggressive experience modified the sensitivity of animals to pharmacological effect of the drug. However the direction of the effect seems to depend on the psychoemotional status of animals and the neurochemical background of diazepam effect, and these states are different in animals with short and long aggressive experience. Without discussing in detail this latter assumption, we can assert that aggression during the first confrontations is often uncontrollable and associated with a potent stress reaction. The emotional component is lower in male mice with long aggressive experience, and aggressiveness is largely realized via ritual forms of behavior [10]. Moreover, it was shown that long aggressive experience modifies the state of serotonin, dopamine, and opiate receptors, which results in modification of the reaction to agonists and antagonists of these receptors in comparison with animals with short aggressive experience (T3 aggressors). This indicates modulation of neurochemical activities of these systems under the effect of long aggressive experience.

We recently studied the antiaggressive and antianxious effects of anxiolytic buspirone (5-HT_{1A} receptor agonist) according to an analogous protocol. After two confrontations buspirone, similarly to diazepam, produced pronounced antiaggressive and anxiogenic effects in aggressive animals. On the other hand, in T20 aggressors buspirone did not affect aggressive behavior in antagonistic confrontations or EPM behavior. These results suggest that the efficiency of anxiolytics with different mechanisms of action (in our case buspirone and diazepam) is different in aggressive animals. Both anxiolytics had a negative (anxiogenic) effect in animals with short-term aggressive experience and therefore are not recommended for correcting pronounced aggressiveness against the background of potent stress reaction, which is believed to develop in male mice with short aggressive experience. It is noteworthy that other scientists obtained similar results: benzodiazepines produced an anxiogenic effect under stress conditions [6]. Diazepam more effectively reduced aggressiveness in animals with long aggressive experience, this decrease being observed in the presence of anxiolytic effect of the drug. However it should be noted that diazepam decreased intense attacks, but had no effect on aggressive motivation: aggressive grooming became more intensive and parameters of indirect aggression remained virtually unchanged (throwing partner's bedding, which was regarded as hostile behavior inflicting harm to the partner's "property"). This is also confirmed by an increase in the number of animals demonstrating rotations, which was regarded as behavior intimidating the conquered opponent.

The study was supported by the Russian Foundation for Basic Research (grant No. 00-04-49541) and INTAS YSF grant 01/1-0066.

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