THE EFFECTS OF BUSPIRONE ON THE BEHAVIOUR OF CONTROL AND STRESSED MICE

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The effects of buspirone on the locomotor activity and behaviour in the plus-maze and hole-board tests were studied in control and small platform stressed mice. Small platform stress for 24 hours increased the locomotor activity of mice and induced anxiolytic-like effect in the plus-maze and hole-board tests. Administration of buspirone either did not affect (2.0 and 4.0 mg/kg) or inhibited (8.0 mg/kg) locomotions in control animals. The inhibition of locomotor activity by buspirone was greater in small platform stressed mice. In control mice buspirone in doses 2.0 and 4.0 mg/kg exerted anxiolytic effect in the plus-maze and hole board test that was reflected by an increase in the percentage of entries onto and the percentage of time spent on the open arms of the plus-maze and increased number of head-dippings in the hole-board test. In contrast, in small platform stressed mice, buspirone did not induce anxiolytic action in the plus-maze and hole-board tests at any dose tested. In doses 2.0 and 4.0 mg/kg buspirone produced a sedative effect that was reflected by a decrease in the total number of entries made onto the open and into the closed arms of the plus-maze and a decrease in the number of head-dippings and rearings in the hole-board test. These data suggest that small platform stress induces a sensitization of mice to the motor depressant effect of buspirone. At the same time small platform stress induces hyposensitivity to the anxiolytic effect of buspirone. It is proposed that these changes might be due to alterations in the serotonergic transmission or to changes in the release of corticosterone.

Key words: stress, sleep deprivation, locomotor activity, plus-maze, hole-board, buspirone

INTRODUCTION

In the platform technique or flowerpot technique, animals (usually rats or mice) are forced to stay on a small platform surrounded by water. According to the original idea, depending on the diameter of the platform, animals will be selectively deprived of REM sleep (1, 2).

As the animal enters a REM sleep episode, neck muscle tonus is lost and its head touches the water, thereby waking the animal. During the slow wave sleep
sufficient muscle tonus is maintained, which allows sleep to continue on the platform.

However, considering that besides its specific effect on sleep this technique involves other factors of stress such as isolation, immobilization and falling into the water, this technique must be considered a model of stress where REM sleep deprivation is one factor (3, 4).

In our previous experiments we have shown that small platform stress for 24 hours induces profound behavioural and neurochemical changes in laboratory animals. In our previous works we have shown that exposure of mice to 24 hour small platform stress produces increased locomotions and anxiolytic-like behaviour in the plus-maze test (5—7); and also alterations in the behavioural effects of drugs acting at the $\text{GABA}_A$ receptor-chloride ionophore complex — hyposensitivity to the anxiolytic effects of diazepam and hypersensitivity to the anxiogenic effect of flumazenil (5, 7). In neurochemical experiments an increase in the number of benzodiazepine diazepam-sensitive receptors without changes in their affinity and a reduction in GABA and muscimol-stimulated $^{36}\text{Cl}^-$ uptake are observed (7, 8).

Buspirone is a atypical anxiolytic with a structure and mechanism of action different from classical anxiolytic-sedative drugs. While benzodiazepines, barbiturates and meprobamate exert their action by interacting with $\text{GABA}_A$ receptor-chloride ionophore complex and enhancing GABAergic function (9, 10) buspirone acts as a partial agonist of $5\text{HT}_{1A}$ receptors (11, 12). In man, buspirone is effective in the treatment of anxiety disorders (13, 14), reducing aggressive behaviour in developmentally retarded patients (15) and in the treatment of depression (16).

However, the data about the action of buspirone in the laboratory animals are controversial. The most inconsistent effects of buspirone have been observed in the plus-maze test. In the plus-maze test buspirone has produced: 1. anxiolytic effect (17—20), 2. no effect at all (21) 3. or even anxiogenic effect (22—24). Controversial results can be explained with differences in used doses, animal species (mice vs. rats) and administration regimen (acute vs. chronic administration).

Buspirone has anxiolytic effect in conflict situations (25), in social competition test (26), in light-dark compartment transition test (27) and two-compartment black and white test box (28). In an extensive study of Matto et al. (29) buspirone in dosage range from 0.04 to 5.0 mg/kg did not change the behaviour of rats in the elevated zero-maze.

The main aim of our work was to study whether the effects of atypic anxiolytic buspirone are changed in small platform stressed mice.

The second aim of our work was to investigate whether the small platform stress has impact on the behaviour of mice in the hole-board test.
MATERIALS AND METHODS

Animals

Naive male albino mice (NMRI strain, Grindex Breeding Center, Riga, Latvia) weighting 25—35 grams were used throughout the study. Mice were maintained at 20±2°C; water and standard laboratory food were available ad libitum. Mice were housed 20 per cage and exposed to a 12/12 hour light/dark cycle. Lights were on from 7.00 a.m. to 7.00 p.m. All experiments were carried out between 10.00 a.m. and 2.00 p.m.

Drugs

Buspirone was suspended in saline with a drop of Tween-80. Buspirone was administered intraperitoneally (i.p.) at the end of stress exposure and behavioural testing was carried out 30 minutes later. Injection volume was adjusted with saline to 0.1 ml per 10 grams of body weight in all experiments. Control animals were given vehicle (saline with a drop of Tween-80) 30 minutes before behavioural testing.

The small platform technique

The following groups of mice were used.

Group I — naive control, animals were kept grouped in their home cages for 24 hours.

Group II — small platform-induced stress. Animals were exposed to the small platform according to the method described previously (30) with some modifications. Mice were placed individually for 24 hours on the small platform (3 cm high, 3.5 cm in diameter) which was fixed at the center of a plastic chamber (20 cm diameter, 40 cm high) and surrounded by water (1 cm deep) at 22°C. When drug or vehicle injections were carried out mice were returned to the platforms until behavioural testing. Behavioural experiments were carried out at the end of 24-hour stress presentation.

To eliminate the effect of stress due to handling each animal was handled twice a day for seven days before the start of experiments.

BEHAVIOURAL METHODS

1. Measurement of the locomotor activity

Locomotor activity was measured in an actometer constructed at the Tartu University workshop. The actometer consisted of 16 individual cylindrical cages with an inner diameter of 40 cm. Each cage was equipped with 2 photocells located in the walls 1.5 cm above the floor. The number of the beam interruptions made by animal (counts) was registered by electronical counter. Animals were placed individually in the cages and the locomotor activity was measured during 60 minutes.

2. Plus-maze test

The plus-maze test was carried out according to File et al. (31). The plus-maze consisted of two open (8 × 17 cm) and two closed arms (8 × 17 × 30 cm), which were connected by a central platform (8 × 8 cm). Mice were placed on the central platform facing an open arm. During 5 minutes the
number of entries made onto the open and into the closed arms and the time spent on the open and in the closed arms were measured. On the basis of these data the percentage of entries made onto the open arms and the percentage of time spent on the open arms were calculated.

3. The hole-board test

The hole-board test was carried out according to the method of Durcan and Lister (32), slightly modified in our laboratory. The hole-board was made of plexiglas (30 x 30 x 18 cm) and had 16 holes (d = 1.4 cm) in the floor. During 5 minutes the number of head-dippings and the number of rearings made by mice were recorded.

Statistical analysis

The data concerning locomotor activity and the behaviour of animals in the plus-maze and hole-board tests were analyzed using two-way analysis of variance (ANOVA), where stress and drug treatment were used as categorical factors. Post-hoc statistical analysis was made by Bonferroni test.

RESULTS

The effect buspirone on the locomotor activity of control and small platform stressed mice.

After 24 hour small platform stress exposure the animals did not fall asleep as it could be expected. In contrast, an increase of locomotor activity was observed (Table 1). Increase in locomotor activity lasted for about 60 minutes and then returned to control level.

Table 1. The effect of buspirone on the locomotor activity of control and small platform stressed (SP) mice.

<table>
<thead>
<tr>
<th>Drug, dose (mg/kg)</th>
<th>Control (counts during 60 minutes)</th>
<th>SP (counts during 60 minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>471.8 ± 32.0 (%)</td>
<td>669.1 ± 64.9** (100)</td>
</tr>
<tr>
<td>Buspirone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>357.3 ± 87.8 (%)</td>
<td>140.8 ± 18.9** (75.7)</td>
</tr>
<tr>
<td>4.0</td>
<td>353.4 ± 74.1 (%)</td>
<td>159.5 ± 65.6** (74.9)</td>
</tr>
<tr>
<td>8.0</td>
<td>219.6 ± 39.7** (%)</td>
<td>66.7 ± 25.9** (46.5)</td>
</tr>
</tbody>
</table>

The total number of beam interruptions in the actometer during 60 minutes. Values are mean ±SEM from groups of 8 mice.

**p < 0.01 vs. control/vehicle;
***p < 0.01 vs. small platform stressed/vehicle (Bonferroni test).
In control mice buspirone in doses 2.0 and 4.0 mg/kg did not statistically significantly affect the locomotor activity and in a dose 8.0 mg/kg significantly decreased the locomotor activity (Table 1).

Buspirone counteracted the enhancement of locomotor activity caused by the small platform stress exposure. In small platform stressed mice buspirone significantly decreased the locomotor activity of mice in all doses used (Table 1). Two-way ANOVA revealed significantly stronger inhibitory action of buspirone on the locomotor activity of small platform stressed mice (F(3,57) = 6.237; p<0.01).

The effect buspirone in the plus-maze test

Small platform stress in vehicle-treated mice significantly increased the percentage of entries made onto the open arms, the percentage of time spent on the open arms and total number of entries made onto the open and into the closed arms (Fig. 1A, 1B, 1C).

In control mice buspirone dose-dependently increased the percentage of entries made onto and the percentage of time spent on the open arms, this effect was statistically significant at a dose 4.0 mg/kg (Fig. 1A, 1B). Buspirone did not affect the total number of entries in control mice in any dose used (Fig. 1C).

By contrast, in small platform stressed mice buspirone had no effect on the percentage of entries made onto and the percentage of time spent on the open arms. However, in all doses used buspirone decreased the total number of entries made onto the open and into the closed arms of the plus-maze (Fig. 1A, 1B, 1C).

The effect buspirone in the hole-board test

In the hole-board test small platform stress significantly increased the number of head-dippings (Table 2) (F(1,106) = 4.570; p<0.01) and rearings (F(1,106) = 32.728; p<0.01). In control mice buspirone dose-dependently increased the number of head-dippings, this effect was statistically significant with doses 2.0 and 4.0 mg/kg. Buspirone did not affect the number of rearings in control mice in any dose used.

By contrast, in small platform stressed mice buspirone in all doses used decreased both the number of head-dippings and the number of rearings (Table 2).
Fig. 1A, 1B and 1C.
The effect of buspirone on the behaviour of control and small platform stressed mice in the plus-maze test.
A: The % of entries made onto the open arms.
B: The % of time spent on the open arms.
C: The total number of entries. Values are mean ±SEM from groups of 24 mice.
*p<0.05, **p<0.01 vs. control: vehicle; ++p<0.01 vs. small platform stressed/vehicle (Bonferroni test).
Table 2. The effect of buspirone on the behaviour of control and small platform stressed (SP) mice in the hole-board test.

<table>
<thead>
<tr>
<th>Drug, dose (mg/kg)</th>
<th>Head-dippings</th>
<th>Rearings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>SP</td>
</tr>
<tr>
<td>Vehicle</td>
<td>13.77 ± 0.76</td>
<td>21.58 ± 0.79**</td>
</tr>
<tr>
<td>Buspirone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>15.14 ± 0.51</td>
<td>19.86 ± 0.77</td>
</tr>
<tr>
<td>2.0</td>
<td>16.57 ± 0.81*</td>
<td>16.75 ± 2.22**</td>
</tr>
<tr>
<td>4.0</td>
<td>20.23 ± 1.51**</td>
<td>15.09 ± 0.62**</td>
</tr>
</tbody>
</table>

The number of head-dippings and rearings in the hole-board test. Values are mean ±SEM from groups of 10 mice.

*p<0.05, **p<0.01 vs. control/vehicle;
"*p<0.01 vs. small platform stress/vehicle (Bonferroni test).

DISCUSSION

As in our previous works small platform stress caused significant increase in the locomotor activity of mice. Buspirone in doses of 2.0 and 4.0 mg/kg had no effect and in a dose of 8.0 mg/kg decreased the locomotor activity of control mice. These data are in line with the data of Lee and Rodgers (19) who got significant suppression of locomotor activity with a dose of 10 mg/kg but not with doses 0.1 and 1.0 mg/kg. Buspirone, when given to small platform stressed mice, produced stronger inhibition of locomotor activity as compared to control animals. These data show sensitization of mice to the motor depressant effect of buspirone.

Small platform stress induced an increase in the exploratory activity of mice in the plus-maze test, as evidenced by an increase in the percentage of entries made onto and the percentage of time spent on the open arms. Since the validation of plus-maze test in rats (33) and mice (34) it has been repeatedly shown that anxiolytic drugs increase the percentage of entries made onto and the percentage of time spent on the open arms and anxiogenic drugs decrease these measures (35, 36). The anxiolytic-like effect of small platform stress can be explained with hypothesis expressed in the literature that "boldness" in the face of aversive cues would have survival value (37). In control mice buspirone produced anxiolytic action in the plus-maze test as it was evidenced by an increase in the percentage of entries made onto and the percentage of time spent on the open arms. This effect was evident at a dose 4.0 mg/kg, whereas with lower doses of buspirone (1.0 and 2.0 mg/kg) this effect was not significant. These data are consistent with data in the literature
showing buspirone's anxiolytic effect in the plus-maze test (17—20). Buspirone in doses 1.0, 2.0 and 4.0 mg/kg did not change the total number of entries made by control mice in the plus-maze test.

In small platform stressed mice buspirone had no effect on percentage of entries made onto the open arms or on the percentage of time spent on the open arms. In small platform stressed mice buspirone in all doses used significantly decreased the total number of entries. This might reflect buspirone's sedative action, which is more pronounced in small platform stressed mice.

One explanation to the absence of buspirone's anxiolytic effect in stressed mice is so called ceiling effect. However, since the percentage of time spent on the open arms and the percentage of entries made onto the open arms by the small platform stressed and vehicle treated mice were not extremely high (9.5 ± 1.1% and 18.5 ± 1.7%, respectively), this explanation is not probable.

On the basis of plus-maze data we can say that small platform stress induces hyposensitivity to the anxiolytic and hypersensitivity to the sedative effect of buspirone in the plus-maze test.

The assumption that small platform stress also induced hyposensitivity to the anxiolytic effect of buspirone is supported by the fact that the anxiolytic effect of buspirone was also absent in the hole-board test.

In the hole-board test small platform stress significantly increased the number of head-dippings and the number ofrearings. Since the classical works of File and Wardwill (38) and Lister (39) a number of drugs have been tested in the hole-board. It has been demonstrated that exploratory activity directed at the holes (head-dipping) is dissociated from the general activity in the hole-board — ambulation and rearing (40). Anxiolytic drugs increase, whereas anxiogenic drugs and stress decrease exploratory head-dipping in the hole-board test (41, 42). Therefore it could be proposed that small platform stress results in the reduction of anxiety as well as in the increase of locomotor activity in the hole-board test.

In the hole-board test buspirone in doses 2.0 and 4.0 mg/kg dose dependently increased the number of head-dippings in control mice that shows its anxiolytic effect. In small platform stressed mice buspirone in doses 2.0 and 4.0 mg/kg decreased both the number of head-dippings and the number of rearings. These data reflect buspirone's sedative effect.

There are two possible explanations of this hyposensitivity to the anxiolytic effect of buspirone in the plus-maze and hole-board tests.

First of all this can be caused by changes in the activity of serotonergic system. This hypothesis is supported by the fact that the activity of serotoninergic system is changed after small platform stress (43, 44).

Hyposensitivity to the anxiolytic effect of buspirone can also be explained with its effect on pituitary-adrenal axis. Buspirone, in doses similar to those
used in the study, has been shown to increase plasma corticosterone levels (45). It has also been shown that while benzodiazepines attenuate stress-induced alterations in corticosterone concentrations, buspirone is not effective in attenuating the stress-elicited rise of corticosterone (46, 47). Also, there are data in the literature, indicating that buspirone may have its anxiolytic action reduced through its release of corticosterone (48). Therefore it is possible that the summation of the effects of buspirone and stress on corticosterone release can totally counteract the anxiolytic effect of buspirone.

In conclusion, we can propose that small platform stress induces a hyposensitivity of mice to the anxiolytic and hypersensitivity to the sedative effect of buspirone in the plus-maze and hole-board test.

We can also conclude that small platform stress induces an anxiolytic effect in the hole-board test.

Buspirone generally is thought to cause markedly less sedation than benzodiazepines (49) or even no sedation at all (50), however on the basis of our data we can also propose that in certain circumstances (stress, sleep deprivation) its anxiolytic effect can be diminished and sedative effect more pronounced.

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REFERENCES


35. Dawson GR, Crawford SP, Collinson N, Iversen SD, Tricklebank MD. Evidence that the anxiolytic-like effects of chlordiazepoxide on the elevated plus maze are confounded by increases in locomotor activity. *Psychopharmacology* 1995; 118: 316—322.


