Short communication

Nefazodone attenuates the stress-induced facilitation of wet dog shaking behaviour but not the facilitation of sexual behaviour in female rats

Lori A. Brotto, Laura A. Hanson, Boris B. Gorzalka * 
Department of Psychology, The University of British Columbia, Vancouver, BC, Canada V6T 1Z4

Received 28 July 1999; accepted 3 August 1999

Abstract

The effects of chronic stress both alone and in combination with the antidepressant, nefazodone, which possesses antagonistic activity at the 5-HT3A receptor, were examined on the 5-HT2A receptor-mediated behaviour, wet dog shaking and sexual behaviour. Ovariectomized female rats received either a chronic stressor or no stress for 30 days, and half of each group received concurrent nefazodone treatment (100 mg/kg/day). Following treatment with either estrogen, or estrogen combined with progesterone, sexual behaviour and wet dog shaking were recorded. Chronic stress alone was found to facilitate sexual behaviour and increase wet dog shaking, while nefazodone administration alone was without effect. Furthermore, nefazodone completely attenuated the stress-induced facilitation of wet dog shaking, but not sexual behaviour. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Nefazodone; Chronic stress; 5-HT, Serotonin; 5-HT3A receptor; Sexual behaviour; Lordosis

1. Introduction

Recent data have demonstrated that chronic stress exposure significantly increases 5-HT2A receptor density in the parietal cortex (McKittrick et al., 1995) as well as increases the display of behaviours that are mediated by the 5-HT2A receptor. For example, it has been demonstrated that chronic stress increases both wet dog shaking behaviour and female sexual behaviour in rats (Gorzalka et al., 1998).

Wet dog shaking behaviour in rats consists of a shuddering movement of the head, neck and trunk (Bedard and Pycock, 1977) and appears to be mediated primarily by activity at the 5-HT2A receptor (Schreiber et al., 1995). In previous studies, wet dog shaking has been used as a behavioural assay of 5-HT2A receptor activity in vivo (Watson and Gorzalka, 1990; Essman et al., 1994). Sexual behaviour in the female rat appears to be mediated by activity at the 5-HT2A receptor (Wilson and Hunter, 1985) while antagonists decrease (Mendelson and Gorzalka, 1985, 1986) sexual receptivity.

It has been suggested that the effects of stress on both wet dog shaking and female sexual behaviour are mediated by alterations in 5-HT2A receptor activity (Gorzalka et al., 1998). Moreover, it has been suggested that stress induces these changes in 5-HT2A receptor-mediated behaviours by increasing plasma levels of corticosterone (McKittrick et al., 1995; Gorzalka et al., 1998). This suggestion is based on evidence that chronic administration of high doses of corticosterone increases 5-HT2A receptor density (Kuroda et al., 1992; Takao et al., 1997), increases wet dog shaking behaviour (Kuroda et al., 1992; Berendsen et al., 1996; Takao et al., 1997; Hanson et al., 1998), and increases female sexual behaviour (Hanson et al., 1998). Additionally, we have demonstrated that the facilitation of both wet dog shaking and female sexual behaviour by corticosterone is attenuated by the antidepressant, nefazodone (Hanson et al., 1998) which exhibits antagonistic activity at the 5-HT2A receptor and inhibits serotonergic reuptake (Taylor et al., 1995).

It remains to be determined whether both chronic stressors and chronic corticosterone treatment exert their facilitatory effects on wet dog shaking and sexual behaviour through a common mechanism. In the current study, we will attempt to attenuate the behavioural effects of stress through the chronic administration of nefazodone.
2. Materials and methods

2.1. Subjects

Forty female and 20 male Long–Evans rats were obtained from Charles River, Montreal, Canada. All females were bilaterally ovariectomized, at 3 months of age, using standard surgical procedures while anesthetized with 75 mg/kg ketamine HCl and 7 mg/kg xylazine. Females were 6 months of age at testing. At this time, males were screened for copulatory proficiency, and those displaying robust copulatory behaviour were chosen to serve as stud males for the testing of the female subjects. All rats were housed in groups of three or four, by sex, in triple wire mesh cages in a colony kept at 21°C ± 1°C. Colony lights were set on a 12 h dark/12 h light cycle with lights off at 0900 h, and animals were provided with Purina Rat Chow and tap water, ad libitum.

2.2. Stress and injection procedure

Female subjects were randomly assigned to one of four treatment groups: no stress and saline; no stress and 100 mg/kg nefazodone; stress and saline; or stress and 100 mg/kg nefazodone. This dose of nefazodone was chosen as it has been previously shown to decrease cortical 5-HT2A receptor site density (Eison et al., 1990). Females assigned to the stress group (n = 20), were subjected to 30 min of stress exposure per day for 30 days. In brief, the stress procedure consisted of crowding females into a wooden arena (118 cm × 118 cm × 30 cm in height) covered with San-i-cel, while simultaneously exposing them to strobe lighting and white noise (100 ± 5 dB) in a dark room (Williams et al., 1992). Females in the no-stress group (n = 20) were removed from their cages and subsequently returned, without exposure to the stress regimen.

Nefazodone (Pfizer, Groton, USA) was dissolved in 0.9% saline (50 mg/ml) and was injected subcutaneously (2 ml/kg, s.c.) for 30 days in half of the females from each of the stress and no-stress conditions. The remaining females received an injection of the equivalent volume of 0.9% saline. All injections were performed either immediately following stress administration, or after removal from the home cage.

2.3. Behavioural testing procedure

2.3.1. Experiment 1A

On days 23, 28, and 29, all female subjects were injected s.c. with 0.8 μg estradiol benzoate dissolved in 0.1 cm³ peanut oil.

After habituating to the Plexiglas testing chambers (30 cm × 30 cm × 45 cm in height) for 5 min, male rats were exposed to non-experimental, receptive females for a few minutes. These females were then removed, and replaced by a female subject, at which point data collection began.

Receptivity was measured using the lordosis quotient: the proportion of full lordoses displayed in response to 10 mounts with full pelvic thrusting by a male. Proceptive behaviour was calculated as the frequency of solicitations (ear wiggling and darting). The frequency of wet dog shaking and sexual rejection was also recorded. Any overt defensive display of behaviour (including kicking, boxing, and rolling onto back) was scored as a rejection response. Solicitations, rejection, and wet dog shaking were divided by the total test time to obtain a score per minute. Data collection terminated once the female received 10 mounts by a male rat.

Testing was carried out in the middle third of the dark cycle by trained observers who were blind to the experimental conditions of the animals. Data were analyzed using a two-way analysis of variance (ANOVA) with a significance criterion set at P < 0.05.

2.3.2. Experiment 1B

Immediately following testing in Experiment 1A, females were injected with 50 μg progesterone (s.c.) and tested 3–4 h later. Receptivity, proceptivity, rejection, and wet dog shaking were scored as in Experiment 1A. One female was excluded from Experiment 1B due to illness.

3. Results

3.1. Experiment 1A

The means and standard errors for receptivity, proceptivity and wet dog shaking are presented in Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Receptivity</th>
<th>Proceptivity</th>
<th>Wet dog shaking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline–no stress</td>
<td>7.00 ± 3.35</td>
<td>0.13 ± 0.07</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>Nefazodone–no stress</td>
<td>8.00 ± 5.54</td>
<td>0.26 ± 0.15</td>
<td>0.11 ± 0.05</td>
</tr>
<tr>
<td>Saline–stress</td>
<td>7.00 ± 7.42</td>
<td>0.98 ± 0.20</td>
<td>0.65 ± 0.07</td>
</tr>
<tr>
<td>Nefazodone–stress</td>
<td>48.00 ± 8.41</td>
<td>1.57 ± 0.32</td>
<td>0.14 ± 0.06</td>
</tr>
</tbody>
</table>

There was a main effect of stress, F(1,36) = 41.5, P < 0.001; a main effect of nefazodone treatment, F(1,36) = 15.0, P < 0.001; and a significant interaction between the two, F(1,36) = 35.3, P < 0.001 on wet dog shaking. Chronic stress increased the display of wet dog shaking and this was completely attenuated by treatment with nefazodone. Females treated with nefazodone alone did not differ on the display of wet dog shaking from control females.

For receptive behaviour, analyses of main effects indicate that stress significantly increased the lordosis quotient, F(1,36) = 65.8, P < 0.001, and nefazodone treatment did...
not significantly reduce the level of receptivity, $F(1,36) = 3.2, P = 0.084$. There was no stress by drug interaction on receptivity, although it approached statistical significance, $F(1,36) = 3.7, P = 0.061$.

Chronic stress significantly increased proceptive behaviour, $F(1,36) = 27.1, P < 0.001$. The administration of nefazodone, alone, had no effect on proceptivity. In addition, statistical analysis did not reveal a significant interaction between stress and nefazodone treatment on proceptive behaviour.

There were no significant differences between groups in the frequency of sexual rejections.

### 3.2. Experiment 1B

The means and standard errors for receptivity, proceptivity, rejection and wet dog shaking are presented in Table 2.

As in Experiment 1A, there was a significant main effect of stress, $F(1,35) = 17.2, P < 0.001$; a significant main effect of nefazodone treatment, $F(1,35) = 9.2, P < 0.01$; and a significant interaction between the two, $F(1,35) = 13.6, P < 0.001$ for wet dog shaking. The stress-induced increase in wet dog shaking was completely blocked by nefazodone treatment but nefazodone alone had no effect on the display of wet dog shaking.

There was a significant main effect of stress on the lordosis quotient, $F(1,35) = 75.4, P < 0.001$, such that stressed females displayed higher lordosis quotient scores than non-stressed females. Nefazodone administration alone had no effect on receptivity. Statistical analysis revealed no significant interaction between stress and nefazodone treatment on receptivity.

Results for proceptivity were similar to results obtained for receptivity. There was a significant main effect of stress with stressed females showing significantly more proceptive behaviour than non-stressed females, $F(1,35) = 27.9, P < 0.001$, and nefazodone treatment alone having no effect. There was no significant interaction between stress and nefazodone treatment.

There was a main effect of stress on rejection behaviour. Statistical analysis confirmed that stressed females displayed lower rejection behaviour (mean 0.19, S.E.M. 0.09) than non-stressed females (mean 0.93, S.E.M. 0.17), $F(1,35) = 14.6, P < 0.001$. Nefazodone administration did not alter the display of rejection behaviour nor was there a significant interaction between stress and nefazodone treatment.

### 4. Discussion

The present study demonstrated that nefazodone completely attenuated the stress-induced facilitation of wet dog shaking but failed to prevent the increase in sexual behaviour. Since nefazodone has been demonstrated to block the corticosterone-induced facilitation of sexual behaviour (Hanson et al., 1998), it is unlikely, as previously proposed, that the stress-induced facilitation of sexual activity is mediated exclusively by either elevations in corticosterone or alterations at the 5-HT$_{2A}$ receptor. However, the near-significant ability of nefazodone to attenuate stress-induced receptivity suggests that the effects of stress on receptivity may be regulated by 5-HT$_{2A}$ receptor activity, and that an increase in sample size would help clarify this possibility.

It remains to be determined how nefazodone attenuates the stress-induced facilitation of wet dog shaking but not the facilitation of sexual behaviour. Apparently, the effects of stress on female sexual behaviour and wet dog shaking are mediated by different mechanisms. Chronic stress is known to produce an elevation in all adrenal hormones (Nelson, 1980), including corticosterone. For example, estrogens, progestins, and androgens, which are released by the adrenal cortex, may be influencing sexual behaviour without stimulating 5-HT$_{2A}$ receptor activity. Also, the mineralocorticoid, deoxycorticosterone, has been shown to increase receptivity (Gorzalka and Whalen, 1977). Furthermore, stress enhances β-endorphin release which has also been demonstrated to facilitate receptivity at high doses (Pfaus and Gorzalka, 1987). Therefore, if other adrenal or non-adrenal hormones are contributing to the increase in sexual behaviour, and their effect does not involve 5-HT$_{2A}$ receptor activity, then it follows that nefazodone, which specifically antagonizes the 5-HT$_{2A}$ receptor and inhibits serotonergic reuptake, would not attenuate those effects.

The present findings are consistent with previous evidence that chronic stress increases sexual behaviour and wet dog shaking in female rats treated with estrogen and progesterone. However, in contrast to previous studies (Williams et al., 1992; Gorzalka et al., 1998), stress facilitated sexual behaviour in animals treated with estrogen alone. This is likely due to differing modes of estrogen administration. In the present study, estrogen was administered subchronically whereas in previous studies, it was given acutely.

Overall, the results replicate previous findings that chronic stress increases sexual behaviour and wet dog shaking in the female rat (Gorzalka et al., 1998). The

### Table 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Receptivity</th>
<th>Proceptivity</th>
<th>Wet dog shaking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline–no stress</td>
<td>13.33 ± 11.06</td>
<td>0.04 ± 0.03</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Nefazodone–no stress</td>
<td>32.00 ± 12.63</td>
<td>0.21 ± 0.12</td>
<td>0.09 ± 0.06</td>
</tr>
<tr>
<td>Saline–stress</td>
<td>96.00 ± 4.00</td>
<td>2.69 ± 0.58</td>
<td>1.03 ± 0.24</td>
</tr>
<tr>
<td>Nefazodone–stress</td>
<td>98.00 ± 2.00</td>
<td>2.53 ± 0.70</td>
<td>0.15 ± 0.05</td>
</tr>
</tbody>
</table>
current observations are the first to show that nefazodone completely attenuates the stress-induced increase in wet dog shaking. This is consistent with the 5-HT\textsubscript{2A} antagonistic properties of nefazodone. The ability of nefazodone to block the corticosterone-induced (Hanson et al., 1998), but not the stress-induced facilitation of sexual activity indicates that further research is necessary to elucidate the mechanism by which chronic stress facilitates female sexual behaviour.

Acknowledgements

This research was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) grant to B.B. Gorzalka. L.A.B. is the recipient of a NSERC Predoctoral Fellowship and L.A.H. is the recipient of an Izaak Killam Memorial Predoctoral Fellowship.

References


