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Short communication

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

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Abstract

The effects of chronic stress both alone and in combination with the antidepressant, nefazodone, which possesses antagonistic activity at the 5-HT_{2A} receptor, were examined on the 5-HT_{2A} 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.

Keywords: Nefazodone; Chronic stress; 5-HT, Serotonin; 5-HT_{2A} receptor; Sexual behaviour; Lordosis

1. Introduction

Recent data have demonstrated that chronic stress exposure significantly increases 5-HT_{2A} receptor density in the parietal cortex (McKittrick et al., 1995) as well as increases the display of behaviours that are mediated by the 5-HT_{2A} 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-HT_{2A} receptor (Schreiber et al., 1995). In previous studies, wet dog shaking has been used as a behavioural assay of 5-HT_{2A} 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-HT_{2A} receptor in that 5-HT_{2A} receptor agonists increase (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-HT_{2A} receptor activity (Gorzalka et al., 1998). Moreover, it has been suggested that stress induces these changes in 5-HT_{2A} 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-HT_{2A} 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-HT_{2A} receptor and inhibits serotoninergic 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.

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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 $21° \pm 1°C$. 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-HT_{2A} 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 cm^3 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.

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

Table 1
Effects of stress and nefazodone on receptivity (lordosis quotient), proceptivity (solicitations per minute) and wet dog shaking in ovariectomized rats treated with a subchronic level of estrogen
Values represent means ± S.E.M.s.

	Receptivity	Proceptivity	Wet dog shaking
Saline–no stress	7.00 ± 3.35	0.13 ± 0.07	0.01 ± 0.01
Nefazodone–no stress	8.00 ± 5.54	0.26 ± 0.15	0.11 ± 0.05
Saline–stress	72.00 ± 7.42	0.98 ± 0.20	0.65 ± 0.07
Nefazodone–stress	48.00 ± 8.41	1.57 ± 0.32	0.14 ± 0.06

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.

Table 2
Effects of stress and nefazodone on receptivity (lordosis quotient), proceptivity (solicitations per minute), sexual rejection and wet dog shaking in ovariectomized rats treated with a subchronic level of estrogen and progesterone
Values represent means ± S.E.M.s.

	Receptivity	Proceptivity	Wet dog shaking
Saline–no stress	13.33 ± 11.06	0.04 ± 0.03	0.00 ± 0.00
Nefazodone–no stress	32.00 ± 12.63	0.21 ± 0.12	0.09 ± 0.06
Saline–stress	96.00 ± 4.00	2.69 ± 0.58	1.03 ± 0.24
Nefazodone–stress	98.00 ± 2.00	2.53 ± 0.70	0.15 ± 0.05

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 serotoninergic 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

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_{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.

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References

Bedard, P., Pycock, C.J., 1977. Wet-dog shaking behaviour in the rat: a possible quantitative model of central 5-hydroxytryptamine activity. Neuropharmacology 16, 663–670.

Berendsen, H.H.G., Kester, R.C.H., Peeters, B.W.M.M., 1996. Modulation of 5-HT receptor subtype-mediated behaviours by corticosterone. Eur. J. Pharmacol. 308, 103–111.

Eison, A.S., Eison, M.S., Torrente, D.P., Wright, R.N., Yocca, F.D., 1990. Nefazodone: preclinical pharmacology of a new antidepressant. Psychopharmacol. Bull. 26, 311–315.

Essman, W.D., Singh, A., Lucki, I., 1994. Serotoninergic properties of cocaine: effects on a 5-HT_2 receptor-mediated behavior and on extracellular concentrations of serotonin and dopamine. Pharmacol. Biochem. Behav. 49, 107–113.

Gorzalka, G.G., Whalen, R.E., 1977. The effects of progestins, mineralocorticoids, glucocorticoids, and steroid solubility on the induction of sexual receptivity in rats. Horm. Behav. 8, 94–99.

Gorzalka, B.B., Hanson, L.A., Brotto, L.A., 1998. Chronic stress effects on sexual behavior in male and female rats: mediation by 5-HT_{2A} receptors. Pharmacol. Biochem. Behav. 61, 405–412.

Hanson, L.A., Gorzalka, B.B., Brotto, L.A., 1998. The antidepressant, nefazodone, attenuates corticosterone-induced increases in 5-HT_{2A}-mediated behaviours in the female rat. Eur. J. Pharmacol. 342, 163–165.

Kuroda, Y., Mikuni, M., Ogawa, T., Takahashi, K., 1992. Effect of ACTH, adrenalectomy and the combination treatment on the density of 5-HT_2 receptor binding sites in neocortex of rat forebrain and 5-HT_2 receptor-mediated wet-dog shake behaviors. Psychopharmacology 108, 27–32.

McKittrick, C.R., Blanchard, D.C., Blanchard, R.J., McEwen, B.S., Sakai, R.R., 1995. Serotonin receptor binding in a colony model of chronic social stress. Biol. Psychiatry 37, 383–393.

Mendelson, S.D., Gorzalka, B.B., 1985. A facilitatory role for serotonin in the sexual behavior of the female rat. Pharmacol. Biochem. Behav. 22, 1025–1033.

Mendelson, S.D., Gorzalka, B.B., 1986. Serotonin type 2 antagonists inhibit lordosis behavior in the female rat: reversal with quipazine. Life Sci. 38, 33–39.

Nelson, D., 1980. The Adrenal Cortex: Physiological Function and Disease. Saunders, Philadelphia.

Pfaus, J.G., Gorzalka, B.B., 1987. Opioids and sexual behavior. Neurosci. Biobehav. Rev. 11, 1–34.

Schreiber, R., Brocco, M., Audinot, V., Gobert, V., Veiga, S., Millan, M.J., 1995. 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane-induced head-twitches in the rat are mediated by 5-hydroxytryptamine (5-HT)$_{2A}$ receptors: modulation by novel 5-$HT_{2A/2C}$ antagonists, D1 antagonists, and 5-HT_{1A} agonists. J. Pharmacol. Exp. Ther. 273, 101–112.

Takao, K., Nagatani, T., Kitamura, Y., Yamawaki, S., 1997. Effects of corticosterone on 5-HT_{1A} and 5-HT_2 receptor binding and on the receptor-mediated behavioural responses of rats. Eur. J. Pharmacol. 333, 123–128.

Taylor, D.P., Carter, R.B., Eison, A.S., Mullins, U.L., Smith, H.L., Torrente, D.P., Wright, R.N., Yocca, F.D., 1995. Pharmacology and neurochemistry of nefazodone, a novel antidepressant drug. J. Clin. Psychiatry 56, 3–11.

Watson, N.V., Gorzalka, B.B., 1990. Relation of spontaneous wet dog shakes and copulatory behavior in male rats. Pharmacol. Biochem. Behav. 37, 825–829.

Williams, G.W., McGinnis, M.Y., Lumia, A.R., 1992. The effects of olfactory bulbectomy and chronic psychosocial stress on serum glucocorticoids and sexual behavior in female rats. Physiol. Behav. 52, 755–760.

Wilson, C.A., Hunter, A.J., 1985. Progesterone stimulates sexual behaviour in female rats by increasing 5-HT activity on 5-HT_2 receptors. Brain Res. 333, 223–229.