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Corticosterone Regulation of 5-HT_{2A} Receptor-Mediated Behaviors: Attenuation by Melatonin

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GORZALKA, B. B., L. A. BROTTO AND J. J. HONG. *Corticosterone regulation of 5-HT_{2A} receptor-mediated behaviors: Attenuation by melatonin*. PHYSIOL BEHAV **67**(3) 439–442, 1999.—The effects of chronic corticosterone treatment on sexual behavior and on wet-dog shakes (WDS), a serotonergic type 2A (5-HT_{2A}) receptor-mediated behavior, were explored in the male rat. In addition, the effects of acute melatonin treatment, both alone and in combination with corticosterone, were investigated. Chronic injections of corticosterone resulted in an overall decrease in consummatory measures of sexual behavior, and an increase in WDS. Furthermore, although an acute injection of melatonin alone had no effect on any recorded behavior, it attenuated the effects of corticosterone on sexual behavior and WDS. The data suggest that in the context of 5-HT_{2A} receptor-mediated behaviors, melatonin has possible implications as a 5-HT_{2A} antagonist. © 1999 Elsevier Science Inc.

Melatonin Corticosterone Serotonin 5-HT_{2A} receptor Sexual behavior

IN the male rat, serotonergic type 2A (5-HT_{2A}) receptor activity has been reliably found to modulate an inhibition of sexual behavior (15). Furthermore, the frequently employed behavior known as "wet-dog shakes" (WDS) has been found to be largely mediated by 5-HT_{2A} activity, with activation of the 5-HT_{2A} receptor producing a robust facilitation of WDS in the rat (29). WDS, which consist of a rotational shudder of the upper body (2), resemble the purposeful movement, as seen in dogs, and have reliably been used as a behavioral assay of 5-HT_{2A} receptor activity (29). A strong, inverse correlation exists between male sexual behavior and WDS, such that activation of the 5-HT_{2A} receptor produces an increase in WDS, and a concomitant decrease in sexual behavior (27). As a result, spontaneously occurring WDS have been frequently employed as a noninvasive index of 5-HT_{2A} activity during sexual behavior.

Unique to the 5-HT_{2A} receptor is its "nonclassical" mechanism of regulation (24). The receptor's relative resistance to upregulation in response to serotonergic manipulation has triggered an interest in nonserotonergic methods of its regulation. For example, the adrenal hormone, corticosterone, has been explored in this context, and has been found to exert some regulatory effects on the 5-HT_{2A} receptor. Chronic corticosterone treatment has been demonstrated to significantly increase the density of central 5-HT_{2A} receptors (9,21,26), and to significantly facilitate 5-HT_{2A} receptor-mediated behaviors (3,13,18).

The hormone, melatonin, has also been explored in the context of 5-HT_{2A} receptor regulation. Eison et al. (8) demonstrated a dose-dependent attenuation by melatonin of the increase in WDS induced by a 5-HT_{2A} receptor agonist. Moreover, radioligand binding techniques demonstrated that melatonin reduced the concentration-dependent 5-HT_{2A}-mediated phosphoinositide (PI) hydrolysis response to 5-HT_{2A} agonists without altering central 5-HT_{2A} receptor density (8). Furthermore, investigations that demonstrate a similarity in the behavioral effects of melatonin to 5-HT_{2A} antagonists (10) are consistent with melatonin's putative 5-HT_{2A} antagonism.

Given the apparently opposing effects of corticosterone and melatonin on 5-HT_{2A} receptor activity, it seems reasonable to predict that concurrent administration of melatonin would attenuate the behavioral effects of corticosterone treatment. Furthermore, because drugs that antagonize 5-HT_{2A} receptor activity, including ritanserin, pirenperone, ketanserin,

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and nefazodone, exert no effects on sexual behavior (28) and WDS (18), it is expected that neither should melatonin, a putative 5-HT_{2A} antagonist, exert effects on these measures when administered alone. The aim of the present study is to investigate the effects of corticosterone, both alone and in combination with melatonin, on sexual behavior and WDS, and to explore the possibility of 5-HT_{2A} antagonism as a result of melatonin treatment.

MATERIALS AND METHODS

Subjects

Eighty Long–Evans male rats (Charles River Canada Inc., Quebec), were obtained at 5 weeks of age. Prior to testing, males were screened for copulatory proficiency, and those displaying consistently vigorous sexual activity were selected for the study. The screening procedure resulted in 68 males being employed in the study. At the time of testing, males were 4.5 months of age and 475 g on average. In addition, 18 sexually experienced female rats were used to elicit sexual behavior in the males. Females were previously bilaterally ovariectomized at 3 months of age using standard surgical procedures while anesthetized with ketamine HCl (75 mg/kg) and xylazine (7 mg/kg) obtained from the UBC Animal Care Centre, Vancouver, Canada.

All rats were housed in same-sex groups of three or four, in standard wire mesh cages, and were allowed free access to Purina Rat Chow and water. Colony conditions were maintained at 21 ± 1°C, and animals were kept on a reverse 12/12-h light cycle (lights off at 0900 h).

Injection Procedure

Corticosterone-21-acetate (Sigma Chemical Co., Chicago, IL) was suspended in propylene glycol (20 mg/mL) and melatonin (Sigma Chemical Co) was dissolved in a solution of 20% dimethyl sulfoxide (DMSO) and saline (6 mg/mL). Corticosterone or the vehicle, propylene glycol, was injected subcutaneously for 14 days (1 mL/kg). On the 15th day, animals received an intraperitoneal injection of either melatonin (1 mL/kg), or the vehicle, DMSO, 45 min prior to testing. In addition, given previous evidence that the frequency of spontaneously occurring WDS is quite low in males engaging in copulatory behavior (4), all animals received as injection (1 mL/kg) of the 5-HT_{2A} receptor agonist, (+)1-(2,5 dimethyl-4-iodophenyl)-2-aminopropane (DOI; Research Biochemicals International), to amplify this effect. DOI was dissolved in 0.9% saline (1.25 mg/mL), 30 min prior to testing. Male subjects were randomly assigned to one of four treatment groups: 1) propylene glycol and DMSO, $n = 18$; 2) propylene glycol and melatonin, $n = 20$; 3) corticosterone and DMSO, $n = 15$; and 4) corticosterone and melatonin, $n = 15$.

Behavioral Testing Procedure

Females were injected subcutaneously with 10 μg estradiol benzoate (Sigma Chemical Co.) 2 days before testing, and 500 μg progesterone (Sigma Chemical Co.), 4 h before testing. Hormones were dissolved in 0.1 mL peanut oil. Males were tested in Plexiglas chambers (30 × 30 × 45 cm in height) covered with contact bedding.

Males were given 5 min to habituate to the chambers before being presented with a receptive female. Measures of sexual behavior included: mount, intromission, and ejaculation frequencies and latencies, and the postejaculatory interval. In addition, the frequency of WDS was tallied for a 30-min observation period.

All testing was conducted during the middle third of the dark cycle by trained observers blind to the experimental conditions of the animals. A priori predictions that 1) melatonin should attenuate the effects of corticosterone, 2) melatonin alone should not exert any behavioral effects, and 3) corticosterone alone should produce behavioral effects that differ from the other three conditions, and that are similar to each other, permit use of the statistical method of nondirectional Planned Contrasts (11), with a significance level preset at $p < 0.05$.

RESULTS

Table 1 suggests that corticosterone inhibited sexual behavior and increased WDS, and that these effects were attenuated by melatonin. Planned comparisons revealed that corticosterone significantly decreased the frequency of ejaculations compared to the control, melatonin-treated, and corticosterone combined with melatonin-treated groups, as a whole, $t(64) = 2.03$, $p = 0.047$. Also, melatonin significantly attenuated the effects of corticosterone on ejaculations, $t(64) = 2.17$, $p = 0.034$. Melatonin alone did not affect ejaculation frequency, $p > 0.05$.

A similar pattern was observed for ejaculation latency. Melatonin significantly blocked the effects of corticosterone, $t(64) = -2.24$, $p = 0.028$. Although not statistically significant, corticosterone increased the ejaculation latency compared to the other three conditions as a group, $t(64) = -1.848$, $p = 0.069$. Again, melatonin alone had no effect on ejaculation latency, $p > 0.05$.

For mounting behavior, corticosterone significantly increased the frequency of mounts compared to the other three conditions as a group, $t(33) = -2.503$, $p = 0.017$. Whereas melatonin appeared to block the effects of corticosterone on mounts, this effect did not quite reach statistical significance, $p > 0.05$. Although similar trends were observed on some other measures of sexual behavior, there was no significant effect of either corticosterone or melatonin treatment on mount latency, intromission latency, intromission frequency, and postejaculatory interval.

Corticosterone significantly increased WDS relative to the other three conditions as a group, $t(64) = -3.969$, $p = 0.001$. In addition, melatonin significantly attenuated the effects of corticosterone on WDS, $t(64) = -3.803$, $p = 0.001$. Again, melatonin alone had no effect on WDS, $p > 0.05$.

DISCUSSION

The present results support previous findings of a corticosterone-induced inhibition of sexual behavior in the male rat (13) and facilitation of WDS (3,18). The chronic corticosterone regimen employed in these studies results in plasma corticosterone levels that are similar to those produced after a chronic stressor (22), and various stressors have been found to inhibit sexual behavior and facilitate WDS in the male rat (4,14). Although acute melatonin treatment alone exerted no effect on male sexual activity, it completely reversed the corticosterone-induced inhibition of ejaculatory behavior. These are the first reported data to suggest that melatonin may protect against the debilitating effects of chronic stress on male sexual behavior.

The observed behavioral responses to chronic corticosterone treatment are consistent with a 5-HT_{2A} receptor-medi-

TABLE 1

EFFECTS OF CHRONIC CORTICOSTERONE TREATMENT AND ACUTE MELATONIN TREATMENT ON FREQUENCIES OF MOUNTS, INTROMISSIONS, AND EJACULATIONS, LATENCIES OF MOUNTS (ML), INTROMISSIONS (IL), AND EJACULATIONS (EL) IN SECONDS, POSTEJACULATORY INTERVAL (PEI) IN SECONDS, AND FREQUENCY OF WET DOG SHAKES (WDS) IN MALE RATS

	No Corticosterone		Corticosterone	
	No Melatonin	Melatonin	No Melatonin	Melatonin
Mounts*	3.25 ± 0.75	4.58 ± 1.06	8.33 ± 1.73	5.55 ± 1.07
Intromissions	9.00 ± 1.20	9.33 ± .83	10.67 ± .92	8.27 ± .82
Ejaculations*	1.17 ± 0.33	1.50 ± 0.34	0.67 ± 0.23	1.73 ± 0.37
ML	914.4 ± 187.0	877.9 ± 178.8	1108.0 ± 188.5	601.1 ± 199.6
IL	970.4 ± 195.9	896.2 ± 176.9	1165.7 ± 193.2	724.6 ± 197.2
EL†	1077.6 ± 196.1	854.5 ± 179.3	1276.7 ± 176.5	641.7 ± 194.7
PEI	1181.3 ± 168.1	974.4 ± 166.9	1301.5 ± 166.6	853.1 ± 194.4
WDS*	13.1 ± 2.1	13.4 ± 1.7	25.7 ± 5.0	9.2 ± 2.5

Values represent means ± SEMs.

*Denotes that the group tested with corticosterone alone differed significantly from the other three groups, $p < 0.05$.

†Denotes that the group treated with corticosterone alone differed from the group treated with both corticosterone and melatonin, $p < 0.05$.

ated mechanism suppressing male sexual behavior while increasing WDS (27). Both chronic corticosterone treatment (9,21,26) and corticosterone elevation induced by a chronic stressor (22) significantly increase the density of central 5-HT$_{2A}$ receptors. WDS provide an index of 5-HT$_{2A}$ receptor activity (29); therefore, the present observation of increased WDS after corticosterone administration supports the notion of a corticosterone–5-HT$_{2A}$ receptor interaction.

Melatonin significantly attenuated the effects of corticosterone treatment on measures of sexual behavior and WDS, albeit in the presence of the 5-HT$_{2A}$ receptor agonist, DOI. This observation further supports previous observations that implicate melatonin as a 5-HT$_{2A}$ antagonist. Melatonin has previously been shown to attenuate agonist-induced WDS (8), and radioligand investigations revealed that it blocks the 5-HT$_{2A}$ second messenger system. In addition, administration of the nonselective 5-HT$_{2A}$ antagonist methysergide has been reported to induce behavioral changes similar to those found after treatment with melatonin (10). Opposite effects between melatonin and 5-HT$_{2A}$ antagonists have also been discovered. For example, pretreatment with the antidepressant, mianserin, a 5-HT$_{2A}$ antagonist, prevents the effects of intraaccumbens melatonin administration on locomotor activity and sniffing behavior (7). However, this effect of mianserin may be attributable to activity at 5-HT$_2$ receptors other than the 5-HT$_{2A}$ subtype, and does not necessarily generalize to sexual behavior. Therefore, it remains plausible that melatonin exerts some effects via a 5-HT$_{2A}$ receptor-mediated mechanism.

Although there have been extensive investigations as to the functional interaction between melatonin and serotonin, corticosterone has been reported to have little or no effect on 5-HT metabolism (5). Therefore, the increase in WDS and decrease in male rat sexual behavior following corticosterone administration are likely due to a specific receptor mechanism rather than an effect on serotonin, per se. Because these effects were blocked to varying degrees by melatonin, this further supports an action on the 5-HT$_{2A}$ receptor, and not on levels of serotonin. Moreover, radioligand studies have demonstrated that chronic corticosterone treatment induces 5-HT$_{2A}$ receptor upregulation independent of changes in 5-HT levels (21). The previous suggestion that hormones of the HPA axis may play a major role in the regulation of 5-HT$_{2A}$ receptor density (4) may also be extended to include melatonin (8).

A melatonin–corticosterone interaction has been widely investigated, and melatonin has been suggested to exert antiglucocorticoid effects (1). Melatonin has been implicated as protecting against the detrimental effects of elevated glucocorticoids on the hypothalamic–pituitary–adrenal axis (19,20). In addition, Chuang and Lin (6) have reported that the thermal stress-induced increases in locomotor activity are effectively attenuated by acute melatonin treatment. However, melatonin has no effect on corticosterone-induced thymus regression (16). Therefore, it remains possible that antiglucocorticoid effects of melatonin may not become apparent behaviorally until pharmacological doses are administered, and that at physiological levels, there may be little or no coupling between these hormones. These antiglucocorticoid effects of melatonin may be mediated, in part, by a 5-HT$_{2A}$ receptor antagonism.

Elevated circulating glucocorticoids are one physiological response to stress in humans, and stress has been identified as one of the predisposing factors for major depression (12). Postmortem studies on the brains of depressed suicide victims have found an increase in 5-HT$_{2A}$ receptor density (23), and the therapeutic onset of most antidepressants occurs with the concurrent downregulation of the 5-HT$_{2A}$ receptor (25). Melatonin has been implicated as a mood enhancer, and as possessing some possible therapeutic benefits to individuals with depression (17). As a preliminary hypothesis, melatonin may play a role in mood elevation by acting as an antagonist at the 5-HT$_{2A}$ receptor.

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