

Corticosterone attenuates the antidepressant-like effects elicited by melatonin in the forced swim test in both male and female rats

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Abstract

Melatonin has been demonstrated to increase activity in the forced swim test (FST), a putative model of antidepressant efficacy, indicating that it may possess antidepressant-like qualities. It has been suggested that corticosterone can interfere with the efficacy of antidepressants, an effect that has previously been demonstrated in the FST. This experiment examined the effects of melatonin and corticosterone, independently and in combination, on the behaviours of both male and female rats in the FST. Corticosterone, melatonin, combined vehicles or a combined melatonin/corticosterone regimen were administered for 20 days, after which the animals were observed in the FST. As seen in previous research, melatonin elicited an antidepressant-like effect in the FST by reducing immobile behaviour ($P < .01$) and increasing active behaviour ($P < .01$). Corticosterone was found to reduce activity ($P < .01$) and increase immobility ($P < .01$), as well as attenuate the anti-immobility effects of melatonin ($P = .03$). These findings suggest that while melatonin may possess antidepressant-like qualities, high levels of corticosterone seem capable of attenuating these effects.

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1. Introduction

The forced swim test (FST) was designed to assess the efficacy of antidepressant drugs on behavioural despair in rodents (Porsolt et al., 1978). Therapeutic agents with antidepressant properties, such as fluoxetine, venlafaxine and desipramine (Reneric and Lucki, 1998) have been shown to decrease the amount of immobility and increase the prevalence of active behaviour within the FST. The finding that the effectiveness of antidepressants in reducing immobility in the FST is highly correlated with their clinical efficacy and potency (Borsini and Meli, 1988) has helped to establish the FST as a model of antidepressant potential.

The FST has been shown to be sensitive to hormones implicated in the etiology and exacerbation of depression.

The hormone cortisol in humans, has been consistently linked to symptoms of depression (Murphy, 1991). Chronic administration of corticosterone to rats, a protocol that resembles the hypercortisolemia seen in humans with depression (Peeters and Broekkamp, 1994), produces behavioural deficits that are linked to depression including diminished memory (Bodnoff et al., 1995), decreased motor functioning (Fernandes et al., 1997) and reduced expression of motivated behaviours such as copulation (Gorzalka and Hanson, 1998). Furthermore, behavioural immobility in the FST seems to be contingent upon the presence of corticosterone, as elimination of the effects of corticosterone through an adrenalectomy (Jeffreys et al., 1983), inhibition of corticosterone synthesis (Baez and Volosin, 1994) or antisense treatment of the glucocorticoid receptor (Korte et al., 1996) results in a decrease in the occurrence of immobility. Paradoxically, a recent study demonstrated an antidepressant effect of corticosterone in that chronic administration led to decreased immobile behaviour in the FST (Brotto et al., 2001a). This novel finding may be due to the use of female rats, given that all prior research which has examined the

Abbreviations: ACTH, adrenocorticotropic hormone; EB, estradiol benzoate; FST, forced swim test; GABA, γ -amino butyric acid; HPA, hypothalamo–pituitary–adrenal; 5-HT, serotonin.

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effects of corticosterone in the FST has employed male rats (Baez and Volosin, 1994; Korte et al., 1996) and directional sex differences have been reported for other behavioural effects of corticosterone (Hanson and Gorzalka, 1999). Thus, while most of the data suggest that corticosterone decreases activity in the FST, this finding may depend on the sex of the animal.

The hormone melatonin has received much widespread attention for its putative stress-attenuating (Brotto et al., 2001b; Kopp et al., 1999), and antidepressant properties (Halbreich, 1997), and from anecdotal claims of its mood-enhancing effects. These and other behavioural effects may in fact be due to its properties as a homeostatic regulatory system or as a pharmacological buffer to the effects of stress (Brotto et al., 2000; Kopp et al., 1999). Corroborating this claim are reports of melatonin's ability to attenuate the effects of stress-induced gastric ulceration (Kato et al., 1997), hypothalamo-pituitary-adrenal (HPA) axis dysregulation (Konakchieva et al., 1998) and stress-induced sexual dysfunction (Brotto et al., 2001b). A role for melatonin as an antidepressant agent is supported by the finding that melatonin (Brotto et al., 2000) and the melatonin agonist S-20304 (Overstreet et al., 1998) decrease immobile behaviour in the FST.

The common link between corticosterone, melatonin and depression may be a serotonergic mechanism. Chronic corticosterone treatment has been shown to up-regulate the 5-HT_{2A} receptor (Kuroda et al., 1992) and potentiate the behavioural responses to a 5-HT_{2A} receptor agonist (Gorzalka and Hanson, 1998; Gorzalka et al., 1999). 5-HT₂ receptor density has been shown through SPECT imaging to be up-regulated in the parietal cortex of depressed patients (D'haenen et al., 1992), through autoradiography to be up-regulated in the post mortem frontal cortex of suicide victims (Arango et al., 1990; Arora and Meltzer, 1989; Hrdina et al., 1993; Mann et al., 1986) and also to be up-regulated in the platelets of depressed patients (Biegon et al., 1990). It has been demonstrated that chronic antidepressant treatment results in a down-regulation of the 5-HT_{2A} receptor (Blackshear and Sanders-Bush, 1980; Peroutka and Snyder, 1980) and this down-regulation corresponds temporally to when antidepressants become clinically effective (Peroutka and Snyder, 1980). Taken together, these findings all suggest that increased activity at the 5-HT_{2A} receptor could be involved in the behavioural manifestation of depressive symptomatology, whereas reducing the activity at this receptor is a mechanism common to antidepressants. This idea supports the findings that melatonin may exert antidepressant-like effects as melatonin has been shown to be a putative 5-HT_{2A} receptor antagonist. For example, melatonin inhibits the phosphoinositide hydrolysis induced by activation of the 5-HT_{2A} receptor (Eison et al., 1995). Further, melatonin counteracts the hypophagia, HPA axis activation and the stereotypical "wet dog shakes" induced

by administration of a 5-HT_{2A} agonist (Eison et al., 1995; Gorzalka et al., 1999; Raghavendra and Kulkarni, 2000).

This experiment intends to examine if melatonin does elicit antidepressant-like effects, if there are sex differences in the effects and further if these effects can be attenuated by corticosterone treatment, an effect that has been seen with other antidepressants that primarily affect the serotonergic system (Takamori et al., 2001).

2. Methods

2.1. Animals

Thirty-nine male Long-Evans rats (Charles River, Canada) with a mean weight of 675 g and 37 female Long-Evans rats (Charles River, Canada) with a mean weight of 550 g were used. At the time of testing all animals were 18 months of age, and had been used previously as control subjects in other behavioural studies, but not been exposed to other experimental treatments. At 3 months of age female rats were bilaterally ovariectomized using standard surgical procedures, ketamine hydrochloride (75 mg/kg), and xylazine (7 mg/kg). Ovariectomies were performed so that endogenous fluctuations of ovarian steroids could be controlled for. All rats were housed by sex in groups of three in triple mesh wire cages with Purina Rat Chow and tap water available ad libitum. The colony room was maintained under an automatically regulated 12:12-h reverse light/dark cycle (lights off at 0900 h) and at a standard temperature of 21 ± 1 °C. All research was conducted under the ethical procedures of the Canadian Council on Animal care.

2.2. Apparatus

Hormone administration was delivered via 26-gauge 1/2-in. stainless steel needles. Two Plexiglas cylindrical containers (diameter 35 cm and height 45 cm) were used during forced swim testing, and allowed rats to be tested in pairs. Given the documented influence of water depth on FST behaviours (Abel, 1994), each container was filled to 30 cm so that an animal could only touch the bottom with the tip of its tail. A video camcorder (SONY TRV99) was positioned such that the two containers, which were separated by a screen, could fit in the field of view at one time. Stopwatches and standard recording sheets were used to document the occurrence and duration of immobile behaviour. Plastic maternity bins (30 × 30 × 60 cm) were employed to transport the rats from the colony rooms to the testing rooms.

2.3. Hormone administration

Corticosterone-21-acetate (Sigma, Chicago, IL) was dissolved in propylene glycol and injected at a concentra-

tion of 20 mg/ml/kg. Melatonin (Sigma) was dissolved in equal quantities of 0.9% saline and propylene glycol and injected at 10 mg/ml/kg. Estradiol benzoate (Sigma; EB) was dissolved in peanut oil at a dose of 4 μ g /0.1 ml.

2.4. Experimental procedure

All animals were randomly assigned to one of four hormonal conditions: corticosterone/saline (male $n=9$; female $n=10$); melatonin/propylene glycol (male $n=10$; female $n=10$); corticosterone/melatonin (male $n=10$; female $n=8$); and saline/propylene glycol (male $n=10$; female $n=9$). All animals received these injections subcutaneously during the first third of the dark cycle for 20 consecutive days. Furthermore, on testing days, injections for that day occurred immediately following the behavioural test.

Consistent with the usual method of testing in the FST (Porsolt et al., 1978), each rat was exposed to the FST on two separate occasions –once on Day 19 and the second time on Day 20. Each female rat was given an injection of 4 μ g EB prior to testing to approximate physiologically relevant estrogen levels at the time of testing (Pare and Redei, 1993). Forced swim behaviours were scored on Day 19, during the first third of the dark cycle and following a procedure adapted from prior research (Hansen et al., 1997). Cylinder water was maintained at a constant 21 ± 1 °C. Once a rat was placed in the chamber, behaviour was recorded for a 15-min period, after which it was removed with the help of a wire mesh ladder. Rats were then placed in maternity bins, dried off with paper towels, and returned to another room for their daily injection before being returned to their home cage. To remove the influence of potential alarm substances on behaviours in the FST (Abel, 1991), fresh water was introduced prior to each test. FST testing on Day 20 was identical in procedure to that employed on Day 19, except that a duration of 10 min was employed instead of 15 min. All videotapes were later scored by two trained observers. Immobility was defined as when the rat was stationary and only made the minimal movements necessary to stay afloat, whereas any movement where the rat actively moved its limbs was considered active, escape directed behaviour, and both behaviours were scored accordingly (Porsolt et al., 1978).

2.5. Statistical analyses

A three-factor analysis of variance was used to analyze FST and data with corticosterone, melatonin, and sex as fixed-factor conditions. Two- and one-way analyses of variance were employed. Data from the second day of FST testing were analyzed given that antidepressant drugs affect behaviour on the second, and not the first day, of FST testing (Porsolt et al., 1978). A P level of .05 was employed in all analyses.

3. Results

3.1. Active behaviour

The three-way interaction between sex, melatonin and corticosterone approached statistical significance, $F(1,68)=3.154$, $P=.08$, for active behaviour. The interaction between melatonin and corticosterone was statistically significant, $F(1,68)=4.704$, $P=.034$ such that corticosterone was found to attenuate the increases in activity induced by melatonin treatment. The interaction between melatonin and sex approached statistical significance, $F(1,68)=3.562$, $P=.06$. That is melatonin appeared to produce a relatively greater increase in activity in females than males (Fig. 1). There was no significant interaction between corticosterone and sex, $F(1,68)=0.037$, $P>.05$. There was a significant main effect of melatonin, $F(1,68)=10.930$, $P=.002$, and a significant main effect of corticosterone, $F(1,68)=8.273$, $P=.005$, with the former enhancing and the latter reducing active behaviour. The main effect of sex was also significant, with males showing higher levels of activity than females, $F(1,68)=8.167$, $P=.006$, as shown in Fig. 1.

3.2. Immobility

The three way interaction between corticosterone, melatonin and sex showed a trend towards significance, $F(1,68)=3.154$, $P=.08$, on immobility behaviour. There was a signif-

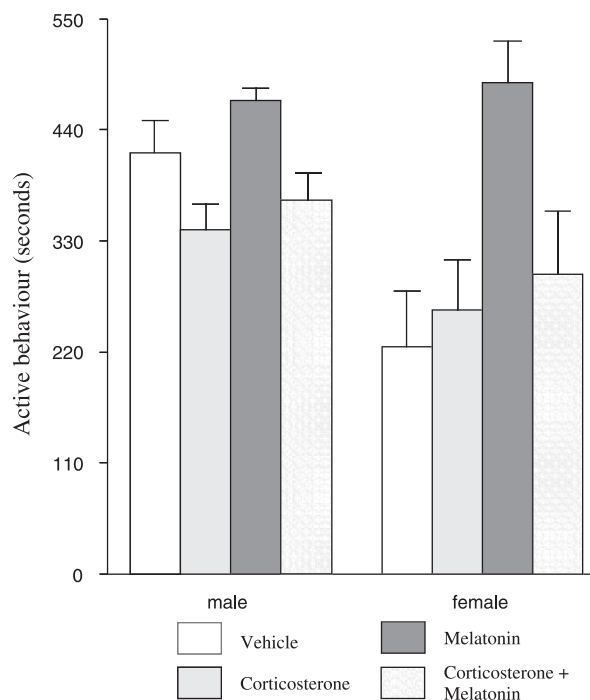


Fig. 1. Effects of corticosterone, melatonin, and the combination on active behaviour in male ($N=39$) and female ($N=37$) rats. Data represent means \pm S.E. The effects of melatonin, corticosterone, and sex were each statistically significant ($P<.01$), and there was a significant interaction between corticosterone and melatonin ($P<.05$).

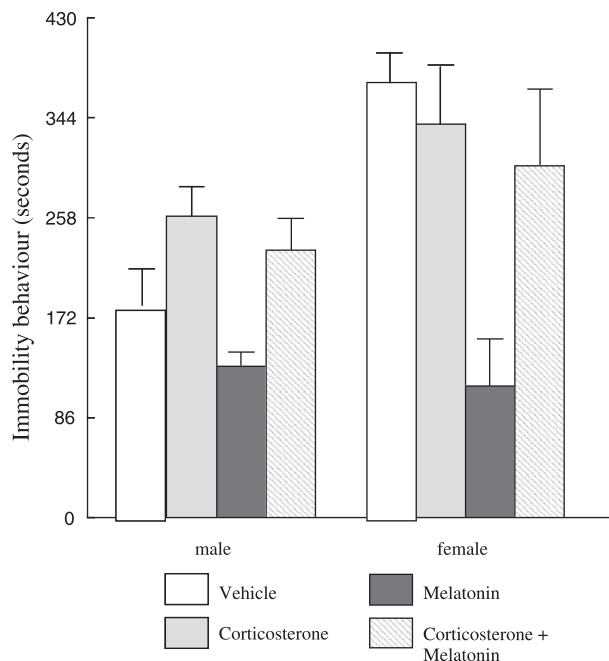


Fig. 2. Effects of corticosterone, melatonin, and the combination on immobility behaviour in male ($N=39$) and female ($N=37$) rats. Data represent means \pm S.E. The effects of melatonin, corticosterone and sex were each statistically significant ($P < .01$), and there was a significant interaction between corticosterone and melatonin ($P < .05$).

icant interaction between corticosterone and melatonin on immobility behaviour, $F(1,68) = 4.704$, $P = .034$. The interaction between sex and melatonin approached significance, $F(1,68) = 3.562$, $P = .063$, such that the effects of melatonin were much more pronounced in females than males (Fig. 2). There was no significant interaction between corticosterone and sex for immobility, $F(1,68) = 0.037$, $P = .847$. There was a significant main effect of sex, $F(1,68) = 8.167$, $P = .006$ in that females demonstrated higher levels of immobility than males. There were significant main effects of melatonin, $F(1,68) = 10.930$, $P = .002$ and corticosterone, $F(1,68) = 8.273$, $P = .005$. As shown in Fig. 2, melatonin reduced and corticosterone facilitated behavioural immobility.

4. Discussion

Rats administered melatonin exhibited less behavioural immobility and more active behaviours in the FST. These effects of melatonin on immobility occurred in both sexes but a statistical trend suggested that they were more pronounced in the female. With respect to corticosterone, statistical analyses showed that in general corticosterone increased time spent in the immobile position. However, inspection of Fig. 2 indicates that the effects of corticosterone alone were primarily seen in the male. Additionally, corticosterone significantly blocked the effect of melatonin on both immobility and activity, and this effect was seen in both sexes. Statistical analyses of sex differences showed

that males in general were more active than females, whereas females spent significantly more time in the immobile position than males. While one cannot rule out that the effects seen in this study were a reflection of changes in motor activity, this is unlikely based on previous findings. For example, our data demonstrated that females have lower baseline activity levels than males in the FST, however in motor tasks such as an open field test, females consistently demonstrate higher levels of activity than males (Brotto et al., 2000; Masur et al., 1980). Also, melatonin is known to exert central nervous system depressant-like effects, and decrease locomotor activity (Isobe et al., 2002; Shaji and Kulkarni, 1998), whereas the present results indicated increased active behaviour in the FST following melatonin treatment. Further, rats receiving corticosterone in this study were less active in the FST, although chronic corticosterone treatment does not decrease activity in other locomotor tasks (Brotto et al., 2001a; Ehlers et al., 1992).

4.1. Sex differences

Sex differences in activity levels in the FST have previously been reported (Pare and Redei, 1993). Females tend to have higher basal levels of corticosterone than males (Handa et al., 1994), and this may partially account for the sex difference. Prior research has demonstrated that the occurrence of immobility appears to be contingent upon the presence of corticosterone (Baez and Volosin, 1994; Jeffreys et al., 1983; Korte et al., 1996), and the current study demonstrated that corticosterone administration lead to increased levels of immobility. Taken together, these findings suggest that sexually dimorphic differences seen in FST behaviours, such as increased immobility in females, may be related to females having higher levels of corticosterone. The effect could also be due to sexually dimorphic patterns of learning. Female rats have been shown to learn faster than males (Shors et al., 2000), and females may consolidate information faster, and thus in this model, demonstrate behavioural despair at a more accelerated rate and have a lower level of activity upon second exposure to the FST.

4.2. The effects of corticosterone and melatonin

The findings that melatonin suppressed immobility behaviour and increased escape-directed activity supports earlier claims of its antidepressant-like effects as measured in the FST (Brotto et al., 2000; Raghavendra et al., 2000). The fact that this effect was seen in both sexes is consistent with the notion that melatonin may exert antidepressant-like effects, regardless of sex, although the effect appeared to be more pronounced in females.

Previous research investigating the effects of glucocorticoids on behaviours in the FST has produced somewhat paradoxical results. Administration of metyrapone, a cor-

ticosterone synthesis inhibitor, prevented the increase of immobility seen in the FST (Baez and Volosin, 1994). This suggests that corticosterone has an immobility enhancing effect, and further support for this concept comes from research demonstrating that impairment of the glucocorticoid receptor via antisense treatment results in decreased immobility displayed in the FST (Korte et al., 1996). In contrast to this, it was recently demonstrated that corticosterone decreased immobility in females but not males (Brotto et al., 2001a). In the present study, corticosterone produced a significant decrease in general activity, and this effect was not dependent on the sex of the animal. Unlike the effects of corticosterone on copulatory activity (Gorzalka and Hanson, 1998; Hanson and Gorzalka, 1999), which appear to be sexually dimorphic, the effects of corticosterone on active behaviours in the FST are not.

4.3. Potential mechanism of interaction between corticosterone and melatonin and implications for depression

Across both sexes, corticosterone was found to attenuate the activity-enhancing effects of melatonin, as well as significantly attenuate the anti-immobility effect of melatonin. One implication of this finding is that corticosterone can interfere with the efficacy of the antidepressant-like action of melatonin, possibly via a serotonergic mechanism. Chronically elevated corticosterone levels, following ACTH treatment, have been shown to up-regulate the density of 5-HT_{2A} receptors (Kuroda et al., 1992), and a high density of 5-HT_{2A} receptors has been implicated in the etiology and exacerbation of depression (Arango et al., 1990; Arora and Meltzer, 1989). Melatonin treatment alone resulted in an increase in activity and a decrease in immobility in the FST, arguing for an antidepressant effect of the hormone. In the presence of corticosterone, however, these potentially enhancing effects of melatonin, manifested in increased active behaviour, are attenuated. The antidepressant effects of melatonin are likely mediated by the 5-HT_{2A} receptor, as biochemically (Eison et al., 1995) and behaviourally (Gorzalka et al., 1999; Raghavendra and Kulkarni, 2000) melatonin has been shown to act as a 5-HT_{2A} antagonist. Furthermore, decreases in the occurrence of immobility in the FST have been linked directly to a reduction in activity at the 5-HT_{2A} receptor, and it has been suggested that antidepressant-like effects seen in the FST are ultimately regulated by the 5-HT_{2A} receptor (Sibelle et al., 1997). However, it should be noted that the antidepressant-like actions of melatonin could be mediated by the melatonin receptor itself (Overstreet et al., 1998), or by interactions with other neurotransmitter systems, such as the GABAergic system (Golombek et al., 1996; Raghavendra et al., 2000).

Since one of the common mechanisms of most antidepressants is to ultimately reduce 5-HT_{2A} receptor

density (Blackshear and Sanders-Bush, 1980; Peroutka and Snyder, 1980; Skrebuhova et al., 1999; Yatham et al., 1999), and the period in which their clinical efficacy becomes apparent corresponds temporally to that of down-regulation of the 5-HT_{2A} receptor (Peroutka and Snyder, 1980; Yates et al., 1990), it is possible that melatonin's effects may be mediated by its effects on 5-HT_{2A} receptor activity. One explanation for the sensitivity of melatonin to the presence of corticosterone may be that whereas typical antidepressants eventually lead to a down-regulation of 5-HT_{2A} receptors (Peroutka and Snyder, 1980), melatonin treatment reduces 5-HT_{2A} receptor transmission, but does not actually down-regulate the receptor (Eison et al., 1995). Given the finding that chronically elevated corticosterone levels result in an up-regulation of the 5-HT_{2A} receptor (Kuroda et al., 1992), the melatonin-induced decrease in 5-HT_{2A} receptor transmission may be insufficient to counter the density changes from corticosterone. The fact that other antidepressants typically down-regulate the 5-HT_{2A} receptor (Peroutka and Snyder, 1980; Yatham et al., 1999) could explain why they are more resilient to the effects of corticosterone. Although recent research has shown that chronic ACTH treatment can attenuate the antidepressant effects of imipramine and desipramine in the FST (Takamori et al., 2001), antidepressants were administered acutely in that study and so would not have had the opportunity to down-regulate the 5-HT_{2A} receptor and ultimately counteract the opposing effects induced by the elevated glucocorticoids. Further research is needed to clarify if the antidepressant-diminishing effects of chronically elevated glucocorticoids are still present after chronic antidepressant treatment.

5. Conclusion

This study demonstrated that melatonin elicits antidepressant-like effects in the FST, in that it increases the occurrence of active, escape-directed behaviours. Corticosterone was found to induce immobile behaviour, and to attenuate all of the antidepressant-like effects elicited by melatonin. Females were found to have a higher level of baseline immobility than males, which may in part be due to their higher levels of basal corticosterone. Moreover, there was a trend toward a more pronounced effect of melatonin in the female. We propose that the mechanism of the antidepressant-like effects of melatonin seen in the FST, may be mediated by a reduction in activity at the 5-HT_{2A} receptor. Furthermore, an up-regulation of the 5-HT_{2A} receptor by chronically elevated corticosterone levels may explain the attenuation of the behavioural effects of melatonin. Despite the stress-attenuating effects of melatonin, the present data argue against its use as a conventional antidepressant when glucocorticoid levels are elevated.

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