CHAPTER 3

Chronic systemic administration of serotonergic ligands flibanserin and 8-OH-DPAT enhance HPA axis responses to restraint in female marmosets

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ABSTRACT

Background. Flibanserin, a novel serotonin (5-HT)$_{1A}$ agonist and 5-HT$_{2A}$ antagonist, has been shown to increase sexual desire and reduce distress in women with Hypoactive Sexual Desire Disorder (HSDD). In marmoset monkeys, flibanserin has demonstrated pro-social effects on male-female pairmates, while the classic 5-HT$_{1A}$ agonist 8-OH-DPAT suppresses female sexual behavior and increases aggressive interactions between pairmates. Activation of 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors is known to stimulate the hypothalamic-pituitary-adrenal (HPA) axis. This study aims to characterize the effects of repeated flibanserin and 8-OH-DPAT administration on the marmoset HPA axis and to elucidate endocrine correlates of altered marmoset pair behavior.

Methods. Adrenocorticotropic hormone (ACTH) and cortisol were examined at baseline and during 5-HT$_{1A}$ agonist and restraint challenges in 8 female marmoset monkeys receiving daily flibanserin (15 mg/kg) and an additional 8 female marmosets receiving 8-OH-DPAT (0.1 mg/kg) for 15-16 weeks. Corresponding vehicle treatments were administered in a counterbalanced, within-subject design. All females were housed in stable male-female pairs. Treatment-induced changes in ACTH and cortisol levels were correlated with previously assessed marmoset pair behavior.

Results. While morning basal cortisol levels and HPA responses to a 5-HT$_{1A}$ agonist challenge were not altered by chronic flibanserin or 8-OH-DPAT, both treatments increased the responsiveness of the marmoset HPA axis to restraint. Enhanced ACTH responses to restraint correlated with reduced sexual receptivity and increased aggression in 8-OH-DPAT-, but not in flibanserin-treated female marmosets.

Conclusions. Unaltered HPA responses to a 5-HT$_{1A}$ agonist challenge after chronic flibanserin and 8-OH-DPAT treatments indicate little or no desensitization of the HPA axis to repeated 5-HT$_{1A}$ manipulation. Chronic 8-OH-DPAT, but not flibanserin, leads to aggravated ACTH responses to stress that may contribute to anti-sexual and anti-social behavior between 8-OH-DPAT-treated females and their male pairmates. Despite similar flibanserin and 8-OH-DPAT induced ACTH responses to restraint stress, flibanserin-treated females show unchanged cortisol profiles. This is possibly due to flibanserin's regional selectivity in 5-HT$_{1A}$ activation and concurrent 5-HT$_{2A}$ inhibition. The contrasting restraint-related cortisol responses emulate contrasting behavioral phenotypes of diminished pair-bond of 8-OH-DPAT-treated females compared to the more affiliative pair-bond of flibanserin-treated females.

INTRODUCTION

Hypoactive sexual desire disorder (HSDD), a distressing condition affecting an estimated 10% of women (Clayton, 2010), has no approved pharmacological treatment. Recently, flibanserin (2H-benzimidazol-2-one, 1,3-dihydro-1-[2-[4-[3-(trifluoromethyl) phenyl]-1-piperazinyl]ethyl]), an agonist of serotonin $(5\text{-HT})_{1A}$ and antagonist of 5-HT_{2A} receptors (Borsini et al., 1995a; Borsini et al., 2002), demonstrated an ability to stimulate female sexual behavior in rats (Gelez et al., 2010) and to improve sexual desire in women with either HSDD (Derogatis et al., 2012; Thorp et al, 2012) or major depression (Kennedy, 2010). We have tested the long-term effects of flibanserin on sexual and social behavior in female common marmoset monkeys *(Callithrix jacchus)* housed with long-term male pairmates, and compared flibanserin-induced behavioral outcomes with those induced by *R*-(+)-8-hydroxy-2-(di-n-propylamino)-tetralin hydrobromide (8-OH-DPAT), a classic 5-HT_{1A} agonist (Arvidsson et al., 1981). We found that flibanserin-treated females attracted more sexual interest from male pairmates and enhanced the frequency of grooming interactions between pairmates. In contrast, 8-OH-DPAT-treated females showed increased rejection of male sexual advances and increased aggression with male pairmates (Aubert et al., 2011).

Pharmacological manipulations of the central serotonin neurotransmitter system alter functioning of the hypothalamic-pituitary-adrenal (HPA) axis (Fuller, 1996; Jørgensen, 2007). Such HPA changes play important roles in behavioral modulation (de Kloet 2000; Sapolsky et al., 2000) and may contribute to the alteration of sexual and social behavior observed in our flibanserin and 8-OH-DPAT studies. Stress glucocorticoid levels generally inhibit female reproduction and sexual behavior in most species (De Catanzaro and Gorzalka, 1980; Sapolsky et al., 2000). Interestingly, stress is one of the most commonly perceived factors that contribute to low sexual desire in women diagnosed with HSDD (Maserejian et al., 2010), and disorders of the HPA axis are frequently associated with altered sexual desire and arousal in women (Starkman and Schteingart, 1981; Erichsen et al., 2010).

The central 5-HT system generally exerts a stimulatory function on HPA axis activity (Feldman et al., 1987; Dinan, 1996; Jørgensen, 2007). Activation of 5-HT_{1A} and 5-HT_{2A} receptor subtypes, which are both expressed on corticotropin-releasing hormone (CRH) containing neurons in the paraventricular hypothalamic nucleus (PVN) (Liposits et al., 1987), stimulates the HPA axis to release adrenocorticotropic hormone (ACTH) and subsequently glucocorticoids (Rittenhouse et al., 1994; Osei-Owusu, 2005; Van de Kar et al., 2001). Conversely, the responsiveness of the HPA axis to stress is reduced following 5-HT depletion (Feldman and Weidenfeld, 1998).

5-HT induced regulation of HPA axis activity may thus underlie glucocorticoid influence on female sexual behavior.

The effects of chronic flibanserin administration on HPA axis functioning are not known. Repeated injections of 8-OH-DPAT for 21 days elevate basal circulating corticosterone, but not ACTH levels, in rats (Owens et al., 1990). A single systemic injection of 8-OH-DPAT acutely increases circulating ACTH and corticosterone levels (Owens et al., 1990; Raap et al., 2002) and inhibits sexual behavior in female rodents (Uphouse et al., 1991). No information regarding primate HPA axis function following chronic 8-OH-DPAT administration is currently available.

In female marmosets housed with long-term male pairmates, we previously reported pro-social outcomes during chronic flibanserin treatment in contrast to anti-social and anti-sexual effects of chronic 8-OH-DPAT administration (Aubert et al., 2012). The present study aims to investigate the effects of chronic flibanserin and 8-OH-DPAT (1) on the well-established stimulatory effect of 5-HT1A agonism on HPA function and (2) on the HPA response to restraint stress. Aim (1) will thus determine whether the responsiveness of the HPA axis to 5-HT_{1A} activation is impacted by chronic 5-HT_{1A} activation/5-HT_{2A} inhibition (flibanserin), or by chronic 5-HT_{1A} activation alone (8-OH-DPAT), while aim (2) will determine how flibanserin and 8-OH-DPAT affect marmoset HPA function during restraint stress. Such changes in HPA function may contribute to accompanying changes in female interactions with established male pairmates.

METHODS

Study animals

This study was conducted in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act and its subsequent amendments. All animal procedures were reviewed and approved by the Graduate School Animal Care and Use Committee of the University of Wisconsin-Madison. The Wisconsin National Primate Research Center (WNPRC) is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care as part of the University of Wisconsin-Madison Graduate School. Sixteen adult (age 2-5 yr) nulliparous captive-born common marmoset *(Callithrix jacchus)* females were pair housed with similarly aged male partners at the WNPRC for 8-20 months before onset of this study. Eight of the 16 females were assigned to test the effects of flibanserin, while the remaining 8 females were assigned to test the effects of 8-OH-DPAT. Females were housed with the same male partner for the entire study and

were ovariectomized and primed with either mid-follicular phase estradiol levels or no estradiol before study onset. Estradiol status remained the same throughout the study for each individual animal. Surgical procedures, estradiol priming and study design with regard to estradiol priming were performed as described in Barnett et al. (2006).

Experimental design

A counterbalanced, cross-over study, that applied within-subject comparisons, was designed to examine the effects of chronic (15-16 weeks) daily (12:00h-14:00h) administration of flibanserin (n=8; 15 mg/kg, orally (PO) in 1ml/kg vehicle; Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany) or 8-OH-DPAT (n=8; 0.1 mg/kg in 0.4ml/kg vehicle, injected subcutaneously (SC); Sigma-Aldrich St. Louis, MO, USA), with respective vehicles (for flibanserin, 98.5% of 0.5% hydroxycellulose solution and 1.5% of 1% polysorbate 80 solution, 1.0 ml/kg PO; for 8-OH-DPAT, 0.4 ml saline, SC). The study focused on three aspects of HPA axis functioning: (1) morning basal levels of cortisol, (2) following an acute 5-HT_{1A} agonist challenge, and (3) during and after acute restraint-induced increases in plasma ACTH and cortisol levels.

Blood sampling and hormone assays

Within 3 min of home-cage entry, blood samples were collected by femoral puncture during brief restraint in a marmoset restraint tube (Hearn, 1977). This minimizes circulating ACTH and cortisol responses to the sampling procedures (Saltzman et al., 1994). Samples to be analyzed for ACTH were stored at -80°C while those for cortisol and estradiol were stored at -20°C until hormone assay.

Hormone assays were fully validated previously for use with marmoset plasma (Saltzman et al., 1994; Saltzman et al., 1998; Saltzman et al., 2004). Plasma concentrations of ACTH and cortisol were determined by radioimmunoassay (RIA), and for estradiol by RIA following extraction with 5 ml ethyl ether and celite column chromatography. Assay sensitivity was 0.05 ng/tube (1.0 ng/ml) for ACTH, 18.3 pg/tube (1.8 µg/dl) for cortisol, and 4.6 pg/tube (30.4 pg/ml) for estradiol. Intra- and inter-assay assay coefficients of variation (CVs), respectively, were 4.6% and 12.4% for ACTH, 3.6% and 17.2% for cortisol, and 5.0% and 14.0% for estradiol.

Morning basal cortisol levels (08:45-09:15h)

Basal circulating cortisol was assessed prior to treatment (0 wk) and at 3 and 6 weeks of daily flibanserin, 8-OH-DPAT or respective vehicle treatment.

Neuroendocrine tests

Neuroendocrine tests assessing both cortisol and ACTH responses were performed during week 7 (5-HT_{1A} agonist challenge test) and week 9 (restraint test) of daily treatment, at least 22 h after the previous daily administration of 8-OH-DPAT, flibanserin, or vehicle. The time between 5-HT_{1A} agonist challenge and restraint tests was 13 ± 0.5 days (mean ± SEM).

a) **5-HT_{1A} agonist challenge test.** Blood samples (0.3ml) were taken 0, 15 and 180 min after a SC injection of 8-OH-DPAT (0.1 mg/kg), or vehicle (0.4ml/kg saline), administered at 12:00h-13:00h.
b) **Restraint test.** Animals were restrained for 30 min. A baseline blood sample (0.3ml, 0 min) was drawn immediately prior to restraint onset, and additional samples were taken at t=15 and t=30 min during restraint, and 3 h after return to the home cage (210 min).

Behavioral testing

Sexual and social behavior of the pairmates was observed after a 90-minute separation. Four 30-minute behavioral tests were conducted at 07:00h-13:00h (16-24 hours after daily administration of active serotonergic ligand/vehicle), 5-6 weeks after treatment onset. Pair behavior was stable during the 2 weeks of testing. Procedural details are described in Aubert et al. (2012).

Data analysis

Analyses of circulating ACTH, cortisol and estradiol levels were performed on untransformed data when normality of data distribution was confirmed, or on log transformed data to normalize data distribution. To assess morning basal levels, a 2-way ANOVA with Drug Treatment (test compound, vehicle) and Time (0 wk, 3 wk, 6 wk) as within-subject factors was applied. For the 5-HT_{1A} agonist challenge test, a 3-way ANOVA was employed, with Drug Treatment (test compound, vehicle), Challenge Type (5-HT_{1A} agonist challenge, saline challenge) and Time (0 min, 15 min, 180 min) as within-subject factors. For the restraint test, a 2-way ANOVA was used, with Drug Treatment (test compound, vehicle) and Time (0 min, 15 min, 30 min, 210 min) as within-subject factors. Significant main effects of Time were specified post-hoc by contrast analysis. Data are presented as mean ± S.E.M.

Initial analyses were performed using the same mixed design ANOVA with Estradiol supplementation and Order of treatment as additional between-subject factors. As both factors consistently failed to affect ($p>0.05$) any test variable, they were omitted in the final analyses reported here.

To investigate the relationship between flibanserin and 8-OH-DPAT induced

behavioral changes (Aubert et al., 2012) and HPA axis function, all behaviors that were significantly altered by flibanserin or 8-OH-DPAT were post-hoc correlated to significantly altered endocrine responses, using two-tailed Pearson's tests on the differential [serotonergic ligand – respective vehicle] for each selected variable, after normality of the bivariate distributions was confirmed.

For all results, a p-value below 0.05 was considered significant. Behaviors analyzed are described in Aubert et al. (2012).

RESULTS

Chronic effects of 8-OH-DPAT and flibanserin on HPA axis functioning

Morning basal cortisol

Neither chronic flibanserin (Treatment: $F(1,7) = 0.17$, $p = .694$; Treatment x Time interaction: $F(2,14) = 0.36$, $p = .704$) nor chronic 8-OH-DPAT (Treatment: $F(1,7) = 2.76$, $p = .140$; Treatment x Time interaction: $F(2,14) = 0.99$, $p = .397$) altered morning basal cortisol levels compared to respective vehicle (Tab. 1).

Table 1. Morning basal cortisol levels. Plasma concentrations of cortisol (µg/dl; mean ± SEM) at 0, 3 and 6 weeks of daily flibanserin vehicle, flibanserin, 8-OH-DPAT vehicle and 8-OH-DPAT treatment.

	Flibanserin vehicle	Flibanserin	8-OH-DPAT vehicle	8-OH-DPAT
0 wk	221 ± 30.4	200 ± 27.0	167 ± 19.3	175 ± 23.9
3 wk	234 ± 16.8	235 ± 25.6	156 ± 13.3	196 ± 22.9
6 wk	202 ± 15.4	203 ± 21.9	160 ± 22.9	203 ± 24.0

5-HT$_{1A}$ agonist challenge test

The 5-HT$_{1A}$ agonist challenge test elicited ACTH and cortisol responses in all female groups (Fig. 1A), without influence of chronic daily flibanserin or 8-OH-DPAT. In response to a 5-HT$_{1A}$ agonist challenge, flibanserin-, 8-OH-DPAT- and vehicle-treated female marmosets all exhibited increased plasma ACTH levels at 15 min post-challenge compared to a saline challenge. Plasma cortisol levels were also elevated following 5-HT$_{1A}$ agonist challenge, at 180 min post-challenge (Fig. 1B), without influence of chronic daily flibanserin or 8-OH-DPAT.

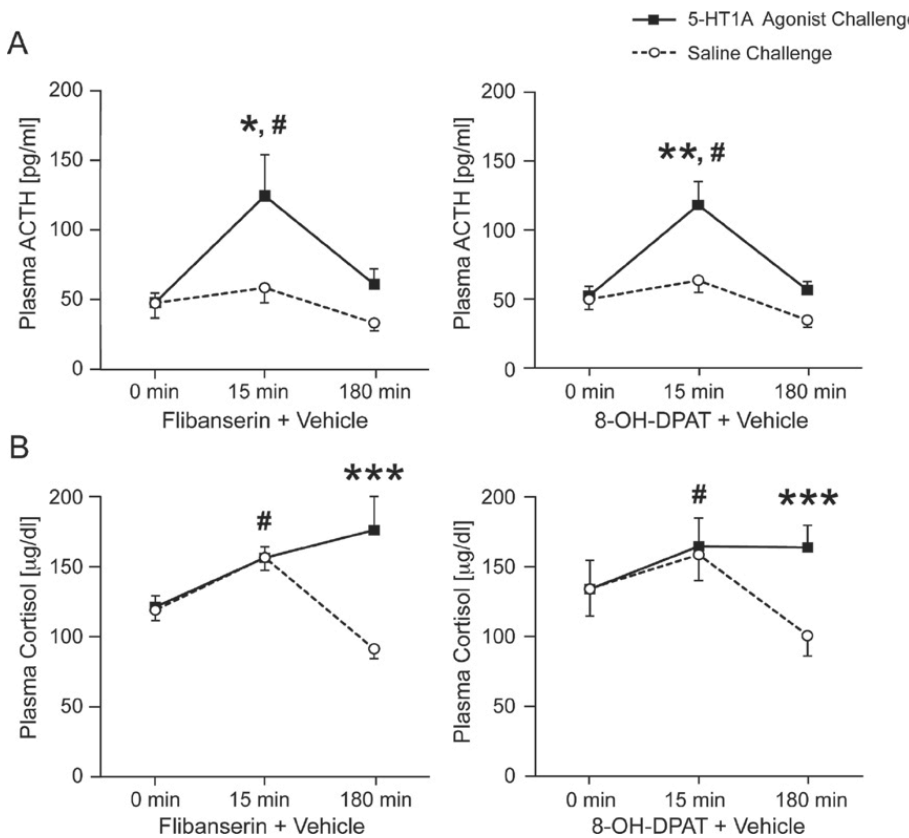

Figure 1. 5-HT$_{1A}$ challenge test, effect of Challenge. Plasma concentrations of **(A)** ACTH (pg/ml; mean±SEM) and **(B)** cortisol (µg/dl; mean±SEM) at 0, 15, and 180 min following an acute 5-HT$_{1A}$ challenge (square dots) at 7 weeks of daily flibanserin, 8-OH-DPAT or corresponding vehicle treatment. ACTH flibanserin group (upper left graph): p=0.045 vs. saline challenge (circular dots; F(1,7) = 6.0); ACTH 8-OH-DPAT group (upper right graph): p=0.008 vs. saline challenge (F(1,7) = 13.5); cortisol flibanserin group (lower left graph): p=0.014 vs. saline challenge (F(1,7) = 8.1), p<0.001 Challenge x Time interaction (F(2,14) = 17.8); cortisol 8-OH-DPAT (lower right graph): p=0.038 vs. saline challenge (F(1,7) = 6.5), p<0.001 Challenge x Time interaction (F(2,14) = 13.9); * p<0.05, ** p<0.01, *** p<0.001 vs. saline challenge (circular dots); # p<0.05 vs. 0min (Time effect). ACTH, adrenocorticotropic hormone; 8-OH-DPAT, *R*-(+)-8-hydroxy-2-(di-n-propylamino)-tetralin.

Restraint test

Thirty minutes of restraint led to acute increases in plasma ACTH levels 15 min and 30 min following restraint onset. Both chronic daily flibanserin

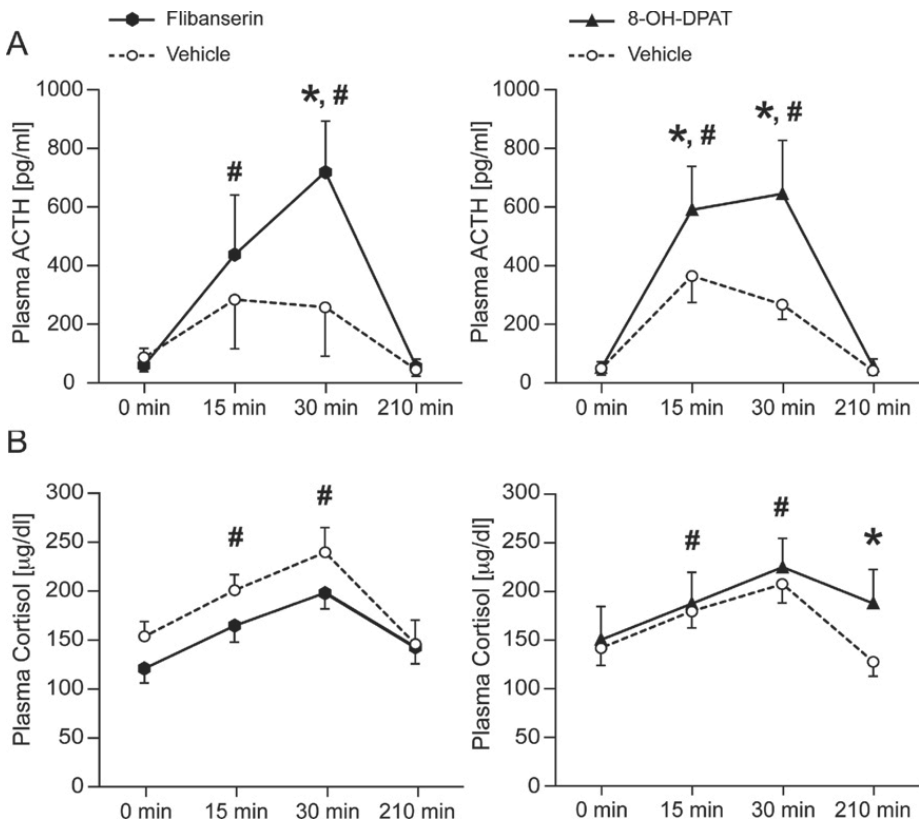

Figure 2. Restraint test. Plasma concentrations of **(A)** ACTH (pg/ml; mean±SEM) and **(B)** cortisol (µg/dl; mean±SEM) at 0, 15, 30 and 210 min during and following 30-min restraint at 0-30 min at 9 weeks of daily flibanserin, 8-OH-DPAT (solid line) or corresponding vehicle (dotted line) treatment. ACTH flibanserin group (upper left graph): p=0.033 Treatment x Time interaction (F(3,21) = 3.5); ACTH 8-OH-DPAT group (upper right graph): p=0.041 Treatment effect (F(1,7) = 6.3), p=0.029 Treatment x Time interaction (F(3,21) = 3.6); cortisol flibanserin group (lower left graph): Treatment x Time interaction non-significant; cortisol 8-OH-DPAT group (lower right graph): p<0.001 Treatment x Time interaction (F(3,21) = 9.5); * p<0.05 vs. corresponding time point control; # p<0.05 vs. 0min (Time effect). ACTH, adrenocorticotropic hormone; 8-OH-DPAT, *R*-(+)-8-hydroxy-2-(di-n-propylamino)-tetralin.

and chronic daily 8-OH-DPAT enhanced restraint-induced increase in plasma ACTH levels (Fig. 2A). 8-OH-DPAT increased ACTH levels at both 15 and 30 minutes of restraint, while flibanserin increased ACTH at 30 minutes only. At 210 min, or 3 h after the animals were returned to their home cages following

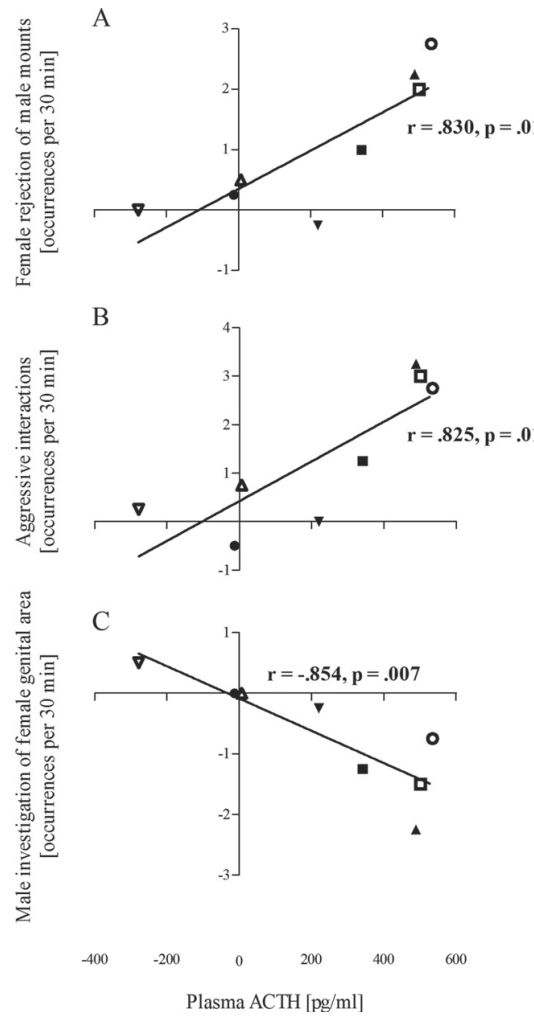

Figure 3. Behavioral correlates of enhanced ACTH response to restraint after 8-OH-DPAT. The magnitude of the 8-OH-DPAT-induced elevation in ACTH responses at 15 min of restraint is positively correlated to female rejection of male mount attempts and mounts **(A)** and aggression between pairmates **(B)**, and negatively correlated to male investigation of the female's genital area **(C)**. The graphs show the differential [8-OH-DPAT – vehicle; n=8] of both ACTH levels (x-axis) and behavior scores (y-axis). Each symbol indicates the same individual female marmoset receiving estradiol (solid symbols) or no estradiol (open symbols). ACTH, adrenocorticotropic hormone; 8-OH-DPAT, *R*-(+)-8-hydroxy-2-(di-n-propylamino)-tetralin.

restraint, plasma ACTH levels in both flibanserin and 8-OH-DPAT treated females were comparable to respective vehicle treated controls.

In flibanserin treated females, elevated ACTH levels at 30 minutes of restraint (the only significantly different time point from vehicle control responses) were not correlated to any behavior altered by flibanserin, including female genital area self-grooming, male investigation of the female's genital area, and allogrooming between pairmates. In 8-OH-DPAT treated females, however, elevated ACTH levels at 15 minutes of restraint were positively correlated to female rejection of male mount attempts and mounts (r = .830, p = .011; Fig. 3A), to unsuccessful male mount attempts (r = .721, p = .044), and to aggressive interactions between pairmates (r = .825, p = .012; Fig 3B),

while they were negatively correlated to male investigation of the female's genital area (r = -.854, p = .007; Fig. 3C). Such correlations were absent at 30 minutes of restraint.

Restraint also led to a stress-induced release of cortisol in all animals. 8-OH-DPAT chronic daily treatment, however, resulted in a significant Treatment x Time interaction, indicating that chronic treatment with 8-OH-DPAT maintained elevated plasma cortisol levels at 210 min, after animals had already been returned to their home cages for 3 h, following cessation of restraint. Chronic daily flibanserin treatment, in contrast, did not induce prolonged elevation of cortisol following cessation of restraint (Fig. 2B). Significantly elevated cortisol levels in 8-OH-DPAT treated females were not correlated to any behavior altered by 8-OH-DPAT.

Estradiol supplementation, during both chronic daily flibanserin and 8-OH-DPAT administration, was without effect on either ACTH or cortisol responses to 5-HT_{1A} agonist challenge or restraint.

DISCUSSION

Comparisons to previous studies

Despite the prevalence of HSDD in women (Clayton, 2010), little is known about the neural and endocrine bases of this disorder. Flibanserin, a novel 5-HT_{1A} agonist and 5-HT_{2A} antagonist, has demonstrated pro-sexual (humans and rodents; Gelez et al., 2010; Kennedy 2010; Stahl et al., 2010) and pro-social (marmosets; Aubert et al., 2011) effects on female behavior, while the classic 5-HT_{1A} agonist 8-OH-DPAT suppresses female sexual behavior in rodents and marmosets (Uphouse et al., 1991; Aubert et al., 2011) and increases aggressive interactions between male-female pairmates of marmosets (Aubert et al., 2011).

In the present study, we demonstrate an increased responsiveness of the female marmoset HPA axis to restraint during chronic flibanserin and 8-OH-DPAT treatments, suggesting that selective manipulation of 5-HT_{1A} receptors, with or without respective 5-HT_{2A} receptor manipulation, sensitizes HPA reactivity. Morning basal cortisol levels, however, are not affected by either treatment and contrast with a study in rats that reports elevated basal plasma corticosterone levels after 21 days of daily 8-OH-DPAT injections (Owens et al., 1990). Differences in species (marmoset vs. rat), dosing (0.1 mg/kg vs. 1.0 mg/kg), route of administration (SC vs. IP) and time of blood sampling in relation to diurnal/nocturnal rhythm (beginning vs. end of active phase in marmoset and rat, respectively), however, make it difficult to directly compare studies. There are no previous studies on flibanserin's effects on HPA axis

function. Both flibanserin and 8-OH-DPAT are agonists on postsynaptic 5-HT_{1A} receptors (Borsini et al., 1995a; Palego et al., 2000), suggesting that the sensitization of the HPA axis to restraint-type of stressors may be mediated by chronic activation of the 5-HT_{1A} receptor in projection areas of 5-HT neurons.

Interestingly, enhanced ACTH responses to restraint correlate with increased aggression and reduced sexual receptivity in 8-OH-DPAT treated female marmosets. In a study in female rats, repeated exposure to a stressful environment is associated with reduced sexual receptivity and increased aggressive behavior (Yoon et al., 2005). ACTH and corticosteroid levels, however, were not measured in the rodent study.

The lack of estradiol effects on HPA axis responses in both flibanserin and 8-OH-DPAT treated marmosets might be somewhat surprising in light of well-documented interactions of estrogens with the central serotonin system in female mammals (Bethea et al., 2002). Estradiol replacement was also without effects on sexual behavior (Aubert et al., 2012). This is in line with our previous studies indicating that follicular phase estradiol supplementation has no effect on circulating cortisol levels (Saltzman et al., 2006) and marmoset female sexual behavior (Barnett et al., 2006), but contrasts with a study conducted by Kendrick and Dixson (1985). Kendrick and Dixson (1985), however, applied mid-cycle levels of estradiol supplementation equivalent to pre-ovulatory peak levels (~940 pg/mL). They also showed that pre-ovulatory estradiol levels are associated with elevated cortisol levels and frequent display of proceptive sexual behavior (Kendrick and Dixson, 1984). The lower follicular phase circulating estradiol levels in our study (~396 pg/mL), chosen to facilitate a modest baseline of sexual behavior, may be responsible for the lack of obvious estradiol-induced HPA-axis and behavioral changes in the present study.

Potential mechanisms of HPA axis sensitization

A neuroendocrine 5-HT_{1A} challenge test, serving as peripheral indicator of central 5-HT_{1A} receptor function (Power and Cowen, 1992; Van de Kar, 1997; Cowen, 2000), was applied to test the responsiveness of the HPA axis to an acute activation of hypothalamic 5-HT_{1A} receptors. It is well known that activation of hypothalamic 5-HT_{1A} receptors stimulates the HPA axis (Przegaliński et al., 1989; Raap et al., 2002; Osei-Owusu et al., 2005). While we show that both circulating ACTH and cortisol levels increase immediately following an injection of 0.1 mg/kg 8-OH-DPAT (SC), neither chronic flibanserin nor chronic 8-OH-DPAT treatments alter ACTH and cortisol responses in this neuroendocrine test (Fig. 1). Thus, repeated administration of flibanserin and 8-OH-DPAT, both agonists at post-synaptic 5-HT_{1A} receptors, did not alter

sensitivity and function of hypothalamic 5-HT_{1A} receptors and the downstream HPA axis response.

Alterations in modulatory (descending) or activating (ascending) systems that regulate CRH/AVP release from the PVN may contribute to stress responses elicited by restraint, as is the case for the limbic system comprising amygdala and hippocampus (Van de Kar et al., 1991; De Kloet et al., 1998), as well as the catecholaminergic system of the brainstem comprising noradrenergic and adrenergic neurons of the nucleus of the solitary tract (Pacák et al., 1993; Pacák and Palkovits, 2001) and locus coeruleus (Vermetten and Bremner, 2002). All of these brain areas express 5-HT_{1A} receptors (Azmitia et al., 1996; Wang et al., 1997; Popova et al., 1998) and project to the PVN (De Kloet et al., 1998; Pacák and Palkovits, 2001), thus becoming potential substrates to flibanserin- and 8-OH-DPAT-induced alterations that modulate HPA axis responsiveness to restraint. In rats, both flibanserin and 8-OH-DPAT have been shown to alter neuronal activity in addition to serotonin, norepinephrine and dopamine neurotransmitter levels in a brain region-specific manner (Lejeune and Millan, 2000; Allers et al., 2010). Chronic flibanserin and 8-OH-DPAT treatments could sensitize the descending and ascending systems described above, leading to enhanced activation of CRH-containing neurons of the PVN during restraint and consequently to the observed exaggerated HPA axis response.

Flibanserin vs 8-OH-DPAT: Differences in pharmacological profiles and HPA responses to restraint

Although both flibanserin and 8-OH-DPAT enhance the excitability of the HPA axis during restraint, subtle differences in ACTH and cortisol response profiles exist between flibanserin and 8-OH-DPAT treated females. Both treatments enhance ACTH responses to the 30-minute restraint stressor, while chronic 8-OH-DPAT, but not flibanserin, also elevates cortisol levels. Increases in circulating ACTH levels relative to vehicle control values occur at both 15 and 30 minutes of restraint in 8-OH-DPAT-treated females, while only at 30 minutes in flibanserin-treated females, indicating a durational difference in the stress response distinguishing a more prolonged HPA axis activation during chronic 8-OH-DPAT compared to chronic flibanserin exposure.

Differences in HPA responses to restraint likely reflect the differences in pharmacological profiles between flibanserin and 8-OH-DPAT. Both flibanserin and 8-OH-DPAT are 5-HT_{1A} agonists. 8-OH-DPAT, however, activates both pre- and postsynaptic 5-HT_{1A} receptors (Palego et al., 2000), while flibanserin activates postsynaptic 5-HT_{1A} receptors (Borsini et al., 1995b) without activating presynaptic 5-HT_{1A} receptors in the raphé nuclei (Marazziti et al.,

2002). Flibanserin thus lacks presynaptic inhibition of 5-HT neurotransmission (Borsini et al., 2002). Flibanserin is additionally an antagonist on 5-HT_{2A} receptors and is possibly a weak partial agonist on dopamine D_4 receptors (Borsini et al. 1995a; Borsini et al., 2002). 8-OH-DPAT is devoid of 5-HT_{2A} (Borsini et al., 1995a) and dopaminergic (Arvidsson et al., 1981) actions, but shows additional 5-HT_7 agonist properties (Shen et al., 1993).

These differences in receptor specificity result in fundamental differences in pharmacology and in abilities of flibanserin and 8-OH-DPAT to induce functional changes in a brain region-specific manner. For example, flibanserin inhibits forskolin-stimulated cAMP formation in the cortex, while 8-OH-DPAT does not affect cortical cAMP accumulation (Borsini et al., 1995a). Flibanserin decreases neuronal firing rate in the rat cortex regardless of whether the presynaptic receptor-containing dorsal raphé nucleus is intact, while the effects of 8-OH-DPAT are dependent upon intact raphé serotonergic neurons (Borsini et al., 1995b). Taken together, flibanserin and 8-OH-DPAT display different regional selectivity in the brain, and they differentially affect neuronal function in 5-HT projection sites.

It is unclear how differences in receptor binding profiles between flibanserin and 8-OH-DPAT translate into a prolongation of cortisol and acceleration of ACTH responses to restraint stress induced by 8-OH-DPAT compared to flibanserin. Additional activation of 5-HT_7 receptors by 8-OH-DPAT may have a stimulatory effect on the HPA axis (Jørgensen et al., 1999), which could explain the increased efficacy in HPA activation by 8-OH-DPAT compared to flibanserin. Alternatively, differences in negative feedback mechanisms may account for the observed differences in hormone levels. The restoration of basal cortisol levels after a stress response is achieved by negative feedback mediated by cortisol binding to glucocorticoid receptors (GR) in limbic structures, PVN of the hypothalamus, and anterior pituitary (De Kloet et al., 1998). Onset of negative feedback, however, varies depending on the modulatory input from the limbic system to the PVN. Genomic GR effects in the hippocampus suppress excitatory β-adrenergic actions and enhance inhibitory effects of 5-HT on the HPA axis (De Kloet et al., 2008). Due to their distinct actions on 5-HT receptor subtypes, flibanserin and 8-OH-DPAT thus may differentially modulate the GR-mediated hippocampal input to the PVN. Indeed, activation of 5-HT_7 increases GR expression in primary hippocampal cell cultures (Laplante et al., 2002), supporting the hypothesis that enhanced cortisol release after restraint may be mediated by chronic 5-HT_7 activation in 8-OH-DPAT-treated females. Another explanation could involve peripheral mechanisms. It is suggested that systemic effects of 5-$HT_{2A/2C}$ agonists may be partially mediated by 5-HT_{2A} receptors in the adrenal cortex (Rittenhouse et al.,

1994; Welch and Saphier, 1994), or through a sympathetic catecholaminergic mechanism (Welch and Saphier, 1994). Flibanserin may reduce the sensitivity of peripheral 5-HT_{2A} receptors to endogenous 5-HT during restraint, or may diminish sympathetic catecholaminergic stimulation of the adrenal cortex, thus preventing a comparable degree of cortisol elevation following restraint induced by 8-OH-DPAT.

Behavioral correlates of enhanced stress reactivity in 8-OH-DPAT-treated marmosets

The difference in ACTH response dynamics between flibanserin and 8-OH-DPAT is of particular significance given the correlation analysis with behavior. 8-OH-DPAT-treated females with the greatest ACTH response at the early measurement point of the restraint test (at 15 minutes) also experienced the most between-partner aggression, attracted the least genital investigation by their male pairmates, and most frequently rejected the pairmate's sexual advances, causing an increased number of unsuccessful male mount attempts (Fig. 3). These associations were absent at the later measurement point of the restraint test (at 30 minutes). Thus, 8-OH-DPAT treated females that rapidly develop aggravated endocrine markers of stress (i.e. elevated ACTH beyond vehicle control values at 15 minutes) also display symptoms of aversive pair behavior proportional to the magnitude of 8-OH-DPAT-induced increase in ACTH responses. Enhanced stress reactivity may thus contribute to decreased female sexual receptivity and heightened state of aggression in 8-OH-DPAT-, but not in flibanserin-treated female marmosets.

While exposure to a stressful environment is associated with impaired sexual behavior and elevated aggression in female rats (Yoon et al., 2005), and personal distress and distress in partner relations are hallmarks of HSDD in women (DSM-IV-TR, 2000), the link between HPA axis activity and aggression is not clear. In some human studies high aggression is associated with low HPA axis activity (Gordis et al., 2006), but others do not confirm such an inverse relationship between aggression and stress hormone levels (Schulz et al., 1997). It is therefore not clear whether the behavioral associations with increased HPA responsiveness to restraint in the current study reflect HPA axis hormone-mediated changes in behavior, or whether both behavior and HPA hormones serve as separate and reliable biomarkers of 8-OH-DPAT-mediated action.

CONCLUSIONS

Our findings are the first to demonstrate enhanced HPA axis responsiveness to a restraint-type stressor after chronic serotonergic modulation in a nonhuman

primate. Considering the receptor binding profiles of flibanserin and 8-OH-DPAT, similarities in their effects on HPA axis sensitization are likely mediated by their shared postsynaptic 5-HT_{1A} agonist property. Chronic 8-OH-DPAT, but not flibanserin, however, leads to rapid aggravated ACTH responses to stress that may contribute to anti-sexual and anti-social behavior between the 8-OH-DPAT-treated female and her male pairmate. Flibanserin, in contrast, dissociates such ACTH-marked stress reactivity from prolonged cortisol responses and aversive pair behavior possibly due to its unique regional selectivity in 5-HT_{1A} receptor activation, combined with concurrent 5-HT_{2A} antagonist activity. Thus, despite endocrine similarities in terms of enhanced ACTH responses to restraint stress, flibanserin-treated females show regular cortisol profiles and strengthening of the affiliative pair-bond with their male pairmates in contrast to the HPA axis and behavioral phenotypes of 8-OH-DPAT-treated females.

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