Diminished Cortisol Levels in Subordinate Female Marmosets Are Associated with Altered Central Drive to the Hypothalamic-Pituitary-Adrenal Axis

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Background: *Female marmosets offer a promising primate model of stress-related hypocortisolism, as they undergo chronic reductions in circulating cortisol after becoming subordinate in a social group. In this study, we treated dominant and subordinate female marmosets with the cortisol synthesis inhibitor metyrapone and corticotropin-releasing factor (CRF) to characterize the effects of subordination on central regulation of the hypothalamic-pituitary-adrenal (HPA) axis.*

Methods: *Seven dominant and six subordinate female marmosets received CRF (6 μg/kg, intravenous [IV]), following pretreatment with metyrapone (75 mg/kg, by mouth [PO]) or water. Plasma adrenocorticotropic hormone (ACTH) and cortisol concentrations were determined before metyrapone or water treatment and before, 1 hour after, and 2 hours after CRF treatment.*

Results: *Following metyrapone treatment, subordinates had similar cortisol levels to dominants but significantly higher ACTH levels. During CRF challenges, cortisol concentrations were lower and ACTH concentrations higher in subordinates, although net integrated responses to CRF did not differ. Cortisol-to-ACTH ratios were consistently lower in subordinates.*

Conclusions: *These results confirm previous findings of low cortisol concentrations and blunted adrenal responsiveness in subordinates and suggest that when differences in cortisol levels are eliminated, subordinates exhibit exaggerated hypothalamic drive to the pituitary. These neuroendocrine alterations in subordinate marmosets resemble those in posttraumatic stress disorder patients and adult survivors of child abuse.*

Key Words: Metyrapone, adrenocorticotropic hormone, corticotropin-releasing factor, stress, posttraumatic stress disorder, subordination

Hypocortisolism, or insufficient secretion of cortisol by the adrenal cortex, is a pathophysiological condition associated with numerous stress-related disorders, including posttraumatic stress disorder (PTSD), chronic fatigue syndrome, fibromyalgia, and atypical depression (Gold et al 2002; Heim et al 2000; Kasckow et al 2001; Yehuda 2002). Although its exact significance in these conditions is not clear, chronic hypocortisolism is well known to produce such symptoms as weakness, fatigue, and myalgias, and has been suggested to play a key role in the pathogenesis of stress-related disorders (Heim et al 2000; Orth and Kovacs 1998; Raison and Miller 2003). In spite of the potentially broad clinical relevance of hypocortisolism and the growing appreciation of its prevalence, however, few animal models are available for investigation of its underlying neuroendocrine mechanisms and clinical consequences.

One potential model of stress-related hypocortisolism is the socially subordinate female common marmoset (*Callithrix jacchus*). Both in the field and in captivity, these small-bodied New World monkeys live in small social groups in which, typically, only a single, behaviorally dominant female reproduces (reviewed by French 1997; Saltzman 2003). Behaviorally subordinate females usually fail to breed as a consequence of socially induced anovulation and inhibition of sexual behavior and instead provide alloparental care for the infants of the dominant female marmoset (Abbott et al 1997; French 1997; Saltzman 2003). Anovulatory subordinate females also undergo pronounced, chronic reductions in circulating cortisol concentrations (Abbott et al 1997; Johnson et al 1996; Saltzman et al 1994, 1998, 2004, Saltzman et al, in press). When groups of unrelated adult males and females are formed in the laboratory, a dominance hierarchy is usually established in each sex within 1 week (Abbott 1986; Saltzman et al 1994), during which time plasma cortisol levels may rise precipitously in females that are actively competing for dominance status (Saltzman et al 1994). Thereafter, circulating basal cortisol returns to pregroup formation levels in newly dominant females but drops well below pre-group formation levels within 6 to 7 weeks in newly subordinate females (Saltzman et al 1994). These socially induced reductions in cortisol may persist for months to years (Saltzman et al 1998) but are not known to have any pathophysiological consequences.

The mechanisms underlying diminished cortisol levels in subordinate female marmosets are not fully understood. Circulating basal adrenocorticotropic hormone (ACTH) concentrations do not differ between dominants and subordinates (Johnson et al 1996; Saltzman et al 2004), suggesting that adrenocortical responsiveness to ACTH is lower in subordinates than dominants. Consistent with this possibility, we have found that subordinate female marmosets secrete less cortisol than dominant females in response to exogenous ACTH (Saltzman et al 2000).

Indirect evidence suggests that central regulation of the hypothalamic-pituitary-adrenal (HPA) axis may also be altered in subordinate female marmosets. The finding that these animals do not have elevated circulating ACTH levels in association with their low cortisol levels suggests either that ACTH secretion is restrained by enhanced sensitivity to cortisol negative feedback (i.e., steroid-dependent suppression) or that the pituitary corticotropes receive decreased stimulation from the brain or are hyporesponsive to secretagogs, independent of cortisol negative feedback (i.e., steroid-independent suppression). In a series of dexamethasone suppression tests, we found no evidence of differential efficacy of glucocorticoid negative feedback on

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ACTH or cortisol (Saltzman et al 2004). Thus, reduced basal cortisol levels in subordinate female marmosets may instead be mediated by altered central release of corticotropin-releasing factor (CRF) or other ACTH secretagogs (e.g., vasopressin) or by altered pituitary responsiveness to these secretagogs. Decreased stimulation of ACTH release by the pituitary might be masked by the reduced negative-feedback signal stemming from low endogenous cortisol concentrations, resulting in maintenance of normal ACTH levels in conjunction with diminished cortisol levels (e.g., Yehuda 2002).

In this study, we tested the hypothesis that subordinate female marmosets exhibit impaired pituitary responsiveness to CRF, by characterizing the ACTH and cortisol responses of dominant and subordinate females to exogenous CRF. We performed CRF stimulation tests both with and without pretreatment with metyrapone, an inhibitor of glucocorticoid biosynthesis, to discern the role of endogenous cortisol in restraining ACTH secretion and to minimize the effects of cortisol negative feedback on responses to CRF.

Methods and Materials

Animals

We used 13 captive-born, adult female common marmosets: 7 socially dominant females undergoing regular ovulatory cycles and 6 anovulatory subordinate females. Dominant and subordinate females did not differ ($p > .05$) in body mass (419 ± 13 vs. 436 ± 20 g, respectively; mean ± SEM) or age (29.8 ± 2.4 vs. 25.2 ± .9 months, respectively) at the outset of data collection. They were housed in groups containing two or three unrelated females and one or two gonadally intact adult males. Social groups were formed as described previously (Saltzman et al 1998) ≥2 months prior to data collection. Dominance hierarchies in such groups typically are established within 1 week and may remain stable for several years or more (Abbott 1986; Abbott and George 1991; Saltzman et al 1994). Assessment of dominant and subordinate status, based on directionality of submissive behaviors (Saltzman et al 1994, 1996), was confirmed by the occurrence of ovulatory cycles in dominant female marmosets and anovulation in subordinate females, based on plasma progesterone concentrations in blood samples collected twice each week (Saltzman et al 1994). Subordinates were anovulatory for ≥70 days prior to data collection and had not exhibited elevated plasma progesterone concentrations (>10 ng/mL), characteristic of the luteal phase of the ovarian cycle or early pregnancy, for ≥62 days.

Marmosets were housed indoors at the National Primate Research Center at the University of Wisconsin–Madison, with lights on from 0630 to 1830 hours, ambient temperature at approximately 27°C, and humidity at approximately 50%. Most of the animals occupied aluminum and wire mesh cages measuring 61 × 91 × 183 cm, 122 × 61 × 183 cm, or 61 × 61 × 183 cm; however, one dominant and one subordinate female were housed in a larger room measuring 310 × 141 × 250 cm. Animals were fed Mazuri Callitrichid High Fiber Diet 5M16 (Purina Mills, St. Louis, Missouri) supplemented with fruit, cereal, nuts, and miniature marshmallows. Marmosets were fed once daily between 1230 and 1430 hours, and water was available ad libitum. Additional information on marmoset housing and husbandry is provided by Saltzman et al (1998).

CRF Stimulation Tests

Each marmoset underwent two CRF stimulation tests: one following pretreatment with metyrapone, an 11β-hydroxylase inhibitor that blocks the final step in cortisol biosynthesis (metyrapone condition), and one following pretreatment with water (control condition). At least 30 days elapsed between the two tests on each animal, and the order of tests was approximately balanced across animals.

For each CRF stimulation test, animals were given 75 mg/kg metyrapone (Sigma-Aldrich, St. Louis, Missouri) in approximately .3 mL sterile water or an equivalent volume of water without metyrapone, by oral gavage at 0700 to 0730 hours, followed by a second, identical treatment 3 hours later. Three hours after the second metyrapone or water treatment, ovine CRF (National Hormone and Peptide Program, Torrance, California) (6 μg/kg in approximately .08 mL sterile saline) was injected intravenously (IV). Metyrapone and CRF doses were based on results of a published study (Johnson et al 1996) and pilot tests (Saltzman et al, unpublished data). Blood samples (.4 mL) were collected immediately before the first metyrapone or water treatment, immediately before CRF treatment (= 3 hours after the second metyrapone or water treatment), and 60 and 120 minutes following CRF treatment. All samples were assayed for ACTH and cortisol. Marmosets were returned to their home cages after each metyrapone or water treatment, but following CRF injection, each animal was kept individually in a nestbox from its home cage until the end of data collection.

For collection of blood samples, animals were briefly restrained in a marmoset restraint tube (Hearn 1977), to which they had been adapted extensively, while blood was collected in a heparinized syringe by femoral venipuncture. Blood samples were immediately placed on ice and subsequently processed as described by Orth (1979) for subsequent ACTH assay: samples were centrifuged at 4200 rpm for 15 minutes at 4°C, and the plasma fraction was removed and centrifuged again at 9000 rpm for 10 minutes at 4°C. The plasma fraction was again removed and stored at −80° until assayed (Saltzman et al 2004).

Monitoring and Control of Ovarian Function

Progesterone was assayed in samples collected twice weekly, at 3- to 4-day intervals, from each female marmoset. Ovulation was considered to have occurred on the day before a sustained (≥2 consecutive blood samples) elevation of progesterone above 10 ng/mL (Harlow et al 1983; Saltzman et al 1994). As commonly practiced in our laboratory, to prevent term pregnancies and to ensure that all dominant female marmosets were in the early to mid-follicular phase of the ovarian cycle during CRF stimulation tests, we gave each dominant animal an intramuscular (IM) injection of 1.0 μg cloprostenol sodium (Estrumate, Mobay Corporation, Shawnee, Kansas, and Cayman Chemicals, Ann Arbor, Michigan), a prostaglandin F2α analog, 14 to 45 days after each ovulation and, correspondingly, 3 to 4 days before each CRF stimulation test. This treatment causes luteolysis and termination of the luteal phase or early pregnancy (Summers et al 1985). Subordinate female marmosets were treated with the same dose of cloprostenol 3 to 4 days before each CRF stimulation test to control for any potential effects of cloprostenol on HPA activity. This treatment has not been found to alter baseline cortisol levels in marmosets (Saltzman et al 1998).

Hormone Assays

All hormone assays were fully validated for use with marmoset plasma (Saltzman et al 1994, 2004). Plasma cortisol concentrations were determined in duplicate aliquots by radioimmunoassay (RIA) using an antibody-coated-tube kit (GammaCoat, DiaSorin Corporation, Stillwater, Minnesota), as described previ-

ously (Saltzman et al 1994). Assay sensitivity at 90% binding was .1 ng, and intra-assay and interassay coefficients of variation (CVs) of a plasma pool assayed in quadruplicate in each assay (33% binding) were 6.0% and 13.4%, respectively ($n = 6$ assays).

Plasma progesterone levels were measured in duplicate aliquots using a heterologous enzyme immunoassay (Saltzman et al 1994). Assay sensitivity at 90% binding was 5.2 pg, and intra-assay and interassay CVs of a marmoset plasma pool (39.5% binding) were 4.8% and 20.6%, respectively.

Plasma ACTH concentrations were determined by RIA, as previously described (Saltzman et al 2004). Assay sensitivity at 90% binding was .61 pg. Intra-assay and interassay CVs of a marmoset plasma pool assayed in quadruplicate in each assay (31.3% binding) were 2.5% and 18.5%, respectively.

Analysis

Data were normally distributed and therefore were analyzed by analysis of variance (ANOVA), with group (dominant vs. subordinate) treated as a between-groups factor and treatment (metyrapone vs. water) and time of sample (before first metyrapone or water treatment, after second metyrapone or water treatment, 1 hour after CRF, 2 hours after CRF) treated as within-groups factors. Post hoc analyses were performed by univariate F tests. For each hormone, we initially performed two repeated-measures ANOVAs: one examining the response to metyrapone or water, using only the blood samples collected immediately before and after metyrapone or water treatment, and one examining the response to CRF, using the blood samples collected immediately before, 1 hour after, and 2 hours after CRF treatment. To evaluate net integrated cortisol and ACTH responses to CRF, we determined area under the curve (AUC) using the method of Pruessner et al (2003) for AUC with respect to increase. Finally, to evaluate whether adrenal responsiveness to ACTH was blunted in subordinate female marmosets, we determined the ratio of cortisol concentration to the log of ACTH concentration in the same blood sample and compared this ratio between dominants and subordinates across the four blood samples collected in the control condition. The cortisol:log ACTH ratio has been used as a measure of adrenal responsiveness to ACTH in studies of rats (Wilkinson et al 1981), monkeys (Lyons et al 1995), and humans (Sherman et al 1985), based on findings that adrenal glucocorticoid concentrations and secretion rates increase linearly with logarithmic increases in ACTH in bioassay systems (see Lyons et al 1995 and references therein). Analyses were performed using Systat v. 5.2 for the Macintosh (SPSS, Chicago, Illinois), and results were evaluated at the .05 level (two-tailed).

Results

Cortisol

Response to Metyrapone. Overall, circulating cortisol concentrations were higher in dominant female marmosets than subordinate females [main effect of group: $F(1,11) = 36.880$, $p = .0001$] and declined from approximately 0700 hours (before the first metyrapone or water treatment) to approximately 1300 hours (after the second metyrapone or water treatment) [main effect of time: $F(1,11) = 77.514$, $p < .0001$; Figure 1]. Metyrapone accentuated this decline in dominant but not subordinate females [group × time interaction: $F(1,11) = 9.130$, $p = .012$]. Post hoc tests revealed that dominant females had significantly higher basal cortisol levels than subordinates prior to metyrapone ($p = .0003$) or water treatment ($p = .007$) and after water treatment ($p = .014$) but not after metyrapone treatment ($p = .212$). Thus, metyrapone caused a greater decrease in circulating cortisol levels in dominant than subordinate female marmosets, from a higher baseline level to a comparable metyrapone-suppressed level.

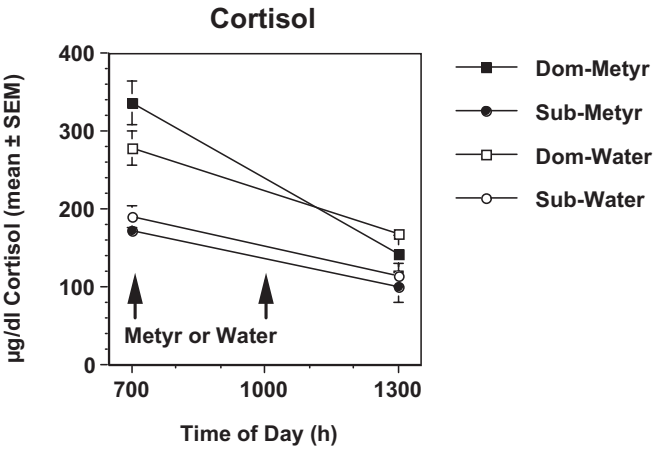

Figure 1. Plasma cortisol concentrations (mean ± SEM) immediately before the initial treatment (approximately 0700 hours) and 3 hours after the second treatment (approximately 1000 hours) with metyrapone (75 mg/kg in .3 mL water, PO) or water alone (.3 mL, PO) in seven socially dominant and six socially subordinate female marmosets. See text for statistical results. PO, by mouth.

Response to CRF. A repeated-measures three-way ANOVA comparing cortisol levels between dominant and subordinate female marmosets immediately before, 1 hour after, and 2 hours after CRF injection in the metyrapone and control conditions indicated that metyrapone blocked the cortisol response to exogenous CRF [treatment × time interaction: $F(2,22) = 37.895$, $p < .0001$. As shown in Figure 2, cortisol levels rose progressively in response to CRF after pretreatment with water but not after pretreatment with metyrapone. Dominant females had higher cortisol levels than subordinates [main effect of group: $F(1,11) = 21.041$, $p = .0008$] across all three time points (group × time interaction: $p > .9$) and both treatment conditions (group × treatment interaction: $p > .7$; group × treatment × time interaction: $p > .9$). Net integrated cortisol responses to CRF were significantly greater in the control than the metyrapone condition [142.41 vs. −2.07 µg/dL − hour, respectively; $F(1,11) = 68.329$, $p < .0001$] and did not differ between dominant and subordinate females (main effect of group: $p > .6$; group × condition interaction: $p > .9$).

ACTH

Response to Metyrapone. Circulating ACTH concentrations were significantly higher in the metyrapone condition than in the control condition [main effect of treatment: $F(1,11) = 26.138$, $p = .0003$], and this effect differed between the two time points [before vs. after metyrapone or water treatment; treatment × time interaction: $F(1,11) = 7.272$, $p = .021$] and, marginally, between dominant and subordinate female marmosets [group × treatment interaction: $F(1,11) = 4.711$, $p = .053$; Figure 3]. Post hoc tests revealed that although subordinate females tended to have higher ACTH concentrations than dominant females both before and after metyrapone or water treatment, this difference was significant only after treatment with metyrapone ($p = .050$). Thus, metyrapone caused a greater increase in circulating ACTH concentrations in subordinate than dominant female marmosets,

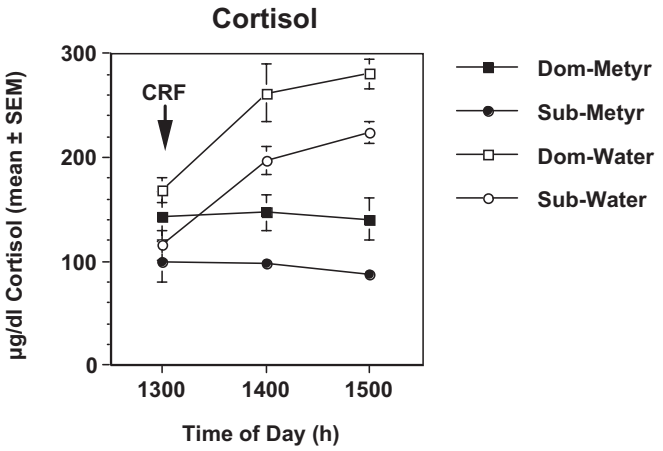

Figure 2. Plasma cortisol concentrations (mean ± SEM) immediately before, 1 hour after, and 2 hours after CRF injection (6 µg/kg, IV; approximately 1300 hours) in seven socially dominant and six socially subordinate female marmosets, following pretreatment with either metyrapone or water at approximately 0700 and 1000 hours. Note that the first time point in this figure corresponds to the second time point in Figure 1. See text for statistical results. CRF, corticotropin-releasing factor; IV, intravenous.

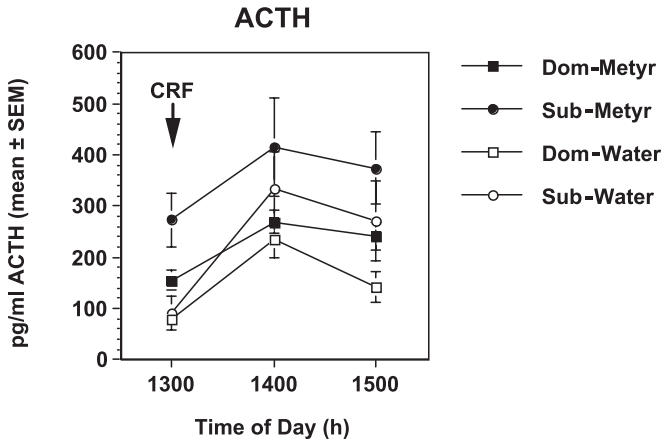

Figure 4. Plasma ACTH concentrations (mean ± SEM) immediately before, 1 hour after, and 2 hours after CRF injection (6 µg/kg, IV; approximately 1300 hours) in seven socially dominant and six socially subordinate female marmosets, following pretreatment with either metyrapone or water at approximately 0700 and 1000 hours. Note that the first time point in this figure corresponds to the second time point in Figure 3. See text for statistical results. ACTH, adrenocorticotropic hormone; CRF, corticotropin-releasing factor.

from a comparable baseline level to a higher post-metyrapone level.

Response to CRF. Corticotropin-releasing factor injection significantly elevated plasma ACTH concentrations [main effect of time: $F(2,22) = 25.178$, $p < .0001$], with ACTH levels peaking 1 hour after CRF treatment and declining by 2 hours after treatment (Figure 4). Across all three time points, ACTH levels were higher in the metyrapone condition than in the control condition [main effect of treatment: $F(1,11) = 4.807$, $p = .051$] and were higher in subordinate than dominant female marmosets [main effect of group: $F(1,11) = 7.126$, $p = .0218$]. None of the interactions among group, treatment, and time were significant. Net integrated ACTH responses to CRF did not differ between groups ($p > .1$) or between treatments ($p > .2$; group × treatment interaction: $p > .4$).

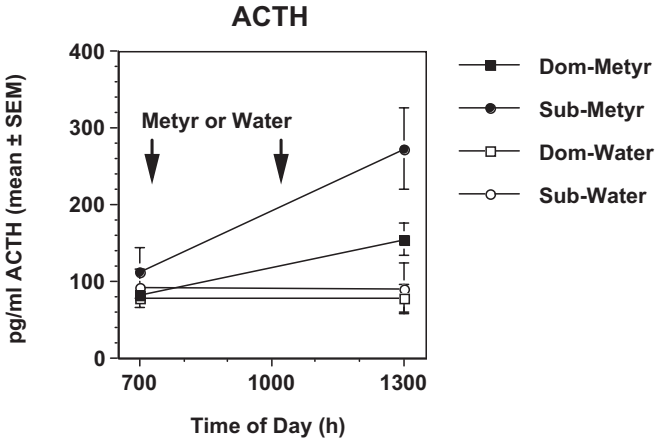

Figure 3. Plasma ACTH concentrations (mean ± SEM) immediately before the initial treatment (approximately 0700 hours) and 3 hours after the second treatment (approximately 1000 hours) with metyrapone (75 mg/kg in .3 mL water, PO) or water alone (.3 mL, PO) in seven socially dominant and six socially subordinate female marmosets. See text for statistical results. ACTH, adrenocorticotropic hormone; PO, by mouth.

Cortisol:Log ACTH Ratio

The ratio of cortisol concentration to the log of ACTH concentration, a measure of adrenocortical responsiveness to ACTH, changed over time in the control condition [main effect of time: $F(3,33) = 13.342$, $p < .0001$], as ratios declined significantly from 0700 hours to 1300 hours ($p = .002$), remained low at 1400 hours (1 hour after CRF treatment; $p = .004$ vs. 0700 hours), and increased by 1500 hours (2 hours after CRF treatment; $p = .002$ vs. 1400 hours; Figure 5). Cortisol:log ACTH ratios across all four time points were lower in subordinate females than in dominant female marnosets [main effect of group: $F(1,11) = 17.517$, $p = .002$; group × time interaction: $p > .6$].

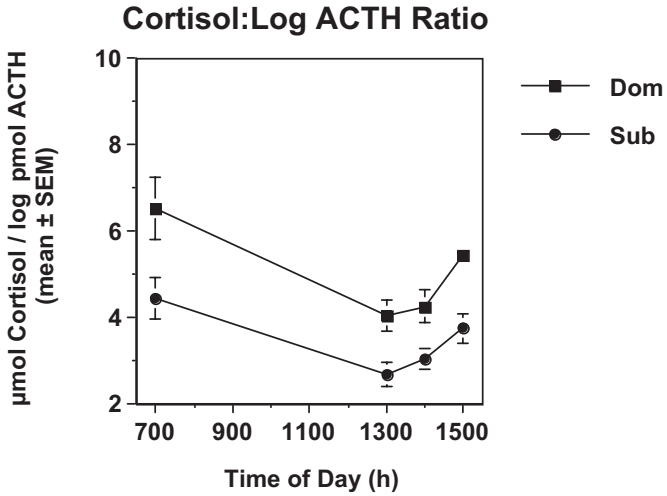

Figure 5. Ratio of plasma cortisol concentrations to the log of plasma ACTH concentrations (mean ± SEM) in the control condition, immediately before water treatment (approximately 0700 and 1000 hours), and immediately before, 1 hour after, and 2 hours after CRF injection (6 µg/kg, IV; approximately 1300 hours) in seven socially dominant and six socially subordinate female marmosets. See text for statistical results. ACTH, adrenocorticotropic hormone; IV, intravenous; CRF, corticotropin-releasing factor.

Discussion

The results of this study provide the first clear evidence that chronically reduced basal cortisol levels in socially subordinate female marmosets may be associated with altered central regulation of the HPA axis. Similar to previous findings by ourselves and others (Abbott et al 1997; Johnson et al 1996; Saltzman et al 1994, 1998, 2004, Saltzman et al, in press), under baseline conditions, subordinate females in this study had lower circulating cortisol concentrations than dominant females but similar ACTH concentrations. Following administration of the cortisol synthesis inhibitor metyrapone, however, cortisol dropped to comparable levels in the two groups, while ACTH rose to significantly higher levels in subordinates. Thus, when differences in the endogenous glucocorticoid milieu were removed, subordinate female marmosets appeared to have exaggerated hypothalamic stimulation of ACTH secretion, as compared with dominant females. Moreover, subordinate females maintained higher ACTH levels than dominant female marmosets following CRF administration, either with or without metyrapone pretreatment. Interpretation of these latter findings, however, is complicated by the fact that differences in cortisol concentrations between dominants and subordinates re-emerged during CRF stimulation tests, so that ACTH responses to CRF by dominant females may have been attenuated in their relatively higher cortisol levels.

We have previously demonstrated that subordinate female marmosets have blunted cortisol responses to exogenous ACTH as compared with dominant females (Saltzman et al 2000), confirmed by our findings of reduced cortisol:log ACTH ratios in subordinates in the present study. Furthermore, we have suggested that dominant and subordinate females do not differ in the efficacy of glucocorticoid negative feedback, as ACTH and cortisol responses to dexamethasone, a synthetic glucocorticoid, did not differ between the two groups (Saltzman et al 2004). In contrast, the present finding that metyrapone caused a smaller decrease in cortisol but a greater increase in ACTH in subordinates than in dominants suggests that the HPA axis may be under stronger negative feedback control in subordinates. Because dexamethasone penetrates the brain poorly and acts preferentially at the pituitary (De Kloet 1997), it is possible that our previous study (Saltzman et al 2004) failed to detect centrally mediated differences in glucocorticoid negative feedback and that subordinate females do, in fact, show enhanced negative feedback effects in the brain compared with dominants. Enhanced negative feedback alone, however, would not explain why ACTH levels were significantly higher in subordinates than in dominants following metyrapone treatment, when cortisol levels did not differ between the two groups.

An alternative explanation for our findings is that subordinate female marmosets have exaggerated steroid-independent hypothalamic drive to the pituitary, compared with dominant females. Such CRF hyperdrive would be expected to induce downregulation of pituitary CRF receptors and therefore to reduce pituitary responsiveness to CRF (Heim et al 2000; Fries et al 2005). The resulting decline in ACTH secretion would likely be masked by the reduced negative feedback signal stemming from low endogenous cortisol concentrations (e.g., Yehuda 2002; see also Parker et al 2003). We did not find clear evidence of altered ACTH responses to CRF in subordinate females: although ACTH levels were consistently higher in subordinates than dominants across CRF stimulation tests, net integrated responses to CRF did not differ. Because dominant females had higher cortisol levels than subordinate females during CRF stimulation tests, however, it is possible that differences in pituitary responsiveness were obscured by differences in the cortisol negative feedback signal. Indeed, the finding that subordinate females did not exhibit enhanced ACTH responses to CRF, in spite of their low cortisol levels, may be interpreted as evidence that pituitary responsiveness to CRF is blunted in subordinates. This hypothesis will require further testing in additional studies.

As in previous studies of marmosets and other small-bodied New World primates (Coe et al 1992), plasma cortisol concentrations were an order of magnitude higher than those in Old World primates, including humans. Free cortisol levels are likely to be equally high, as marmosets have extremely low circulating levels of corticosteroid-binding globulin (Klosterman et al 1986; Pugeat et al 1984; Robinson et al 1985). These high levels of free cortisol appear, however, to be compensated for by partial glucocorticoid resistance (Chrousos et al 1986). Relative resistance to glucocorticoids in New World primates is associated with overexpression of the intracellular FK506-binding protein (FKBP51), which inhibits glucocorticoid binding to the glucocorticoid receptor (Denny et al 2000; Scammell et al 2001), and possibly with diminished functionality of the glucocorticoid receptor (Her et al 2005).

The results of this study suggest that the neuroendocrine mechanisms underlying hypocortisolism in subordinate female marmoset monkeys may be similar, although not identical, to those in posttraumatic stress disorder (PTSD: reviewed by Kasckow et al 2001; Yehuda 2002). Numerous studies have found that individuals with PTSD have low circulating or urinary cortisol concentrations. As in subordinate female marmosets, low cortisol in these patients is not associated with abnormalities in basal ACTH levels but may be associated with blunted adrenocortical responsiveness to ACTH (Kanter et al 2001) and elevated ACTH responses to metyrapone (Yehuda et al 1996; but see Kanter et al 2001). In contrast to subordinate female marmosets, however, PTSD patients typically exhibit exaggerated negative feedback effects of dexamethasone on ACTH and cortisol. Posttraumatic stress disorder patients have also been reported to exhibit elevated cerebrospinal fluid CRF concentrations (Baker et al 1999; Bremner et al 1997) and blunted, augmented, or normal ACTH responses to exogenous CRF (Kellner et al 2003; Rasmusson et al 2001; Smith et al 1989). Together, the results from humans have been interpreted as evidence that hypocortisolism in PTSD is associated with hypothalamic hypersecretion of CRF, which leads to downregulation of pituitary CRF receptors and impaired pituitary responsiveness to CRF (Kasckow et al 2001; Yehuda 2002). In contrast, hypocortisolism in several other stress-related disorders, such as atypical depression, chronic fatigue syndrome, and fibromyalgia, appears to result from hyposecretion rather than hypersecretion of hypothalamic CRF (Claes 2004; Gold et al 2002; Parker et al 2001).

Our results in subordinate female marmosets are also similar to findings from women who were abused during childhood but who do not suffer from major depressive disorder (reviewed by Shea et al 2004). These women, either with or without PTSD, may exhibit basal hypocortisolism but normal basal ACTH levels, blunted cortisol responses to exogenous ACTH, and elevated ACTH but diminished cortisol levels following CRF treatment (Heim et al 2001; Newport et al 2004). Moreover, in contrast to PTSD patients but similar to subordinate female marmosets, nondepressed survivors of child abuse show normal responsiveness to standard and low-dose dexamethasone suppression tests, as compared with nonabused, psychiatrically healthy women (Newport et al 2004). The mecha-

nisms underlying HPA dysregulation in subordinate female marmosets may, therefore, mirror those in adult survivors of child abuse. It is not yet known whether subordinate female marmosets exhibit other neuroendocrine alterations seen in PTSD patients and/or victims of child abuse, such as altered responsiveness to acute stress, elevated cerebrospinal concentrations of CRF, or increased activation of the sympathetic nervous system (Penza et al 2003; Yehuda 2000, 2002).

In female marmosets, as in PTSD patients and adult survivors of child abuse, dysregulation of the HPA axis appears to be triggered by an identifiable, possibly traumatic event: the onset of social subordination during the acute period of group formation (Saltzman et al 1994). Establishment of an intrasexual dominance hierarchy during this period may be accompanied by transient elevations in circulating cortisol levels in dominant as well as subordinate females, possibly associated with engagement in wounding aggression, but leads, within 6 to 7 weeks, to diminished basal cortisol levels only in subordinates (Saltzman et al 1994). Also, as with PTSD and child abuse (Penza et al 2003; Yehuda 2000), the development and maintenance of HPA dysregulation does not depend on persistence of the initial triggering event. Indeed, we have argued, based on the typically low frequency and low intensity of aggression, high level of social support available, and absence of classic stress-related physical and physiological symptoms (i.e., low body mass, hyperprolactinemia), that subordinate female marmosets in laboratory groups are not subject to chronic stress (Abbott et al 1997, 2003). Instead, we have proposed that reproductive suppression, and perhaps also cortisol diminution, in subordinate female marmosets is maintained by a fear conditioning process, in which subordinates initially undergo physiological suppression in response to aggression, harassment, and intimidation received from the dominant female during the acute phase of group formation. Subordinates subsequently learn to associate olfactory and perhaps visual cues from the dominant female marmoset with this aggression and intimidation, and these cues then become conditioned stimuli that maintain long-term physiological suppression in subordinates (Abbott et al 1997). Interestingly, fear conditioning has also been implicated in the pathogenesis of PTSD (Bremner et al 1995; Li et al 2005).

In summary, the results of this study indicate that socially induced reductions of basal cortisol levels in subordinate female marmosets may be associated with enhanced central drive to the HPA axis, possibly resulting in diminished pituitary responsiveness to CRF. Based on these results as well as our previous findings (Saltzman et al 2000, 2004), we propose that social subordination induces two regulatory changes in the HPA axis of female marmosets, thereby leading to chronic reductions in basal cortisol levels: 1) diminished adrenal responsiveness to ACTH and 2) exaggerated CRF secretion by the hypothalamus. The opposing effects of a decreased cortisol negative feedback signal and increased CRF stimulation of the pituitary, possibly accompanied by blunted pituitary responsiveness, may permit the maintenance of normal basal ACTH concentrations in association with markedly reduced basal cortisol concentrations. These patterns are similar to those seen in PTSD patients and adult survivors of child abuse, suggesting that the subordinate female marmoset may be a valuable primate model for investigations of the neuroendocrine causes and consequences of stress-related hypocortisolism.

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