PERGAMON

Psychoneuroendocrinology 25 (2000) 463–477

PNEC

www.elsevier.com/locate/psyneuen

Reduced adrenocortical responsiveness to adrenocorticotropic hormone (ACTH) in socially subordinate female marmoset monkeys

Wendy Saltzman [a, b,*], Shelley L. Prudom [a], Nancy J. Schultz-Darken [a], David H. Abbott [a, b, c]

[a] *Wisconsin Regional Primate Research Center, University of Wisconsin-Madison, Madison, WI 53715-1299, USA*

[b] *Endocrinology–Reproductive Physiology Program, University of Wisconsin-Madison, Madison, WI 53715-1299, USA*

[c] *Department of Obstetrics and Gynecology, University of Wisconsin-Madison, Madison, WI 53715-1299, USA*

Received 25 August 1999; accepted 7 December 1999

Abstract

Socially subordinate female common marmoset monkeys undergo pronounced, chronic reductions in basal plasma cortisol levels, which appear to result both from socially induced suppression of reproductive hormones and from direct effects of social subordination. In this study, we tested the hypothesis that this cortisol suppression is mediated by reduced adrenocortical responsiveness to adrenocorticotropic hormone (ACTH). Dominant, subordinate, and ovariectomized females were given dexamethasone (5 mg/kg, IM), followed the next morning by human $ACTH_{1–39}$ (10 μg/kg, IV) or sterile saline (0.5 ml/kg, IV); blood samples were collected at −20 through 150 min from ACTH or saline treatment and assayed for cortisol. ACTH, but not saline, caused a marked elevation of plasma cortisol levels. Prior to ACTH treatment, dominant females tended to have higher dexamethasone-suppressed cortisol levels than subordinate and ovariectomized females. After ACTH treatment, dominant females had significantly higher cortisol concentrations, as well as higher peak and net integrated cortisol responses to ACTH, than did subordinate and ovariectomized animals; the latter two groups showed comparable cortisol responses to ACTH. These results suggest that dampened adrenocortical responsiveness to ACTH contributes to chronic reductions in cortisol levels in subordi-

* Corresponding author. Tel.: +1-608-263-3515; fax: +1-608-263-4031.
E-mail address: saltzman@primate.wisc.edu (W. Saltzman).

PII: S0306-4530(00)00003-2

nate female marmosets and may be mediated by suppression of reproductive hormones.

Keywords: ACTH; Cortisol; Adrenal cortex; Marmosets; Social subordination; Ovariectomy

1. Introduction

Chronic dysfunction of the hypothalamo–pituitary–adrenal (HPA) axis is associated with numerous psychopathological conditions. For example, elevations of HPA activity frequently accompany major depressive disorder (Chrousos et al., 1987), panic disorder (Abelson and Curtis, 1996), and anorexia nervosa (Licinio et al., 1996), whereas suppression of circulating glucocorticoid levels may be associated with post-traumatic stress disorder (Yehuda, 1998) and chronic fatigue syndrome (Demitrack et al., 1991). Studies of HPA dysregulation in human psychopathology have typically focused on centrally mediated mechanisms, such as altered release of corticotropin-releasing hormone (CRH) from the hypothalamus or altered feedback of glucocorticoids on the brain. While these centrally mediated effects contribute to chronic changes in glucocorticoid secretion, direct effects at the level of the adrenal cortex have also been implicated (e.g. Amsterdam et al., 1983; Charlton and Ferrier, 1989; Ehrhart-Bornstein et al., 1998; Jaeckle et al., 1987; Scott et al., 1999). The specific role of adrenocortical disruptions in psychopathology has received little attention, and little is known of their mechanisms.

One novel, naturally occurring model of chronic alterations in HPA function is found in the common marmoset (*Callithrix jacchus*), a small New World monkey that exhibits pronounced social regulation of both HPA and reproductive function. Social groups may contain 2–4 adult females, but typically only the dominant female produces offspring. In both wild and captive groups, behaviorally subordinate females usually fail to breed and instead provide care for the offspring of the dominant female (reviewed by French, 1997). These subordinate females are frequently anovulatory and hypoestrogenemic as a result of inadequate pituitary release of luteinizing hormone (LH; Abbott et al., 1981, 1988). The endocrine consequences of subordination are both rapid and reversible: when a cycling female is introduced into a social group in which she becomes behaviorally subordinate, plasma LH levels decline precipitously in 1–4 days and ovulatory cycles soon cease. Upon removal from the social group, anovulatory subordinate females show an equally rapid reversal of these endocrine changes and usually ovulate within 2–3 weeks (Abbott and George, 1991; Abbott et al., 1988). Socially subordinate females can remain anovulatory and hypoestrogenemic for 2 years or more (Abbott and George, 1991; unpublished data).

In contrast to several other primate species, in which subordinate individuals have elevated HPA activity and appear to be subjected to greater stress than dominants, socially subordinate female marmosets undergo pronounced suppression of plasma cortisol levels (Abbott et al., 1997; Johnson et al., 1996; Saltzman et al., 1994, 1998). When females undergoing ovulatory cycles are introduced into a new social group

in which they become behaviorally subordinate and anovulatory, morning basal cortisol levels drop significantly within 6–7 weeks, to approximately 45% of the levels typical of cycling females (Abbott et al., 1997; Saltzman et al., 1994, 1998; see also Johnson et al., 1996); females that become dominant in new groups, in contrast, do not show sustained changes in plasma cortisol levels (Abbott et al., 1997; Saltzman et al., 1994). The dramatic suppression of cortisol levels in subordinates can persist for months to years and appears to be mediated in part by suppression of reproductive hormones (Saltzman et al., 1994, 1998; unpublished data): females that are anovulatory but not subordinate to other females also exhibit cortisol suppression (Saltzman et al., 1994). Moreover, plasma cortisol levels in female marmosets change systematically across the ovarian cycle, suggesting that HPA activity is modulated by reproductive hormones in this species (Saltzman et al., 1998). Social subordination per se, however, may also exert a direct inhibitory influence on HPA activity. We have recently found that plasma cortisol levels of ovary-intact subordinate females are suppressed not only as compared to dominant females undergoing ovulatory cycles, but also as compared to pair-housed ovariectomized females of comparable ages and body weights (Saltzman et al., 1998; unpublished data). These ovariectomized animals are neither dominant nor subordinate to other females and have significantly lower plasma estrogen levels than ovary-intact dominants and subordinates (Saltzman et al., 1998).

In spite of the pronounced differences in basal cortisol levels between dominant and subordinate females, initial studies have found no differences in basal plasma adrenocorticotropic hormone (ACTH) levels (Abbott et al., 1998; Johnson et al., 1996; Saltzman et al., 1997; unpublished data). These findings suggest that subordinate females may have dampened adrenocortical responsiveness to ACTH. In this study, therefore, we tested the hypothesis that suppression of basal cortisol levels in subordinate female marmosets is mediated, at least in part, by reduced cortisol responses to ACTH. To do this, we characterized plasma cortisol levels before and after injection of a standardized dose of ACTH, following treatment with dexamethasone the previous day to suppress endogenous ACTH and cortisol secretion. We compared subordinate females' cortisol levels with those of both dominant females and long-term ovariectomized animals, to assess the effects of both subordinate social status and low gonadal hormone levels on adrenal responsiveness to ACTH.

2. Methods

2.1. Animals

We used a total of 28 captive-born, adult female common marmosets (*Callithrix jacchus jacchus*), including 11 socially dominant females undergoing ovulatory cycles, eight anovulatory subordinates, and nine long-term ovariectomized, pair-housed females. For the ACTH Challenge condition, in which we compared cortisol responses to exogenous ACTH among groups, we used eight dominant, six subordinate, and eight ovariectomized females. Dominant, subordinate, and ovariectomized

animals in this condition did not differ significantly in body mass (394.9±19.0 vs. 422.2±22.5 vs. 424.9±18.6 g, respectively; mean±SE; $F[2,19]=0.74$, $p=0.49$) but did differ in adult age at the time of the study ($F[2,19]=3.87$, $p<0.05$): ovariectomized females (54.20±5.76 months) were significantly older than subordinate females (35.10±3.16 months, $p<0.05$, Tukey test), whereas dominant females (42.92±4.29 months) were intermediate in age and did not differ significantly from the other two groups. For the Saline Control condition, we used three dominant, two subordinate, and three ovariectomized females, including two ovariectomized animals that had been used previously in the ACTH Challenge condition.

Dominant and subordinate animals were housed in groups containing two or three unrelated adult females and one or two gonadally intact adult males. Groups had been formed as described previously (Saltzman et al., 1998), 11.78±1.75 months prior to data collection. Dominance hierarchies in such groups typically are established within 1–2 weeks and may remain stable for several years or more (Abbott, 1986; unpublished data). Behavioral assessments of dominant and subordinate status, based on directionality of submissive behaviors (Saltzman et al., 1994, 1996), were confirmed by the occurrence of ovulatory cycles in dominant females and anovulation in subordinate females, based on plasma progesterone concentrations in blood samples collected twice weekly (Saltzman et al., 1994; see below). Subordinates had not ovulated (see below) for at least 73 days prior to data collection and had not exhibited elevated plasma progesterone concentrations (>10 ng/ml; see below), characteristic of the luteal phase of the ovarian cycle, for at least 66 days.

Ovariectomized females had been pair-housed with a gonadally intact male for 23.62±5.71 months prior to data collection and had undergone bilateral removal of the ovaries and Fallopian tubes at least 6.94 months before the study began (23.93±5.23 months). Ovariectomy was performed by midline incision under Saffan anesthesia (8.1 mg alphaxalone:2.7 mg alphadolone acetate, IM; Pitman-Moore, Harefield, Uxbridge, Middlesex, UK). Ovariectomized females had not undergone ovarian hormone-replacement treatment at any time.

Animals were housed indoors, with lights on from 06:00–18:00 h, 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, three of the animals were housed in a larger room (363×212×218 cm). Animals were fed once daily between 13:00 and 15:00 h, and water was available ad libitum. Additional information on marmoset housing and husbandry is provided by Saltzman et al. (1998).

2.2. Experimental design

To ensure that all dominant females were in the early to mid-follicular phase of the ovarian cycle during the experiment, we gave each dominant animal an IM injection of 1.0 μg cloprostenol sodium (Estrumate, Mobay Corp., Shawnee, KS), a prostaglandin F2α analog, 3–4 days before data collection, which was timed to occur 15–45 days following the previous ovulation. This treatment causes luteolysis and termination of the luteal phase or early pregnancy (Summers et al., 1985). We also

injected the ovariectomized females and subordinate females with the same dose of cloprostenol 3–4 days before data collection, to control for any possible effects of cloprostenol on HPA activity. This treatment has not been found to alter baseline cortisol levels in marmosets (Saltzman et al., 1998).

On the day prior to data collection, each animal was weighed and given an IM injection of 5 mg/kg dexamethasone sodium phosphate (DEX; American Regent Laboratories, Shirley, NY) at approximately 15:20–16:00 h to suppress endogenous ACTH and cortisol secretion. This dose of DEX has been found to suppress plasma cortisol and ACTH levels in female marmosets for 2–3 days (unpublished data). On the day of data collection, each animal received an IV injection of either 10 μg/kg synthetic human $ACTH_{1–39}$ (Sigma, St. Louis, MO) in 0.5 ml/kg sterile saline (ACTH Challenge condition) or an equivalent volume of sterile saline without ACTH (Saline Control condition) at 09:00–09:20 h. Previous studies have shown that this dose of ACTH produces a robust but sub-maximal cortisol response in female marmosets (unpublished data). Blood samples (0.1–0.3 ml) were collected by femoral puncture at −20 (baseline), 20, 40, 60, 80, 100, 120, and 150 min from the ACTH or saline injection and immediately placed on ice. 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 into a heparinized syringe by femoral puncture. Between samples, each marmoset remained alone in a stainless steel nestbox, which also served as a transport cage. Blood samples were centrifuged at 2000 rpm for 10 min, and the plasma fraction was separated and stored at −20°C until assayed.

Plasma cortisol concentrations in each blood sample were determined in duplicate aliquots by radioimmunoassay using an antibody-coated-tube kit (GammaCoat™, Incstar® Corp., Stillwater, MN) as described previously (Saltzman et al., 1994). Assay sensitivity at 90% binding was 0.1 ng per tube (1.0 μg/dl), and intra- and inter-assay coefficients of variation (CVs) of a plasma pool assayed in quadruplicate in each assay (40% binding) were 4.03% and 9.96%, respectively (n=7 assays).

2.3. Monitoring of ovarian function

To monitor ovarian function in the months prior to this experiment, we collected blood samples twice each week, at 3- to 4-day intervals, from all intact females, for measurement of plasma progesterone levels (Saltzman et al., 1994). Animals were manually captured from the home cage and placed in a restraint tube while 0.1–0.3 ml blood was collected by femoral puncture into a heparinized syringe.

Plasma progesterone levels in twice-weekly blood samples were measured in duplicate aliquots using a heterologous enzyme immunoassay (Saltzman et al., 1994). Assay sensitivity at 90% binding was 3.6 pg per tube (2.7 ng/ml), and intra- and inter-assay CVs of a marmoset plasma pool (38% binding) were 6.13% and 20.85%, respectively (n=1193 assays). Ovulation was considered to have occurred on the day before a sustained (≥2 consecutive blood samples) elevation of progesterone levels above 10 ng/ml (Harlow et al., 1983). To prevent term pregnancies, we gave each

dominant female an IM injection of 0.75–1.0 μg cloprostenol 14–45 days after each ovulation.

2.4. Statistical analysis

To reduce heterogeneity of variance and increase normality of the data, cortisol concentrations were log-transformed (Sokal and Rohlf, 1995). Data from the ACTH Challenge condition were initially analyzed by two-way ANOVA, with groups (dominant, subordinate, ovariectomized) treated as a between-groups factor and time from ACTH injection treated as a within-groups factor. Following significant omnibus ANOVA results, we performed post hoc analyses using the Tukey HSD test and univariate F tests. To determine net integrated cortisol responses to ACTH across time, we calculated the area under the curve following ACTH treatment, using the trapezoid method. Net integrated and peak cortisol responses to ACTH, and cortisol responses to saline in the Saline Control condition, were log-transformed and analyzed by one-way ANOVA.

Statistical analyses were performed using Systat version 5 for the Macintosh. For all analyses, significance was assessed at the .05 level. Cortisol data are presented as back-transformed means ±95% confidence limits.

3. Results

3.1. ACTH Challenge condition

Plasma cortisol levels differed markedly among groups ($F[2,19]=11.56$, $p<0.001$) and across time from ACTH injection ($F[7,133]=255.70$, $p<0.001$). As shown in Fig. 1, ACTH caused a rapid and pronounced elevation of cortisol in all three groups of animals. Post hoc univariate F tests revealed that for all three groups combined, cortisol levels at all seven timepoints following ACTH injection were significantly higher than those at −20 min (p values<0.001). Moreover, post hoc comparisons of consecutive pairs of timepoints indicated that after ACTH treatment, cortisol levels rose steadily from 20 to 80 min (p values<0.05) and plateaued at 80–100 min, before declining from 100 to 150 min (p values<0.05; see Fig. 1). Finally, Tukey tests revealed that, overall, cortisol levels of dominant females were significantly higher than those of both subordinate and ovariectomized females (p values<0.005), whereas the latter two groups did not differ from one another ($p=0.82$). Consistent with previous findings, plasma cortisol levels of marmosets were approximately 5–10 times higher than those of humans and other Old World primates (Coe et al., 1992).

To further characterize differences among groups in the adrenal response to ACTH, we compared the peak cortisol level from each animal (Fig. 2). Peak cortisol concentrations differed significantly among groups ($F[2,19]=5.99$, $p<0.01$) and were significantly higher in dominant females than in either subordinate ($p=0.01$) or ovariectomized ($p<0.05$) animals; the latter two groups did not differ from one another

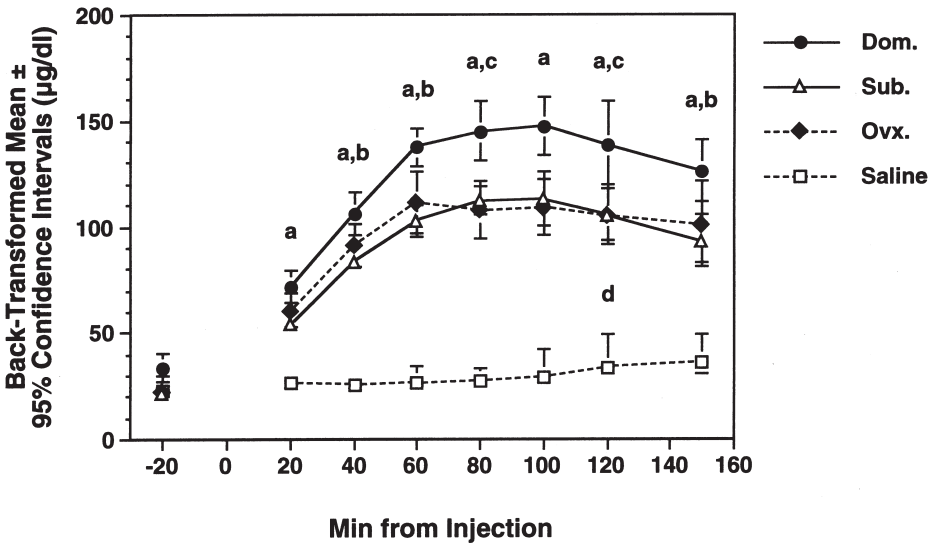

Fig. 1. Plasma cortisol levels (back-transformed means±95% confidence intervals) of dominant (n=8), subordinate (n=6) and ovariectomized (n=8) female marmosets before and after injection of human $ACTH_{1-39}$ (10 µg/kg, IV), or of a total of eight female marmosets before and after injection of physiological saline (0.5 ml/kg, IV). Dexamethasone (5 mg/kg, IM) was administered the previous day to suppress endogenous cortisol and ACTH secretion. For the ACTH Challenge condition: a, $p<0.001$ vs. −20 min; b, $p<0.001$ vs. preceding timepoint; c, $p<0.05$ vs. preceding timepoint. For the Saline Control condition: d, $p<0.05$ vs. preceding timepoint.

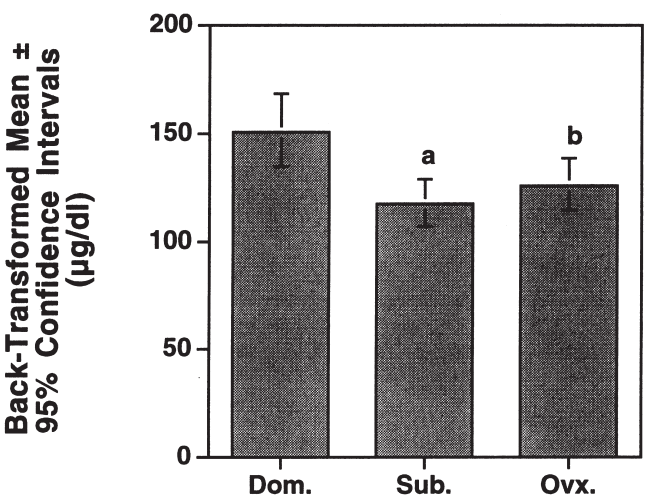

Fig. 2. Peak plasma cortisol responses (back-transformed means±95% confidence intervals) of dominant (n=8), subordinate (n=6) and ovariectomized (n=8) female marmosets to human $ACTH_{1-39}$ (10 µg/kg, IV), following suppression with dexamethasone (5 mg/kg, IM) the previous day: a, p=0.01 compared with dominants; b, $p<0.05$ compared with dominants.

(p=0.66). The time from ACTH injection at which cortisol levels peaked did not differ significantly among groups (94±6 min, F[2,19]=0.12, p=0.89).

To evaluate possible differences in net integrated cortisol responses to ACTH over time, we compared the area under the curve for cortisol levels following ACTH injection (Fig. 3). This measure revealed significant differences among groups (F[2,19]=4.24), $p<0.05$): dominant females had higher integrated cortisol responses to ACTH than both subordinate and ovariectomized females, but these pairwise differences were only marginally significant (p values=0.0579, 0.0532, respectively). Again, subordinate and ovariectomized animals did not differ reliably from one another (p=0.99).

The finding that cortisol levels showed a significant main effect of group, but not a group×time interaction, in the omnibus ANOVA indicated that cortisol levels differed among groups both before and after ACTH injection (Fig. 1). This was confirmed by a separate one-way ANOVA, which revealed a significant difference among groups in cortisol levels at −20 min (F[2,19]=3.69, $p<0.05$). Dominant females tended to have somewhat higher cortisol levels prior to ACTH injection than both subordinate (p=0.07) and ovariectomized (p=0.08) females, but these pairwise differences were not statistically significant. We next performed a series of Pearson correlations to determine whether each animal's peak cortisol response to ACTH was related to its cortisol concentration 20 min before ACTH treatment. These analyses did not reveal a significant relationship between peak cortisol responses to ACTH and initial DEX-suppressed cortisol levels (dominant: r=0.48, df=6, p=0.23; subordinate: r=−0.04, df=4, p=0.93; ovariectomized: r=−0.14, df=6, p=0.74; all groups combined: r=0.38, df=20, p=0.08). Similarly, additional Pearson correlations did not

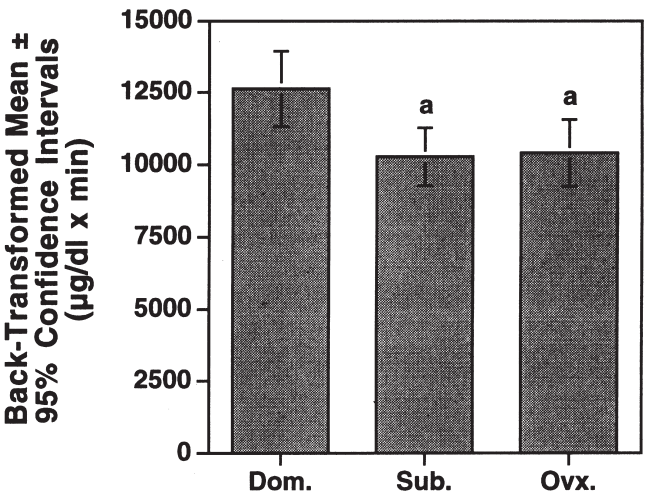

Fig. 3. Net integrated cortisol responses (back-transformed means±95% confidence intervals) of dominant (n=8), subordinate (n=6) and ovariectomized (n=8) female marmosets to human $ACTH_{1-39}$ (10 µg/kg, IV), following suppression with dexamethasone (5 mg/kg, IM) the previous day: a, $0.05<p<0.06$ compared with dominants.

reveal a significant relationship between each animal's net integrated cortisol response to ACTH and its cortisol concentration prior to ACTH treatment (dominant: $r=-0.12$, df=6, $p=0.77$; subordinate: $r=-0.25$, df=4, $p=0.63$; ovariectomized: $r=-0.41$, df=6, $p=0.32$; all groups combined: $r=0.09$, df=20, $p=0.69$).

To determine whether the relatively older ages of the ovariectomized females may have contributed to their low cortisol levels, we examined possible age effects on morning basal plasma cortisol concentrations in a total of nine female marmosets, including five dominant females in the early to mid-follicular phase of the ovarian cycle and four long-term ovariectomized, pair-housed animals. For each animal, we compared basal cortisol levels in two blood samples collected 14–28 months apart (mean±SE: 19.60±1.38 months, comparable to the maximum age difference between groups in the ACTH Challenge condition—see Section 2; age range of these nine animals: 26.18–68.19 months at first sample, 44.61–92.50 months at second sample). Cortisol levels did not change significantly across time (main effect of time: $F[1,7]=0.19$, $p=0.68$; group×time interaction: $F[1,7]=0.23$, $p=0.65$). Using a different data set, we additionally performed Pearson correlations to determine whether basal morning plasma cortisol levels were correlated with age in 14 dominant females in the early to mid-follicular phase of the ovarian cycle (age range: 26.33–65.33 months), 16 anovulatory subordinates (17.99–53.06 months), and 10 long-term ovariectomized females (42.14–96.94 months). Correlations for each group were weak and non-significant (r values: -0.28–0.01, p values>0.3), further indicating that basal plasma cortisol levels do not change across the age range of animals used in this study.

3.2. Saline Control condition

Cortisol levels showed a significant, but small, change across time during the Saline Control condition ($F[7,49]=3.17$, $p<0.01$; Fig. 1). In contrast to the clear pattern of changes across time following ACTH treatment in the ACTH Challenge condition, however, post hoc univariate F-tests revealed that the only significant pairwise difference was between cortisol levels at 100 and 120 min following saline injection ($p<0.05$).

To further clarify the difference between cortisol responses to ACTH and saline, we performed treatment conditions (ACTH vs. saline) × time ANOVAs for dominant females and for subordinate and ovariectomized females together, since these latter two groups showed no differences from one another. Each of these analyses revealed a highly significant effect of treatment condition (dominant: ($F[1,9]=43.38$, $p<0.001$); subordinate+ovariectomized: ($F[1,17]=69.06$, $p<0.001$)) and of time (dominant: ($F[7,63]=49.47$, $p<0.001$); subordinate+ovariectomized: ($F[7,119]=47.08$, $p<0.001$)) as well as a treatment condition × time interaction (dominant: ($F[7,63]=49.37$, $p<0.001$); subordinate+ovariectomized: ($F[7,119]=17.81$, $p<0.001$)). Subsequent t-tests revealed that cortisol levels did not differ prior to injection of ACTH and saline (dominant: ($T[9]=0.56$, $p=0.59$); subordinate+ovariectomized: ($T[17]=0.51$, $p=0.61$)) but that mean cortisol levels were significantly higher after

ACTH than after saline injection (dominant: (T[9]=7.69, $p<0.001$); subordinate+ovariectomized: (T[17]=8.11, $p<0.001$)).

4. Discussion

Female common marmosets show pronounced, chronic suppression of basal circulating cortisol levels as a consequence of becoming behaviorally subordinate in a social group and undergoing social suppression of reproductive function (Abbott et al., 1997; Johnson et al., 1996; Saltzman et al., 1994, 1998). This adrenocortical suppression can be mimicked, to some extent, by ovariectomizing non-subordinate females, indicating that withdrawal of reproductive hormones, as well as direct effects of social subordination, contributes to chronic suppression of adrenocortical activity in these animals (Saltzman et al., 1998).

Results of the present study indicate that in both subordinate and ovariectomized females, cortisol suppression may be mediated, at least in part, by reduced responsiveness of the adrenal cortex to ACTH. Compared with socially dominant females in the follicular phase of the ovarian cycle, both subordinate and ovariectomized animals had significantly lower peak cortisol responses to exogenous ACTH and marginally significantly lower net integrated responses. Importantly, however, no differences were found between cortisol responses of subordinate and ovariectomized females.

Interpretation of the present results is complicated by the finding that cortisol levels differed somewhat among groups before ACTH injection, at −20 min. The most obvious explanation for this finding is that dominant, subordinate, and ovariectomized females may have differed in their negative-feedback sensitivity to the high dose of DEX that was administered. However, a separate series of studies, in which we examined morning plasma cortisol before and after DEX treatment, indicated that dominant females may show reduced cortisol responsiveness to a low dose of DEX (0.5 mg/kg), compared with subordinate and ovariectomized females, but do not appear to be more resistant to cortisol suppression in response to higher doses of DEX (1.0 or 5.0 mg/kg; Saltzman et al., 1997; unpublished data). Moreover, if the observed differences among groups in cortisol responses to ACTH reflected differences in the degree of HPA suppression initially induced by DEX, then we would expect to find a significant positive correlation between marmosets' initial, DEX-suppressed cortisol levels, prior to ACTH treatment, and their overall (peak, net integrated) responses to ACTH. The absence of such a relationship in the present study strongly suggests that reduced cortisol responses to ACTH in subordinate and ovariectomized females were not caused by or closely linked to these animals' relatively low pre-ACTH cortisol levels.

Another possible explanation for the observed differences in cortisol levels is that subordinate and ovariectomized females had reduced endocrine responses to the capture and handling procedures used in our experiment, compared with dominants. This is unlikely, however, as DEX strongly inhibits ACTH and glucocorticoid responses to stressors in several species (e.g. dog: Keller-Wood et al., 1988; rat:

Nicholson et al., 1984). Furthermore, we have shown previously that the procedures involved in capture and blood sampling are not a potent stressor to the animals in our colony (Saltzman et al., 1994). Thus, differences in the endocrine stress–response are unlikely to account for the present findings.

Because subordinate and ovariectomized females showed virtually identical cortisol responses to exogenous ACTH (although both differed from dominant females), altered reproductive function, rather than social subordination per se, may be the critical factor reducing adrenocortical responsiveness. One possibility is that adrenal steroidogenesis is dampened by chronic reductions in circulating estrogen levels. Plasma estrogen concentrations are significantly reduced in both subordinate and ovariectomized female marmosets, as compared to females undergoing ovulatory cycles (Kendrick and Dixson, 1984; Saltzman et al., 1998), and both anatomical and physiological evidence supports a direct effect of estrogen on the adrenal cortex. In several species, the adrenal cortex expresses estrogen receptors (e.g. rat: Calandra et al., 1980; brushtail possum: Weiss and Xu, 1990; see also Müller and Wotiz, 1978), and in rhesus monkeys, estrogen receptor expression is particularly high in the zona fasciculata, the adrenocortical zone primarily responsible for secretion of glucocorticoids (Hirst et al., 1992). Moreover, in vitro studies have indicated that estrogen can stimulate adrenal steroidogenesis. For example, adrenal slices from oophorectomized female rats produced less corticosterone than those from intact females, and produced more corticosterone following in vivo or in vitro estradiol treatment than in the absence of estradiol (Kitay, 1963a,b). Estrogen also potentiated the steroidogenic effects of ACTH on adrenal slices and adrenal homogenates from hypophysectomized female rats; however, estrogen did not alter adrenal steroidogenesis in the absence of ACTH in these preparations (Colby and Kitay, 1974), suggesting that stimulatory effects of estrogen on the adrenal cortex are mediated by altered ACTH responsiveness.

Another reproductive hormone that might influence adrenocortical steroidogenesis is LH. In a recent study, we found that plasma concentrations of LH, but not estradiol, estrone, or progesterone, were a significant predictor of morning basal cortisol levels in ovariectomized and subordinate female marmosets (Saltzman et al., 1998). Plasma LH levels of both ovariectomized and subordinate females are significantly lower than those of cycling females in either the periovulatory or luteal phase of the ovarian cycle but not the follicular phase (Saltzman et al., 1998; see also Abbott et al., 1988). Effects of LH on HPA activity have not been studied extensively; however, a growing body of evidence indicates that LH can alter glucocorticoid secretion, and that these effects are mediated, at least in part, by direct stimulatory effects on the adrenal. For example, LH can raise circulating corticosterone levels in rats in vivo (Phillips and Poolsanguan, 1978), and can stimulate glucocorticoid release from both rat and opossum adrenal tissue in vitro (Vinson and Renfree, 1975; Vinson et al., 1976). Similarly, human chorionic gonadotropin (CG), which is structurally and functionally homologous to LH, increases cortisol release from isolated guinea-pig adrenal cells (O'Connell et al., 1994). LH has also been implicated in causing hypertrophy of cellular organelles in the zona fasciculata and zona glomerulosa, and in increasing adrenal steroidogenic capacity in rats (Chung, 1978). Moreover, LH/CG receptor

transcripts and receptor protein have recently been identified in the zona fasciculata of human adrenal glands (Pabon et al., 1996), and LH receptors have recently been identified in adrenal tumors from transgenic mice expressing the Simian Virus 40 T-antigen (Rilianawati et al., 1998).

In contrast to the present study, in which subordinate and ovariectomized females showed no differences in adrenal responsiveness to ACTH, previous studies have revealed significantly lower basal plasma cortisol levels in subordinates than in ovariectomized animals (Saltzman et al., 1998; unpublished data). This suggests that suppression of basal cortisol levels in subordinate female marmosets may involve at least two mechanisms. One of these mechanisms appears to involve reduced adrenocortical responsiveness to ACTH and may result directly from hypogonadotropic hypogonadism and chronic hypoestrogenism secondary to social subordination. The second mechanism may be independent of altered gonadotropin and ovarian hormone levels and may result in an additional lowering of cortisol concentrations below those of ovariectomized females. This mechanism is likely to be a direct neural consequence of social subordination mediated by the central nervous system, possibly resulting in altered hypothalamic release of CRH, arginine vasopressin, or other neuroendocrine factors influencing HPA function. Consistent with this possibility, preliminary data suggest that ovariectomized marmosets may have higher basal plasma ACTH levels than either dominant or subordinate females (unpublished data). Thus, ovariectomized, but not subordinate, females may compensate for reduced cortisol secretion with increased pituitary release of ACTH, suggesting that subordinate females may undergo suppression at the level of the pituitary and/or hypothalamus in addition to the adrenal cortex. Alternatively, suppression of cortisol in subordinates below levels seen in ovariectomized animals may result from a direct neural effect on the adrenal cortex. In numerous mammalian species, sympathetic innervation of the adrenal cortex can modulate adrenal steroidogenesis, including responsiveness to ACTH (reviewed by Ehrhart-Bornstein et al., 1998; Engeland, 1998). It is intriguing to speculate that social subordination in female marmosets may result in altered sympathetic activation of adrenal steroidogenesis.

Acknowledgements

This research was supported by NIH grants RR00167 and HD07678, and NSF grant IBN-9604321. We thank S.A. Brice, E.L. Duhr, and J.J. Haegele for maintenance of the marmoset colony; F.H. Wegner and D.J. Wittwer for assistance with hormone assays; J.C. Ramer, C.M. O'Rourke, and J.A. Vanderloop for veterinary care; T. Garland, Jr., for statistical advice; I.M. Bird for valuable discussion of the data and manuscript; and two anonymous reviewers for helpful comments on the manuscript. Preparation of the manuscript was facilitated by the staff and resources of the Wisconsin Regional Primate Research Center (WRPRC) Library. Marmosets were maintained in accordance with the recommendations of the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*, and experimental protocols were reviewed and approved by the Graduate School Animal Care and

Use Committee of the University of Wisconsin-Madison. The WRPRC is accredited by AAALAC as part of the UW-Madison Graduate School. This is publication number 39-020 of the WRPRC.

References

Abbott, D.H., 1986. Social suppression of reproduction in subordinate marmoset monkeys *(Callithrix jacchus jacchus*). In: De Mello, M.T. (Ed.), A Primatologia No Brasil-2. Anais do 2nd Congresso Brasileiro de Primatologia. Sociedade Brasileira de Primatologia, Brasilia, pp. 15–31.

Abbott, D.H., George, L.M., 1991. Reproductive consequences of changing social status in female common marmosets. In: Box, H.O. (Ed.), Primate Responses to Environmental Change. Chapman and Hall, London, pp. 295–309.

Abbott, D.H., Hodges, J.K., George, L.M., 1988. Social status controls LH secretion and ovulation in female marmoset monkeys (*Callithrix jacchus*). J. Endocrinol. 117, 329–339.

Abbott, D.H., McNeilly, A.S., Lunn, S.F., Hulme, M.J., Burden, F.J., 1981. Inhibition of ovarian function in subordinate female marmoset monkeys (*Callithrix jacchus jacchus*). J. Reprod. Fertil. 63, 335–345.

Abbott, D.H., Saltzman, W., Schultz-Darken, N.J., Smith, T.E., 1997. Specific neuroendocrine mechanisms not involving generalized stress mediate social regulation of female reproduction in cooperatively breeding marmoset monkeys. Ann. New York Acad. Sci. 807, 219–238.

Abbott, D.H., Saltzman, W., Schultz-Darken, N.J., Tannenbaum, P.L., 1998. Adaptations to subordinate status in female marmoset monkeys. Comp. Biochem. Physiol. C 119, 261–274.

Abelson, J.L., Curtis, G.C., 1996. Hypothalamic–pituitary–adrenal axis activity in panic disorder: 24-hour secretion of corticotropin and cortisol. Arch. Gen. Psychi. 53, 323–331.

Amsterdam, J.D., Winokur, A., Abelman, E., Lucki, I., Rickels, K., 1983. Cosyntropin (ACTHα_{1-24}) stimulation test in depressed patients and healthy subjects. Am. J. Psychiat. 140, 907–909.

Calandra, R.S., Lüthy, I., Finocchiaro, L., Terrab, R.C., 1980. Influence of sex and gonadectomy on sex steroid receptors in rat adrenal gland. J. Steroid Biochem. 13, 1331–1335.

Charlton, B.G., Ferrier, I.N., 1989. Hypothalamo–pituitary–adrenal axis abnormalities in depression: a review and a model. Psych. Med. 19, 331–336.

Chrousos, G.P., Luger, A., Avgerinos, P.C., Kling, M.A., Oldfield, E.H., Nieman, L.K., Schürmeyer, T., Loriaux, D.L., Gold, P.W., 1987. Corticotropin releasing hormone: relevance to human physiology and pathophysiology. In: D'Agata, R., Chrousos, G.P. (Eds.) Recent Advances in Adrenal Regulation and Function. Raven Press, New York, pp. 121–135.

Chung, K.W., 1978. Effects of tropic hormones on ultrastructure and synthesis of androgens in adrenals of male pseudohermaphrodite rats. Cell. Tiss. Res. 189, 469–477.

Coe, C.L., Savage, A., Bromley, L.J., 1992. Phylogenetic influences on hormone levels across the Primate order. Am. J. Primatol. 28, 81–100.

Colby, H.D., Kitay, J.I., 1974. Interaction of estradiol and ACTH in the regulation of adrenal corticosterone production in the rat. Steroids 24, 527–536.

Demitrack, M.A., Dale, J.K., Straus, S.E., Laue, L., Listwak, S.J., Kruesi, M.J.P., Chrousos, G.P., Gold, P.W., 1991. Evidence for impaired activation of the hypothalamic–pituitary–adrenal axis in patients with chronic fatigue syndrome. J. Clin. Endocrinol. Metab. 73, 1224–1234.

Ehrhart-Bornstein, M., Hinson, J.P., Bornstein, J.P., Scherbaum, W.A., Vinson, G.P., 1998. Intraadrenal interactions in the regulation of adrenocortical steroidogenesis. Endocrinol. Rev. 19, 101–143.

Engeland, W.C., 1998. Functional innervation of the adrenal cortex by the splanchnic nerve. Horm. Metab. Res. 30, 311–314.

French, J.A., 1997. Proximate regulation of singular breeding in Callitrichid primates. In: Solomon, N.G., French, J.A. (Eds.) Cooperative Breeding in Mammals. Cambridge University Press, Cambridge, pp. 34–75.

Harlow, C.R., Gems, S., Hodges, J.K., Hearn, J.P., 1983. The relationship between plasma progesterone and the timing of ovulation and early embryonic development in the marmoset monkey (*Callithrix jacchus*). J. Zool., London 201, 273–282.

Hearn, J.P., 1977. Restraining device for small monkeys. Lab. Anim. 11, 261–262.
Hirst, J.J., West, N.B., Brenner, R.M., Novy, M.J., 1992. Steroid hormone receptors in the adrenal glands of fetal and adult rhesus monkeys. J. Clin. Endocrinol. Metab. 75, 308–314.
Jaeckle, R.S., Kathol, R.G., Lopez, J.F., Meller, W.H., Krummel, S.J., 1987. Enhanced adrenal sensitivity to exogenous cosyntropin (ACTH α^{1-24}) stimulation in major depression: relationship to dexamethasone suppression test results. Arch. Gen. Psychi. 44, 233–240.
Johnson, E.O., Kamilaris, T.C., Carter, C.S., Calogero, A.E., Gold, P.W., Chrousos, G.P., 1996. The biobehavioral consequences of psychogenic stress in a small, social primate (*Callithrix jacchus jacchus*). Biol. Psychi. 40, 317–337.
Keller-Wood, M., Leeman, E., Shinsako, J., Dallman, M.F., 1988. Steroid inhibition of canine ACTH: in vivo evidence for feedback at the corticotrope. Am. J. Physiol. 255, E241–E246.
Kendrick, K.M., Dixson, A.F., 1984. Ovariectomy does not abolish proceptive behaviour cyclicity in the common marmoset (*Callithrix jacchus*). J. Endocrinol. 101, 155–162.
Kitay, J.I., 1963a. Effects of estradiol on pituitary–adrenal function in male and female rats. Endocrinology 72, 947–954.
Kitay, J.I., 1963b. Pituitary–adrenal function in the rat after gonadectomy and gonadal hormone replacement. Endocrinology 73, 253–260.
Licinio, J., Wong, M.-L., Gold, P.W., 1996. The hypothalamic–pituitary–adrenal axis in anorexia nervosa. Psychiatr. Res. 62, 75–83.
Müller, R.E., Wotiz, H.H., 1978. Estrogen-binding protein in mouse and rat adrenal glands. J. Biol. Chem. 253, 740–745.
Nicholson, S., Campbell, E., Torrellas, A., Beckford, U., Altaher, R., Sandford, R., Scraggs, R., Gillham, B., Jones, M., 1984. Recovery of the hypothalamo–pituitary–adrenocortical axis in the rat after long-term dexamethasone treatment. Neuroendocrinology 39, 343–349.
O'Connell, Y., McKenna, T.J., Cunningham, S.K., 1994. The effect of prolactin, human chorionic gonadotropin, insulin and insulin-like growth factor 1 on adrenal steroidogenesis in isolated guinea-pig adrenal cells. J. Steroid Biochem. Mol. Biol. 48, 235–240.
Pabon, J.E., Li, X., Lei, Z.M., Sanfilippo, J.S., Yussman, M.A., Rao, Ch.V., 1996. Novel presence of luteinizing hormone/chorionic gonadotropin receptors in human adrenal glands. J. Clin. Endocrinol. Metab. 81, 2397–2400.
Phillips, J.G., Poolsanguan, W., 1978. A method to study temporal changes in adrenal activity in relation to sexual status in the female laboratory rat. J. Endocrinol. 77, 283–291.
Rilianawati, T.P., Kero, J., Zhang, F.-P., Rahman, N., Kananen, K., Huhtaniemi, I., 1998. Direct luteinizing hormone action triggers adrenocortical tumorigenesis in castrated mice transgenic for the murine inhibin α-subunit promoter/Simian Virus 40 T-antigen fusion gene. Mol. Endocrinol. 12, 801–809.
Saltzman, W., Schultz-Darken, N.J., Abbott, D.H., 1996. Behavioural and endocrine predictors of dominance and tolerance in female common marmosets, *Callithrix jacchus*. Anim. Behav. 51, 657–674.
Saltzman, W., Schultz-Darken, N.J., Abbott, D.H., 1997. Social and reproductive influences on plasma cortisol and ACTH levels in female common marmosets: sensitivity to glucocorticoid negative feedback. Am. J. Primatol. 42, 144–145.
Saltzman, W., Schultz-Darken, N.J., Scheffler, G., Wegner, F.H., Abbott, D.H., 1994. Social and reproductive influences on plasma cortisol in female marmoset monkeys. Physiol. Behav. 56, 801–810.
Saltzman, W., Schultz-Darken, N.J., Wegner, F.H., Wittwer, D.J., Abbott, D.H., 1998. Suppression of cortisol levels in subordinate female marmosets: reproductive and social contributions. Horm. Behav. 33, 58–74.
Scott, L.V., Teh, J., Reznek, R., Martin, A., Sohaib, A., Dinan, T.G., 1999. Small adrenal glands in chronic fatigue syndrome: a preliminary computer tomography study. Psychoneuroendocrinology 24, 759–768.
Sokal, R.R., Rohlf, F.J., 1995. Biometry, third ed. W.H. Freeman, New York.
Summers, P.M., Wennink, C.J., Hodges, J.K., 1985. Cloprostenol-induced luteolysis in the marmoset monkey (*Callithrix jacchus*). J. Reprod. Fertil. 73, 133–138.
Vinson, G.P., Bell, J.B.G., Whitehouse, B.J., 1976. Production of testosterone and corticosteroids by the rat adrenal gland incubated *in vitro* and the effects of stimulation with ACTH, LH and FSH. J. Steroid Biochem. 7, 407–411.

Vinson, G.P., Renfree, M.B., 1975. Biosynthesis and secretion of testosterone by adrenal tissue from the North American opossum, *Didelphis virginiana*, and the effects of tropic hormone stimulation. Gen. Comp. Endocrinol. 27, 214–222.

Weiss, M., Xu, R.J., 1990. Estrogen receptors in the adrenal cortex of the possum (*Trichosurus vulpecula*). Comp. Biochem. Physiol. 96, 375–380.

Yehuda, R., 1998. Psychoneuroendocrinology of post-traumatic stress disorder. Psychiatr. Clin. N. Am. 21, 359–379.