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Social suppression of cortisol in female marmoset monkeys: role of circulating ACTH levels and glucocorticoid negative feedback

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Abstract

Behaviorally subordinate female common marmoset monkeys (*Callithrix jacchus*) exhibit pronounced, chronic reductions of circulating cortisol levels. Cortisol suppression in these animals is mediated in part by adrenocortical hyporesponsiveness to adrenocorticotropic hormone (ACTH). In addition, we hypothesized that social subordination may activate a central, neurally mediated mechanism to further inhibit hypothalamo-pituitary-adrenal function. In this study, therefore, we evaluated basal plasma cortisol and ACTH concentrations, as well as cortisol and ACTH responses to dexamethasone (DEX), in dominant and subordinate females to initially characterize such a mechanism. Morning plasma cortisol and ACTH levels were determined before, and 1, 2, and 3 days following administration of DEX (0.5, 1.0, or 5.0 mg/kg, IM) or saline. Baseline cortisol concentrations prior to DEX treatment were significantly lower in subordinate females than in dominants, as previously reported. However, ACTH concentrations in the same blood samples did not differ between the two groups. Furthermore, dominant and subordinate females showed similar cortisol and ACTH responses to DEX. These results indicate that reduced circulating cortisol levels in subordinate females are not associated with either altered circulating ACTH concentrations or enhanced responsiveness to glucocorticoid negative feedback. However, the finding that basal ACTH levels are not elevated in subordinate females as compared to dominants, in spite of low circulating cortisol

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concentrations, suggests that ACTH secretion in subordinate females is restrained by a steroid-independent inhibitory mechanism operating at the level of the brain or pituitary.

Keywords: Marmoset; Dexamethasone; Adrenocorticotropic hormone; Negative feedback; Cortisol; Subordination

1. Introduction

Social subordination in a number of animal species is associated with elevated circulating levels of glucocorticoid hormones, presumably reflecting high levels of stress in subordinate individuals (e.g. mice: Louch and Higginbotham, 1967; rats: Blanchard et al., 1993; golden hamsters: Huhman et al., 1991; tree shrews: von Holst, 1997; sugar gliders: Mallick et al., 1994; baboons: Sapolsky, 1982; talapoin monkeys: Eberhart et al., 1983; rainbow trout, brown trout: Sloman et al., 2001; Arctic charr: Elofsson et al., 2000). In recent years, however, subordinate animals in an increasing number of species have been found to undergo significant reductions in circulating or excreted glucocorticoid levels, as compared to dominant individuals (white-browed sparrow weaver: Wingfield et al., 1991; common marmoset monkey: Saltzman et al., 1994, 1998; Johnson et al., 1996; Abbott et al., 1997; ring-tailed lemur: Cavigelli, 1999; Japanese macaque: Barrett et al., 2002; African wild dog, dwarf mongoose: Creel et al., 1996, 1997). Investigation of these contrasting socioendocrine profiles may be particularly informative both in understanding the psychosocial, physical, and physiological sequelae of social status that influence endocrine function (Abbott et al., in press), and in identifying the neuroendocrine mechanisms leading to chronic dysregulation of the hypothalamo-pituitary-adrenal (HPA) axis. However, the mechanisms underlying socially induced suppression of HPA activity have not been elucidated clearly in any species.

We have been investigating the mechanisms of social suppression of HPA activity in the common marmoset (*Callithrix jacchus*), a small New World monkey in which endocrine function is profoundly influenced by social status. Both free-living and captive social groups may contain as many as six adult females, but only one or two behaviorally dominant females breed in each group (reviewed by French, 1997; Saltzman, in press). Subordinate females are often anovulatory and hypoestrogenemic as a result of inadequate pituitary secretion of luteinizing hormone (Abbott et al., 1981, 1988). This socially induced infertility can persist for months or even years but is reversed rapidly following separation of the subordinate female from her dominant female groupmate (Abbott et al., 1988; Abbott and George, 1991).

Anovulatory subordinate female marmosets also exhibit profound, chronic suppression of circulating cortisol levels (Saltzman et al., 1994, 1998; Johnson et al., 1996; Abbott et al., 1997). Baseline morning plasma cortisol concentrations decline markedly within 6–7 weeks following the onset of social subordination and anovulation, to levels less than half those of dominant females undergoing ovulatory cycles (Saltzman et al., 1994, 1998; Abbott et al., 1997), and can remain suppressed for

months to years (Saltzman et al., 1998; unpublished data). Attainment of dominant status, in contrast, does not alter cortisol levels (Abbott et al., 1997). Several lines of evidence suggest that the cortisol reduction in subordinates is mediated both by suppression of reproductive hormones and by social subordination per se. Attainment of subordinate status without a corresponding change in reproductive function may not be associated with a decline in cortisol levels (Saltzman et al., 1994). Moreover, ovariectomy of female marmosets, like social subordination, leads to a reduction in circulating cortisol levels; however, this effect is both smaller in magnitude and slower to develop than that seen in anovulatory, hypoestrogenemic subordinates (Abbott et al., 1997; Saltzman et al., 1998).

Recently we demonstrated that low baseline cortisol levels in anovulatory subordinate female marmosets are associated with reduced adrenocortical responsiveness to adrenocorticotropic hormone (ACTH). Following suppression of endogenous cortisol and ACTH with dexamethasone, a potent synthetic glucocorticoid, subordinate females showed impaired cortisol responses to exogenous ACTH, as compared to dominant females in the follicular phase of the ovarian cycle (Saltzman et al., 2000). Adrenocortical responses of ovariectomized (non-subordinate) females were identical to those of intact subordinates, indicating that this adrenocortical impairment might be mediated by withdrawal of reproductive hormones, such as estrogen, rather than by social subordination per se.

Because subordinate females have lower baseline cortisol levels than ovariectomized females, but similar adrenal responsiveness to ACTH, we hypothesize that an additional mechanism, activated directly by social subordination or its psychosocial sequelae, contributes to the greater suppression of basal cortisol levels in subordinates. Such a mechanism is likely to be neurally mediated and therefore would be expected to involve altered hypothalamic release of corticotropin-releasing hormone, arginine vasopressin, or other secretagogs, resulting in altered pituitary release of ACTH. Enhanced responsiveness to glucocorticoid negative feedback at the brain and/or pituitary (steroid-dependent inhibition), or tonic inhibitory neuroendocrine input to the corticotropes, independent of glucocorticoid negative feedback (steroid-independent inhibition), may underlie such a neural basis for cortisol suppression. In this paper we report our initial attempts to identify such a neural mechanism by characterizing: (1) baseline circulating ACTH levels; and (2) ACTH and cortisol responses to negative feedback by dexamethasone in anovulatory subordinate females as compared to dominant females undergoing ovulatory cycles.

2. Methods

2.1. Animals

We used a total of 28 captive-born, adult female common marmosets (*Callithrix jacchus jacchus*), including 14 socially dominant females undergoing ovulatory cycles and 14 anovulatory subordinates. Each animal was tested no more than once with each dose of dexamethasone (DEX) or saline, but individual animals were tested

with one ($N = 12$), two ($N = 11$), three ($N = 2$) or four ($N = 3$) different doses. At least 45 days elapsed between successive periods of data collection on individual marmosets. The numbers and ages of dominant and subordinate animals tested with each dose of DEX are presented in Table 1. Among the animals tested with each dose, dominant and subordinate females did not differ significantly in body mass ($P > 0.1$). Among the animals tested with the highest dose of DEX, but not the other doses, subordinate females were significantly younger than dominants ($T[11] = 5.56$, $P < 0.001$; see Table 1).

Dominant and subordinate animals were housed in groups containing two to three unrelated adult females and one to two gonadally intact adult males. Groups had been formed as described previously (Saltzman et al., 1998), at least 2 months (13.9 ± 1.1 months, mean ± SEM) 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). 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 females and anovulation in subordinate females, as determined by plasma progesterone concentrations in blood samples collected twice weekly (Saltzman et al., 1994; see below). Subordinates had not ovulated (see below) for at least 75 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 63 days.

Animals were housed indoors, with lights on from 06:00–18:00 h, ambient temperature at approximately 27°C, and humidity at approximately 40%. Most of the animals occupied aluminum and wire mesh cages measuring 61 × 91 × 183 cm or 122 × 61 × 183 cm; however, three dominants and three subordinates were housed in a larger room (363 × 212 × 218 cm). Animals were fed once daily between 13:00

Table 1
Numbers and ages (mean ± SEM, range) of dominant and subordinate female marmosets tested with each DEX dose

DEX dose (mg/kg)	No. dom.	No. sub.	Age (mo.) dom.	Age (mo.) sub.
0.0 (saline)	6	4	42.6 ± 3.6 (30.7−57.3)	32.7 ± 4.1 (20.4−37.4)
0.5	8	8	39.7 ± 4.2 (29.2− 65.3)	40.4 ± 3.1 (29.3−53.1)
1.0	8	7[a]	42.4 ± 3.8 (29.3−62.7)	40.9 ± 2.6 (30.5−50.4)
5.0	8	5	38.7 ± 2.1 (32.3−52.2)	23.9 ± 1.6[b] (18.8−26.8)

[a] Cortisol data were available from only 6 subordinate females.
[b] $P < 0.001$ vs. dominant females.

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 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, 4 days before data collection commenced and 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 subordinate females with the same dose of cloprostenol 4 days before data collection commenced, to control for any possible effects of cloprostenol on HPA activity. Cloprostenol treatment does not alter baseline cortisol levels in marmosets (Saltzman et al., 1998).

On the first day of data collection (day 0), a basal blood sample was collected from each animal (see below) at 08:45–09:15 h, and the animal was weighed. That afternoon, each animal was given an IM injection of 0.5, 1.0, or 5.0 mg/kg dexamethasone sodium phosphate (0.09–0.63 ml; American Regent Laboratories, Inc., Shirley, NY) or 0.25 ml/kg sterile saline at 16:30–17:15 h. Blood samples were subsequently collected at 08:45–09:15 h on each of the next 3 days (days 1, 2, 3). DEX doses were based on preliminary studies (George and Abbott, unpublished) and on work by others (Johnson et al., 1996).

For collection of blood samples, marmosets were transported from their home cage and briefly placed in a marmoset restraint (Hearn, 1977) while 0.4 ml blood was collected from the femoral vein into a heparinized syringe. Animals received a liquid treat (Ensure, Abbott Laboratories, Columbus, OH) and were returned to their home cage within 5–10 min. No more than 3.5 min elapsed from initial disturbance to the animal to collection of the blood sample, and 98.6% of samples were collected in $\leq$3 min (mean ± SEM latency for all samples: 110 ± 1 s). These blood-sampling procedures are well tolerated by marmosets and do not elevate plasma cortisol levels (Saltzman et al., 1994).

Blood samples were immediately divided into two aliquots and placed on ice. Approximately 0.3 ml whole blood was processed as described by Orth (1979) for subsequent ACTH assay. These samples were initially centrifuged at 4200 r.p.m. for 15 min at 4°C, and the plasma fraction was removed and centrifuged again at 9000 r.p.m. for 10 min. The plasma fraction was again removed and frozen at −80°C until assayed for ACTH. The remaining 0.1 ml whole blood from each sample was centrifuged at 2000 r.p.m. for 10 min, and the plasma fraction was separated and stored at −20°C until assayed for cortisol and, in some cases, progesterone.

2.3. Monitoring of ovarian function

To monitor ovarian function prior to and during this experiment, we collected blood samples twice each week, at 3- to 4-day intervals, for measurement of plasma

progesterone levels (Saltzman et al., 1994). Animals were transported from their home cage and placed in a marmoset restraint while 0.1–0.3 ml blood was collected by femoral puncture into a heparinized syringe. Samples were placed on ice, centrifuged at 2000 r.p.m. for 10 min, and the plasma fraction extracted and frozen at −20°C until assayed for progesterone. 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 0.75–1.00 μg cloprostenol 15–45 days after each ovulation.

2.4. Hormone assays

Plasma cortisol concentrations 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/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 4.0% and 10.0%, respectively.

Plasma ACTH concentrations were determined by radioimmunoassay using methods modified from those described by Clarke et al. (1994). Plasma samples, reference standards, and quality-control pools were diluted with diluent to a final volume of 100 μl, and 100 μl of primary antibody was added to each tube. ^{125}I-labeled human $ACTH_{1\text{-}39}$ (Diagnostic Products Corp., Los Angeles, CA) was added at a concentration of 9000 c.p.m. (approximately 125 pg of peptide) per tube. Because of the large plasma volumes required, only a single aliquot (75–100 μl) of each sample was assayed. Serial dilutions of a marmoset plasma pool ($N = 7$) were parallel to the standards, with no significant difference in slopes, and accuracy was 102.1 ± 3.26% (mean ± SEM). Assay sensitivity was 0.5 pg/tube, and intra- and inter-assay CVs of a marmoset plasma pool assayed in triplicate in each assay were 4.2% and 12.9%, respectively. For samples below the sensitivity limit of the assay (0.5 pg/tube), we assigned a value of 6.7 pg/ml (0.5 pg/tube) for statistical analyses.

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/tube (2.7 ng/ml), and intra- and inter-assay CVs of a marmoset plasma pool (38% binding) were 6.1% and 20.9%, respectively.

2.5. Analysis

Hormone values were log-transformed prior to analysis to increase normality of the data (Sokal and Rohlf, 1995); values presented are antilogs of the mean + 95% confidence intervals. Statistical analyses were performed using Systat 5 for the Macintosh. Initially, to compare baseline cortisol and ACTH levels between groups using maximal numbers of animals, we calculated the mean day-0 cortisol and ACTH level for each dominant and subordinate female, using data from each experiment

in which the animal was used; unpaired T-tests were performed to compare mean day-0 hormone levels between the two groups.

We next analyzed cortisol and ACTH levels for each DEX and saline dose separately, using two different approaches. First, to determine whether dominant and subordinate animals responded differently to DEX across the 4-day period, we performed a two-way ANOVA with groups treated as a between-groups factor and days from DEX as a within-groups factor. Post hoc comparisons were performed using univariate F tests following significant main effects of days and using analysis of simple effects following significant interactions (Keppel, 1982). Second, because we predicted that the response to DEX would be most pronounced on day 1 after treatment, we determined the ratio of cortisol or ACTH levels on day 1 to those on day 0 for each animal and performed an unpaired T-test to compare these ratios in dominant and subordinate animals.

Finally, to compare the groups' responses across the different doses of DEX, we performed 3-way ANOVAs (groups × doses × days) for cortisol and ACTH, using data from the three DEX doses excluding the saline control condition. Because the greatest responses to DEX occurred on day 1 for all doses used, we used hormone levels from only day 0 and day 1 in these analyses. We treated the data from each animal in each dose as independent, although many of the animals were tested with two or three DEX doses.

3. Results

3.1. Baseline cortisol and ACTH

We initially compared baseline (day-0) plasma cortisol and ACTH concentrations between dominant and subordinate animals, using data from all four DEX doses combined. As anticipated, baseline cortisol levels were significantly lower in subordinate females than in dominants ($T[23.8] = 3.78$, $P < 0.001$; Fig. 1). Baseline ACTH levels, in contrast, were nearly identical in the two groups ($P > 0.7$).

3.2. DEX effects on cortisol

3.2.1. Saline control

As expected, plasma cortisol levels did not change reliably across days in response to saline injection ($P > 0.3$). Across all 4 days, however, cortisol levels were significantly higher in dominant animals than in subordinates (antilog of mean [95% confidence inervals]: 223.9 μg/dl [311.9, 160.7] vs. 104.7 μg/dl [146.9, 74.7]; $F[1,8] = 9.46$, $P = 0.015$).

3.2.2. 0.5 mg/kg DEX

In response to the lowest dose of DEX, cortisol levels showed a highly significant change across days ($F[3,42] = 41.23$, $P < 0.001$; Fig. 2), as well as a significant difference between groups ($F[1,14] = 7.73$, $P = 0.015$) and a marginally significant

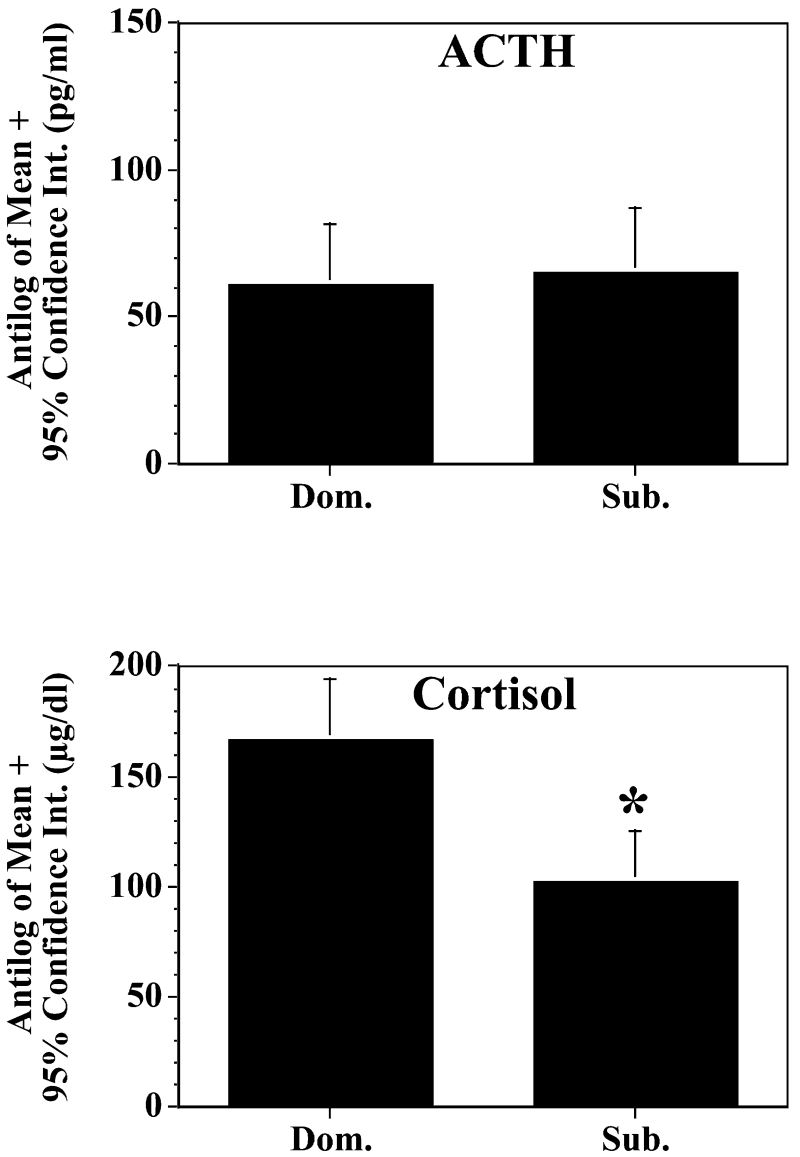

Fig. 1. Morning baseline concentrations of plasma ACTH and cortisol, prior to DEX or saline treatment, for all doses combined, in dominant female marmosets during the follicular phase of the ovarian cycle ($N = 14$) and anovulatory subordinate females ($N = 14$). As typically found in studies of marmosets and other small-bodied New World monkeys, plasma cortisol values were approximately 5–10 times higher than those of humans and other Old World primates (Coe et al., 1992; Saltzman et al., 1994, 1998). $*P < 0.001$, dominant vs. subordinate.

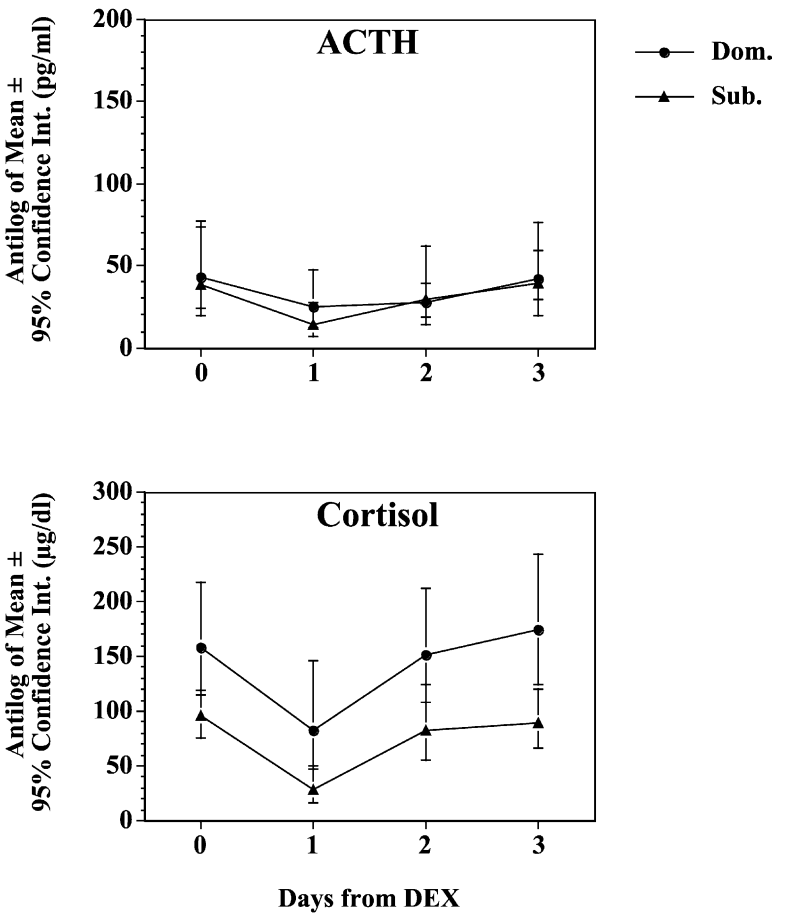

Fig. 2. Plasma ACTH and cortisol concentrations before and after treatment with 0.5 mg/kg DEX in dominant ($N = 8$) and subordinate ($N = 8$) female marmosets. Concentrations of both hormones were significantly altered by DEX; this response differed significantly between groups for cortisol but not for ACTH. See text for further details.

groups × days interaction ($F[3,42] = 2.86$, $P = 0.048$). For each group considered separately, cortisol levels were lower on day 1 after DEX than on day 0 but recovered to baseline levels by day 2 (Table 2). Dominant females additionally showed a near-significant elevation of cortisol above baseline levels on day 3 ($P = 0.06$), which might have reflected the beginning of the periovulatory cortisol elevation (Saltzman et al., 1998). Dominant females had significantly higher cortisol levels than subordinates on each of the 4 days ($P < 0.05$). Consistent with the groups × days interaction, comparison of the ratio between day-1 and day-0 cortisol values revealed a near-significant difference between groups ($T[14.0] = 2.00$, $P = 0.067$), indicating that subordinate females tended to show a somewhat greater decline in cortisol levels immediately following this dose of DEX.

Table 2
P-values for pairwise post hoc comparisons between hormone concentrations by days from DEX treatment, for dominant and subordinate animals combined

Hormone	DEX dose	Day 0 vs. 1	Day 0 vs. 2	Day 0 vs. 3
Cortisol	0.5 mg/kg	Dom: 0.01[a] Sub: <0.001[a]	Dom: NS[a] Sub: NS[a]	Dom: 0.06[a] Sub: NS[a]
Cortisol	1.0 mg/kg	<0.001	<0.001	NS
Cortisol	5.0 mg/kg	<0.001	<0.001	0.001
ACTH	0.5 mg/kg	<0.01	0.05	NS
ACTH	1.0 mg/kg	<0.001	<0.005	NS
ACTH	5.0 mg/kg	<0.001	<0.001	NS

[a] Post hoc analyses were performed separately for dominant and subordinate females because of a significant groups × days ANOVA interaction.

3.2.3. 1.0 mg/kg DEX

The intermediate dose of DEX yielded a highly significant change in plasma cortisol levels across days ($F[3,36] = 137.43$, $P < 0.001$; Table 2; Fig. 3). Dominant females had significantly higher cortisol levels than subordinates overall ($F[1,12] = 5.75$, $P = 0.034$), but the cortisol response to DEX over time did not differ between the groups ($P > 0.7$). For both groups combined, cortisol levels were significantly lower on days 1 and 2 after DEX than on day 0, but recovered to baseline levels by day 3. The ratio of day-1 to day-0 cortisol levels did not differ between groups ($P > 0.4$).

3.2.4. 5.0 mg/kg DEX

Again, plasma cortisol levels changed significantly across days ($F[3,33] = 33.38$, $P < 0.001$; Table 2; Fig. 4). However, cortisol did not show either a significant main effect of group ($P > 0.2$) or a significant groups × days interaction ($P > 0.6$). For dominant and subordinate females combined, cortisol levels were significantly lower on each of the 3 days following DEX treatment than on day 0. The ratio of day-1 to day-0 cortisol levels did not differ between dominant and subordinate animals ($P > 0.7$).

3.2.5. Comparison of doses

A 3-way ANOVA comparing day-0 and day-1 cortisol levels between dominant and subordinate females across the three DEX doses (excluding saline control) confirmed that cortisol levels were higher in dominants than subordinates ($F[1,37] = 14.80$, $P < 0.001$) and higher on day 0 than on day 1 after DEX treatment ($F[1,37] = 269.19$, $P < 0.001$]. Furthermore, the difference between the two days was reliably influenced by DEX dose ($F[2,37] = 9.44$, $P < 0.001$). However, the effects of dose, day, and the dose × day interaction did not differ between dominant and subordinate animals ($P > 0.2$).

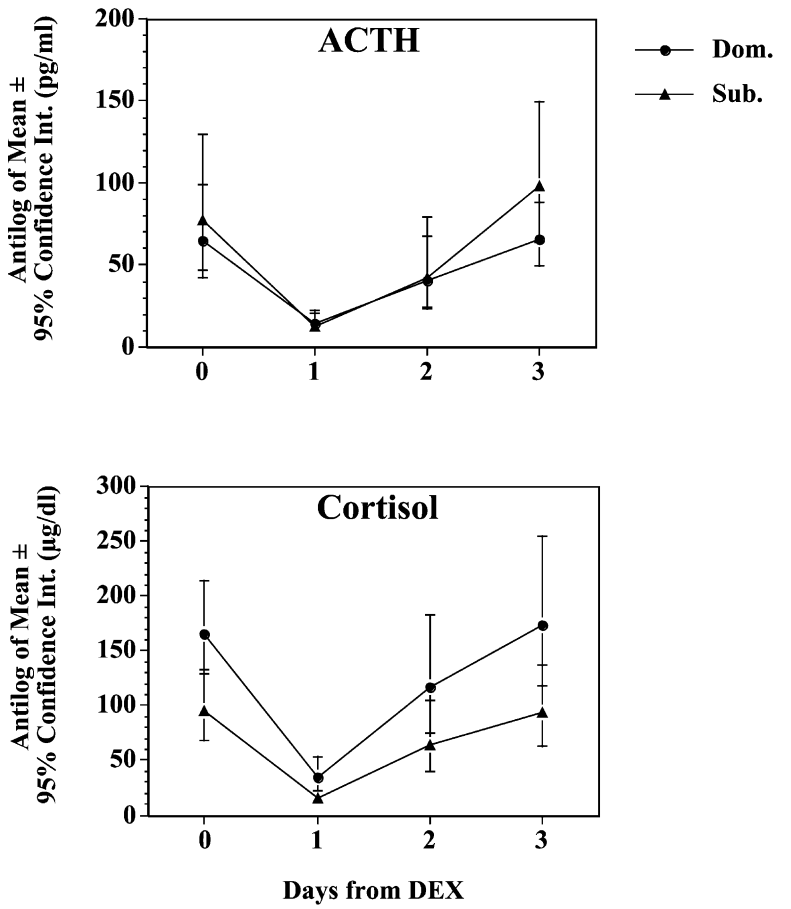

Fig. 3. Plasma ACTH and cortisol concentrations before and after treatment with 1.0 mg/kg DEX in dominant ($N = 8$) and subordinate ($N = 7$ for ACTH, 6 for cortisol) female marmosets. Concentrations of both hormones were significantly altered by DEX but did not differ reliably between groups. See text for further details.

3.3. DEX effects on ACTH

3.3.1. Saline control

ACTH levels did not change reliably across days following saline treatment ($P > 0.6$) and did not differ between dominant and subordinate females (main effect of groups: $P = 0.2$; groups × days interaction: $P > 0.9$).

3.3.2. 0.5 mg/kg DEX

In response to the lowest dose of DEX, plasma ACTH concentrations changed significantly across days ($F[3,42] = 9.75$, $P < 0.001$; Table 2; Fig. 2) but showed no differences between groups (main effect of groups: $P > 0.6$, groups × days inter-

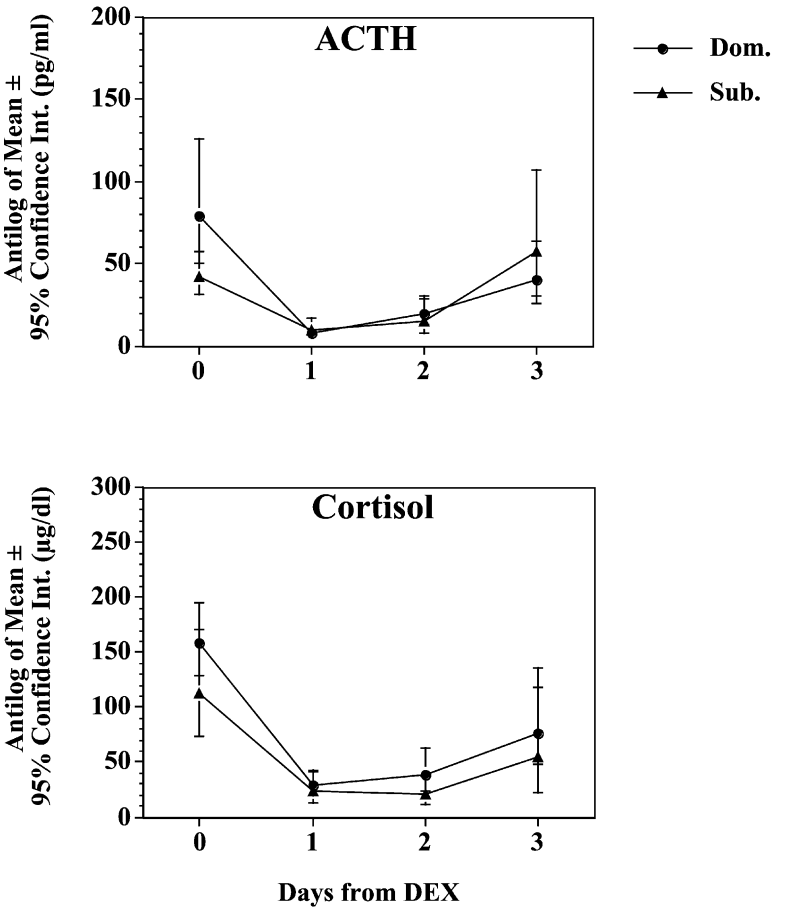

Fig. 4. Plasma ACTH and cortisol concentrations before and after treatment with 5.0 mg/kg DEX in dominant ($N = 8$) and subordinate ($N = 5$) female marmosets. Concentrations of both hormones were significantly altered by DEX but did not differ reliably between groups. See text for further details.

action: $P > 0.2$). For both groups combined, ACTH concentrations were significantly lower on days 1 and 2 after DEX than on day 0 but returned to baseline levels by day 3. The ratio of day-1 to day-0 ACTH levels did not differ between groups ($P > 0.3$).

3.3.3. 1.0 mg/kg DEX

This dose of DEX again caused a significant change in ACTH levels across days ($F[3,39] = 77.77$, $P < 0.001$; Table 2; Fig. 3); however, no differences were found between dominant and subordinate animals (main effect of groups: $P > 0.6$, groups × days interaction: $P > 0.2$). For the two groups combined, ACTH levels were significantly lower on days 1 and 2 after DEX than on day 0 but returned to

baseline levels by day 3. The ratio of day-1 to day-0 ACTH levels did not differ between dominant and subordinate females ($P > 0.3$).

3.3.4. 5.0 mg/kg DEX

ACTH levels again differed reliably across days ($F[3,33] = 31.69$, $P < 0.001$; Table 2; Fig. 4) but not between groups (main effect of groups: $P > 0.6$; groups × days interaction: $P > 0.1$). ACTH levels the day after DEX treatment were undetectable in our assay in nine of the 13 animals. For dominant and subordinate females combined, ACTH concentrations were significantly lower than baseline levels on days 1 and 2 following DEX treatment but not on day 3. The ratio of day-1 to day-0 ACTH levels did not differ between groups ($P > 0.1$).

3.3.5. Comparison of doses

Three-way ANOVA confirmed that ACTH levels were significantly lower on day 1 after DEX than on day 0 ($F[1,38] = 139.99$, $P < 0.001$) and that this effect differed across the three DEX doses ($F[2,38] = 8.27$, $P = 0.001$). Once again, however, no differences were found between dominant and subordinate animals ($P \geq 0.1$).

4. Discussion

Anovulatory, socially subordinate female marmosets exhibit pronounced and persistent reductions in circulating basal cortisol levels (Saltzman et al., 1994, 1998; Johnson et al., 1996; Abbott et al., 1997). Previously we showed that one mechanism of this cortisol suppression may be reduced adrenocortical responsiveness to ACTH, which in turn might be mediated by suppression of reproductive hormones: anovulatory subordinates and ovariectomized females exhibited diminished, but identical, cortisol responses to exogenous ACTH (Saltzman et al., 2000). Because basal cortisol levels decline more rapidly and more precipitously in subordinates than in ovariectomized females (Abbott et al., 1997; Saltzman et al., 1998), however, we hypothesized that an additional mechanism must also contribute to cortisol suppression in subordinates. This mechanism is likely to be neurally mediated and directly activated by social subordination or its psychosocial sequelae. In the present experiment, therefore, we evaluated both circulating basal ACTH levels and the ACTH and cortisol responses to dexamethasone to begin to characterize such a mechanism.

Comparison of baseline hormone levels using animals from all four DEX or saline doses, prior to treatment, confirmed our previous findings that morning basal cortisol concentrations are significantly lower in anovulatory subordinate females than in dominant females in the follicular phase of the ovarian cycle (Saltzman et al., 1994, 1998; Abbott et al., 1997). Mean basal cortisol levels of subordinates in this study were approximately 60% of those of dominants. In contrast, ACTH levels in the same blood samples were virtually identical in dominants and subordinates. Similar results were reported by Johnson et al. (1996), who found that subordinate female marmosets in stable social groups had lower basal circulating cortisol levels but similar ACTH levels as dominant females. Moreover, in a separate study, we found

that basal ACTH levels of dominant and subordinate females did not differ reliably around either the peak (09:00 h, comparable to the present study) or trough (17:00 h) of the circadian cycle (Saltzman, unpublished data). Thus, the low cortisol levels of subordinate females do not appear to result directly from a comparable suppression of ACTH.

Furthermore, our results suggest that low cortisol levels in subordinate female marmosets may not result from enhanced responsiveness to glucocorticoid negative feedback. Both dominant and subordinate animals showed suppression of ACTH and cortisol in response to all three doses of DEX. Moreover, day-1 ACTH and cortisol responses to DEX were dose-dependent, as was the duration of the cortisol response. However, subordinate females did not show a different pattern of ACTH or cortisol responses across the DEX doses than dominant females and did not show greater suppression of ACTH in response to any dose of DEX. Dominant and subordinate females did exhibit somewhat different cortisol responses to the lowest dose of DEX (0.5 mg/kg), but the initial (day-1) cortisol response to this dose showed only a non-significant trend between groups. Thus, we found no clear evidence that social subordination enhances responsiveness to glucocorticoid negative feedback in female marmosets.

The finding that subordinate females have lower circulating cortisol levels than dominant females in spite of similar ACTH levels is consistent with our previous finding that an identical dose of ACTH stimulates lower cortisol output in subordinates than in dominants (Saltzman et al., 2000). Because the present results indicate that glucocorticoid negative feedback on ACTH appears to operate normally in subordinate females, however, it is not immediately clear why their reduced endogenous cortisol levels do not lead to a compensatory elevation in ACTH concentrations above levels typical for dominant females. Basal endogenous glucocorticoids normally exert strong negative-feedback effects on ACTH (Dallman et al., 1994), and severe or moderate reductions in circulating cortisol levels, for example due to primary adrenal insufficiency (Oelkers, 1996), congenital adrenal hyperplasia (New, 1992), or treatment with metyrapone, an 11β-hydroxylase inhibitor (Veldhuis et al., 2001), are associated with elevated plasma ACTH concentrations. Our findings suggest, therefore, that an inhibitory drive to the pituitary restrains ACTH secretion in subordinate female marmosets, independently of glucocorticoid negative feedback.

One interpretation of our present and previous findings is that social subordination activates two inhibitory mechanisms in the HPA axis of the female marmoset. One mechanism apparently is mediated by suppression of estrogen or other reproductive hormones and dampens adrenocortical responsiveness to ACTH (Saltzman et al., 2000). This adrenal hyporesponsiveness, in turn, produces a decline in circulating cortisol levels and, hence, a diminished glucocorticoid negative-feedback signal to the brain and pituitary. The second mechanism may act on the brain to tonically inhibit release of corticotropin-releasing hormone (CRH), arginine vasopressin (VP), or other secretagogs, leading to a decline in circulating ACTH levels. Because glucocorticoid negative feedback is apparently unaffected by social subordination, the low circulating cortisol levels resulting from adrenocortical hyporesponsiveness would then lead to a compensatory rise in ACTH to levels similar to those of dominant

females. Thus, according to this scenario, central inhibitory influences on ACTH secretion in subordinates are at least partly masked by maintenance of glucocorticoid negative feedback, which allows ACTH levels to rise in response to low cortisol concentrations. Another possibility is that social subordination chronically elevates, rather than inhibits, hypothalamic release of CRH, VP, or other secretagogs, leading to receptor down-regulation in the pituitary and, consequently, diminished ACTH release, as has been suggested to occur in posttraumatic stress disorder (Yehuda et al., 1995; Kasckow et al., 2001); again, reductions in ACTH concentrations could be masked by the reduced cortisol negative-feedback signal. To evaluate these possibilities, it will be necessary to eliminate endogenous cortisol pharmacologically, in order to characterize hypothalamic drive to the pituitary and determine the extent to which endogenous cortisol restrains ACTH secretion.

Central inhibition of HPA function in subordinate female marmosets could be mediated by the reproductive consequences of social subordination. Anovulatory subordinate females have significantly lower plasma estradiol levels than dominant females undergoing ovulatory cycles (Abbott et al., 1988; Saltzman et al., 1998), and estradiol is well-known to increase HPA output through a variety of actions, including effects on the brain and pituitary. In female rats and primates, estrogen has been shown to increase circulating and pituitary ACTH levels (Kitay, 1963a,b; Coyne and Kitay, 1969; Viau and Meaney, 1991; Giussani et al., 2000; but see Young et al., 2001), stimulate CRH gene transcription and elevate CRH mRNA levels in the hypothalamus (Vamvakopoulos and Chrousos, 1993; Roy et al., 1999), and alter expression and function of corticosteroid receptors in the brain and pituitary (Peiffer and Barden, 1987; Turner, 1990; Ferrini and De Nicola, 1991; Burgess and Handa, 1992, 1993; Carey et al., 1995), as well as to alter glucocorticoid negative feedback (Redei et al., 1994; Almeida et al., 1997). Thus, central inhibition of the HPA axis could well be mediated by hypoestrogenism in subordinate female marmosets. This scenario, however, would not explain the more pronounced suppression of cortisol in subordinates than in ovariectomized females (Abbott et al., 1997; Saltzman et al., 1998).

Another possibility is that central inhibition of HPA function in female marmosets is mediated by the psychosocial correlates, rather than the reproductive consequences, of subordination. In several species, elevated cortisol levels in subordinates are associated with altered central regulation of the HPA axis. Subordinate male tree shrews (*Tupaia belangeri*), for example, exhibit drastically elevated urinary cortisol levels as well as altered numbers and affinities of CRH binding sites in the brain and pituitary, as compared to control males (Fuchs and Flugge, 1995). Socially subordinate male baboons (*Papio anubis*) exhibit hypercortisolism and glucocorticoid negative-feedback resistance, which appear to be centrally mediated (Sapolsky, 1989, 1995). Clearly, however, subordinate male tree shrews and baboons differ from subordinate female marmosets in exhibiting elevated, rather than diminished, cortisol levels, which has been attributed to psychosocial stress (Sapolsky, 1989, 1995; Fuchs and Flugge, 1995). Subordinate female marmosets do not appear to be subjected to any increased (or decreased) stress, as compared to dominants, and the concept of stress may not be particularly useful in understanding socially induced cortisol sup-

pression in these animals (Abbott et al., 1997). Marmosets, as well as several other species in which subordinates exhibit low baseline glucocorticoid levels (white-browed sparrow weaver: Wingfield et al., 1991; African wild dog, dwarf mongoose: Creel et al., 1996, 1997), are singular cooperative breeders: typically, only a single, socially dominant female breeds in each social group, while subordinate females and other group members help to provide care for her offspring. Thus, dominant and subordinate females occupy distinct social niches characterized by sharply different behavioral and physiological demands and constraints. Suppression of HPA activity in subordinates may therefore represent an adaptive response to the subordinate, nonreproductive condition rather than a pathological response to stress (Abbott et al., 1998; Saltzman et al., 1998)

Chronic suppression of cortisol in subordinate female marmosets superficially appears to resemble cortisol alterations in several human neuropsychiatric disorders, including posttraumatic stress disorder (PTSD: Yehuda, 1998), chronic fatigue syndrome (CFS: Parker et al., 2001), and atypical depression (Kasckow et al., 2001; Gold et al., 2002). However, our results suggest that the underlying mechanisms of these cortisol reductions differ between marmosets and humans. PTSD patients, for example, exhibit reduced urinary and plasma cortisol levels and, possibly, adrenocortical hyporesponsiveness, as compared to controls, but no differences in basal plasma ACTH levels (reviewed by Yehuda et al., 1995; Yehuda, 1998; Kanter et al., 2001). In contrast to marmosets, however, maintenance of 'normal' ACTH levels in PTSD patients despite low cortisol concentrations is associated with enhanced responsiveness to glucocorticoid negative feedback (reviewed by Yehuda, 2000; but see Kanter et al., 2001). These symptoms in PTSD are thought to result from hypothalamic hypersecretion of CRH, leading to down-regulation of CRH receptors in the pituitary (Yehuda, 1998; Kasckow et al., 2001). In contrast, low cortisol levels in CFS patients may be associated with enhanced responsiveness to cortisol negative feedback (Gaab et al., 2002) as well as with elevated basal ACTH levels, increased adrenocortical sensitivity to low doses of ACTH, and reduced adrenocortical secretory capacity (Demitrack et al., 1991; Scott et al., 1998), secondary to decreased hypothalamic release of CRH (reviewed by Parker et al., 2001). Corticosteroid-binding globulin (CBG) levels are elevated in both PTSD (Kanter et al., 2001) and CFS (Demitrack et al., 1991) patients, leading to further reductions in free cortisol levels. We have not examined this latter possibility in marmosets because this species has been reported to have extremely low levels of CBG, so that virtually all cortisol circulates unbound or weakly bound to albumin (Pugeat et al., 1984; Robinson et al., 1985; Klosterman et al., 1986).

Although the results of the present study provide no clear evidence for altered glucocorticoid negative feedback in subordinate female marmosets, several caveats should be mentioned. First, we were unable to assess circulating DEX concentrations in our animals, due to low blood volumes, and therefore cannot rule out the possibility that dominant and subordinate females differed in their bioavailability or pharmacokinetics of DEX, as has been reported for humans (Lowy and Meltzer, 1987). Second, DEX shows different affinities for corticosteroid receptor subtypes than do endogenous glucocorticoids, binding preferentially to GR rather than MR (De Kloet

et al., 1975; Cole et al., 2000). Thus, DEX does not perfectly mimic cortisol binding or, presumably, cortisol feedback effects on ACTH. Third, DEX does not readily cross the blood-brain barrier and thus exerts negative-feedback effects preferentially at the pituitary rather than the brain, whereas endogenous glucocorticoids act at both sites (De Kloet, 1997; Meijer et al., 1998; Cole et al., 2000). Therefore, possible differences between dominant and subordinate female marmosets in responsiveness to cortisol negative feedback, especially at the level of the brain, may not have been detected.

Similarly, we cannot definitively rule out possible differences in ACTH secretion between dominants and subordinates. ACTH release is a dynamic process that fluctuates over multiple time scales, including both circadian and ultradian rhythms (Gudmundsson and Carnes, 1997). Thus, ACTH concentrations in individual blood samples must be interpreted cautiously. More precise characterization of the temporal patterning and secretory dynamics of ACTH release will be necessary to confirm that ACTH secretion does not differ between dominant and subordinate female marmosets.

In summary, the results of this study confirm previous findings that morning basal cortisol levels are suppressed in socially subordinate female marmoset monkeys and indicate that this suppression is not reliably associated with altered responsiveness to glucocorticoid negative feedback. Instead, our findings suggest that socially induced cortisol suppression may be mediated in part by steroid-independent inhibition at the level of the brain or pituitary, which restrains ACTH secretion in spite of low circulating cortisol concentrations. In contrast to suppression of HPA activity in human neuropsychiatric conditions, cortisol suppression in female marmosets may be an adaptation to social subordination in a cooperatively breeding primate (Abbott et al., 1998). Elucidation of the mechanisms underlying socially induced HPA suppression in these animals may therefore advance our understanding of the adaptive plasticity of the HPA axis under different psychosocial conditions.

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