Dehydroepiandrosterone sulfate levels reflect endogenous luteinizing hormone production and response to human chorionic gonadotropin challenge in older female macaque (*Macaca fascicularis*)

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Abstract

Objective: We propose that the adrenal gland of an older higher primate female animal model will respond to human chorionic gonadotropin (hCG) hormone challenge by secreting additional dehydroepiandrosterone sulfate (DHEAS). Such a response in surgically and chemically castrated animals will provide proof of concept and a validated animal model for future studies to explore the rise in DHEAS during the menopausal transition of women.

Methods: Twenty-four 18- to 26-year-old female cynomolgus monkeys were screened for ovarian function and then either ovariectomized (n = 4) or treated with a gonadotropin-releasing hormone agonist (GnRHa; n = 20) to block ovarian steroid production. After a recovery period from surgical procedure or down-regulation, a single-dose challenge (1,000 IU/animal, IM) of hCG was then administered to determine if luteinizing hormone (LH)/chorionic gonadotropin could accelerate circulating DHEAS production. Serum DHEAS, bioactive LH, and urinary metabolites of ovarian sex steroids were monitored before, during, and after these treatments.

Results: Circulating LH bioactivity and immunoreactive DHEAS concentrations were suppressed in all animals 14 days postadministration of GnRHa. Urinary metabolites of estradiol and progesterone remained low after the surgical procedure or a flare reaction to GnRHa. Circulating DHEAS levels were increased after hCG administration, and the increase in individual animals was proportional to the pretreatment DHEAS at baseline. Circulating DHEAS concentrations were positively correlated to endogenous LH bioactive concentrations prior to hCG challenge and were subsequently further elevated by the hCG challenge while no concomitant change in ovarian steroid hormone excretion was observed.

Conclusions: These data demonstrate a positive adrenal androgen response to LH/chorionic gonadotropin in older female higher primates and suggest a mechanism for the rise in adrenal androgen production during the menopausal transition in women. These results also illustrate that the nonhuman primate animal model can be effectively used to investigate this phenomenon.

Key Words: Dehydroepiandrosterone sulfate – Menopause – Adrenal androgens – Luteinizing hormone/human chorionic gonadotropin.

Humans, along with other higher primates, are unique among animal species in having adrenal glands that secrete large amounts of dehydroepiandrosterone (DHEA) and especially dehydroepiandrosterone sulfate (DHEAS). Although it is generally accepted that the DHEAS secretion rate decreases with age in humans and can be used as an index for somatic aging in men, recent observations suggest that this is not true for women. Data indicate that circulating levels of DHEAS, DHEA, androstenediol (Adiol), androstenedione (Adione), and testosterone increase during the menopausal transition (MT) in most women, with differences in absolute levels observed in different ethnic groups.[1-5] Similarly, increased variability in DHEAS levels[5] and a small increase in DHEAS levels are observed as female laboratory macaques progress through the MT.[6] These important observations are relevant to ovarian senescence, decreased estrogen production,

and production of androgens as the adrenal gland becomes the primary source of sex steroids in women approaching and transitioning through menopause.[7] This life stage-specific increase in DHEAS concentrations may alter the balance between androgens and estrogens and may therefore explain some of the health outcomes that are not explained by the analysis of estradiol and testosterone concentrations.

The relationship between a decline in ovarian cycles and an observed rise in adrenal androgens remains to be explained. It is probably mediated through the hypothalamus-pituitary axis. However, whether pituitary luteinizing hormone (LH) and/or adrenocorticoid-stimulating hormone is involved is not known, and several scenarios are possible. The first and simplest scenario is that the rise in adrenal androgens is a response to increasing LH due to a direct withdrawal of negative feedback on the pituitary that is exerted by primary ovarian sex steroids. A second scenario is that the classic adrenotropic drive results in a compensatory increase in adrenocorticoid-stimulating hormone due to shunting of pregnenolone from the $\Delta 4$ pathway to the $\Delta 5$ pathway. However, such dissociation between the production of cortisol and the adrenal androgens[8] requires a mechanism for the initial $\Delta 4$-$\Delta 5$ pathway shunting. Testing either scenario in humans would be challenging owing to the requirement for recruiting appropriate study cohorts. Still, there is a strong rationale for conducting such experiments because there is indirect evidence that gonadotropin-releasing hormone (GnRH) pulse amplitude is increased at this time in women[9-13] and in the nonhuman primate animal model.[14] This increased GnRH-mediated drive would probably lead to elevations in circulating LH[15] that could be the stimulus for adrenal androgens.

Elevated LH increases DHEAS/DHEA production in postmenopausal women,[16] and more recent data indicate that the source of the rise in weak androgens during the MT is the adrenal gland.[3] Ample evidence supports the concept that the primate adrenal gland can respond to LH. Thirty-five years ago, human chorionic gonadotropin (hCG), an LH-like molecule, was able to stimulate DHEAS secretion from the human adrenal fetal zone in perfusion experiments,[17,18] suggesting for the first time that the human adrenal gland may have functional LH/hCG or LH/chorionic gonadotropin (CG)–like receptors. It was later confirmed that the human adrenal gland expresses LH/hCG receptors in the reticularis and fasciculata zones,[19] and this was further corroborated in a human adrenal cell line.[20] However, the functionality of the LH/CG receptor in the human adrenal gland remains a matter of debate, as it has no identified major role in the regulation of adrenal androgen production in healthy women.[21]

Taken together, the putative presence of LH receptors in the adrenal gland and the decline in negative feedback on LH production during the MT provide the basis for hypothesizing a mechanism for increased adrenal androgen production in middle-aged women. The primary purpose of the current study was to first test our primary hypothesis—that increased LH drive contributes to DHEAS production in a higher primate animal model. A second objective was to validate a noninvasive animal model for future studies to investigate adrenal steroid production in middle-aged women. Two experiments directed at these objectives were carried out using the cynomolgus macaque.

METHODS

Animals

Two approaches were used to meet all of the goals of the study described here. First, a small group of animals (n = 4) was used in an invasive pilot study to establish proof of concept. These animals were surgically castrated to rule out ovarian steroid contribution. A more conservative noninvasive approach was used in a larger group of animals (n = 20) that were chemically castrated to prevent ovarian activity. Cynomolgus macaques (*Macaca fascicularis*) aged 18 to 26 years and weighing between 2.7 and 5.9 kg were selected for this study. Animals were housed indoors in individual cages at the California National Primate Research Center (CNPRC). Animal rooms were maintained on a 12-hour light/12-hour dark cycle (lights on at 06:00 AM) at a constant temperature of approximately 22°C and 60% relative humidity. Animals had ad libitum access to tap water and were fed standard Purina Monkey Chow (Ralston-Purina Co., St Louis, MO) twice daily, supplemented with fresh seasonal fruit. The CNPRC is fully accredited by the American Association for the Accreditation of Laboratory Animal Care. The University of California Davis Animal Use and Care Committee, the Campus Veterinarian, and the CNPRC approved the research protocol and procedures used for the study described in this report.

Urine samples and hormone analyses

Daily urine samples were collected from each animal for 30 days starting at least 10 days before the initiation of experimental treatments and continuing until at least 6 days posthCG challenge. Urine samples were stored at −20°C until assayed for ovarian steroid hormone metabolites, estrone conjugate (E_1C), and pregnanediol glucuronide (PdG) by immunoassay using the automated ACS-180 chemiluminescent immunoassay analyzer (Bayer Diagnostics Corp., Norwood, MA), as previously described.[22] Briefly, urine was prediluted in distilled water and incubated with the following specific reagent pairs: R6590 rabbit-anti-E_1C polyclonal antiserum and E_1C-horseradish peroxidase-dimethyl acridinium ester, or R13904 rabbit-anti-PdG polyclonal antiserum and PdG-horseradish peroxidase-dimethyl acridinium ester.[22] In both cases, the ACS-180 autoanalyzer performed all sample/reagent addition, incubation, and chemiluminescent signal-reading steps. Creatinine (CR) concentration was measured in all urine samples following a previously described method.[23] The E_1C and PdG results are expressed as nanograms per milligram of CR for each sample. The ovarian cycle phase was determined by the urinary steroid metabolites (E_1C and PdG) profile in retrospect. The CNPRC maintains a menses calendar database that was used to determine if the animals were menstruating (an indicator of ovarian cycle activity) for 1 year before the study.

Gonadotropin-releasing hormone agonist treatment

Gonadotropin-releasing hormone agonist (GnRHa) is commonly used to disrupt the hypothalamus-pituitary-ovary axis and to stop ovarian activity by decreasing gonadotropin.[24] Leuprolide has been used in the past to stimulate[25] and down-regulate pituitary response to GnRH.[26,27] Based on literature, a single dose (0.25 mg/kg of body weight) of leuprolide acetate (TAP Pharmaceutical Products Inc., Lake Forest, IL) was administered intramuscularly. Blood samples (3 mL) were collected by venipuncture of the femoral vein before, 2 days after, and 14 days after GnRHa injection.

Surgical procedure

Animals were sedated with 10 mg/kg of ketamine IM, given 0.04 mg/kg of atropine SC, intubated, placed on isoflurane anesthesia, and positioned in dorsal recumbency. A ventral caudal midline abdominal incision was made to visualize the body of the uterus and both ovaries. Ovarian vessels and the fallopian tubes were isolated, ligated, and severed. The abdominal wall was then closed in three layers, with two layers of 2/0 absorbable suture and a final subcuticular layer with 3/0 absorbable suture. The animals were then allowed to recover in the CNPRC surgical recovery unit for 3 days under immediate postoperative analgesia (1.5 mg/kg of oxymorphone, IM) three times daily, followed by a 2-week complete recovery period.

LH/CG challenge

Two weeks after surgical procedure or GnRHa treatment, an LH challenge experiment was performed to study the direct effect of LH/hCG on DHEAS production in the adrenal glands. A commercial hCG preparation (NOVAREL; Ferring Pharmaceuticals, Suffern, NY) was administered intramuscularly (1,000 IU/animal) to supplement the loss of endogenous LH after GnRHa treatment.

Serum samples and hormone analyses

A midmorning blood sample (3 mL) was taken from restrained, nonanesthetized animals immediately before and then 3, 6, 24, and 48 hours after hCG treatment. Sera were obtained by centrifugation of the blood samples and stored at −20°C until analysis. DHEAS was measured by radioimmunoassay using a commercially available kit (Coat-A-Count; Diagnostic Products Corp., Los Angeles, CA). Circulating bioactive LH/CG was measured using a stably transfected cell line, as previously described.[28]

Data analysis

Descriptive statistics and individual hormone profiles were used to identify individual differences at baseline and after treatment. Grouped data are expressed as mean ± SE in all figures. For nonparametric values, significant statistic differences among the groups were analyzed by Friedman repeated-measures analysis of variance (ANOVA) on ranks, followed by an all-pairwise multiple comparison procedure using the Student-Newman-Keuls method. Mann-Whitney rank sum test was used to determine differences in hormone levels at baseline, 2 days after, and 14 days after GnRHa treatment between monkeys with and without menstrual cycle activity. Pearson's product moment correlation was used to analyze the relationships among baseline hormone levels and different posttreatment time points. All statistical analyses were performed using Sigma Stat software (Systat, San Jose, CA). Significance was set at $P < 0.05$.

RESULTS

Animals

Twenty-four animals were recruited for both studies, and 10 of these were experiencing cyclic ovarian activity, as indicated by urinary metabolite excretion in the immediate 10 days before the initiation of the study. Another 10 animals had normal menstrual flow during the previous year, as verified by a routine check of the colony for vaginal bleeding. Only four animals were considered noncyclic or postmenopausal.

Pilot study

Three of the four surgically castrated animals exhibited a rise in DHEAS levels in response to the hCG challenge that followed a fall in mean DHEAS levels from 12.6 ± 3.8 µg/dL before surgical procedure to 8.5 ± 3.0 µg/dL immediately before hCG challenge 2 weeks later. Mean DHEAS levels then rose immediately to 13.4 ± 4.9 µg/dL ($P > 0.05$) in response to hCG 6 hours postinjection. Daily E_1C and PdG levels were consistently low in all animals after recovery from surgical procedure (data not shown). The average E_1C value was below 25 ng/mg of CR, whereas the mean PdG value was below 28 ng/mg of CR, with no difference in either E_1C or PdG levels before or after hCG treatment. These steroid hormone metabolite profiles were compared with corresponding data from previous studies in which cyclic, noncyclic, and castrated animals were characterized using the same methods.

Principal study

Baseline DHEAS concentrations ranged from nondetectable (analytical limit of detection [LOD] = 1.1 µg/dL) to a maximum of 12.7 µg/dL in 20 animals. Five of 20 animals in the principal study had no detectable levels of DHEAS and were not responsive to any manipulations during the entire study period; thus, they were not included in further analysis. For all the data collected from the remaining 15 animals, there were eight time points where DHEAS concentrations were below LOD (8 of 105 data points) and replaced with LOD divided by $\sqrt{2}$, a standard replacement technique.[29] The basal DHEAS level was 4.8 ± 0.98 µg/dL. GnRHa treatment decreased DHEAS mean concentrations at 14 days posttreatment to 3.4 ± 0.59 µg/dL (median: 2.8 vs 2.2 µg/dL, respectively, $P < 0.05$, Friedman repeated-measures ANOVA on ranks; Fig. 1). Daily E_1C levels significantly increased 2 days after GnRHa treatment (pretreatment: 72.9 ± 6.17 vs 101.5 ± 12.3 ng/mg of CR, respectively, $P < 0.05$, one-way repeated-measures ANOVA; Fig. 2), reaching the peak value on post-GnRHa treatment day 4 (119.4 ± 17.8 ng/mg of CR) and then decreasing steadily to posttreatment day 14 (37.5 ± 4.7 ng/mg of CR, $P < 0.05$, one-way repeated-measures ANOVA).
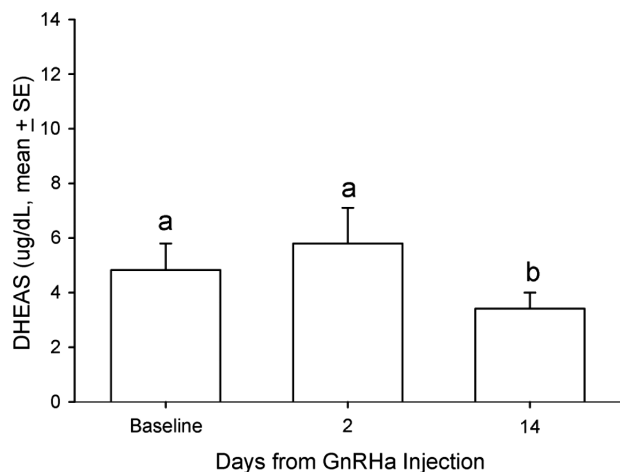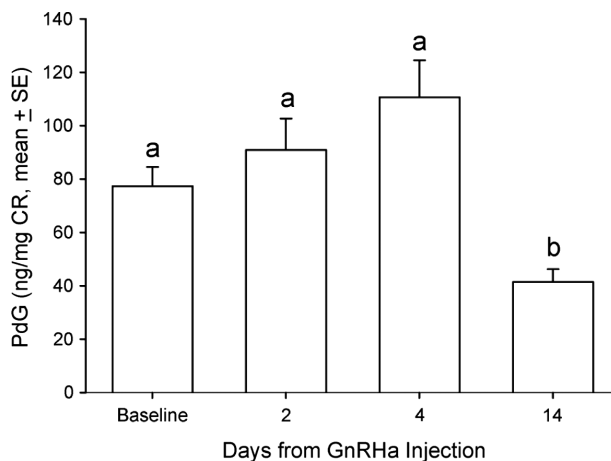
FIG. 1. Mean serum DHEAS concentration relative to GnRHa treatment in aging female macaques. A single dose (0.25 mg/kg of body weight) of GnRHa (leuprolide acetate) was administered intramuscularly. DHEAS was measured in serum obtained before (baseline), 2 days after, and 14 days after the GnRHa injection. Friedman repeated-measures analysis of variance on ranks was conducted for comparison. Levels not linked by the same letter indicate a statistically significant difference ($P < 0.05$). DHEAS, dehydroepiandrosterone sulfate; GnRHa, gonadotropin-releasing hormone agonist.

A similar pattern was observed for PdG levels, which reached the peak value on post-GnRHa treatment day 4, followed by a significant decrease on post-GnRHa treatment day 14 (pretreatment: 77.3 ± 7.2 ng/mg of CR; post-GnRHa treatment day 4: 110.6 ± 14.4 ng/mg of CR; post-GnRHa treatment day 14: 41.3 ± 4.8 ng/mg of CR, $P < 0.05$, one-way repeated-measures ANOVA; Fig. 3). The average E_1C value was below 27 ng/mg of CR, whereas the mean PdG value was below 31 ng/mg of CR before hCG challenge and remained consistently low in all animals after hCG challenge (Figs. 4 and 5). After
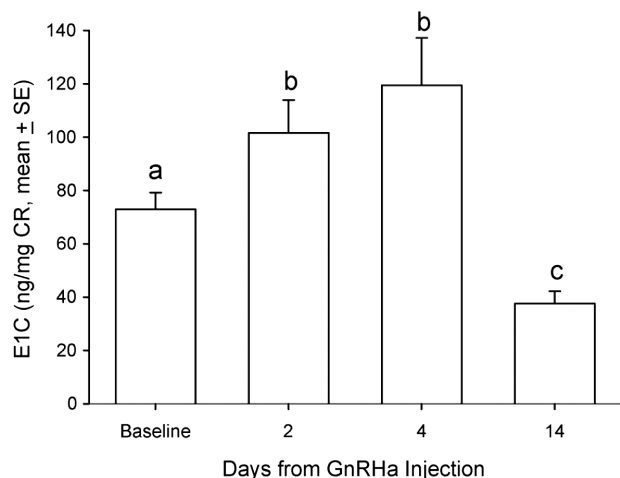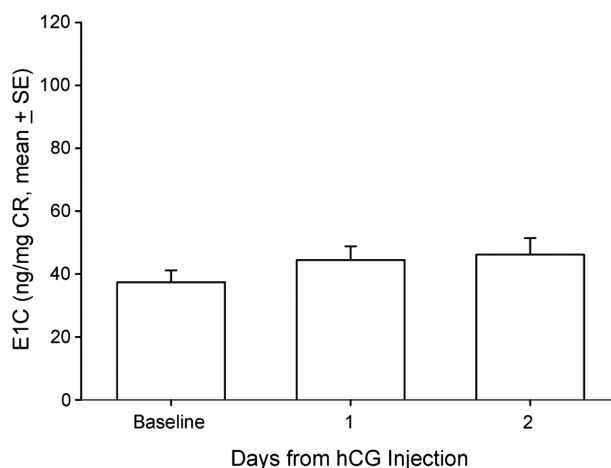

FIG. 2. Mean urinary estrogen metabolite (estrone conjugate [E_1C]) concentration indexed by CR concentration before and after GnRHa treatment in aging female macaques. A single dose (0.25 mg/kg of body weight) of GnRHa (leuprolide acetate) was administered intramuscularly. Friedman repeated-measures analysis of variance on ranks was conducted for comparison. Levels not linked by the same letter indicate a statistically significant difference ($P < 0.05$). CR, creatinine; GnRHa, gonadotropin-releasing hormone agonist.
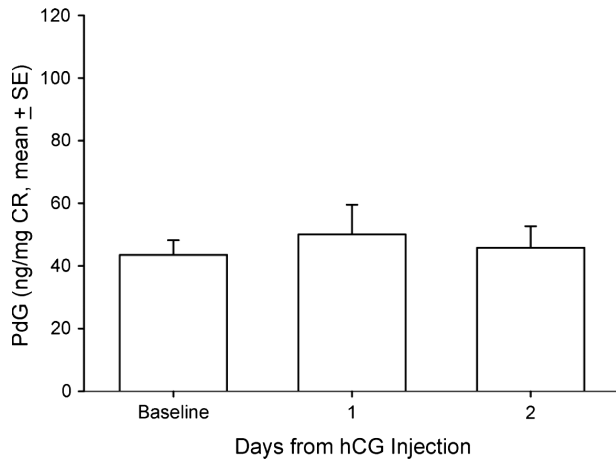

FIG. 3. Mean urinary progesterone metabolite (pregnanediol glucuronide [PdG]) concentration before and after GnRHa treatment in aging female macaques. A single dose (0.25 mg/kg of body weight) of GnRHa (leuprolide acetate) was administered intramuscularly. Friedman repeated-measures analysis of variance on ranks was conducted for comparison. Levels not linked by the same letter indicate a statistically significant difference ($P < 0.05$). CR, creatinine; GnRHa, gonadotropin-releasing hormone agonist.

hCG treatment, the mean DHEAS significantly increased to 5.9 ± 1.2 μg/dL at 3 hours and remained elevated for at least 48 hours after treatment (means: 10.9 ± 1.9 μg/dL [6 h]; 8.04 ± 1.4 μg/dL [24 h]; 7.9 ± 1.4 μg/dL [48 h]; medians: 8.7 μg/dL [6 h]; 6.7 μg/dL [24 h]; 7.2 μg/dL [48 h]; Fig. 6). There was a positive correlation between the concentration of circulating DHEAS levels before and 6 hours after hCG challenge (4.81 ± 0.98 vs 10.9 ± 1.9 μg/dL, $r = 0.63$, $P < 0.05$; Fig. 2). Four animals showed no cyclic activity; among them, only one animal had consistent nondetectable DHEAS regardless of any manipulations. There were no differences observed for DHEAS levels when animals with and without apparent menstrual activity (based on colony records) were compared at any of

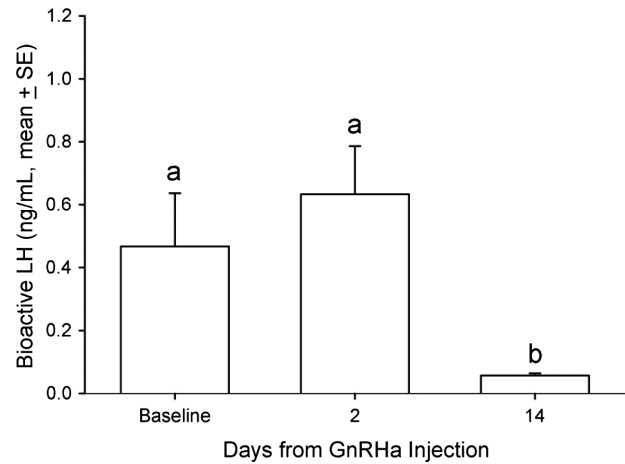**FIG. 4.** Mean urinary estrogen metabolite (estrone conjugate [E_1C]) concentration indexed by CR concentration before, 1 day after, and 2 days after hCG treatment in aging female macaques. A single dose (1,000 IU/animal) of hCG (NOVAREL) was administered intramuscularly 14 days after GnRHa treatment. CR, creatinine; GnRHa, gonadotropin-releasing hormone agonist; hCG, human chorionic gonadotropin.

FIG. 5. Mean urinary progesterone metabolite (pregnanediol glucuronide [PdG]) concentration before, 1 day after, and 2 days after hCG treatment in aging female macaques. A single dose (1,000 IU/animal) of hCG (NOVAREL) was administered intramuscularly 14 days after GnRHa treatment. CR, creatinine; GnRHa, gonadotropin-releasing hormone agonist; hCG, human chorionic gonadotropin.
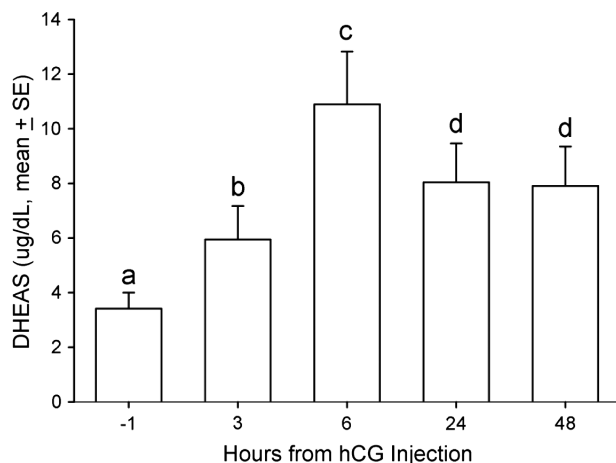

FIG. 7. Mean bioactive LH concentration relative to GnRHa treatment in aging female macaques. A single dose (0.25 mg/kg of body weight) of GnRHa (leuprolide acetate) was administered intramuscularly. Bioactive LH in the three serum samples before the hCG challenge was measured. Friedman repeated-measures analysis of variance on ranks was conducted for comparison. Levels not linked by the same letter indicate a statistically significant difference ($P < 0.05$). GnRHa, gonadotropin-releasing hormone agonist; LH, luteinizing hormone.

the time points analyzed before or after the treatments ($P > 0.05$).

There was no observed correlation between DHEAS and PdG levels at baseline ($r = -0.18$, $P > 0.05$) or at 6 hours after hCG treatment ($r = 0.12$, $P > 0.05$). Serum LH/CG bioactivity levels were observed to have a modest increase 2 days post-GnRHa treatment, followed by a substantial and significant decrease 14 days post-GnRHa treatment (basal level: 0.47 ± 0.17 ng/mL; post-GnRHa treatment day 2: 0.63 ± 0.15 ng/mL; post-GnRHa treatment day 14: 0.06 ± 0.01 ng/mL, $P < 0.05$, one-way repeated-measures ANOVA; Fig. 7). The animals

lacking recent or regular menstrual activity (4 of 20) had circulating LH levels greater than those with normal menstrual calendars at baseline (median: 1.80 vs 0.09 ng/mL; mean: 1.85 ± 0.19 vs 0.16 ± 0.05 ng/mL, $P < 0.05$, Mann-Whitney rank sum test) and at 2 days post-GnRHa treatment (median: 1.73 vs 0.31 ng/mL; mean: 2.12 ± 0.64 vs 0.42 ± 0.11 ng/mL, respectively, $P < 0.05$, Mann-Whitney rank sum test). Although all animals were suppressed, there was still a significant difference in circulating bioactive LH between cyclic and noncyclic animals at 14 days post-GnRHa.

DISCUSSION

To avoid as much stress-induced adrenal steroid production as possible, we used urinary monitoring in this study whenever appropriate and handled animals as little as possible. In one group of animals, the natural source of gonadotropin support was withdrawn by down-regulating the GnRH receptor by a single injection of GnRHa. This "castration effect" of GnRHa treatment has been observed to be 100% effective in most models, yet it is reversible and not associated with significant adverse effects beyond the reduction of gonadotropin secretion. In the other group of animals, complete recovery from ovariectomy was allowed before hCG challenge. Thus, the minimization of physical manipulations and the collection of sequential urine (rather than blood samples) for endocrine evaluations in our experimental design obviated most concerns regarding a stress-induced increase in adrenal steroid production. The gonadotropin challenge in this study was delivered as a single treatment with exogenous hCG, which is known to have no effect beyond the induction of signal transduction mediated through LH/CG receptors. We hypothesized that DHEAS production could be accelerated using a noninvasive experimental design with a nonhuman

FIG. 6. Mean serum DHEAS concentration relative to hCG treatment in aging female macaques. A single dose (1,000 IU/animal) of hCG (NOVAREL) was administered intramuscularly 14 days after GnRHa treatment. DHEAS was measured in serum samples before and at several time points after hCG treatment. Friedman repeated-measures analysis of variance on ranks was conducted for comparison. Levels not linked by the same letter indicate a statistically significant difference ($P < 0.05$). DHEAS, dehydroepiandrosterone sulfate; GnRHa, gonadotropin-releasing hormone agonist; hCG, human chorionic gonadotropin.

primate animal model. This was based on the premise that DHEAS is primarily an adrenal product[30,31] and that LH/CG receptors may be present and functional in the adrenal cortex of older female primates. However, as Havelock et al[16] demonstrated, the postmenopausal ovary and perhaps the premenopausal ovary may also have the capacity to respond to LH/hCG and produce Δ5 steroids. This possibility was ruled out by ovariectomy in the current design.

GnRHa treatment induced an initial modest increase in DHEAS concentration followed by a significant decrease, supporting the concept that circulating LH maintains adrenal DHEAS production because LH concentrations follow the same trajectory as DHEAS after GnRHa treatment. Furthermore, the hCG challenge triggered a rapid response in circulating DHEAS concentrations, which was sustained for at least 48 hours. GnRHa treatment also induced a transient increase in E_1C and PdG, followed by a significant decrease in both ovarian steroid metabolites. This supports the concept that GnRHa is effective in decreasing ovarian steroidogenesis after a flare reaction (Figs. 2 and 3).

These experimental data indicate that the nonhuman primate animal model is appropriate for studies relating to the endocrinology of the MT. The explanation that LH receptors are responsible for the rise in adrenal androgen production in later life is based largely on observations,[32] partly on speculations, and even less on experimental evidence. In fact, few of the mechanisms for androgen production in older women have been well characterized, and the source of androgens is still a controversial issue.[30] This uncertainty makes the broad use of DHEAS therapy controversial, and mounting evidence indicates that such supplements may not provide the expected benefit for women who already exhibit increased DHEAS production and healthy aging. Such issues may be best resolved by dissecting the problem empirically using an appropriate animal model, as we have attempted to do here.

Five important observations arise from this study. First, GnRHa treatment decreased DHEAS concentrations consistently in all animals, demonstrating the involvement of the pituitary-adrenal axis signal pathway in the production of androgens of adrenal and/or ovarian origin. Second, suppressed DHEAS secretion, either spontaneously or caused by GnRHa, was consistently stimulated after hCG treatment in all animals, including three animals that had baseline DHEAS levels below assay sensitivity before hCG challenge. This suggests a potential up-regulation of adrenal sensitivity in response to gonadotropin challenge after reduced LH support. Third, DHEAS was detected immediately in most animals at 6 hours post-hCG challenge (Fig. 6), suggesting that the adrenal response to gonadotropin stimulation persists during pituitary down-regulation and that all of the animals responded in a consistent temporal fashion to the gonadotropin challenge even though they had different, and sometimes undetectable, basal circulating DHEAS concentrations. Fourth, there was conspicuous absence of a short-term increase in levels of ovarian steroid metabolites (E_1C and PdG) immediately after the hCG challenge. This suggests that the observed DHEAS elevation

was of adrenal origin, with little or modest involvement of the ovary in the chemically castrated group. Fifth, there is a positive correlation between DHEAS levels before and at different time points after hCG treatment, as well as a proportionality of the response to hCG treatment with baseline DHEAS levels. These data suggest different sensitivities to endogenous LH and exogenous hCG stimuli among the animals, producing different levels at baseline and after the hCG challenge, respectively. The pulsatile nature of LH secretion precludes any expectation of a stronger correlation among basal levels of LH and corresponding DHEAS levels. The significance of these observations, however, is that they suggest that DHEAS secretion in these animals was mediated by the GnRH/LH pathway and was stimulated through LH/CG receptors in the adrenal gland that mediated a consistent response. Thus, the individual differences in circulating DHEAS levels were quantitatively but not qualitatively different.

To our knowledge, there has been no previous evidence that hCG can stimulate adrenal steroid production in vivo in either middle-aged women or laboratory macaques; however, the current data suggest that a change in ovarian status (by ending negative feedback on gonadotropins) may contribute indirectly to increased adrenal sensitivity to an LH-like stimulation. It is possible that the increased levels of DHEAS observed in this study were due not only to direct LH/CG stimulation but also to decreased metabolic clearance. However, the fact that DHEAS decreased similarly after GnRHa treatment in all animals argues strongly against this possibility.

Recently, Study of Women's Health Across the Nation investigators have shown that neither cardiovascular disease nor cardiovascular risk factors are positively related to anovulation or longer menstrual cycles in middle-aged women.[33] However, a significant negative correlation between these two health factors and increased urinary excretion of estrogen and progesterone metabolites was found when daily hormone patterns were examined. This observation suggests that maintenance of normal ovarian steroid production may prevent the MT rise in LH and the attendant increase in adrenal androgen production. In this study, the mean LH/CG bioactivity and DHEAS profiles followed similar patterns (Figs. 1 and 7), and the decrease in DHEAS levels after GnRHa treatment (Fig. 1) suggests that the pituitary drive is responsible for the increase in DHEAS secretion. One possible explanation for not showing a direct positive relationship between circulating LH/hCG and DHEAS concentrations may be that differences in DHEAS production primarily reflect differences in the adrenal sensitivity to modestly increased LH concentrations. This seems improbable because most of the animals responded to the hCG challenge in a qualitatively similar fashion (Fig. 6). A more attractive possibility—and one that is most consistent with all observations—is that a tropic compound that is LH or structurally similar to LH, produced by the pituitary, and under the control of GnRH is capable of stimulating adrenal or ovarian DHEAS production. The cognate receptor in the adrenal gland for this tropic compound would be the LH receptor or could resemble the LH receptor.

CONCLUSIONS

These results are consistent with recent findings that the adrenal glands of middle-aged women have the capacity to increase the production of Δ4 adrenal steroids. The increase in adrenal Δ5 steroids seems to be under the control of LH. In addition, the results reported here indicate that this increase in Δ5 adrenal steroids during the MT is independent of increased ovarian steroidogenesis.

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