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Circulating Levels of Allopregnanolone in Humans: Gender, Age, and Endocrine Influences

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ABSTRACT

Allopregnanolone is a neuroactive steroid involved in modulating behavioral functions, stress, and neuroendocrine axes in rats. Changes in plasma allopregnanolone levels throughout the menstrual cycle have been reported in healthy women, but there exists no information on the possible gender or age-related changes or on the source(s) of circulating allopregnanolone. The aim of the present study was to assess serum allopregnanolone concentrations according to gender, menstrual cycle, age, and menopause in normal men and women; serum progesterone (P) and dehydroepiandrosterone (DHEA) levels were evaluated in the same specimens. In addition, the possible source of circulating allopregnanolone in fertile women was investigated by using stimulatory and inhibitory endocrine tests acting on the ovary and/or adrenal cortex.

The present study included 189 fertile women, 112 postmenopausal women, and 46 men. Serum steroid levels were determined after extraction, using specific RIAs. Allopregnanolone levels in fertile women in the follicular phase were similar to those in age-matched men; no significant difference was found between fertile women in the follicular phase and postmenopausal women. The highest levels were found in fertile women during the luteal phase ($P < 0.01$). An age-related decrease was observed in men ($P < 0.01$), but not in women. P and DHEA levels were significantly higher in women than in men and were higher in fertile women than in postmenopausal women ($P < 0.01$). Both P and DHEA showed an age-related decrease in men and women ($P < 0.01$).

Serum allopregnanolone and P, but not DHEA, significantly increased in response to a GnRH test, whereas corticotropin-releasing factor and ACTH tests elicited a significant increase in allopregnanolone, P, and DHEA levels ($P < 0.01$). The suppression of adrenal steroidogenesis by dexamethasone markedly reduced both allopregnanolone and DHEA serum levels ($P < 0.01$).

In conclusion, the present study demonstrated that although men show an age-related decrease, serum allopregnanolone levels in women do not change with age and correlate with P levels during the menstrual cycle and in response to endocrine tests. Ovary and adrenal cortex may be major sources of circulating allopregnanolone in fertile women. (*J Clin Endocrinol Metab* **83:** 2099–2103, 1998)

ALLOPREGNANOLONE, 3α-hydroxy-5α-pregnan-20-one, is a ring A-reduced pregnane derivative of progesterone (P), synthesized in the gonads, adrenal cortex, and central nervous system (1–7). In rats, an ovarian origin of circulating allopregnanolone has been demonstrated by 1) its presence in ovarian tissue, 2) increased circulating levels at estrous, and 3) decreased levels after ovariectomy (8). An adrenal origin is suggested by the disappearance of plasma allopregnanolone in female rats only after combined adrenalectomy and ovariectomy (8).

Experimental studies have shown an involvement of allopregnanolone in stress, mood, and sexual behavior (9–11). The changes in brain allopregnanolone concentration in female rats during the estrous cycle (12) and the effect of intracerebroventricular injection of allopregnanolone or its antiserum on ovulatory rat function (13, 14) suggest a correlation between brain allopregnanolone and reproductive function. The central effect of allopregnanolone is probably mediated by its interaction with γ-aminobutyric acid$_A$ (GABA$_A$) receptors or through an increase in GABA$_A$ receptor sensitivity to endogenous GABA (15–17).

Modifications of circulating allopregnanolone levels throughout the menstrual cycle in humans have been reported (18, 19). High levels have been observed during the luteal phase of the menstrual cycle, with controversial results on the possible involvement of allopregnanolone in the premenstrual syndrome (14, 18). An increase in serum allopregnanolone levels in women during the third trimester of pregnancy has been correlated with an increase in GABA$_A$ receptor sensitivity to GABA agonists and with alterations in mood (15).

Although several studies have focused on the central effects of allopregnanolone, no study regarding physiological changes in serum levels in humans is available at present. Consequently, the aim of the present study was to assess the difference in allopregnanolone concentrations according to gender, menstrual cycle, age, and menopause in normal subjects. In addition, possible sources of allopregnanolone in human circulation were investigated by evaluating the response of allopregnanolone to functional endocrine tests ac-

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tivating ovary (GnRH test) or adrenal cortex [corticotropin-releasing factor (CRF) and ACTH tests]. In all specimens, simultaneous evaluation of serum P and dehydroepiandrosterone (DHEA) levels was also performed in the present study.

Subjects and Methods

Subjects

After giving informed consent, 347 subjects participated in the present study; they were subdivided into the following 3 groups: 1) fertile women (n = 189; follicular phase, n = 81; luteal phase, n = 108), aged 16–39 yr; 2) postmenopausal women (n = 112), aged 40–72 yr; and 3) men (n = 46), aged 19–70 yr. Before entering the study each subject had medical history, physical examination, and routine laboratory tests performed, which disclosed no abnormalities. Subjects with a history of cancer were excluded. None of the subjects was taking psychoactive medications, hormonal drugs (including oral contraceptives), mineral or vitamin supplements, or antiinflammatory drugs. A diagnostic interview did not show current or recent (within the past 2 yr) medical or psychiatric illness, and no subject described pathological changes in mood or behavior in the recent years. All fertile women reported regular menstrual cycle lengths, with serum P values above 20 nmol/L. Informed consent was obtained from each subject after full description of the protocol, which had been approved by the local ethical committee.

Protocol

In each subject a blood sample was drawn between 0700–0900 h. Blood samples were centrifuged and serum stored at −20 C until assayed.

In some of the fertile women the following functional tests were performed: 1) GnRH test (n = 6 in the luteal phase), consisting of a bolus injection of 100 μg GnRH (Ferring, Kiel, Germany); 2) CRF test (n = 6 women in the follicular phase), consisting of an iv bolus injection of 100 μg human CRF (Clinalfa, Laufelfingen, Switzerland); 3) ACTH test (n = 6 in the follicular phase), consisting of an im injection of 0.50 mg ACTH (Synachten, Ciba-Geigy, Huningue, France); and 4) dexamethasone (DXM) test (n = 4 in follicular phase), consisting of the administration of DXM (0.5 mg Decadron, Merck, Sharp, and Dohme, Rome, Italy) at 6-h intervals for 48 h.

In all patients a catheter was inserted into the antecubital vein, and a slow infusion of 0.9% saline solution was begun. Blood samples were taken 0, 15, 30, 45, 60, 90, and 120 min after injection of GnRH or CRF and 0, 30, 60, and 90 min after ACTH injection; blood samples were taken at 0800 h and 24 and 48 h after the first administration of DXM.

Allopregnanolone, P, and DHEA were assayed by using a specific RIA.

Allopregnanolone, P, and DHEA assays

Analytical grade solvents were purchased from Merck (Darmstadt, Germany); C_{18} Sep-Pak cartridges were obtained from Waters Corp. (Milford, MA). Standard allopregnanolone was purchased from Sigma Chemical Co. (St. Louis, MO), and 5α-[9,11,12-N-^{3}H]pregnan-3α-ol-20-one (45 Ci/nmol) was purchased from Amersham (Aylesbury, UK). The polyclonal antisera raised in sheep against allopregnanolone carboxymethyl ether coupled to BSA was provided by Dr. R. H. Purdy. Serum samples (1 mL) were thawed. Standard solutions of allopregnanolone (0, 15.6, 31.2, 62.5, 125, 250, 500, and 1000 pg steroid/tube) in steroid-free serum (pooled human serum, stripped from endogenous steroids by repeated treatment with charcoal: 50 mg/mL), recovery standard of tritium-labeled steroid was added (1200–1500 cpm), and the samples were extracted twice with 8 mL diethyl ether. The organic phases were removed and evaporated to dryness in Universal Vacuum System Plus (Savant, Rome, Italy). The extracts were eluted with assay buffer (20 mmol/L sodium phosphate in saline, containing sodium azide and BSA, 0.1 g/L each) and divided into two aliquots, each of 500 μL, corresponding to 500 μL serum. The samples in duplicate were passed through a C_{18} Sep-Pak cartridge, previously equilibrated with a solution of aqueous methanol (50:50, vol/vol) containing 1% acetic acid. After washing the cartridge with 50% aqueous methanol containing 1% acetic acid and 50% aqueous methanol, the elution of allopregnanolone was performed with absolute methanol (10 mL). The dry extracts were then assayed by RIA as following: the radioligand (7000 cpm) and the antibody (working dilution, 1:4000, vol/vol; 100 μL each) dissolved in the assay buffer were added to the tubes containing samples and standards, and the volume was adjusted to 500 μL with buffer. The samples for determination of nonspecific binding contained only the radioligand. After vortex mixing and overnight incubation at 4 C, bound and free steroids were separated by dextran-charcoal adsorption (0.25 g charcoal and 0.025 g dextran-70 in 100 mL assay buffer, 0.5 mL) followed by centrifugation at 4 C for 10 min. The radioactivity of supernatants was measured in a liquid scintillation spectrometer equipped with a program for counting disintegrations per min and RIA calculation. After extraction with ether, the recovery of labeled allopregnanolone was 96.3 ± 4.8%; after extraction and chromatography, it was 87.5 ± 7.9%; and for the entire procedure, the recovery of unlabeled standard allopregnanolone was 77.5 ± 9.8%. The sensitivity of the assay, expressed as a minimal amount of allopregnanolone distinguishable from the zero sample with 95% probability, was 15–20 pg/tube, and the intra- and interassay coefficients of variation were 7.2% and 9.1%, respectively. A parallelism test was also performed: a sample containing a high concentration of standard unlabeled allopregnanolone (~2000 pg/mL) was diluted with 0 standard, producing the results reported in Table 1.

Serum P was determined, after ether extraction and chromatographic partition on C_{18} Sep-Pak cartridge, by RIA using a commercially available kit (Radim, Pomezia, Italy); the sensitivity of the assay was 50 pg/tube, and the intra- and interassay coefficients of variation were 6.5% and 8.7%, respectively.

Serum samples for the determination of DHEA were extracted with ether, purified through a C_{18} Sep-Pak cartridge, and then assayed by RIA using trade kit (DSL, Webster, TX); the sensitivity was 15 pg/mL, and the intra- and interassay coefficients of variation were 3.1% and 6.9%, respectively.

Statistical analysis

Statistical analysis of the results was performed with a Macintosh personal computer using Abacus Concepts, Stat-View 4.0 program (Berkeley, CA). All results are reported as the mean ± SEM. Related measures ANOVA was used for comparison of hormone levels between times, and Scheffe's F test was used to determine the locations of significant differences in mean values.

Results

Mean ± SEM serum allopregnanolone levels did not show any significant difference between young fertile women during the follicular phase (n = 81) and age-matched men (0.79 ± 0.30 *vs.* 0.75 ± 0.08 nmol/L). In women (n = 108), allopregnanolone levels were significantly higher (3.69 ± 0.96 nmol/L) during the luteal phase than those during the follicular phase or those found in men ($P < 0.001$; Fig. 1). Serum P levels in women during both follicular (5.32 ± 2.10 nmol/L) and luteal (34.41 ± 7.80 nmol/L) phases were significantly higher than those in men (1.9 ± 0.08 nmol/L; $P < 0.001$; Fig. 2). Also, DHEA levels were higher in women than in men (33.39 ± 3.89 nmol/L in the follicular phase and 31.33 ± 6.06 nmol/L in the luteal phase *vs.* 16.33 ± 0.61 nmol/L in men; $P < 0.01$; Figs. 1 and 2).

TABLE 1. Recovery of allopregnanolone after dilution

Dilution	Expected	Measured	Recovery (%)
Undiluted		1982	
1:2	991.00	931.54	94
1:4	495.50	431.08	87
1:8	247.75	222.97	90
1:16	123.87	115.20	93
1:32	61.93	50.78	82

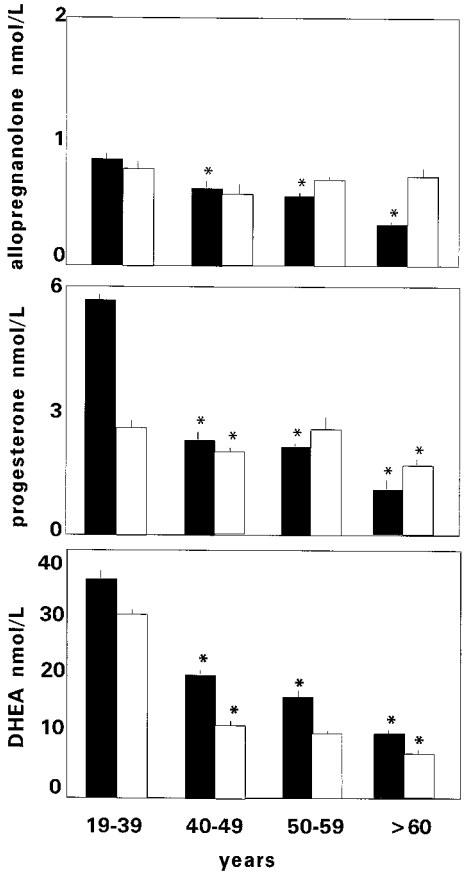

FIG. 1. Age-related changes in allopregnanolone, P, and DHEA levels. In each age group, the *black column* is the men's values, and the *white column* is the women's values (fertile women were in the follicular phase).

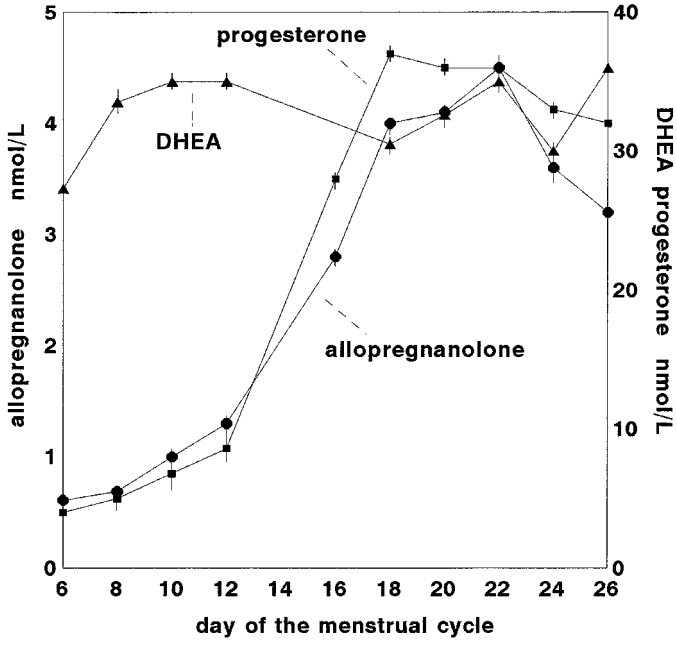

FIG. 2. Allopregnanolone (●), P (■), and DHEA (▲) levels according to the phase of the menstrual cycle.

Although allopregnanolone levels did not change with age in women over 40 yr old, a progressive decrease was observed in groups of men more than 40 and more than 50 yr of age, showing the lowest value at more than 60 yr of age ($P < 0.01$; Fig. 1). Both P and DHEA levels in women and men showed a significant decrease according to age ($P < 0.01$; Fig. 2). When data were evaluated on the basis of menopausal age, mean ± SEM serum allopregnanolone levels in postmenopausal women (0.66 ± 0.34 nmol/L) resulted in the same range of values as those in age-matched men and fertile women in the follicular phase (Fig. 2). P and DHEA levels in postmenopausal women (1.59 ± 1.13 and 9.59 ± 4.43 nmol/L, respectively) were significantly lower than those in fertile women ($P < 0.01$; Fig. 2).

Serum allopregnanolone and P showed a significant increase in response to GnRH test [peak values, 4.11 ± 1.03 nmol/L at 45 min ($P < 0.01$) and 40.7 ± 6.6 nmol/L at 120 min ($P < 0.01$), respectively], whereas there were no significant changes in DHEA (Fig. 3).

When CRF was injected, serum P and DHEA significantly increased, peaking 30 min from the beginning of the test (5.35 ± 0.10 and 37.0 ± 4.3 nmol/L, respectively), whereas allopregnanolone peaked at 60 min (2.53 ± 0.16 nmol/L; $P < 0.01$; Fig. 4).

The ACTH test caused significant increases in serum allopregnanolone (0.84 ± 0.24 nmol/L at 30 min; $P < 0.01$), P (4.58 ± 1.58 nmol/L at 30 min; $P < 0.01$), and DHEA (62.2 ± 9.70 nmol/L at 60 min; $P < 0.01$) levels (Fig. 5).

The DXM test caused a significant decrease in serum allopregnanolone (0.49 ± 0.16 nmol/L after 48 h; $P < 0.01$) and DHEA (2.7 ± 0.5 after 48 h; $P < 0.01$) levels.

Discussion

The present study showed changes in serum allopregnanolone in women and men. No gender-related differences were shown between fertile women in the follicular phase and age-matched man, whereas an age-related decrease in serum allopregnanolone was demonstrated only in men. In fertile women, allopregnanolone closely correlates to P levels during the luteal phase of the menstrual cycle. These data confirm the findings of previous studies (18–21). The correlation disappears in postmenopausal women, and the age-related decrease in P levels in postmenopausal women is not paralleled by a similar decrease in allopregnanolone levels, suggesting that ovarian P is not the major determinant of circulating allopregnanolone.

The responses of allopregnanolone to GnRH test, CRF test, and ACTH test suggest that both ovary and adrenal cortex are possible sources of allopregnanolone. In fact, the GnRH test activating ovarian function as well as the CRF or ACTH test stimulating adrenal cortex function induce a significant increase in allopregnanolone levels. Therefore, ovary and adrenal cortex may both contribute to circulating allopregnanolone. The delayed response of allopregnanolone levels to CRF compared to ACTH is probably related to the time required for CRF to elicit pituitary hormone secretion. However, although the response of P to CRF is similar to its response to ACTH, the allopregnanolone response to CRF is significantly higher than its response to ACTH. The unex-

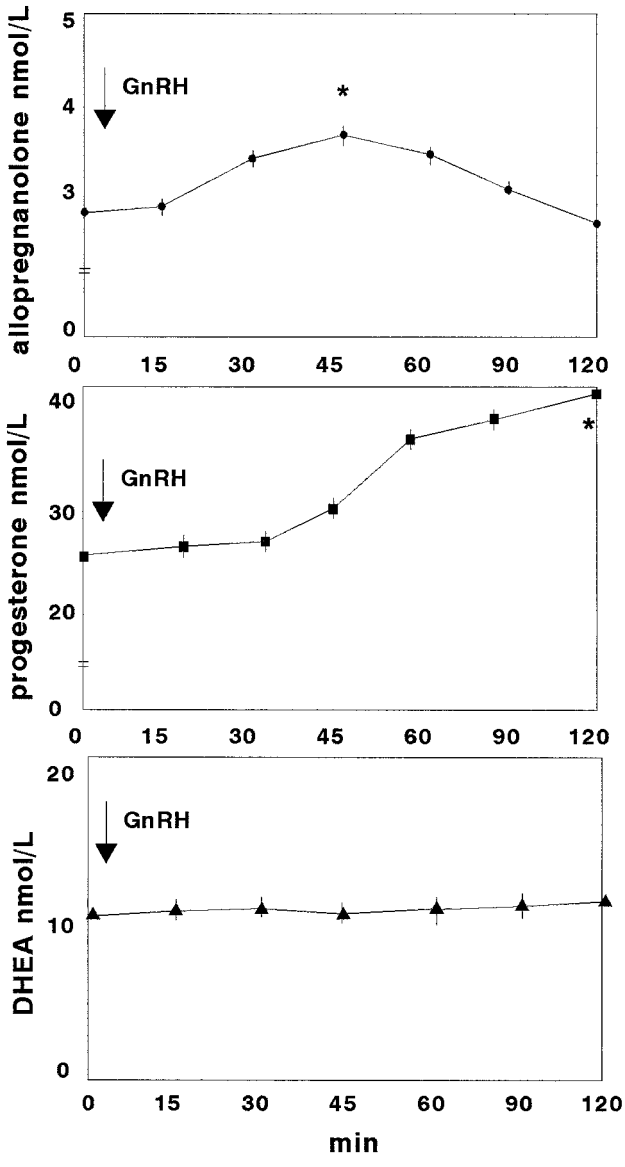

FIG. 3. Mean ± SEM allopregnanolone, P, and DHEA levels in response to the GnRH test. *, $P < 0.01$ *vs.* 0 min.

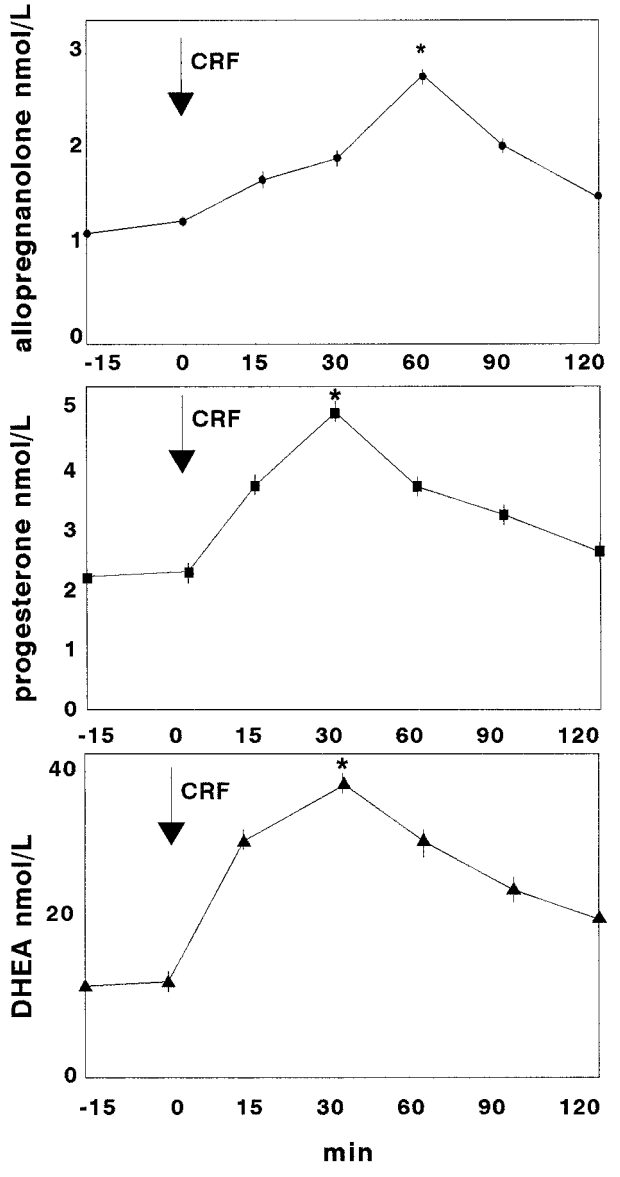

FIG. 4. Mean ± SEM allopregnanolone, P, and DHEA levels in response to the CRF test. *, $P < 0.01$ *vs.* 0 min.

pectedly high response of allopregnanolone to the CRF test compared with that to the ACTH test may be a consequence of a direct and/or indirect stimulation of allopregnanolone synthesis by CRF, independent from the ACTH-mediated stimulation of adrenal steroidogenesis. The observation of a marked reduction in allopregnanolone levels after the DXM test supports the hypothesis that the adrenal cortex largely contributes to its circulating levels, directly or via the synthesis of a precursor converted peripherally in allopregnanolone.

On the other hand, the evidence that the GnRH test determines a significant increase in serum allopregnanolone levels and a limited and delayed increase in serum P levels suggests that steroid secretion from the ovary in response to GnRH is more prone toward allopregnanolone release than toward P release.

In men, allopregnanolone levels show a decrease correlated with age, whereas in women, this decrease is not observed; a GnRH-induced gonadotropin increase may determine an increase in allopregnanolone secretion from the ovary in postmenopausal women. Likewise, the high levels of gonadotropins observed in these subjects may explain the lack of an age-related decrease in allopregnanolone levels, as would be predicted on the basis of age-related P decrease. The age-related decreases in P and DHEA are consistent with previous findings (22, 23).

In conclusion, the present study demonstrated that serum allopregnanolone levels in men decrease with age, whereas no changes are observed in women either after menopause or with advancing age. In fertile women, allopregnanolone levels correlate with P levels during the luteal phase of the menstrual cycle and partially in the follicular phase in response to endocrine tests. The significant increase in serum allopregnanolone in response to CRF and ACTH tests and

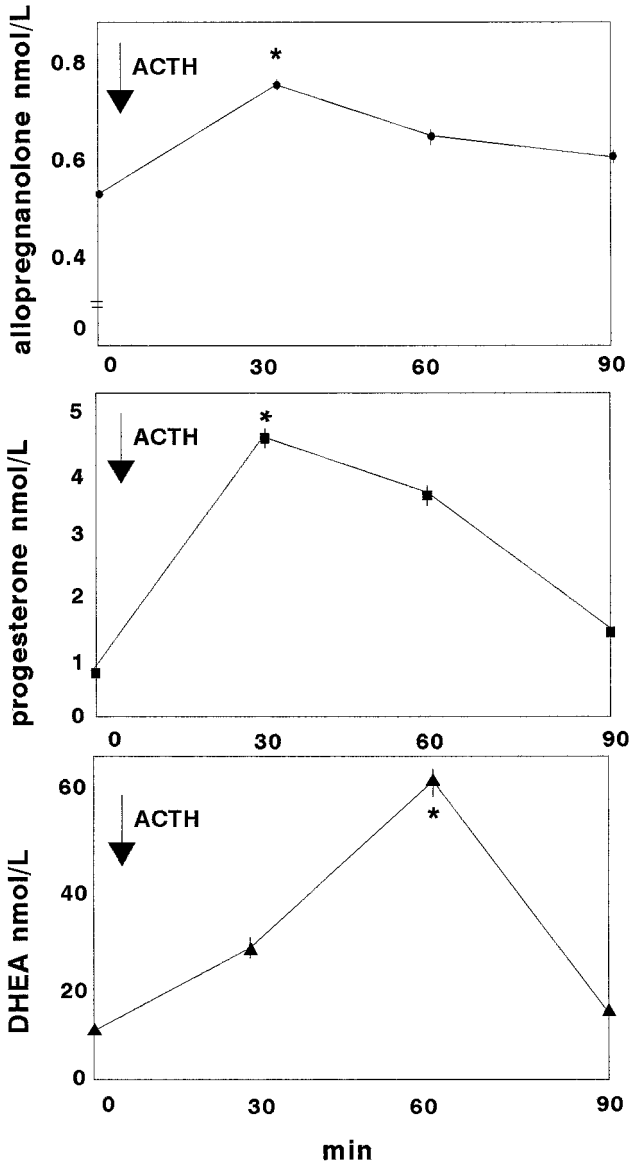

FIG. 5. Mean ± SEM allopregnanolone, P, and DHEA levels in response to the ACTH test. *, $P < 0.01$ *vs.* 0 min.

the reduction in plasma allopregnanolone after DXM-mediated suppression indicate that the adrenal cortex is a site of production of circulating allopregnanolone, directly or indirectly via the synthesis of a precursor. It seems unlikely that P is the precursor of allopregnanolone in men and in nonluteal phase women; therefore, it would be of interest to evaluate pregnenolone sulfate levels in these subjects in correlation with circulating levels of allopregnanolone. In addition, the increase in serum allopregnanolone in response to the GnRH test and the presence of a large amount of serum allopregnanolone not suppressed by DXM indicate that in women, the ovary significantly contributes to circulating allopregnanolone. Therefore, these results indicate that in man, the adrenal cortex is an important site of production of circulating allopregnanolone, and the age-related decrease in steroid levels might be related to an impairment of the adrenal steroidogenic activity. In women, an ovarian contribution to circulating allopregnanolone seems to be present in both fertile women and postmenopausal subjects. The real impact that these preliminary results may have on the physiopathology of female reproductive function has yet to be elucidated.

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