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The Influence of Gender, Age, and the Menstrual Cycle on Plasma Atrial Natriuretic Peptide*

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ABSTRACT. To examine the influence of gender, age, and the menstrual cycle on atrial natriuretic peptide (ANP) levels, we measured daily levels of ANP, aldosterone, estrogen, and progesterone in 13 young women (ages 25–35 yr) during the luteal phase of the menstrual cycle and daily ANP and aldosterone levels in 9 young men (ages 25–43 yr) for 10 consecutive days. In additon, fasting plasma ANP levels were assayed in 12 elderly male (ages 62-86 yr) and 9 elderly female subjects (ages 64–80 yr) on at least two separate occasions.

The average daily ANP levels in the young women were much higher than those in the men (68.1 ± 5.5 *vs.* 39.8 ± 3.4 pmol/L; $P < 0.001$), although no cyclical changes in ANP levels were observed. ANP levels were 94.0 ± 17.9 pmol/L in elderly men and 78.3 ± 19.4 pmol/L in elderly women. Aldosterone levels were higher in women than men during the luteal phase of the menstrual cycle (1154 ± 125 *vs.* 488 ± 42 pmol/L; $P < 0.001$), but not during the periovulatory period (580 ± 103 pmol/L) or during menses (563 ± 61 pmol/L).

In conclusion, ANP levels in young women average approximately twice those in young men, but do not fluctuate with the cyclical changes in estrogen, progesterone, and aldosterone seen during the menstrual cycle. However, ANP levels in postmenopausal women are not greater than those in age-matched elderly men. Thus, gender appears to affect the secretion or metabolism of ANP during the premenopausal years of life. (*J Clin Endocrinol Metab* **70:** 349, 1990)

CARDIAC myocytes produce and release atrial natriuretic peptide (ANP) into the circulation, primarily in response to atrial stretch and volume expansion (1–5). ANP, in turn, produces natriuresis, diuresis, and vasodilation and may play an important role in homeostasis of sodium, water, and blood pressure (4–10). In addition to these direct effects, ANP interacts with other hormonal systems such as renin-aldosterone, vasopressin, catecholamines, and cortisol (4–8).

The female sex hormones are also known to have effects on sodium homeostasis. Progesterone has a natriuretic effect due to aldosterone antagonism, whereas estrogens are associated with sodium retention, although the mechanism is not well understood (11–16). The sex hormones have been implicated in midcycle and premenstrual edema, but, again, the relationship is unclear (11, 15). Administration of exogenous estrogen has been shown to expand plasma volume and increase blood pressure (17, 18). However, this effect is transient, implying some escape phenomenon, such as is seen with mineralocorticoid administration.

Just as ANP has been implicated in the escape from mineralocorticoid-induced sodium retention (3), we wondered if there might be a role for ANP in maintaining sodium homeostasis and blood pressure during the normal menstrual cycle. In this study, we examined the influence of gender and the menstrual cycle on ANP levels in plasma. Our results demonstrate a striking difference between the sexes in basal ANP levels, with levels in premenopausal women approximately twice those in men, but no clear relation in women between the levels of ANP and the time of the menstrual cycle.

Subjects and Methods

We studied 13 women, aged 25–35 yr (mean ± SE, 30.3 ± 0.62), and 9 men, aged 25–43 yr (36.4 ± 2.3 yr). All women had normal menstrual cycles for at least 6 months before this study. All subjects were in good health, had normal body habitus and normal blood pressure, and were taking no medications. There were no significant gender differences in these parameters. All subjects were on a weight-maintaining diet. Daily venous blood samples were taken after a 2-h fast for hormone analysis and placed immediately into prechilled test tubes containing EDTA-2Na (1.5 mg/mL) and Traysolol (400 kallikrein inhibitor units/mL). For the women, an estimated midcycle sample was taken, then samples were obtained during the luteal-premenstrual and menstrual phases from day 22 of 1 cycle through day

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5 of the next cycle. These days were chosen to cover the period during which extremes of change in progesterone and estrogen occur. Since the duration of cycles varied among subjects, for the purposes of subsequent comparison, cycle days were numbered around a reference day 1, which was the first day of menses. Days before menses were numbered −1, −2, *etc.*, and days after the onset of menses were numbered 2, 3, 4, and 5. For the men, samples were obtained for 10 consecutive days. For each subject blood was sampled daily for 10–16 days at approximately the same time of the day (within a 2-h time span).

Blood samples were immediately separated at 4 C, and the plasma was aliquoted and, except for ANP, stored at −20 C for subsequent hormone analysis. For ANP determination 3 mL plasma were acidified (0.25 mL 2 N HCl/mL plasma), extracted using ODS-silica cartridges (Sep-Pak C18, Waters Associates, Milford, MA), and measured with RIA as previously described (19). The lower limit of the assay was 15.4 pmol/L, and the intra- and interassay coefficients of variation were 6.7% and 10.8%, respectively.

Plasma aldosterone, progesterone, and estradiol were determined using RIA kits purchased from Diagnostic Products Corp. (Los Angeles, CA).

In addition to the subjects already described, we assayed fasting morning plasma ANP levels in duplicate in 12 healthy elderly men (aged 62–86 yr), 9 healthy elderly women (aged 64–80 yr), 6 additional healthy young women (aged 19–42 yr), and 13 additional healthy young men (aged 19–45 yr).

All results are described as the mean ± SE. Comparisons were made using Student's t test. Differences were considered statistically significant at $P < 0.05$.

Results

Estrogen and progesterone (Fig. 1)

All women had ovulatory cycles. As anticipated, estrogen levels were highest in the periovulatory period (848 ± 202 pmol/L) and lowest after the onset of menses (95 ± 7 pmol/L). The peak of progesterone occurs later, approximately 8 days before menses (43.9 ± 4.5 nmol/L). Progesterone levels are also lowest after the onset of menses (0.51 ± 0.06 nmol/L).

Aldosterone (Fig. 1)

In addition to these cyclical changes in estrogen and progesterone, there were also cyclical changes in aldosterone. Aldosterone levels were highest during the luteal phase of the cycle at about 1110–1660 pmol/L, and lowest in the periovulatory and menstrual periods at about 550 pmol/L. Thus, aldosterone was highest when progesterone (an aldosterone antagonist) was highest.

In contrast to those in the women, aldosterone levels in the men did not fluctuate significantly from day to day, ranging between approximately 400–550 pmol/L (Fig. 1). Aldosterone levels were higher in women than in men during the luteal phase of the menstrual cycle, but not in the periovulatory period or during menses.

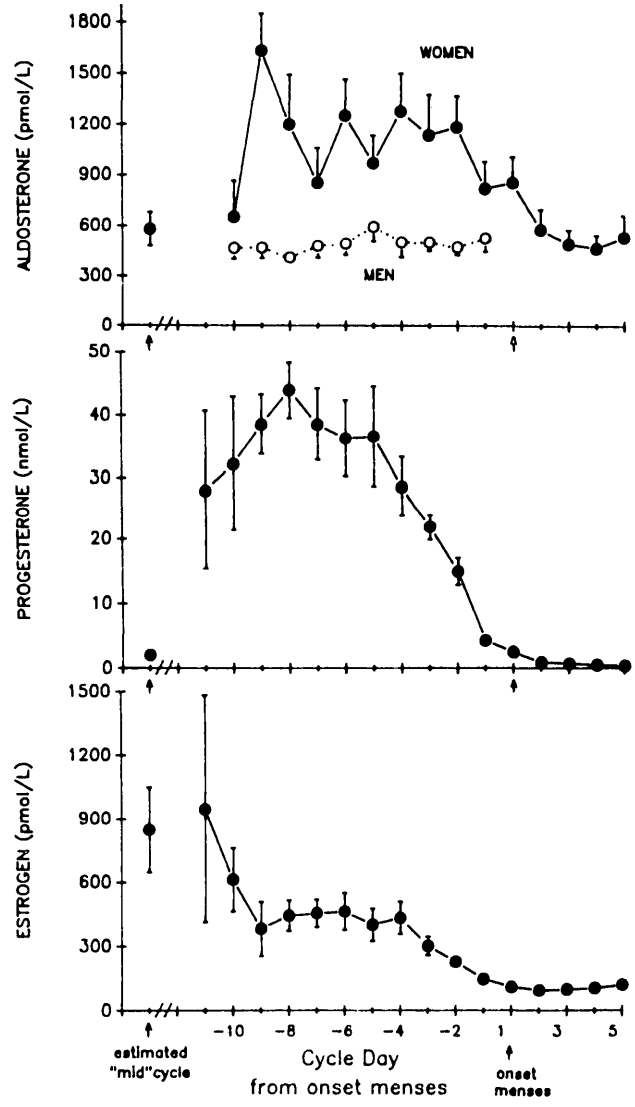

FIG. 1. Aldosterone, estrogen, and progesterone levels. All women had ovulatory cycles, with the anticipated normal change in estrogen and progesterone levels during the luteal phase. Aldosterone levels in women were highest when progesterone levels were highest. Aldosterone levels in men did not change significantly from day to day.

ANP levels

Effect of gender and menstrual cycle. The average daily ANP levels in the women and men are shown in Fig. 2. The striking finding was that throughout the study ANP levels in the women were much higher than those in the men, averaging 68.1 ± 5.5 *vs.* 39.8 ± 3.41 pmol/L, respectively ($P < 0.001$). Although in women ANP levels varied from as low as 53.6 ± 11.1 pmol/L to as high as 102.3 ± 25.3 pmol/L, these differences did not reach the level of statistical significance, and there was no consistent change in ANP levels in the women during the

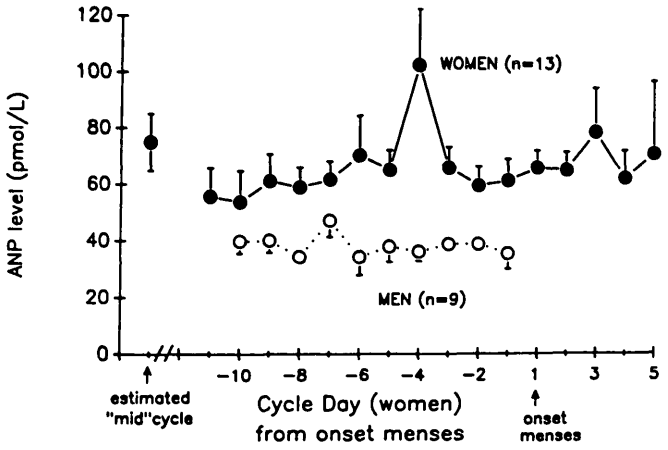

FIG. 2. Average daily ANP levels in women and men. Levels in women are approximately twice those in men, independent of the phase of the menstrual cycle.

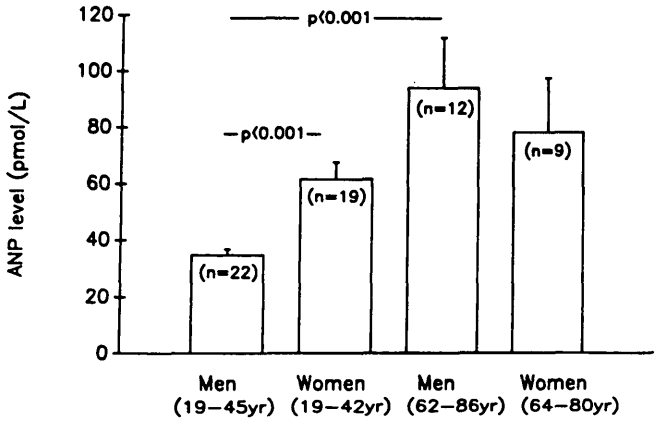

FIG. 3. Mean ANP levels in young and old men and women. The difference in ANP levels between men and women disappears with age.

menstrual cycle. (It should be noted, however, that no significant weight change, edema, or complaints of bloating occurred in these subjects.)

Effect of age (Fig. 3). It has been established that ANP levels increase with age in men (5, 20, 21). In our laboratory, levels in normal healthy young men (19–45 yr) average 34.8 ± 1.9 pmol/L (n = 22) and 93.4 ± 17.9 pmol/L (n = 12) in healthy old men (62–86 yr). In contrast, basal ANP levels in young women (aged 19–42 yr), regardless of ovulatory phase, average 61.6 ± 5.9 pmol/L (n = 19) and 78.3 ± 19.4 pmol/L (n = 9) in healthy old women (aged 64–80 yr). Thus, the difference in ANP levels between men and women disappears with age.

Discussion

These studies clearly demonstrate that plasma ANP concentrations are higher in young women than in young men. The physiological significance of these higher levels, however, is not resolved.

An attractive initial hypothesis was that the difference might be related to gonadal hormone levels. Just as ANP has been implicated in the escape from mineralocorticoid-induced sodium retention (3), it might also act as a counterregulatory hormone to prevent estrogen-induced sodium retention. However, in this study we did not observe a statistically significant cyclical variation in ANP levels during the menstrual cycle. This argues against a direct interaction between estrogen and ANP (such as seen between progesterone and aldosterone).

This was a study of 13 healthy women with normal menstrual cycles, none of whom had complaints of premenstrual or midcycle edema and bloating. Although we do not have a direct measure of plasma volume, there was no clear pattern of premenstrual weight gain. This is consistent with the data of other investigators who report only minor transient changes in plasma volume during the normal menstrual cycle (23). It is unlikely that there is enough estrogen-induced volume retention to induce changes in ANP levels. Our data, however, are only relevant to women with normal menstrual cycles and not to women who develop premenstrual or midcycle edema. We are, therefore, unable to determine if ANP might be a counterregulatory hormone in such women. Further study of women with menstrual related weight gain and edema would be of interest.

To determine whether interference with ovulatory cycling of hormones would affect plasma ANP levels, we measured ANP levels in one subject during a normal ovulatory cycle and during a cycle in which an oral contraceptive (1 mg norethindrone with 0.035 mg ethinyl estradiol) was taken daily. The level of ANP remained relatively constant throughout both cycles (52.4 ± 2.5 *vs.* 54.8 ± 3.7 pmol/L, ovulatory *vs.* nonovulatory cycle), suggesting that the absence of ovulation or physiological amounts of exogenous estrogen do not influence ANP levels. Endogenous estradiol and progesterone levels were similar to those seen during the menstrual phase of the cycle (74.5 ± 18.7 pmol/L and 0.35 ± 0.03 nmol/L, respectively).

The striking finding in this study was a gender difference in the plasma level of ANP. This is unlikely to be secondary to a systematic difference in plasma volume, blood pressure, or salt excretion between men and women as prior studies have demonstrated no such gender difference (24–26). One possible explanation is that young males have increased metabolic clearance of ANP. A tissue endopeptidase (enkephalinase) appears to be important in the clearance of ANP (27, 28). Males have increased levels of other tissue endopeptidases (such as angiotensin-converting enzyme) that seem to be under androgenic control, as tissue levels decrease after castration in male animals (29). Perhaps there is a similar androgenic effect on ANP enkephalinase. Studies of the

half-life of infused ANP in men and women could help clarify this issue.

Further evidence for a sex hormone influence on ANP metabolism is the increase seen with age in men (5, 20, 21). The basal ANP levels in young men and women and elderly men and women demonstrate that the difference in ANP levels between women and men disappears with age (78.3 ± 19.4 *vs.* 93.3 ± 17.9 pmol/L; P = NS) This could be compatible with an influence of female sex hormones, decreased tissue sensitivity to androgens with age, or some other difference in the response of men and women to the aging process.

In summary, ANP levels in young women average approximately twice those in young men, but do not fluctuate with the cyclical changes in estrogen, progesterone, and aldosterone seen during the menstrual cycle. However, ANP levels in postmenopausal women are not greater than those in age-matched elderly men. Gender, thus, appears to affect the secretion or metabolism of ANP during the premenopausal years of life. Further longitudinal studies of ANP secretion and end-organ response in men and women across the lifespan, in postmenopausal women undergoing estrogen repletion, in women with premenstrual edema, and in women with masculinizing syndromes may clarify the nature of this influence.

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