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Plasma Levels of Atrial Natriuretic Peptide Are Increased in Normotensive Postmenopausal Women as a Function of Age

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Abstract. The aim of this study was to assess the changes in atrial natriuretic peptide (ANP) levels before and after menopause and to test whether they depend on age or are an integral part of the hormonal changes in menopause. We measured plasma ANP, plasma renin activity (PRA), plasma aldosterone, serum estradiol-17β and progesterone concentrations in 103 normotensive women, either in premenopause (n = 35; mean age: 24 years), in physiological menopause (n = 34; mean age: 43 years) or surgically induced menopause (n = 34; mean age: 55 years). The last two groups were matched for duration of menopause and were comparable in their estrogen and progesterone status. PRA and plasma aldosterone concentrations decreased in postmenopausal women, whereas systolic blood pressure and ANP increased. These results were not confirmed after adjustment for age by covariance analysis. In all of the groups, plasma ANP concentrations were not significantly correlated with systolic or diastolic blood pressure, nor with plasma aldosterone, estrogen and progesterone concentrations. These correlations were not improved by correction for age. Plasma ANP concentrations were consistently correlated with age. These data suggest that the increase in plasma ANP levels found in postmenopausal women is related with age and that ANP does not play a direct role in the physiological hormonal changes of menopause.

Introduction

Since deBold et al. [1] first described the natriuretic action of atrial extracts, much research concerning the physiology and the pathophysiology of the atrial natriuretic peptide (ANP) has been done. Studies involving healthy human subjects [2–5] indicate that steady-state ANP levels are related to dietary sodium intake and volume status. It is also

well known that a negative correlation exists between plasma renin activity (PRA) and plasma ANP concentrations [6–10].

Estrogens are known to activate the renin-angiotensin-aldosterone system and to produce fluid retention, while ANP tends to increase the urinary output and to return the fluid balance to normal. The levels of ANP are affected by estrogen or progesterone therapy [11] and increase in normal pregnancy [12–14]. Based on these considerations, one might expect different plasma ANP levels in pre- and postmenopausal women, where significant changes in estrogenic activity do occur. On the other hand, no significant interaction of sex on plasma ANP concentrations has been demonstrated [15–17], while different authors report evidence both in favor of [18–20] and against [17] a significant influence of age on plasma ANP concentrations. As far as we know, no studies have focused on the changes in plasma ANP concentrations brought about by menopause.

The aim of this study was to assess the changes in plasma levels of ANP before and after menopause and to test whether they depend on age or are an integral part of the hormonal changes provoked by menopause. In order to do this, we compared normal young women with normal postmenopausal women. Since the latter were obviously older, we assessed the statistical significance of the differences in ANP levels after correction for age by covariance analysis. In order to better discern the possible influence of age from the inherent hormonal changes of menopause, we also studied a second group of women in surgically induced menopause, significantly younger than the first, but matched for duration of menopause and for estrogen and progesterone status.

Materials and Methods

We studied 103 female volunteers, 20–66 years old. All the postmenopausal women had been followed up at the menopause clinic of the St. Anna Hospital since the climacteric. Records of their gynecological history were available prior to the study. All the premenopausal women were either studying or working in the same hospital.

Before the study, the women were carefully examined by two specially trained physicians who excluded cardiac, renal, liver or thyroid disease on the basis of history and normal physical and laboratory examinations, including electrocardiogram and chest roentgenogram. Arterial hypertension (systolic blood pressure > 150 mm Hg and/or diastolic blood pressure > 90 mm Hg) was also a cause of exclusion.

The women included in the study had been without any medication for at least 2 months. They were divided into three groups: (1) women with normal menstrual function ($n = 35$); (2) postmenopausal women with a history of precocious menopause surgically induced for therapeutic reasons ($n = 34$), and (3) postmenopausal women with a history of normally timed climacteric ($n = 34$). Women in group 3 were selected from a larger group in order to accurately match group 2 for duration of menopause.

All women were abulatory and ate their usual diets, except that the sodium intake was limited to 150 mEq/day for 1 week before the study. Blood pressure (standard mercury sphygmomanometer) was determined as the average of three measurements taken from the same arm in the supine position, the first after 10 min of rest then subsequently at 3-min intervals. The diastolic pressure was read at Korotkoff phase V. In group 1, ANP concentrations were determined in the early follicular phase.

After an overnight fast and bed rest for 60 min, blood samples were collected in prechilled tubes containing ethylenediamine tetraacetic acid-2Na (1.5 mg/ml) and aprotinin (500 kIU/ml blood), placed on ice and promptly centrifuged at 3,000 rpm for 30 min at 4 °C. Plasma was then frozen and stored at −80 °C until the assay was performed. All samples were processed in duplicate in the same assay. ANP was measured by radioimmunoassay (RIA) combined with an extraction step using reagents supplied by Immuno Technology Service Production BV (Wijchen, The Netherlands). The intra- and interassay coefficients of variation are 8.6 and 11.6%, respec-

Table 1. Clinical and laboratory details of the groups studied

	Pre-menopause (group 1)	Surgical menopause (group 2)	Physiological menopause (group 3)
Women, n	35	34	34
Age, years	24 (20–34)	43 (32–50)*	55 (50–66)*
Duration of menopause, months	–	48 (10–190)	48 (12–186)
Serum estradiol-17β, pg/ml	37 [31–45]	14 [8–19]*	11 [6–14]
Serum progesterone, ng/ml	0.9 [0.6–1.3]	0.3 [0.1–0.4]*	0.2 [0.1–0.3]
Systolic blood pressure, mm Hg	108 [104–113]	119 [114–125]**	125 [121–130]**
Diastolic blood pressure, mm Hg	72 [69–76]	74 [70–79]	75 [72–79]
PRA, ng/ml/h	1.4 [1.1–1.6]	1.0 [0.6–1.4]**	0.9 [0.6–1.3]**
Plasma aldosterone, ng/dl	13.4 [11–16]	10.5 [6–14]**	10.8 [7–15]**
ANP, pg/ml	20 [17–23]	30 [25–36]**	40 [34–45]***

Values are means ± SEM, except for the number of women (ranges in parentheses; 95% confidence intervals in brackets). * $p < 0.001$ vs. preceding group (χ^2 test); ** $p < 0.01$ vs. group 1 (χ^2 test), but not significant after adjustment for age by covariance analysis; *** $p < 0.01$ vs. group 2 (χ^2 test), but not significant after adjustment for age by covariance analysis.

tively, and the sensitivity is 7 pg/ml. Serum estradiol-17β and progesterone were measured by RIAs using reagents supplied by Diagnostic Products (Los Angeles, Calif., USA). The assay sensitivity is 8 pg/ml for estradiol and 0.05 ng/ml for progesterone. The intra- and interassay coefficients of variation are 5.3 and 6.4% for estradiol, and 7.2 and 7.9% for progesterone, respectively. PRA and plasma aldosterone were measured by RIAs using reagents supplied by Sclavo (Siena, Italy) and by Sorin Biomedica (Saluggia, Italy), respectively. The assay sensitivity is 0.15 ng/ml of angiotensin I for PRA, and 0.8 ng/dl for aldosterone. The intra- and interassay coefficients of variation are 6 and 7% for PRA, and 8.2 and 10.4% for aldosterone, respectively.

To determine the significance of differences between means, analysis of covariance was used in order to account for age. The significance of a relation between two variables was assessed by Pearson's coefficient of correlation and by partial correlations in order to account for possible confounding factors. Statistical analyses were done using a statistical package on a personal computer. In the analysis of covariance, postmenopausal women (both surgically induced and physiological) were compared with premenopausal women. χ^2 analysis was used to test the differences in hormonal levels between pre- and postmenopause. The p value for significance was set at 0.05.

Results

Table 1 gives the clinical and laboratory details of the three study groups. As a result of the selection criteria, the mean age was lowest in premenopause and higher in surgically induced menopause and physiological menopause, respectively. No significant difference was present in the estrogen and progesterone status of groups 2 and 3, which had been matched for duration of menopause.

PRA and aldosterone concentrations decreased in postmenopausal women, whereas systolic blood pressure increased. These re-

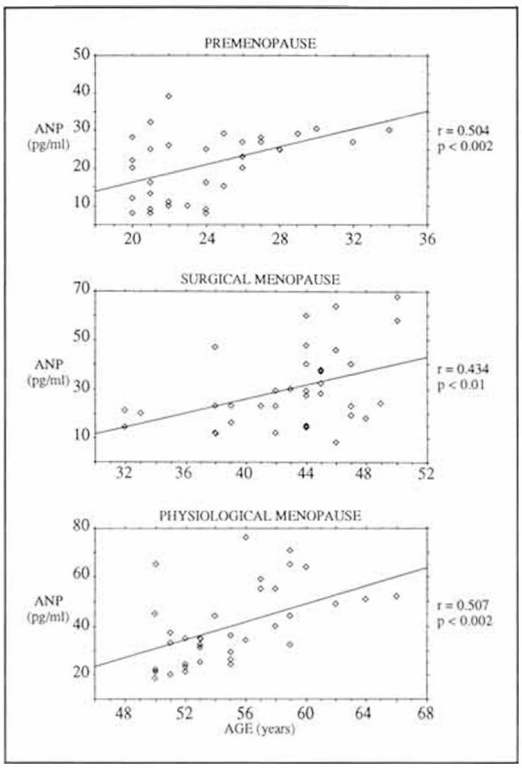

Fig. 1. Age-related increase in plasma ANP concentrations in 35 premenopausal women, 34 women in surgical menopause and 34 women in physiological menopause.

sults were not confirmed after adjustment for age by covariance analysis.

Mean ANP concentrations (± SEM) were 20.1 ± 1.5 in premenopause, 30.3 ± 2.7 in surgical postmenopause and 39.8 ± 2.7 in physiological menopause. The significance of the differences between the three groups disappeared after correction for age. In all groups, plasma ANP concentrations were not significantly correlated with systolic or diastolic blood pressure, nor with plasma aldosterone, estrogen and progesterone concentrations. These correlations were not improved by correction for age. Plasma ANP concentration was negatively correlated with PRA in all groups, though this correlation disappeared after correction for age. On the other hand, plasma ANP concentrations were constantly correlated with age. Figure 1 shows the changes in plasma ANP concentrations with age in the three study groups.

Discussion

In accordance with reports from other authors [15, 17], we found no correlation between ANP concentrations and blood pressure in 103 normotensive healthy women. It appears that, within a normal range, blood pressure per se does not determine ANP release. These findings do not contradict data from other authors who have found increased ANP levels in plasma from patients with hypertension [21–25]. Arterial hypertension is known to cause a deterioration of ventricular and atrial compliance, followed by increased atrial pressure. These morphological and functional changes in the heart are likely to be responsible for the raised plasma ANP concentrations in hypertensive subjects [22].

Several authors have found increased ANP values in the elderly by comparing young and old individuals, either healthy [18–20] or hypertensive [15, 24]. This approach is not appropriate for investigating the influence of age on biochemical variables, since multimorbidity increases with age and cannot be taken into account by such a method. A more appropriate approach is to assess the existence of a significant correlation by regression analysis and the persistence of that correlation after correction for any known interfering factor. A significant

positive correlation of ANP with age has been reported by some authors [4, 22] and is confirmed by our data, while others found no correlation [17]. Experiments in rats show that the ANP content of the heart rises steadily with age and a significant age-dependent decline in biological activity is present [26]. Other studies in humans suggest reduced metabolic clearance rates for ANP [27] and greater ANP release at a given volume expansion in the elderly as compared with the young [28]. Altogether, it appears that age-related morphological and functional changes determine higher plasma ANP concentrations in aged individuals.

Analysis of our data demonstrates the age dependence of the increase in plasma ANP concentrations that we found in postmenopausal women. The difference between pre- and postmenopausal levels of ANP was not significant after correction for age, while a positive correlation with age was present in pre- as well as postmenopause. Even in the two groups of postmenopausal women (matched for duration of menopause and significantly different only at age), the mean levels of ANP showed a difference that disappeared after correction for age.

Our data are also in keeping with the known age dependence of blood pressure and renin-aldosterone activity [28].

In conclusion, our results suggest that ANP is not affected by the hormonal changes of menopause. The physiological estrogen and progesterone variations of normal women occur in much lower ranges than those achieved after exogenous administration. Estrogens and progesterone seem to enhance ANP levels in a dose-dependent way [11] and do not appear to exert any influence on ANP at physiological plasma concentrations.

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