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Atrial Natriuretic Factor in Essential Hypertension: Echocardiographic and Humoral Correlates

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Summary: Aim of this study was to assess the relationship between plasma concentration of atrial natriuretic factor (ANF) and its two-dimensional echocardiographic (left ventricular mass, left atrium diameter) and humoral (plasma renin and aldosterone) variables in essential hypertension (EH). We evaluated 32 patients with uncomplicated mild to moderate EH and 10 controls. They were studied in the supine position after 7 days of constant dietary sodium intake and were off therapy since at least 3 weeks. ANF values overlapped between EH patients and controls (27.8 ± 11.5 vs. 19.5 ± 7.4 pg/ml, p=NS). In EH, no significant correlation was found between ANF values and left ventricular mass ($r = 0.29$), left atrial diameter ($r = 0.04$), mean arterial blood pressure ($r = 0.26$), plasma renin activity ($r = 0.00$), and aldosterone ($r = 0.26$). In EH, ANF values overlapped between the 15 patients with hypertrophy and the 17 patients with normal ventricular mass: 30.3 ± 17 vs. 25.6 ± 10.6 pg/ms (p=NS). We conclude that there is a substantial overlap in plasma ANF values between mild to moderate uncomplicated EH and controls, and left ventricular hypertrophy is not a major independent stimulus to ANF release in EH.

Key words: atrial natriuretic factor, hypertension, echocardiography

Introduction

Conflicting data have been reported on concentration of atrial natriuretic factor (ANF) in essential hypertension (EH).[1–5]

It is now evident that many factors are capable of altering plasma ANF. Among them, left ventricular hypertrophy[6,7] and left atrial distension[8–11]—which can be present to a variable extent in populations of essential hypertensives—have been associated with elevation in ANF concentration. Also humoral factors, such as plasma renin, angiotensin, and aldosterone have been correlated to ANF levels.[12,13]

More data are needed on this subject, with greater attention to confounding factors that may otherwise render results uninterpretable.

Hence, in this study we evaluated ANF levels in normals and in a group of uncomplicated mild to moderate essential hypertensives.

We also examined, in the same population of essential hypertensives, the relationship between ANF values and its humoral (renin and aldosterone plasma levels) and echocardiographic (left ventricular mass, left atrial dimension) correlates.

Subjects and Methods

All subjects gave informed consent. The study group consisted of 10 control subjects and 32 mild to moderate essential hypertensives off therapy since at least 3 weeks.

All patients were within 25% of their ideal body weight.

Clinical and humoral characteristics of the two study groups are summarized in Table I. All hypertensive patients were uncomplicated except for the possible presence of echocardiographically assessed left ventricular hypertrophy (defined according to Devereux as left ventricular mass index > 134 g/m^2 in males and > 110 g/m^2 in females).[14]

Subjects were studied while taking a diet with a controlled constant sodium intake (Table I). Each subject received only a fixed amount of sodium and this goal was

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TABLE I Characteristics of the study patients

	Sex (M/F)	Age (years)	Creat (ml/min)	UNa (mEq/24h)	MBP (mmHg)	ANF (pg/ml)	PRA (ng/ml/h)	Ald (ng%)	LVMI g/m2)
Normals	6/4	40±10	112.4±9.4	130.2±14.6	90±6	19.5±7.2	2.08±1.40	19.1±9.3	107.9±9
Hypertensives	18/14	50±9[a]	115±8.4	127.9±20.1	117±9[a]	27.8±11.5	1.9±2.79	19.7±10.0	125.1±36.1

[a]=p<0.01.

Abbreviations: Ald = serum aldosterone; ANF = atrial natriuretic factor; Creat = creatinine clearance; LVMI = left ventricular mass index; MBP = mean arterial blood pressure; PRA = plasmatic renin activity; UNa = urinary sodium excertion.

achieved by adding an established supplement of sodium chloride to a standard diet containing 10mEq of sodium. The study was started from 6 days after beginning the diet, when the attainment of the equilibrium state was shown by the urinary sodium excretion equaling the intake.[15]

Time-motion and two-dimensional echocardiograms were obtained in each patient with a commercially available instrument (Hewlett Packard, 2.5, and 3.5 MHz transducers) by the same experienced cardiologist-echocardiographer.

Left ventricular mass index was calculated according to the Penn Convention.[14] Left atrial dimension was calculated according to the recommendations of the American Society of Echocardiography.[14] With established approaches,[16] the temporal reproducibility of these measurements is very high, with a variation coefficient consistently less than 5% in our laboratory.

On the study day, between 8:00 and 10:00 A.M., the subject rested supine for 60 minutes. Subjects collected 24-h urine samples for measurement of creatinine excretion in order to calculate the 24-h endogenous creatinine clearance and urinary sodium excretory.

Plasma concentrations of ANF were measured by radioimmunoassay according to a method recently described in greater detail.[17]

Blood for ANF determination was collected in precooled plastic syringes containing 1.5 mg potassium ethylenediaminetetracetate (EDTA) and aprotinin (Trasylol, Bayer, Milan Italy), and transferred to precooled tubes. Samples were immediately centrifuged at 4° C (4,000 rpm for 20 min); plasma was divided into 2.5 ml aliquots and stored at −70° C for a maximum of 8 weeks. Plasma was processed for ANF extraction immediately after thawing at room temperature. Plasma was acidified in HC1 (1N, 0.25 ml/ml plasma) and run slowly through ODS cartridges (Sep-Pak C18, Waters Associates, Milford, Massachusetts), moistened with methanol (3 ml) and washed with 1% trifluoroacetic acid (TFA) (10 ml) in a water solution. Cartridges were coated with 1% polypeptide solution (Polypep, Sigma Chemical Company, St. Louis, Missouri) to minimize nonspecific absorption and washed again in a mixture of methanol/water/TFA (80/19/1%), vol/vol/vol). The sample was applied and the gel washed with a mixture of 5 ml 1% TFA/1% NaCl (1:1 ratio, vol/vol). The absorbed ANF was eluted with 2 ml methanol and evaporated to dryness (Hetovac and Hetotrap CT60, Heto Lab Equipment, A/S, Birkerod, Denmark). The extraction procedure was performed at 4° C using the cartridges once. Extraction efficiency (recovery rate 85.5±5.6%, mean± SD, n = 75) was estimated through labeled ANF (approximately 800 cpm, Amersham, Buckinghamshire, England). The dried residue was frozen at −70° C and reconstituted with 500 µl assay buffer (sodium phosphate 50 mM, 2% bovine albumin, 0.1% Triton-X, EDTA 10 mM, pH 7.4). Samples were incubated overnight (for approximately 15 h) at 4° C with 100 µl antihuman ANF-(1-28) rabbit antiserum (Peninsula Labs., Inc., Belmont, California) for detection of the carboxyl end of ANF and, therefore, also the amino terminal-depleted synthetic hormone used in this study. Labeled ANF in 100 µl assay buffer was then added (approximately 18,00 cpm), and the incubation was prolonged overnight at 4° C. Bound and free ANF were separated by the addition of 100 µl goat antirabbit immunoglobulin antiserum and centrifuged (4,000 rpm for 20 min) after 2 h of incubation at room temperature. Precipitate and supernatant were counted in a gamma counter (LKB 1275, Turku, Finland). Synthetic human ANF-(1-28) (Peninsula Labs., Inc.) was employed for the standard curves. The intra-assay and interassay variation coefficient were consistently less than 15%.[17]

Plasma renin activity (PRA) and aldosterone were measured by radio immunoassay.[18,19] The intra-assay and interassay variation coefficients for all assays were consistently less than 10%.

Unmatched groups were compared by 2-tailed unpaired Student's *t* test. Multiple linear regression analyses were performed with simultaneous entry of all variables.

Data are given as mean±SD; a p value <0.05 was considered significant.

Results

Plasma ANF values were slightly higher in the hypertensive group in comparison with normal subjects (27.8± 11.5 vs. 19.5±7.4 pg/ml, p = NS).

Mean arterial pressure was not significantly correlated with plasma ANF (Table II).

Echocardiographic criteria for LVH were present in 31% of essential hypertensives.

TABLE II Univariate and multiple linear regression correlation coefficient

Independent variable	Normal	Hypertensives
Age	0.642[a]	0.21
Mean arterial pressure	0.268	0.264
Left ventricular mass index	0.443	0.287
Left atrium diameter	0.12	0.041
Plasma renin activity	0.05	0
Aldosterone	0.65	0.26
Multiple correlation coefficient (r)	0.926	0.539
Coefficient of determination (r2)	0.852	0.291

[a] $p<0.05$.
Dependent variable = ANF.

Patients with and without LVH had similar ANF values (30.3 ± 17 vs. 25.6 ± 10, P = NS). There was no significant correlation between plasma ANF and echocardiographic and humoral parameters, such as LV mass, left atrial diameter, PRA and aldosterone (Table II). In normals there was a significant correlation between ANF and age (Table II).

Discussion

In uncomplicated mild to moderate essential hypertensives, plasma ANF concentration overlaps with values obtained in normal subjects.

This finding might apparently conflict with previous reports.[3–5,12] However, we must consider many confounding factors, which are frequently associated with hypertension and may affect ANF concentrations independently: body weight,[13] salt intake,[20–22] volume expansion,[9–23] drugs,[24,25] renal dysfunction,[24,26] and age.[27]

Many of these confounding factors could be minimized in the present study since all patients and controls were within 25% of ideal body weight and off therapy at the time of the study. As an inclusion criterion, all study subjects had mild to moderate essential hypertension and a normal renal function and were on a constant dietary sodium intake at the time of the study. Patients and controls were gender-matched. The mean age was slightly lower in the control group; however, this might have only exaggerated the differences in favor of the older hypertensive group, since there is a tendency of ANF values to rise with age[24,28] which was also found in our group of normal patients.

Removing all these potentially confounding factors, no detectable increase could be found in essential hypertensives versus controls. Although this study cannot exclude the existence of a weak relation between ANF and LV mass, it helps disprove a strong one.

Within the group of essential hypertensives it might appear surprising that no significant correlation was found between ANF values and left ventricular mass and left atrium diameter. In fact, it is well known that left ventricular hypertrophy[6,7] and left atrial distension[8–11] are strongly associated with ANF secretion. However, in those studies higher values and broader ranges of left ventricular hypertrophy and left atrium dilatation were reported, which certainly imply more pronounced changes in left ventricular compliance and left atrial filling pressures. At that more advanced stage of disease, it would be difficult to establish what is primarily due to essential hypertension and what is simply due to secondary changes in left ventricular structure, volume overload, and alterations in kidney function.

In conclusion, there is a substantial overlap in plasma ANF values between uncomplicated mild to moderate essential hypertensives and normal subjects, and left ventricular hypertrophy is not a major independent stimulus to ANF release in essential hypertensives.

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