Correlates of Plasma Atrial Natriuretic Factor in Health and Hypertension

PIERO MONTORSI, GIANCARLO TONOLO, JORGE POLONIA, DAVID HEBURN, AND A. MARK RICHARDS

SUMMARY Plasma concentrations of atrial natriuretic factor (ANF) were compared in normotensive subjects and subjects with untreated, uncomplicated essential hypertension (n = 21 pairs) matched for age, sex, and race. Plasma peptide values were slightly greater (45 ± 3 vs 36 ± 3 pg/ml; p<0.05) in the hypertensive group. On univariate analysis, age (r = 0.52, n = 47, p<0.001) and creatinine clearance (r = −0.30, n = 47, p<0.05) were significantly related to plasma ANF concentrations, but arterial pressure was not (r = 0.14, n = 47), in an extended group of normal subjects. In contrast, plasma ANF values were related to arterial pressure in both an extended group of subjects with untreated essential hypertension (r = 0.54, n = 38, p<0.001) and in our total heterogeneous pool of hypertensive patients (r = 0.46, n = 79, p<0.001), but weak positive associations with age and inverse relationships with creatinine clearance were not statistically significant in either hypertensive group. Similar weak inverse relationships between plasma ANF values and renin-angiotensin-aldosterone system activity were found in both normal and hypertensive subjects. Plasma ANF concentration was related to electrocardiographic scores for left ventricular hypertrophy and to radiological cardiothoracic ratio in subjects with untreated essential hypertension (r = 0.53, n = 34, p<0.01 and r = 0.35, n = 32, p<0.05, respectively) and for all hypertensive subjects combined (r = 0.35, n = 59, p<0.02 for cardiothoracic ratio and r = 0.47, n = 69, p<0.001 for hypertrophy scores). Seventy percent of subjects with left ventricular hypertrophy had plasma ANF values beyond the normal range for our laboratory. Comparison of hypertensive subjects with and without hypertrophy, closely matched for age and arterial pressure (n = 13 pairs), revealed clearly elevated values of plasma ANF in the hypertrophy group (133 ± 24 vs 46 ± 5 pg/ml; p<0.01). (Hypertension 10: 570-576, 1987)

KEY WORDS • atrial natriuretic factor • hypertension • arterial pressure • left ventricular hypertrophy • renal function • age • renin-angiotensin-aldosterone system

PLASMA atrial natriuretic factor (ANF) concentrations have been reported as elevated in essential hypertension, and a positive relationship between arterial pressure and ANF values has been described. However, overlap of ANF levels in hypertensive and normotensive subjects appears considerable, and other workers have found no difference between these groups. To clarify and perhaps reconcile these conflicting reports, we have looked at other factors, aside from arterial pressure, that may help dictate plasma ANF concentrations in hypertension.

These include age, left ventricular hypertrophy (LVH), and renal function. Age and plasma ANF values are positively related, at least in normotensive subjects. Ventricular hypertrophy is associated with decreased ventricular compliance and consequent elevations in mean atrial pressures. Atrial distention remains the primary candidate as the main stimulus for ANF secretion. Plasma ANF concentrations have been shown to correlate with atrial pressure both in normal subjects undergoing acute intravenous saline loading and in patients with cardiac impairment. Hence, the presence of ventricular hypertrophy in hypertension may be a cause of elevated plasma peptide concentrations in this disease. The kidney plays a pivotal role in the regulation of circulating volume. In addition, this organ may be of importance in clearing ANF from plasma. Receptors for ANF exist in renal glomeruli, and there is a pronounced arteriovenous gradient in plasma peptide concentrations across the renal circulation.
more, ANF is markedly elevated in end-stage renal failure, and the half-life of exogenous peptide administered to anephric rats is double that found in normal animals.10,11

Hence, we have examined plasma ANF concentrations and their relationship to arterial pressure, age, LVH, and renal function in normotensive and hypertensive subjects.

Subjects and Methods

The study protocol was approved by the hospital ethical committee, and all subjects gave informed consent. The study groups consisted of 47 normotensive subjects, 38 subjects with untreated essential hypertension, and 41 patients with treated hypertension, in some cases complicated by LVH, renal impairment, or both, who were studied in a standardized fashion. Initial comparison of plasma ANF concentrations was made between normal subjects and subjects with untreated, uncomplicated (including absence of electrocardiographic evidence of LVH) essential hypertension. Normotensive and hypertensive subjects were all within 5% of ideal body weight and were carefully matched for sex, age, race, and creatinine clearance. Subject pairing for this matched analysis was conducted before measurement of plasma ANF. Matching of hypertensive subjects with and without LVH (n = 13 pairs) for arterial pressure, sex, age, and treatment was also conducted without knowledge of plasma ANF values. Details of matched and total normal and hypertensive groups are given in Table 1. Subjects were studied on the second hospital day while taking a free diet. Hypertension was defined as present when at least three consecutive seated or supine blood pressure readings were taken, on separate days, with a diastolic pressure above 90 mm Hg. Subjects with untreated essential hypertension either had never received antihypertensive medication (n = 28) or had stopped treatment at least 1 month before the study.

On the study day, between 0800 and 1000, an intravenous cannula was placed in a forearm vein and the subject rested supine for 30 minutes. Blood was then withdrawn for a routine biochemistry screen, including measurement of serum creatinine and plasma ANF, renin, angiotensin II, and aldosterone concentrations. Three objective indirect blood pressure recordings were obtained at 2-minute intervals from a semiautomatic piezoelectric device with a digital printout facility (Copal, Takeda Medical, Tokyo, Japan). Mean arterial pressure was calculated as the diastolic pressure plus one third pulse pressure. Subjects collected 24-hour urine samples for measurement of creatinine excretion in order to allow calculation of 24-hour endogenous creatinine clearance. Twelve-lead electrocardiograms were obtained on hypertensive patients and scored (before plasma ANF values were known) for LVH according to the criteria proposed by Romhilt et al.12 Subjects also had cardiothoracic ratios measured from recent chest roentgenograms.

Plasma ANF concentrations were measured by radioimmunooassay as previously described.5,7 Briefly, 10 ml of blood was collected into chilled tubes containing potassium EDTA/aprotinin (Trasylol). After separation (1000 g for 20 minutes at 4°C) plasma was stored at −20°C before extraction and radioimmunoassay. ANF was extracted from 3 ml of plasma on C18 reverse-phase columns (Sep-Pak, Waters Associates, Harrow, England). Recovery of synthetic human ANF-(99–126) added to plasma before extraction was consistently greater than 90%. Plasma extracts were reconstituted in sodium phosphate buffer. Serially diluted standard solutions of synthetic ANF (100–0.4 pg) and samples of reconstituted plasma extracts (100 μl) were incubated with 100 μl of a locally raised polyclonal antibody in a final dilution of 1:25,000 and 2 pg of 125I-labeled human ANF-(99–126; Amersham, Cardiff, Wales, UK) in 50 μl of buffer at 4°C for 24 hours. Bound and free radioligand were separated by the addition of dextran-coated charcoal, and the free

Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Subject group</th>
<th>Age (yr)</th>
<th>Age range (yr)</th>
<th>MAP (mm Hg)</th>
<th>ANF (pg/ml)</th>
<th>Renin (μU/ml)</th>
<th>Angiotensin II (pg/ml)</th>
<th>Aldosterone (ng/dl)</th>
<th>Ccr (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matched</td>
<td></td>
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</tr>
<tr>
<td>Normal (n = 21)</td>
<td>45.5 ± 2.3</td>
<td>25–61</td>
<td>87 ± 4</td>
<td>36 ± 3</td>
<td>16.4 ± 8</td>
<td>4.4 ± 0.5 (10)</td>
<td>7.5 ± 1.1 (15)</td>
<td>102 ± 6</td>
</tr>
<tr>
<td>Untreated essential hypertension (n = 21)</td>
<td>46.1 ± 2.6</td>
<td>19–61</td>
<td>125 ± 4*</td>
<td>45 ± 3†</td>
<td>18 ± 3</td>
<td>6.1 ± 1.2 (19)</td>
<td>5.5 ± 0.9 (19)</td>
<td>106 ± 6</td>
</tr>
<tr>
<td>All normal (n = 47)</td>
<td>36.3 ± 1.7</td>
<td>17–61</td>
<td>82 ± 2</td>
<td>27 ± 2</td>
<td>25 ± 3</td>
<td>6.9 ± 1.3 (21)</td>
<td>8.2 ± 0.9 (37)</td>
<td>114 ± 4</td>
</tr>
<tr>
<td>All untreated essential hypertension (n = 38)</td>
<td>48.7 ± 2.0‡</td>
<td>19–71</td>
<td>127 ± 3‡</td>
<td>52 ± 6‡</td>
<td>19 ± 3</td>
<td>7.1 ± 1.1 (31)</td>
<td>6.5 ± 1.1 (32)</td>
<td>100 ± 5</td>
</tr>
<tr>
<td>All hypertension (n = 79)</td>
<td>50.7 ± 1.5‡</td>
<td>19–76</td>
<td>123 ± 2†</td>
<td>62 ± 6‡</td>
<td>57 ± 18‡</td>
<td>8.5 ± 1.3 (62)</td>
<td>8.2 ± 1.1 (66)</td>
<td>81 ± 4‡</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Data were complete for all variables except plasma angiotensin II and aldosterone, for which number of subjects is given in parentheses. MAP = mean arterial pressure; Ccr = creatinine clearance.

* p < 0.001, † p < 0.05, compared with values for normal matched group (by Wilcoxon paired analysis).
‡ p < 0.001, compared with values for the total unmatched normal group (by Mann-Whitney U test).
fraction was counted. The sensitivity of the assay allowed detection of 1 pg of ANF per assay tube.

Plasma concentrations of renin, angiotensin II, and aldosterone were measured by established methods. The intra-assay and interassay coefficients of variation for all assays were consistently less than 10%.

For matched groups, nonparametric Wilcoxon paired signed rank analysis was used. Unmatched groups with nonnormal distribution of data were compared by the Mann Whitney U test. Where data were rendered normal in distribution by logarithmic transformation, correlation coefficients were calculated by the method of least squares. Multiple linear regression analyses were performed with simultaneous entry of all variables. Data are given as means ± SE.

Results

Figure 1 illustrates plasma ANF values found in matched normal subjects and subjects with untreated, uncomplicated essential hypertension. Although values were slightly higher in the hypertensive group (45 ± 3 vs 36 ± 3 pg/ml), all but two fell within the normal range for our laboratory (<65 pg/ml). The difference was significant (p<0.05) by Wilcoxon paired analysis but did not attain statistical significance when tested by the less sensitive unpaired Wilcoxon or parametric Student’s t test. Figure 2 shows plasma ANF concentrations for all normal subjects, all subjects with untreated essential hypertension, and all hypertensive subjects combined. Values in the normal group differed significantly from those observed in both hypertensive categories (27 ± 2 vs 52 ± 6 and 62 ± 6 pg/ml, respectively; p<0.001 for both comparisons, by Mann-Whitney U test). There was considerable overlap between values in normotensive and hypertensive groups, with the latter displaying a skewed distribution, including an eccentric “tail” of values extending above the normal range (see Figure 2).

Electrocardiographic criteria for LVH were present in 27% of our total pool of hypertensive subjects and in half of those with plasma ANF values above the normal range (see Figure 2). Initial analysis of all hypertensive subjects showed that those with LVH (n = 14) had significantly higher plasma ANF values than did those (n = 55) without LVH (129 ± 34 vs 46 ± 43 pg/ml; p<0.001), but this comparison was confounded by the greater mean arterial pressure (134 ± 20 vs 121 ± 17 mm Hg; p<0.05) in subjects with LVH. Hence, hypertensive subjects with and without LVH were then paired as closely as possible for age and blood pressure (n = 13 pairs). The distribution of ANF values was similar within untreated and total hypertensive groups (see Figure 2). Hence, treated patients with LVH were included in this analysis (8 of 13) and were paired with similarly treated hypertensive patients of the same sex without LVH. The group with hypertrophy clearly had greater plasma ANF values (p<0.01; Figure 3), and these fell above the normal range in 70% of such subjects. LVH scores correlated significantly with plasma ANF concentrations in both untreated essential hypertensive and total hypertensive groups (r = 0.53, n = 34, p<0.01 and r = 0.47, n = 69, p<0.001, respectively). Similarly, radiological cardiothoracic ratio correlated with plasma ANF values in both groups (r = 0.35, n = 32, p<0.05 and r = 0.35, n = 59, p<0.02, respectively; Figure 4).

By univariate analysis, mean arterial pressure and log plasma ANF concentrations were significantly correlated both in subjects with untreated essential hypertension (r = 0.54, n = 38, p<0.001) and in all hypertensive subjects combined (r = 0.46, n = 79, p<0.001; Figure 5). Blood pressure and plasma ANF were not significantly related in normal subjects.
Endogenous 24-hour creatinine clearance was insignificantly, inversely related to the log of plasma ANF in untreated essential hypertensive subjects and in all hypertensive subjects (Figure 6). In normal subjects, univariate analysis showed a weak, significant inverse relationship between creatinine clearance and ANF ($r = -0.30, n = 47, p < 0.05$).

As shown in Figure 7, age and log ANF were positively and significantly related in normal subjects ($r = 0.52, p < 0.01$) but not in either untreated hypertensive subjects alone ($r = 0.13$) or in all hypertensive subjects combined ($r = 0.16$).

In both normal and hypertensive groups weak inverse relationships were seen between plasma ANF and renin-angiotensin-aldosterone activity. These achieved significance in the normal group for plasma renin concentrations ($r = -0.47, n = 43, p < 0.01$) only and among all hypertensive subjects combined for plasma aldosterone ($r = -0.26, n = 66, p < 0.05$) alone.
Multiple linear regression analyses were conducted separately for normal and hypertensive subjects, with log ANF as the dependent variable and mean arterial pressure, creatinine clearance, and age as independent variables (Table 2). Age was related, independently of blood pressure or creatinine clearance, to plasma ANF in the normal group. Neither of the other two variables were significantly related to ANF in this group. In contrast, mean arterial pressure was significantly independently related to plasma ANF in subjects with untreated essential hypertension and in all hypertensive subjects combined, while creatinine clearance and age were not. The coefficients of determination derived from these analyses ($R^2$) indicated some 30% of the variation in ANF in normal subjects could be predicted from the three variables considered. For untreated essential hypertension and all hypertension combined, 41 and 40%, respectively, of the variation seen in plasma ANF could be predicted.

**Discussion**

In hypertensive subjects plasma ANF concentrations rose in association with increasing arterial pressure. This relationship (before logarithmic transformation of ANF values) was curvilinear; the rise in peptide concentrations remained gradual until a mean arterial pressure of 140 mm Hg or above was present, at which point values began to rise steeply with further increases in blood pressure. In uncomplicated essential hypertension, without evidence of LVH, the increase in peptide concentrations was slight and, in all but two subjects, values fell within the normal range. Careful matching of normotensive and hypertensive subjects for a number of variables, together with use of a paired form of statistical analysis, demonstrated a significant difference between the ANF values found in these two groups (see Figure 1). Less sensitive analysis by unpaired nonparametric or parametric testing failed to show this difference. This finding emphasizes the sub-
tlety of change in plasma ANF concentrations in untreated, uncomplicated essential hypertension. Lack of similar controlled comparison in previous studies may explain the negative findings of some authors.\textsuperscript{4, 16}

Our data do not allow definition of the biological importance of the very minor increase in peptide values seen in uncomplicated essential hypertension. Whether a sustained difference in plasma ANF of only a few picograms per milliliter exerts effects on renal sodium handling, renin-angiotensin activity, or blood pressure remains unknown. Careful long-term dose-response studies or the advent of a specific antagonist may clarify this issue. This group tended to have milder hypertension of shorter duration than did those hypertensive subjects with clear elevation of peptide levels and thus are probably at an early stage of whatever process elevates plasma ANF in hypertension.

The mechanism underlying the association between plasma ANF concentrations and arterial pressure in hypertension remains uncertain. Conceivably, elevation of ANF levels may be an index of impaired ventricular compliance in hypertension. Decreased ventricular compliance leads to increased atrial work and a rise in mean atrial pressures. Elevated pulmonary artery and atrial pressures have been reported in patients with essential hypertension.\textsuperscript{17-19} Atrial distention remains the strongest candidate as the primary stimulus for ANF secretion. Hence, ANF values may be a marker of the extent to which myocardial structure and function are affected by raised systemic arterial pressure. Consistent with this hypothesis, we observed a striking predominance of hypertensive subjects with peptide concentrations above the normal range among those subjects with evidence of LVH, a condition in which left ventricular compliance is reduced. Furthermore, hypertrophy scores and radiological cardiothoracic ratios were both positively correlated with plasma ANF values. Further studies, preferably incorporating hemodynamic indices including atrial pressure and a more sensitive technique for measurement of LVH, such as echocardiography, should help clarify these issues.

The apparent lack of relationship between arterial pressure and plasma ANF in normal subjects may be a consequence of the rather small range of arterial pressure able to be considered in a group of limited size. Certainly, inspection of Figure 5 reveals a clear continuum of ANF values rising from values in normotensive subjects (see the lower panel in Figure 5) to those observed in hypertensive subjects (see the middle panel in Figure 5).

An index of glomerular filtration rate, endogenous creatinine clearance, was weakly inversely related to plasma ANF in our subjects with untreated essential hypertension and in all the hypertensive subjects combined (see Figure 6). However, this association was not demonstrably significant by univariate or multiple linear regression analysis, even when data from a group with a very broad spectrum of renal function were analyzed (see Table 2). The lack of such a significant relationship in a group of hypertensive subjects that included a number of subjects with quite severe reduction of glomerular filtration rate militates against an important role for other than very severe renal impairment in raising plasma ANF. Renal dysfunction may have to be sufficient to cause frank volume overload or hypertension, or both, with LVH before consistent elevation of plasma peptide concentrations occurs. This is consistent with reports of normal levels of ANF in euvolemic children with chronic renal failure and with evidence that other tissues also contribute to clearance of the peptide from the circulation.\textsuperscript{6, 7, 20}

In contrast to findings in hypertensive subjects, age and creatinine clearance were related to plasma ANF in normal subjects. The relationship to age may reflect the gradual rise in atrial pressures with age in normal subjects\textsuperscript{21} or an age-related impairment of peptide clearance, or both. The relationship of ANF with cre-

\begin{table}[h!]
\centering
\begin{tabular}{llll}
\hline
\textbf{Independent variable} & \textbf{Normal} & \textbf{Untreated essential hypertension} & \textbf{All hypertension} \\
\hline
Mean arterial pressure & 0.14 (47) & 0.54* (38) & 0.46* (79) \\
Univariate & -0.16 (47) & 0.58* (33) & 0.62* (68) \\
Partial & 0.52* (47) & 0.13 (38) & 0.16 (79) \\
Age & 0.58* (47) & 0.08 (33) & 0.07 (68) \\
Creatinine clearance & -0.30* (47) & -0.29 (33) & -0.11 (69) \\
Univariate & -0.06 (47) & -0.15 (33) & -0.13 (68) \\
Partial & 0.546* & 0.644* & 0.631* \\
Multiple correlation coefficient ($r$) & 0.299 & 0.414 & 0.398 \\
Coefficient of determination ($R^2$) & & & \\
\hline
\end{tabular}

Dependent variable = log plasma ANF. The partial correlation coefficients shown all incorporate mean arterial pressure, age, and creatinine clearance as independent variables with log plasma ANF as the dependent variable. The number of subjects in each group is shown in parentheses.

\*p < 0.001, tp < 0.05.
\end{table}
Secretarial and statistical assistance were provided by Mrs. Rose Morton and of the nursing and laboratory staff is much appreciated.

The variables considered in multiple regression analyses accounted for some 30% of the observed variation in plasma ANF values in our normal subjects and about 40% of that occurring in our hypertensive subjects (see Table 2). Other factors that may influence plasma ANF values include interassay and intra-assay variation, dietary sodium intake and sodium balance, and possibly, genetic factors. We have incomplete data for 24-hour urinary sodium excretion. This variable was measured in 80% of the normal group (154 ± 6 [SEM] mmol/day) but in less than half the hypertensive group (136 ± 6 mmol/day). However, from unit records of 24-hour urinary sodium excretion results over the last 3 years, there is no reason to believe that, in our local population, hypertensive subjects consume more dietary sodium than do normal subjects. Therefore, it is unlikely that a major difference in sodium balance biased intergroup comparisons of otherwise matched groups in the current study.

In conclusion, we have found that plasma ANF concentrations rise in association with arterial pressure in subjects with hypertension and that elevated values occur more frequently when LVH is present. In normal subjects ANF levels rise with age.

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