Atrial Natriuretic Factor in Essential Hypertension and Adrenal Disorders

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SUMMARY  Patients with untreated essential hypertension had significantly higher plasma atrial natriuretic factor (ANF) levels (92.9 ± 12.9 pg/ml, mean ± SE) than those of age-matched controls (37.8 ± 6.0 pg/ml; p<0.01). Plasma ANF levels in essential hypertensive patients showed a significant positive correlation with mean arterial pressure (MAP; r = 0.46, p<0.05) and an inverse correlation with plasma renin activity (PRA; r = −0.43, p<0.05). Plasma ANF levels after medication showed significant correlation with the decrease in MAP (r = 0.565, p<0.05). Patients with primary aldosteronism had significantly higher plasma ANF levels (122.4 ± 30.2 pg/ml, n = 8) than those of controls (p<0.05). The levels returned to normal after extirpation of adrenal tumors. The response of plasma ANF levels in patients with primary aldosteronism to volume expansion with infusion of 2 L of physiological saline in 2 hours was greater than in controls. Such exaggerated response disappeared after surgical treatment. Infusion of angiotensin II (Ang II; 20 ng/kg/min) or norepinephrine (200 ng/kg/min) for 30 minutes to normal volunteers (n = 5) resulted in a rise in MAP (24.9 ± 3.3 and 15.8 ± 4.4 mm Hg, respectively) and a twofold increase in plasma ANF level. Infusion of the Ang II antagonist [Sar₁,Der₂]Ang II (600 ng/kg/min) for 30 minutes, resulted in a rise in MAP (18.8 ± 2.1 mm Hg) and more than a twofold increase in plasma ANF level in patients with essential hypertension (n = 6). The increase in the plasma ANF level by the agonistic pressor action of the Ang II antagonist significantly correlated with the increase in MAP (r = 0.760, p<0.05). These results suggest that the plasma ANF level is one of the important clinical parameters for evaluating the hemodynamic state in essential hypertension, primary aldosteronism, and other adrenal disorders. (Hypertension 11 [Suppl I]: I-212-I-216, 1988)

KEY WORDS  • angiotensin II • norepinephrine • blood pressure • angiotensin II analogue • primary aldosteronism • volume expansion

After the discovery of potent natriuretic, diuretic, and vasorelaxant activities in extracts from the rat atrium by de Bold et al., a family of natriuretic polypeptides with high and low molecular weights were isolated from human and rat atrial tissues. These peptides are now called atrial natriuretic factor (ANF) and are implicated in control of body fluid and blood pressure. Using a specific radioimmunoassay (RIA) for human ANF-(99–126), we previously demonstrated that human ANF-(99–126) of 28 amino acids is released from the heart and circulates in the body, and bolus injection of synthetic human ANF-(99–126) into humans results in a rapid increase of natriuresis and diuresis and a rapid fall in blood pressure accompanied by an increase in heart rate, and that the infusion of human ANF-(99–126) in patients with congestive heart failure improves cardiac performance. Also we demonstrated the wide distribution of ANF in the brain by RIA and immunohistochemistry, and its prevalence in the anteroventral third ventricular (AV3V) region, and showed that brain ANF is involved in central cardiovascular control alone or in combination with the brain renin-angiotensin system. These findings indicate that ANF is a possible endogenous antihypertensive agent.

We and others have reported that plasma ANF levels are elevated in patients with essential hypertension (EH) and in hypertensive animal models such as...
spontaneously hypertensive rats and rats with deoxy-corticosterone acetate (DOCA)-salt hypertension. To further elucidate the implication of the ANF system in hypertension, we examined plasma ANF levels in patients with EH and endocrine hypertension, and studied the effects of angiotensin II (Ang II) and norepinephrine (NE) infusion on these levels in normal subjects; we also studied the effects of the infusion of Ang II antagonist (Ang II A; [Sar',Ile8]Ang II) on plasma ANF levels in patients with EH.

**Methods**

**Subjects**

**Measurement of Basal Plasma ANF Level**

Thirty-four patients with untreated EH (22 men and 12 women) aged 24–80 years (50.8 ± 2.7 years) and 20 age-matched healthy control subjects were studied for measurement of plasma ANF levels. According to the World Health Organization classification of organ damage, 13 patients were classified as having Stage I, 14 as stage II, and the remaining 7 as Stage III disease. Blood was taken twice from the 12 patients in Stage I, before and after 3 months of medication with trichlormethiazide, metoprolol tartrate, or nifedipine. In addition, patients with primary aldosteronism (PA; n = 8), Cushing’s syndrome (n = 4), pheochromocytoma (n = 6) and Addison’s disease (n = 3) were studied.

**Infusion Studies**

Five normal male volunteers, aged 28 to 37 years, participated in the volume expansion, Ang II, or NE infusion studies. Two patients with PA were part of the volume expansion study both before and after extirpation of adrenal tumors. The effect of volume expansion in one patient with Addison’s disease was also studied. Six patients with EH participated in the Ang II A infusion study.

Informed consent was obtained from all participants, and the study was approved by the ethical committee on human research of Kyoto University.

**Study Protocol and Blood Sampling**

**Basal Plasma ANF Levels**

After an overnight fast, blood samples from the patients and healthy subjects for the measurement of basal plasma ANF levels were taken at 0900 with participants in a recumbent position. All were consuming a normal sodium diet.

**Volume Expansion Study**

After an overnight fast, 2 L of physiological saline was infused intravenously in 2 hours after 30 minutes of bedrest. Blood samples were taken at 0, 30, 60, 90, 120, and 150 minutes after saline infusion was started.

**Ang II, NE, and Ang II A Infusion Studies**

Synthetic [Val']-angiotensin amide (Hypertensin, CIBA Geigy Japan, Osaka, Japan) was used as Ang II, and synthetic [Sar',Ile8]Ang II (Protein Research Foundation, Osaka, Japan) was used as Ang II A. These peptides were dissolved in 40 ml of physiological saline before use. The chemical nature and content of these peptides were verified by high performance liquid chromatography. After an overnight fast, five normal male subjects remained recumbent for 30 minutes, and Ang II, 20 ng/kg/min, or NE (dl-norepinephrine, Sankyo, Tokyo, Japan), 200 ng/kg/min, in 40 ml of physiological saline was infused intravenously for 30 minutes. Blood samples were taken at 0, 15, 30, and 45 minutes after infusion was started. Six patients with EH were infused with Ang II A (600 ng/kg/min) intravenously for 30 minutes for differential diagnosis of hypertension. Blood samples were also taken at 0, 15, 30, and 45 minutes.

**Measurement of Plasma Hormones**

Plasma ANF levels were measured by the RIA for human ANF-(99–126) reported previously. Plasma renin activity (PRA) and plasma aldosterone concentration (PAC) were measured with commercially available kits (Renin RIA beads, Dainabot, Tokyo, Japan; Aldosterone Test Shionogi, Shionogi, Osaka, Japan).

**Statistical Analysis**

Data are expressed as means ± SE and were statistically analyzed by paired or nonpaired Student’s t test. Linear regression analysis was used to determine correlations between results.

**Results**

**Basal Plasma ANF Levels**

The plasma ANF level in the patients with EH before medication was 92.2 ± 12.9 pg/ml, which was more than twice as high as that in normotensive controls (37.8 ± 6.0 pg/ml; p < 0.01). The level in patients with Stage I disease was 56.0 ± 6.0 pg/ml, significantly higher than that of controls (p < 0.05). Levels in patients with Stage II (96.9 ± 12.9 pg/ml) or III disease (116.6 ± 10.6 pg/ml) were still higher. Basal plasma ANF levels showed no significant correlation with age or heart rate and no difference between sexes. There was a significant correlation in the untreated EH patients between plasma ANF levels and MAP (r = 0.46, p < 0.05). Significant inverse correlation was observed between the plasma ANF level and PRA (r = -0.43, p < 0.05), but not between the plasma ANF level and PAC. Plasma ANF levels of 12 patients with Stage I disease decreased after medication (from 112.2 ± 12.5 to 68.7 ± 10.4 pg/ml; p < 0.05). The decrement showed a significant correlation with that of MAP after medication (r = 0.585, p < 0.05).

Patients with PA showed significantly higher plasma ANF levels (122.4 ± 30.2 pg/ml) than controls (p < 0.05). The levels returned to normal after extirpation of adrenal tumors. Basal plasma ANF levels in the patients with Cushing’s syndrome were 219.8, 142.0, 70.2, and 36.2 pg/ml (mean, 117.1 ± 40.7 pg/ml). Plasma ANF concentrations of patients with pheochromocytoma (41.6 ± 7.3 pg/ml) were not different from those of normal subjects. In one patient...
Aldosteronism
Case 1, pre-op
Case 1, post-op
Aldosteronism
Case 2, pre-op
Case 2, post-opi
Addison’s Disease

**Figure 1.** The influence of volume expansion on plasma ANF levels in primary aldosteronism (Cases 1 and 2), before and after tumor extirpation, and in Addison’s disease. The response of plasma ANF levels in normal subjects (mean ± 2 SD, n = 6) is shown in the shaded area.

with Addison’s disease the level was low (7.5 pg/ml); the mean value of patients with Addison’s disease was 38.4 ± 13.3 pg/ml (n = 3), which was not different from that of normal subjects.

**Volume Expansion Study**

Figure 1 shows the effects of volume expansion on plasma ANF levels in the patients with PA, both before and after operation, and in one patient with Addison’s disease. One patient with PA (Case 1) had an increased basal level (110 pg/ml) and a still higher level in response to volume expansion (maximum, 154 pg/ml at 120 minutes). In the other patient with PA (Case 2), the basal level was normal (22.7 pg/ml), but the response to volume expansion was much greater (maximum, 125 pg/ml at 60 minutes) than those of the normal subjects. After surgical treatment of the adenoma, the basal level of this patient decreased (14.1 pg/ml), and a normal response (maximum, 45.4 pg/ml at 150 minutes) to volume expansion was observed. The patient with Addison’s disease had a low plasma ANF level (7.5 pg/ml) that did not change in response to volume expansion.

**Ang II Infusion Study**

Blood pressure (BP) began to rise within 2 minutes after Ang II infusion was started, reached a maximum at 5 minutes, and remained at the same level until the infusion was stopped. As shown in Table 1, NE infusion resulted in a rise in MAP (15.8 ± 4.4 mm Hg), with a tendency of HR to decrease (64.0 ± 3.5 to 56.4 ± 1.8 beats/min) and about a twofold increase in plasma ANF level. Both PRA and PAC significantly increased after NE infusion.

**NE Infusion Study**

BP began to rise within 2 minutes after NE infusion was started, reached a maximum at 5 minutes, and remained at about the same level until the infusion was stopped. As shown in Table 1, NE infusion resulted in a rise in MAP (15.8 ± 4.4 mm Hg), with a tendency of HR to decrease (64.0 ± 3.5 to 56.4 ± 1.8 beats/min) and about a twofold increase in plasma ANF level. Both PRA and PAC significantly increased after NE infusion.

**Ang II A Infusion Study**

As shown in Table 2, infusion of Ang II A (600 ng/kg/min) for 30 minutes, resulted in a rise in MAP (104.4 ± 4.7 to 123.2 ± 3.7 mm Hg), with the tendency of the HR to decrease and more than a twofold increase in the plasma ANF level in patients with EH. This increase in the plasma ANF level significantly correlated with that in MAP (r = 0.760, p < 0.05). The PRA decreased and PAC increased after infusion of Ang II A.

**Discussion**

We and some groups reported increased ANF levels in patients with EH,16-19 and other groups reported no difference compared with controls.24,25 The reason for the difference in levels reported is not clear at present.

| Table 1. Effects of Angiotensin II or Norepinephrine Infusion in Normal Subjects |
|------------------|------------------|------------------|------------------|
| Hormonal and hemodynamic parameters | Preinfusion | Postinfusion | Preinfusion | Postinfusion |
| ANF (pg/ml) | 40.0 ± 4.9 | 86.5 ± 14.3 * | 49.6 ± 6.3 | 100.8 ± 14.8 † |
| PRA (ng/ml/hr) | 0.90 ± 0.13 | 0.44 ± 0.13 ‡ | 1.01 ± 0.48 | 2.53 ± 0.66 † |
| PAC (pg/ml) | 93.0 ± 7.8 | 158.4 ± 15.4 ‡ | 119.2 ± 20.6 | 133.6 ± 10.0 † |
| MAP (mm Hg) | 79.1 ± 5.9 | 104.2 ± 3.1 † | 77.7 ± 4.0 | 93.5 ± 5.7 † |
| HR (beats/min) | 62.4 ± 3.4 | 55.2 ± 2.1 | 64.0 ± 3.5 | 56.4 ± 1.8 |

*Values are means ± SE. ANF = plasma ANF concentration; PAC = plasma aldosterone concentration; HR = heart rate.

* *p < 0.05, †p < 0.025, ‡p < 0.001, compared with preinfusion values.
TABLE 2.  Effects of Infusion of Angiotensin II Antagonist in Patients with Essential Hypertension

<table>
<thead>
<tr>
<th>Sampling point</th>
<th>ANF (pg/ml)</th>
<th>MAP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>PRA (ng/ml/hr)</th>
<th>PA (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preinfusion</td>
<td>71.1 ± 12.1</td>
<td>104.4 ± 4.7</td>
<td>67.5 ± 4.2</td>
<td>0.36 ± 0.12</td>
<td>73.0 ± 12.4</td>
</tr>
<tr>
<td>Postinfusion</td>
<td>188.5 ± 55.7*</td>
<td>123.2 ± 3.7*</td>
<td>62.4 ± 1.4</td>
<td>0.25 ± 0.11†</td>
<td>94.0 ± 19.0</td>
</tr>
</tbody>
</table>

Abbreviations are the same as in Table 1.

*p < 0.01, †p < 0.05, compared with preinfusion values.

However, it may be accounted for in part by the differences in the populations studied, in sampling conditions, and in the assay methods. The present study further demonstrates that the elevated plasma ANF level in patients with EH decreases after medication. The increased ANF level seen in EH is consistent with increases observed in hypertensive animal models.26

In spontaneously hypertensive rats, left atrial pressure is reported to be more elevated than that in Wistar-Kyoto rats.26 In patients with EH, the total plasma volume is reported to be normal or decreased,27 however, pulmonary capillary wedge pressure is reported to be elevated and is significantly correlated with MAP.28 Thus, in patients with EH as well as in the hypertensive animal model, central hemodynamics are altered. These findings, together with accumulating evidence that atrial stretch is the main factor for ANF secretion from the heart,3 suggest that the increased plasma ANF level in EH is accounted for, at least in part, by the altered central hemodynamics.

Plasma ANF levels were elevated in the patients with PA, which could be explained by the intravascular volume overload. In fact, isotonic volume expansion in these patients resulted in a greater response in their plasma ANF levels than in those of controls. Such exaggerated response disappeared after extirpation of the tumors, when PAC and PRA returned to normal. In the patient with Addison’s disease with contracted plasma volume, the basal plasma ANF level was low (7.5 pg/ml), and it showed little response to the same isotonic volume expansion. Some patients with Cushings syndrome showed elevated plasma ANF levels but in those with pheochromocytoma the levels were not different from those in controls. These results indicate that plasma ANF levels reflect intravascular blood volume in these patients.

The infusion of two different pressor agents, Ang II and NE, was studied in normal subjects in the present study. The plasma ANF level changed in association with the alteration of MAP. As the major regulating factor of ANF secretion from the heart is atrial stretch,4,5 the secretion induced by pressor substances is due to hemodynamic changes that increase atrial stretch. However, the direct actions of Ang II and NE on cardiocytes cannot be ruled out completely at present.

Infusion of Ang II A at a speed of 600 ng/kg/min in normal subjects is reported to elicit about the same pressor response as infusion of Ang II at a speed of 20 ng/kg/min.23 In the present study, infusion of Ang II A in the patients with EH resulted in almost the same or a slightly weaker pressor response as compared with the response to Ang II infusion in normal subjects (18.8 and 25.1 mm Hg, respectively; see Tables 1 and 2). The increase of plasma ANF level, however, was two times higher with the Ang II A infusion in patients with EH than with Ang II infusion in normal subjects. In addition, a significant correlation was observed between increments of MAP and the plasma ANF level. The mechanism of the exaggerated response of plasma ANF in patients with EH is not clear; however, this exaggerated response might contribute partly to the elevated plasma ANF level in EH.

These results indicate that the plasma ANF level is one of the important clinical parameters for evaluating the hemodynamic state in EH, PA, and other adrenal disorders.

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