Plasma Levels of Immunoreactive Atrial Natriuretic Factor in Healthy Subjects and in Patients with Edema

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Abstract

Atrial natriuretic factor (ANF), a recently sequenced cardiac peptide, has been shown to have potent natriuretic, diuretic, and vasodilating effects in several species. We have developed a radioimmunoassay to measure the levels of immunoreactive ANF in human plasma. Plasma levels of ANF in healthy volunteers on a low sodium diet were 9.8±1.4 pmol/liter and increased to 21.9±3.0 on a high sodium diet. The levels of atrial natriuretic factor correlated directly with urinary sodium and inversely with plasma renin activity and plasma aldosterone levels. Patients with marked edema due to congestive heart failure had plasma levels of atrial natriuretic factor five times higher than normal ($P < 0.05$), whereas patients with cirrhosis and edema had levels that were not different from normal. These results suggest that atrial natriuretic factor plays an important role in the adaptation to increased sodium intake.

Introduction

Atrial natriuretic factor (ANF)[1] is the term that is most commonly used to describe a group of vasoactive, natriuretic peptides that have been isolated from mammalian atrial myocytes (1, 2). Because of its potent effects on distant tissues, it has been presumed that ANF acts as a circulating hormone (3, 4). It is found within ultrastructural granules in the atria that anatomically resemble secretory granules of endocrine tissues (5), and is derived from a larger precursor molecule as are many peptide hormones (6).

Three recent reports have described radioimmunoassays (RIAs) for ANF in rat plasma (7–9), and levels of ANF were higher in rats on high sodium diet than on low sodium diet (8).

In this study we report a RIA for measurement of immunoreactive ANF (IR-ANF) in human plasma. The effect of changes in sodium intake on plasma IR-ANF levels and the relationship of IR-ANF to plasma levels of renin activity and aldosterone are described.

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1. *Abbreviations used in this paper:* ANF, atrial natriuretic factor; ANP, atrial natriuretic peptide; IR-ANF, immunoreactive ANF.

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Methods

Subjects. 10 normotensive male volunteers, ages 20–25 yr, were studied. Each volunteer was studied on three occasions; once while on regular diet, once after 3 d of regular diet supplemented with 145 meq of sodium chloride capsules (high sodium diet), and once after 3 d of a 10-meq sodium diet (low sodium diet). During the 3 d of low sodium diet the volunteers ate their meals in the Clinical Research Center of the University of Michigan Hospitals. The order of the high-sodium diet periods and low sodium diet periods was randomized, but these diet periods always followed the regular diet. A 24-h urine sample for sodium and creatinine was collected on the third day of each diet. The samples were drawn on the morning of the fourth day of each diet. Blood was drawn through a butterfly needle after 1 h of standing, and again after 1 h of recumbency.

Seven patients with biventricular congestive heart failure due to ischemic heart disease and four patients with cirrhosis were also studied. Each of these patients had prominent peripheral edema (graded 2^+ or greater) and estimated fluid retention of 10 liters or more. Patients with congestive heart failure had impaired left ventricular ejection fraction (37±7%) measured by radionuclide ventriculography. All edematous patients were treated with furosemide and three of the cirrhotic patients also received spironolactone. Six patients with heart failure were treated with vasodilators. Each patient had one recumbent blood sample drawn. The study was approved by the committee for the protection of human subjects.

Samples for electrolytes, aldosterone, renin activity, and IR-ANF were drawn on each occasion. IR-ANF samples were drawn into EDTA tubes, placed on ice, and separated within 15 min. Plasma samples for ANF were stored at −70°C.

Assay methods. For measurement of IR-ANF, 10 ml of plasma was thawed within 5 d of sampling. Samples were centrifuged and 1 ml of 1 N HCl was added to the supernatant. The acidified plasma was recentrifuged and extracted through C_{18} octadecylsilane cartridges (Sep-Pak; Waters Associates, Milford, MA). Each plasma sample was divided into two 5-ml aliquots and each aliquot was extracted with a separate cartridge. Cartridges were prepared by sequential washing with 5 ml methanol, 5 ml 8 M urea, and 10 ml distilled water. Plasma samples were then added to the cartridge and washed with 10 ml of distilled water followed by 10 ml of 4% glacial acetic acid in distilled water. The samples were then eluted with 10 ml of 90% ethanol plus 4% glacial acetic acid. The eluates were air-dried overnight at room temperature.

Samples were reconstituted in 0.5 ml buffer. Buffer, prepared fresh for each assay, contained 0.01 M K_2HPO_4, 0.15 M NaCl, 0.1% sodium azide, and 0.25% rabbit serum, and was diluted 6:5 with 0.1 M EDTA. Bovine serum albumin, 0.25%, (Miles Scientific Div., Miles Laboratories, Inc., Naperville, IL) was added to the buffer before each assay. The buffer was brought to pH 7.4 at room temperature using 5 N NaOH. Samples were vortexed for 45 s, transferred to polystyrene tubes, and centrifuged for 10 min at 3,300 *g*.

Synthetic ANF standard (atriopeptin III) and antibody against 1–28 α-human ANF were purchased from Peninsula Laboratories, Inc., Belmont, CA. ^{125}I-ANF was prepared using chloramine-T. Monoiodinated

peptide was separated from free iodine and diiodinated peptide by high pressure liquid chromatography using a C_{18} reverse-phase column and a linear gradient of 10–40% acetonitrile. Monoiodinated ANF eluted at 29% acetonitrile.

Plasma extracts or ANF standard, 200 μl, were incubated in duplicate with antibody and ^{125}I-ANF for 18 h at 4°C. Free and bound hormone were separated using 0.5 ml dextran charcoal that was prepared by adding 25 mg T-70 Dextran I (Pharmacia Fine Chemicals, Piscataway, NJ) and 250 mg Norit "A" charcoal (Sigma Chemical Co., St. Louis, MO) to 100 ml of 0.1 M sodium phosphate buffer at pH 7.4. Supernatant was then transferred to another tube for counting. Recovery of synthetic ANF was 56.6±2.4% with a variation coefficient of 12.1%. Recovery was similar in plasma obtained during high and low sodium diet and in plasma from patients with edema. Plasma levels of ANF were calculated in pmol/liter after a correction for recovery.

Intraassay variation was 8.5% and interassay variation was 12.6%. The slope of the dose response curve with logit-log transformation was $2.45\pm0.04 \times 10^{-12}$ M^{-1} (CV = 5.2%) and the midrange (50% of B_0) was 202±8 pmol/liter (CV = 13%). The least detectable concentration was 15.6 pg/tube, which corresponds to 3.0 pmol/liter of plasma for the volume of extraction used. Relative affinity of the antiserum for binding to synthetic ANF peptides was as follows: α-h atrial natriuretic peptide (ANP) (1–28), 100%; hANP(7–28), 95%; β-rANP(17–48), 94.2%; hANP(5–28), 81.6%; atriopeptin III, 74.3%; ANP(13–28), 50.1%; atriopeptin II, 0.26%; atriopeptin I, <0.04%; ANP(1–11), <0.04%. Cross-reactivity with furosemide, angiotensin I, angiotensin II, bradykinin, cosyntropin, α-melanocyte stimulating hormone (MSH), somatostatin, and β-endorphin was less than 0.066%. Cross-reactivity with vasopressin was 0.2%.

Logit-log plots of serial dilutions of plasma samples from normal volunteers and patients with congestive heart failure were closely parallel with the standard curve (Fig. 1). The slope of the unknown samples was $2.46\pm0.6 \times 10^{-12}$ M^{-1} (CV = 7.6%).

Aldosterone and renin activity were measured by radioimmunoassay (10, 11).

Statistical analysis. The data are expressed as mean±SE. Repeated measures analysis of variance (ANOVA) with Newman-Keuls test (α = 0.05) was used for comparison of IR-ANF, plasma renin activity (PRA), and aldosterone levels between different diets and positions. IR-ANF data from edematous patients were compared with normal data using Student's t test, whereas serum sodium and urinary measurements for healthy patients were analyzed using paired t test. Bonferroni protection was used in both instances. Linear regression analysis was used to determine correlation between IR-ANF and other variables (12).

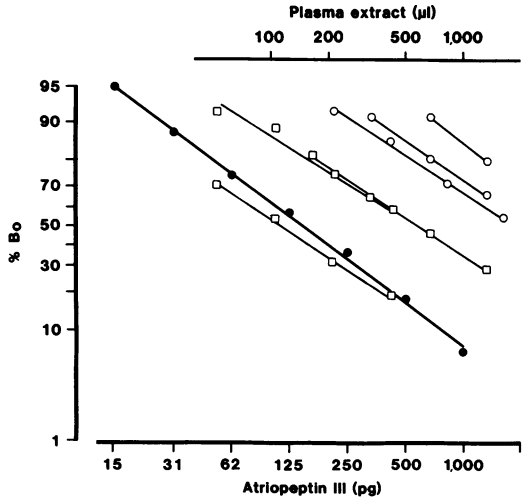

Figure 1. Serial dilution of different plasma samples compared with the standard curve for ANF using logit-log transformation. ○, healthy volunteers; □, patients with congestive heart failure, ●, standard curve.

Results

Aldosterone and renin activity were significantly higher after low sodium diet than after regular or high sodium diet in both supine and upright positions ($P < 0.001$, $\alpha = 0.05$; Table I).

IR-ANF was detectable in all plasma samples except one. This sample, obtained during low sodium diet, was included in our statistical analysis as representing an IR-ANF level at the mean between 0 and the lower limit of detectability. There was no difference in plasma IR-ANF between supine and upright position ($P = 0.98$), but the differences between diets were significant. IR-ANF levels were suppressed by low sodium diet as compared with regular and high sodium diet ($P < 0.001$, $\alpha = 0.05$).

Results of urinary volume and creatinine measurements suggest good compliance in regard to completeness of collection. Urinary sodium varied significantly between each diet (Table I).

A significant correlation was seen between urinary sodium and both upright and supine plasma IR-ANF levels ($r = 0.555$, $P < 0.005$ for upright values; $r = 0.544$, $P < 0.005$ for supine values). Fig. 2 *A* shows the relation between urinary sodium excretion and the mean of upright and supine IR-ANF levels for each diet.

Table I. Changes in Plasma Levels of IR-ANF and Other Variables After Alterations in Dietary Sodium Intake

	Low sodium diet	Regular diet	High sodium diet
IR-ANF (*pmol/liter*)			
Upright	10.5±1.8	19.6±1.8*	20.7±2.7*
Supine	9.1±1.5	18.4±1.9*	23.2±3.8*
Aldosterone (*ng/dl*)			
Upright	47.7±6.8	13.6±1.6*	12.2±1.3*
Supine	20.6±4.3‡	9.6±1.8*	6.1±0.6*
Renin activity (*ng/ml per h*)			
Upright	8.8±0.7	3.1±0.4*	1.9±0.4*
Supine	5.2±0.5‡	2.0±0.3*	1.3±0.3*
Urine volume (*ml/24 h*)	1,385±223	1,307±120	1,728±172
Urine creatinine (*mg/24 h*)	1.88±0.10	2.11±0.15	1.98±0.08
Urine sodium (*meq/24 h*)	26±5	199±22*	306±27§
Serum sodium (*meq/liter*)	140.5±1.0	144.1±0.6*	142.8±1.1

* Significantly different from corresponding low sodium value (α = 0.05 for aldosterone, renin, and IR-ANF; $P < 0.05$ for serum sodium and urinary measurements).
‡ Significantly different from corresponding upright value ($\alpha = 0.05$).
§ Significantly different from both low sodium and regular diet ($P < 0.05$).

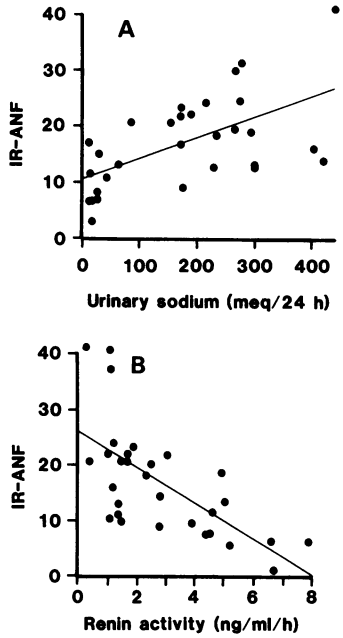

Figure 2. (*A*) Regression analysis of IR-ANF levels (pmol/liter) (mean of upright and supine) and urinary sodium excretion ($r = 0.593$, $P < 0.001$). (*B*) Regression analysis between supine IR-ANF (pmol/liter) and supine plasma renin activity ($r = -0.654$, $P < 0.001$).

There was a significant inverse correlation between both upright and supine renin activity and IR-ANF ($r = -0.493$, $P < 0.01$ for upright values, $r = -0.654$, $P < 0.001$ for supine values, Fig. 2 *B*). There was also a significant inverse correlation between upright aldosterone and IR-ANF levels ($r = -0.451$, $P < 0.025$). For supine aldosterone and IR-ANF levels the correlation was close to significant ($r = -0.349$, $P < 0.06$).

Since inclusion of three samples from each volunteer might influence these regression analyses, the intercorrelations of the IR-ANF levels on the different diets were calculated. There was no significant correlation between these levels, confirming the independence of the observations from the same volunteer.

Finally, a significant positive correlation was found between mean IR-ANF levels and serum sodium ($r = 0.539$, $P < 0.0025$).

The levels of IR-ANF in patients with congestive heart failure and cirrhosis, as well as supine levels in healthy volunteers on regular diet, are shown in Fig. 3. The levels in patients with cirrhosis were similar to those in healthy volunteers, but in patients with congestive heart failure the mean level was five times higher than normal ($P < 0.05$).

In patients with congestive heart failure, there was a significant negative correlation between plasma levels of IR-ANF and left ventricular ejection fraction ($r = -0.857$, $P < 0.025$). The mean plasma renin activity was 5.8±1.1 ng/ml/h and the mean aldosterone level was 17.9±11 ng/dl in these patients.

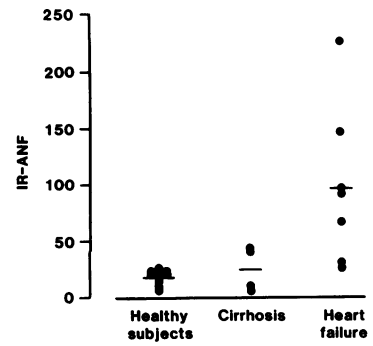

Figure 3. Distribution and mean of supine IR-ANF levels (pmol/liter) in healthy volunteers on regular diet, patients with congestive heart failure, and patients with cirrhosis.

Discussion

Atrial natriuretic factor is a likely regulator of sodium and blood pressure homeostasis. In addition to potent natriuretic and diuretic effects, it causes arterial vasodilation in vitro by blocking the effects of most vasoconstrictors (4, 13), and inhibits secretion of aldosterone in vitro (14). With the recent reports of measurable ANF levels in rat plasma (7–9) and the description of specific membrane receptors for ANF in arterial smooth muscle, renal (15), and adrenal cortical tissue (16), a role for ANF as a circulating hormone seems assured.

Three RIAs for measurement of ANF in rat plasma have been reported. Tanaka et al. (8) found plasma levels of 156 pmol/liter in control animals, and levels of 354 pmol/liter in rats receiving high sodium diet. Gutkowska et al. (7) reported levels of 1.02–1.61 ng/ml in normal rats (356–562 pmol/liter). Lang et al. (9) have reported mean plasma levels in rats of 54.6 pg/ml (21.4 pmol/liter) with a sixfold increase following 30% volume expansion. The present study, using an assay similar to that of Tanaka et al. (8), found plasma levels of IR-ANF in healthy human subjects to be similar to those of Lang et al.

The suppression of IR-ANF levels by low sodium diet and the correlation between urinary sodium excretion and plasma IR-ANF suggest that secretion of ANF is responsive to changes in effective plasma volume. The significant correlation between IR-ANF and plasma aldosterone and renin activity is also consistent with this interpretation.

The mechanisms whereby ANF secretion is regulated by changes in sodium intake can only be speculated upon at present. It has been suggested that increased atrial stretch may directly stimulate ANF secretion (9, 17). Neural or hormonal stimulation of ANF secretion may also occur in response to stimulation of atrial stretch receptors or aortic and carotid baroreceptors.

The lack of change in IR-ANF in response to changes in posture is somewhat surprising. Increased atrial pressure associated with recumbency would be expected to increase secretion of ANF. It is possible that the effects of increased atrial pressure are counterbalanced by other neural or humoral regulators of ANF secretion in this circumstance.

ANF has direct suppressive effects on aldosterone secretion in vitro (14), and blocks the stimulatory effects of angiotensin II and ACTH upon aldosterone secretion in vitro (18). It also suppresses plasma aldosterone and PRA when infused in dogs (19). The suppression of ANF with low sodium diet observed in this study may partially explain the rises in aldosterone and PRA that occur during low sodium diet. In addition, the increased adrenal sensitivity to angiotensin II that occurs during sodium restriction (20) may be mediated by decreases in plasma ANF levels. If this is the case, ANF must be more effective in suppressing aldosterone in vivo in humans than it is in vitro in isolated rat adrenal cells. Concentrations of 10^{-9} M have been required to obtain effects in vitro (14, 18) compared with plasma levels in the present study of 2×10^{-11} M. Binding studies with isolated adrenal membranes, however, have shown 50% occupancy at a concentration of 1.5×10^{-10} M (16), suggesting that lower in vivo concentrations may have important effects.

Elevated levels of IR-ANF in patients with congestive heart failure are likely to be secondary to chronic increases in atrial pressure. The highest levels of IR-ANF were seen in patients with the most severe clinical manifestations of heart failure and the lowest left ventricular ejection fractions. The fact that cir-

rhotic patients have normal levels of IR-ANF also suggests that direct hemodynamic factors are involved, since increased atrial pressure is not a feature of this condition (21).

The negative correlations between plasma IR-ANF and plasma renin activity and aldosterone in normal volunteers were not present in patients with heart failure. Increased secretion of renin and aldosterone in the face of increased atrial pressure is probably due to decreased renal perfusion and stimulation of the juxtaglomerular apparatus in these patients.

The apparent inability of high levels of IR-ANF to cause diuresis and natriuresis in patients with congestive heart failure may also be related to the hemodynamic impairment. It has recently been reported that the natriuretic effects of ANF are blunted in dogs with acute low output failure (22). It is also possible that inactive forms of immunoreactive ANF are secreted in patients with heart failure.

Bioassay measurements of ANF activity in atrial extracts from cardiomyopathic hamsters are low, and ANF deficiency has been suggested as a cause of edema in congestive heart failure (23). The results of the present study suggest that ANF deficiency is not a feature of ischemic congestive heart failure in humans.

Interpretation of these results is limited by the fact that the circulating active forms of ANF are not yet known. Inactive metabolites of ANF may be responsible for some of the immunoreactivity present in plasma. Despite this limitation, the observed alterations in IR-ANF suggest that the measured plasma levels reflect physiologically regulated changes in the secretion of ANF by the heart.

The correlation of plasma IR-ANF with sodium intake suggests a possible role for ANF in the adaptation to increased sodium intake. Sodium restriction suppresses ANF secretion, presumably allowing for more effective sodium retention and vasoconstriction. As dietary sodium intake increases, ANF secretion increases in parallel, leading to natriuresis, diuresis, and vasodilation.

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