Atrial Natriuretic Factor in Hypertension: Bioactivity at Normal Plasma Levels

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To ascertain whether small shifts in plasma atrial natriuretic factor (ANF) exerted biological effects in hypertension, we studied the renal, hemodynamic, and hormonal effects of ANF [human ANF-(99-126)] infused at a dose (0.75 pmol/kg/min for 3 hours) that would induce changes in plasma ANF confined to the normal, resting range, in a group of six young men with uncomplicated, mild essential hypertension. During ANF infusions, the patients excreted 11.8±2.0 mmol (mean±SEM) sodium more than during the time-matched placebo phase natriuresis (p<0.001, mean increase of 53% above placebo values). Urinary excretion of cyclic guanosine monophosphate rose to more than double (212%, p<0.001) placebo values. Plasma renin activity (0.4±0.05 vs. 0.9±0.12 nmol/l/hr, p<0.0001) and aldosterone concentrations (102±4 vs. 184±47 pmol/l, p<0.05) were clearly suppressed during administration of ANF. Plasma norepinephrine also fell significantly below placebo values (268±17 vs. 439±35 pg/ml, p<0.05). Urine volume, the excretion of electrolytes other than sodium, hematocrit, effective renal plasma flow, glomerular filtration rate, and filtration fraction were unaffected by ANF. Plasma concentrations of epinephrine, arginine vasopressin, adrenocorticotropic hormone, and cortisol were unchanged. Blood pressure and heart rate were unchanged. Minor perturbations in plasma ANF concentrations exert clear biological effects in patients with mild essential hypertension. These data suggest that such minor shifts in plasma ANF are of physiological relevance in mild hypertension and probably contribute to volume homeostasis in this condition. (Hypertension 1989;14:261-268)

Atrial natriuretic factor (ANF) continues to attract intense interest as a potentially major regulator of body fluid volumes and arterial pressure. Studies in humans have clearly demonstrated that this peptide may induce natriuresis, suppress the renin-angiotensin-aldosterone system, and lower arterial pressure. 1-3 Recently, we demonstrated that such effects occur in normotensive subjects even with low doses of ANF, which induce perturbations in plasma peptide concentrations entirely within the resting normal range.4 Far less is known concerning the effect of physiological doses of ANF in hypertensive patients. The response to high doses of ANF appears qualitatively similar in both normotensive and hypertensive subjects.5,6 However, data suggest the natriuretic effect of ANF is partially pressure dependent and thus is enhanced in hypertension.5 Conversely, ANF appears to be a relatively poor hypotensive agent when given in brief, high doses to hypertensive patients.5,6 These observations raise the possibility of relative resistance to some of the actions of ANF as a contributing pathogenetic mechanism in hypertension. Such potential ANF resistance may be most pertinent early in the evolution of hypertension and would presumably be evident at the normal or near-normal plasma concentrations of ANF seen in uncomplicated mild essential hypertension.7

The actions of ANF at physiological plasma concentrations have not been previously documented in essential hypertension. We wished to ascertain whether small changes in plasma ANF, previously shown to exert biological effects in normotensive subjects,4 had significant bioactivity in hypertension. Therefore, we studied the renal, hemodynamic, and hormonal effects of ANF, infused at a dose that would induce changes in plasma ANF entirely within the normal range, in a group of young men with uncomplicated borderline or mild essential hypertension.

Subjects and Methods

The experimental protocol was approved by the Hospital Ethical Committee, and six male patients

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(aged 18–40 years, mean 25.5 years; weight 66–83 kg, mean 76 kg) gave informed consent for participation in the study. All six patients had borderline or mild essential hypertension with resting, supine diastolic arterial pressures greater than 90 mm Hg recorded on at least four separate occasions in the 3 months before study. All had been referred by their family practitioners for investigation of hypertension. Four had never received antihypertensive treatment, and two had received enalapril (5 and 10 mg daily) for brief periods. This medication was withdrawn at least 2 months before the studies. No patient had clinical or laboratory evidence of secondary hypertension. In each case, routine hematology, serum electrolytes, creatinine, urea and glucose, renal ultrasound, electrocardiograms, echocardiograms, urinalysis, endogenous creatinine clearance and plasma renin, aldosterone, and catecholamines were normal. In four of the six patients there was a positive family history for hypertension affecting one or more first-degree relatives. The patients were studied on two occasions (1–3 weeks apart) on the fourth day of identical constant sodium (150 mmol/day) and potassium (80 mmol/day) diets.

After eating breakfast and drinking a water load (10 ml/kg distilled water), subjects underwent venous cannulation of both forearms for administration of infusions and withdrawal of blood samples. The nondominant brachial artery was cannulated for measurement of blood pressure by the Oxford continuous recording technique. Subjects were seated throughout the studies except for standing to pass urine every 30 minutes. Urinary indexes measured included volume, sodium, potassium, calcium, magnesium, phosphate, creatinine, and cyclic guanosine monophosphate (cGMP). After each urine collection, the subjects each drank 200 ml distilled water.

A 1.5-hour preliminary period was followed by a 3-hour period of infusion of human ANF-(99–126), in Haemaccel (at a calculated dose of 0.75 pmol/kg/min in a volume of 15 ml/hr) or placebo (Haemaccel, 15 ml/hr), administered in single-blind fashion in random order. Haemaccel (Behring, Marburg, FRG) is an intravenous solution commonly used for plasma volume expansion consisting of 35 g of degraded gelatine polypeptides cross-linked through glutaraldehyde and para-aminohippuran (PAH) clearance techniques. Serial venous blood samples were taken for measurement of plasma concentrations of ANF at −60 and 0 minutes (relative to the start time for ANF or placebo infusions) and then every 30 minutes. Samples for plasma renin activity (PRA), aldosterone, cortisol (enzyme-linked radioassay), catecholamines, hematocrit, and serial standard automated multianalyzer plasma biochemistry profiles were taken hourly at −60, 0, +60, +120, +180, +240, and then +270 minutes. Plasma samples for arginine vasopressin, and adrenocorticotropic hormone (ACTH) were taken at 0, +180 and +270 minutes. Plasma volume was measured by the Evans Blue method within the 30 minutes before completion of active and placebo infusions (n=5).

For all hormone, PAH, and inulin assays, all samples from an individual subject were assayed together. Intra-assay coefficients of variation were 11% or less for all assays.

Data were analyzed by two-way analysis of variance using program P2V of the BMDP statistics package with "treatment" (ANF or placebo) and time as repeated measures.

Results

Studies were completed without mishap and, in particular, no subject incurred symptomatic hypertension or bradycardia. Data collection was complete. Sodium excretion for the 24 hours before studies was 117±8 and 123±10 mmol/day before ANF and placebo infusions, respectively (NS).

ANF infusions induced significant changes in venous plasma peptide concentrations (mean increment of 8 pmol/l above placebo values, p<0.001) entirely within the range established in this laboratory for normal resting subjects (aged 18–60 years) on a free diet and seated for 30 minutes (5–24 pmol/l, Figure 1).

Compared with time-matched placebo data, an ANF-induced natriuresis was apparent within the first half hour of infusion and remained evident for an hour after completion of the infusions (Figure 2). During the 3-hour ANF infusion phase, subjects excreted a mean of 11.8±2.0 mmol (p<0.001) sodium in excess of time-matched placebo phase natriuresis (mean increase of 53% above placebo values). Urinary excretion of cGMP rose to more than double placebo values with infusion of ANF and then returned to baseline levels. The time course of these changes paralleled that for urinary sodium (Figure 2).

Excretion of calcium and magnesium tended to increase with ANF only in the latter part of infusions (NS). No changes in urinary volume, creatinine, potassium, or phosphate excretion were observed (Table 1). No significant changes in effective renal plasma flow, glomerular filtration rate, or calculated renal filtration fraction were detected (Table 2).

Systolic, mean, and diastolic arterial pressure and heart rate were unchanged by ANF (Figure 3).

Plasma renin activity and aldosterone concentrations were clearly suppressed during administration of ANF (Figure 4). Plasma norepinephrine also fell significantly below time-matched placebo values during ANF infusions (p<0.05) (Figure 4). Plasma
cortisol, epinephrine, arginine vasopressin, and ACTH were unchanged (Table 3). Hematocrit and plasma volume were not affected by ANF (Table 2). Similarly, plasma values of albumin, total protein, sodium, potassium, chloride, calcium, magnesium, phosphate, urea, creatinine, urate, glucose, total and conjugated bilirubin, alkaline phosphatase, and osmolality did not differ between active and placebo study phases.

Discussion

These data demonstrate that minor perturbations in plasma ANF concentrations, confined entirely to the resting, normal range, exert clear biological effects in patients with mild essential hypertension. In the present study, particular care was taken to control factors that might hinder detection of subtle effects of low doses of ANF. Complete time-matched placebo data were obtained and both diet and posture were rigidly standardized. In addition, continuous recording of intra-arterial pressure provided complete and continuous information on blood pressure and heart rate for computerized analysis. Under these exacting conditions, we observed that a mean increment of only 8 pmol/l in plasma ANF concentrations resulted in natriuresis, increased urine cGMP, suppression of the renin-angiotensin-aldosterone system and a lowering of plasma norepinephrine concentrations. Significant negative findings included an absence of change in heart rate, blood pressure, hematocrit, and plasma volume.

These observations suggest that the minor elevation of plasma ANF reported by some authors in mild hypertension\(^7\)\(^8\) and the more clear cut increases present in more severe or complicated hypertension\(^7\)\(^8\)\(^9\) are of physiological relevance and are likely to contribute to volume homeostasis in these conditions.

Previous reports of the effects of ANF administered to patients with hypertension have indicated natriuretic responses that were greater than those observed in normotensive subjects who were studied under similar conditions and received similar doses of ANF.\(^5\)\(^6\) Similarly, in comparison with normotensive subjects who underwent an identical experimental protocol,\(^4\) the hypertensive patients in the current study exhibited a proportionally more pronounced natriuretic response. Sodium excretion during the 3-hour ANF infusion phase exceeded that during time-matched placebo infusions by a mean of 53% as compared with a corresponding value of 32% in the normotensive group. In contrast to previous comparisons between normotensive and hypertensive subjects receiving ANF,\(^5\)\(^6\) our groups were well-matched for body weight and the hypertensive group was slightly younger, rather than older, than our normotensive subjects. In addition, baseline plasma ANF values, the increment with ANF infusions, and the proportional increment in urinary excretion of cGMP (232% and 212% above placebo values for the intrainfusion period in normotensive and hypertensive groups, respectively) were all virtually identical in both groups (Figure 4). The comparable changes in plasma ANF induced by these infusions indicate that metabolic clearance of ANF is similar in both normotensive and mildly
hypertensive subjects. Hence, similar changes in plasma ANF and urine cGMP were associated with proportionally enhanced natriuresis in a hypertensive group clearly distinguished from our normotensive subjects solely by the presence of higher arterial pressure (mean arterial pressures, 113 mm Hg vs. 89 mm Hg).

Comparison of data from the normotensive and hypertensive groups must be viewed with some caution because group numbers were small. In addition, the hypertensive group was studied after, rather than contemporaneously with, the normotensive volunteers. Although receiving identical diets and otherwise adhering to an identical study protocol as that used in our normal group, the hypertensive group baseline sodium excretion rates were somewhat lower (4.538±7.34 mmol/30 min vs. 6.560±2.02 mmol/30 min, averaged over preinfusion periods, NS) as were rates of cGMP excretion (10.57±1.7 nmol/30 min vs. 14.9±3.63 nmol/30 min).

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<th>TABLE 1. Urinary Indexes</th>
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<td>Urine magnesium (µmol)</td>
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All values shown are mean 90-minute excretions (mean±SEM) for each variable calculated from serial 30-minute urine collection data. Comparison of active and placebo values revealed no statistically significant differences for any variable. Before, preinfusion data; Infusion, data for first and second intrainfusion 90-minute periods; After, postinfusion data; A, active [0.75 pmol/kg/min atrial natriuretic factor-(99–126) for 3 hours]; P, placebo (vehicle, in same volume as active infusate, for 3 hours).

<table>
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<th>TABLE 2. Hematocrit, Plasma, and Blood Volume and Renal Hemodynamic Indexes</th>
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Before, preinfusion data; Infusion, intrainfusion data; After, postinfusion data; A, active [0.75 pmol/kg/min atrial natriuretic factor-(99–126) for 3 hours]; P, placebo (vehicle, in equal volume as active infusate, for 3 hours); ERPF, effective renal plasma flow; GFR, glomerular filtration rate.

Data for all variables (mean±SEM) are for six subjects except plasma and blood volumes (n=5).

Hematocrits were measured at 0, +180, and +270 minutes. Plasma and blood volume were measured between 150 and 180 minutes. Renal hemodynamic indexes were calculated from plasma samples taken within the 30-minute periods before 0, 90, 180, and 270 minutes.

Comparison of active and placebo values revealed no statistically significant differences for any variable.
NS). This trend probably reflects the increase in seasonal temperature with associated increased pre-study extrarenal sodium losses occurring at the time of the study in some of the hypertensive patients. Thus, the intergroup difference in sodium excretory response to ANF is somewhat obscured by differing baseline rates of urinary sodium excretion. It is clear and statistically significant when viewed as percent, rather than absolute, change in natriuresis (Figure 4). However, baseline sodium status is a powerful determinant of the natriuretic response to ANF. Therefore, the fact that mean absolute sodium excretion in the hypertensive group in the latter 90 minutes of the infusions (6.007±1.025 mmol/30 min) tended to exceed that in the normotensive group (5.17±0.475 mmol/30 min) despite a lower baseline rate is strong evidence of an enhanced natriuretic response in the hypertensive group.

Arterial pressure appears to play a major role in determining the natriuretic response to ANF. The natriuretic effect can be severely curtailed by the onset of major hypotensive effects of the peptide. We have previously observed a close positive correlation between baseline arterial pressure and natriuretic responses to bolus injections of ANF in humans. The pressure dependency of ANF-induced natriuresis has also been confirmed in animal experiments in which renal arterial clamping, to restrict renal perfusion pressure, abolished the natriuresis.

Urinary excretion of calcium and magnesium tended to increase in the latter stages of ANF infusions, and this pattern is similar to that seen at most doses of ANF. Urine volume was not significantly enhanced, and this is consistent with findings in low dose studies in normotensive humans. Possibly, with the relative water loading incorporated in this experiment, the diuresis sustained throughout both placebo and active phases may
TABLE 3. Plasma Concentrations of Epinephrine, Arginine Vasopressin, and Adrenocorticotropin Hormone

<table>
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<tr>
<td>Epinephrine (pg/ml)</td>
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<td>P 82±8</td>
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<td>AVP (pmol/l)</td>
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<td>ACTH (ng/l)</td>
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<td></td>
<td>P ...</td>
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<tr>
<td>Cortisol (nmol/l)</td>
<td>A 356±62</td>
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<td>P 404±65</td>
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</table>

Active and placebo values (mean±SEM) did not differ significantly at any time point for any of the four variables tabled. A, active [0.75 pmol/kg/min atrial natriuretic factor-(99–126) for 3 hours]; P, placebo (vehicle, in same volume as active infusate, for 3 hours); AVP, arginine vasopressin; ACTH, adrenocorticotropin hormone.

have masked any subtle diuretic effect of small changes in plasma ANF.

Urine potassium and phosphorus were unaffected, which is in contrast to findings with bolus injections of ANF[5,6] but in accord with results from constant low dose infusion studies in normotensive volunteers.[3,4] The threshold increment in plasma ANF at which excretion of sodium, and probably also calcium and magnesium, is increased appears to be lower than that for phosphorus. Glomerular filtration rate, excretion of creatinine, effective renal plasma flow, and filtration fraction were not measurably altered by ANF. This concurs with previous findings[3,4] and suggests the changes in electrolyte excretion are independent of major shifts in renal hemodynamics. However, since even meticulously conducted inulin clearance measurements may incorporate an error as large as 10% and since enhanced natriuresis to the degree observed could be attributable to proportionally far smaller (uncountered) shifts in glomerular filtration rate, the possibility of minor but still important shifts in renal hemodynamics cannot be ruled out.

There are varied reports concerning the response of the renin-angiotensin-aldosterone system to ANF administered to subjects with hypertension. Plasma renin has remained unchanged or even increased by ANF.[5,6,24] Plasma aldosterone has been found unchanged or suppressed or has exhibited a “rebound” increase above placebo values once infusions were halted.[5,6] However, it seems likely that the response of the renin-angiotensin-aldosterone system, and plasma renin activity in particular, is modified by the degree of hypotension induced by ANF. Recent data from incremental infusions of ANF in hypertensive patients demonstrated initial suppression of renin but at greater doses of peptide, and coincident with the onset of appreciable hypotensive effects, renin values rose.[25] Thus, the stimulus from hypotension may override the primary suppressive action of ANF on renin release. In the current study, blood pressure was unchanged, renin was clearly and consistently suppressed, and plasma aldosterone also fell (Figure 4). These data match well with those from our normotensive group, and it seems clear that very small increments in plasma ANF result in brisk, sustained suppression of the renin-angiotensin-aldosterone system in both normotensive and hypertensive subjects provided no major hypertensive effect ensues.

As observed in normotensive subjects,[4] plasma concentrations of arginine vasopressin, ACTH, and cortisol were unchanged. ANF, in the dose and duration used, appears to have little effect on the pituitary-adrenal axis. A suppressive effect on arginine vasopressin could conceivably have been masked by the sustained moderate water loading incorporated in the experimental design of this study and others.[1,5,26] With baseline plasma arginine vasopressin thus relatively suppressed, any further reduction in arginine vasopressin secondary to ANF effects may well have been undetectable.

The moderate but significant suppression of plasma norepinephrine observed in the current study was a new and unexpected finding (Figure 4). Previously, little change has been reported in plasma catecholamines or, in the event of substantial falls in blood pressure, an apparent baroreceptor reflex-mediated rise.[5,6] In our normotensive group, plasma catecholamines tended to fall when a similar dose of ANF was given, but this trend failed to achieve statistical significance.[4] Several strands of evidence point to a direct interaction between ANF and the sympathetic nervous system.[27] ANF-like immunoreactivity and specific binding sites for ANF have been demonstrated in sympathetic ganglia.[28,29] In dogs, ANF-induced hypotension was not associated with any increase in heart rate and did not lead to augmented norepinephrine release.[30] Suppression of epinephrine release by ANF has also been described,[30] and we have observed falls in plasma epinephrine concentrations after bolus intravenous doses of ANF in both normotensive and hypertensive subjects.[1,5] Other evidence, from studies in rats, suggests ANF may inhibit renal sympathetic activity.[31] Thus, it is possible that, in the absence of major hemodynamic perturbations that could trigger reflex sympathetic activation, low doses of ANF may exert a subtle primary suppressive effect on the sympathetic nervous system. Such an effect
may be reflected in a lowering of plasma norepinephrine concentrations, as seen in the current study. As always, extrapolation from plasma norepinephrine values to assumptions concerning sympathetic nervous system activity is subject to a number of limitations. This index takes no account of norepinephrine clearance and gives no indication of specific changes in regional sympathetic traffic. Nevertheless, our findings invite further studies to clarify the interaction between ANF and the sympathetic nervous system.

Blood pressure and heart rate were unchanged (Figure 3). This stands in contrast to the modest but significant fall in systolic pressure seen in our normotensive group. Blood pressure is consistently reduced by high doses of ANF. Intermediate and low dose infusion studies have yielded variable results, often with no effect on blood pressure. We used a continuous direct method of blood pressure recording, and computer analysis allowed recall and use of complete pressure data. Thus, we were well-equipped to detect even subtle systematic changes in blood pressure. It seems clear that the threshold at which increases in plasma ANF induce falls in blood pressure is higher than that for enhancement of natriuresis and suppression of the renin-angiotensin-aldosterone system, at least in hypertensive patients. In addition to our current findings, we observed sustained, though minor, lowering of blood pressure in normotensive, but not hypertensive, subjects after bolus injections of ANF. Weidmann et al have commented that, although the decrement in blood pressure in response to most known hypotensive agents is proportional to baseline arterial pressure, ANF given in a high dose, 45-minute infusion caused only minimally greater falls in blood pressure in severely hypertensive patients than in normotensive subjects. Hence, the possibility remains that essential hypertension may be characterized by a relative resistance to the depressor action of ANF. This may be of some pathogenetic significance in hypertension. Extrapolation from the current results is limited by the experimental conditions. It may well be that infusions of greater duration or even of slightly greater doses of ANF may have important effects on arterial pressure in human hypertension. In the only other relatively low dose ANF study in hypertension, ANF, given for 2 hours at 1 pmol/kg/min and then for a further 2 hours at 2 pmol/kg/min, resulted in a substantial and highly statistically significant fall in systolic pressure of 20 mm Hg. ANF, administered for several days in spontaneously hypertensive rats and two-kidney, one clip hypertensive rats, may be reflected in a lowering of plasma ANF concentrations, caused major falls in blood pressure (over days) to near normotensive values. Further controlled studies will be required to document the effect and the therapeutic potential of truly sustained minor elevations in plasma ANF in essential and secondary forms of hypertension.

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