Atrial Natriuretic Factor in Normal Subjects and Heart Failure Patients

Plasma Levels and Renal, Hormonal, and Hemodynamic Responses to Peptide Infusion

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Abstract

We investigated atrial natriuretic factor (ANF) in humans, measuring plasma immunoreactive (ir) ANF (in femtomoles per milliliter), and renal, hormonal, and hemodynamic responses to ANF infusion, in normal subjects (NL) and congestive heart failure patients (CHF). Plasma irANF was 11±0.9 fmol/ml in NL and 71±9.9 in CHF ($P < 0.01$); the latter with twofold right ventricular increment ($P < 0.05$). In NL, ANF infusion of 0.10 μg/kg per min (40 pmol/kg per min) induced increases ($P < 0.05$) of absolute (from 160±23 to 725±198 μeq/min) and fractional (1–4%) sodium excretion, urine flow rate (from 10±1.6 to 20±2.6 ml/min), osmolar (from 3.2±0.6 to 6.8±1.2 ml/min) and free water (from 6.8±1.6 to 13.6±1.6 ml/min) clearances, and filtration fraction (from 20±1 to 26±2%). Plasma renin and aldosterone decreased 33% and 40%, respectively ($P < 0.01$). Systolic blood pressure fell (from 112±3 to 104±5 mmHg, $P < 0.05$) in seated NL; but in supine NL, the only hemodynamic response was decreased pulmonary wedge pressure (from 11±1 to 7±1 mmHg, $P < 0.05$). In CHF, ANF induced changes in aldosterone and pulmonary wedge pressure, cardiac index, and systemic vascular resistance (all $P < 0.05$); however, responses of renin and renal excretion were attenuated. ANF infusion increased hematocrit and serum protein concentration by 5–7% in NL ($P < 0.05$) but not in CHF.

Introduction

The physiologic significance of secretory granules present within mammalian atrial myocytes (1) was elucidated in 1981 by deBold and co-workers (2), who demonstrated that intravenous administration of atrial extracts to intact rats induces profound natriuresis and diuresis. Subsequently, several structurally related peptides, collectively known as atrial natriuretic factor (ANF),[1] have been isolated from rat and human atria, sequenced and synthesized, and shown to mimic the natriuretic and diuretic effect of atrial extracts (3–7). In addition, several other potentially important biological actions of ANF have been demonstrated in vitro and in intact animals, including relaxation of vascular smooth muscle (8, 9), complex renal hemodynamic effects (10, 11), inhibition of renin, aldosterone, and vasopressin release (11, 12), and blood pressure reduction (2, 11). Initial studies in human subjects have confirmed the natriuretic, diuretic, and blood pressure-reducing effects of ANF administration, using bolus or brief infusion techniques (13, 14). It has been postulated that abnormal ANF secretion or responsiveness may contribute to the edema-forming states such as congestive heart failure, where increased circulating immunoreactive ANF levels have been reported (14, 15). There are, however, no studies that have characterized the response to ANF infusion in patients with heart failure.

The present study was designed to address several issues regarding the potential physiologic role of ANF: (*a*) to determine the range of endogenous ANF concentration in normal subjects and patients with congestive heart failure, (*b*) to determine whether constant infusion of exogenous ANF induces sustained natriuresis and diuresis in normal subjects, (*c*) to identify potential renal, hormonal, and systemic hemodynamic mechanisms that may govern the natriuretic and diuretic response to ANF infusion, and (*d*) to determine whether the response to ANF administration is normal or abnormal in patients with heart failure.

Glossary

C_{H_2O}	free water clearance
CI	cardiac index
CosM	osmolar clearance
DBP	diastolic blood pressure
Fe_{Na}	fractional excretion of sodium
FF	filtration fraction
GFR	glomerular filtration rate
HR	heart rate
MAP	mean arterial pressure
PA	plasma aldosterone
PC	plasma cortisol
PAP	pulmonary artery pressure
PRA	plasma renin activity
PVR	pulmonary vascular resistance
PWP	pulmonary wedge pressure
RAP	right atrial pressure
SBP	systolic blood pressure
SVI	stroke volume index
SVR	systemic vascular resistance
U_KV	potassium excretion rate
$U_{Na}V$	sodium excretion rate
$\dot{V}$	urine flow rate

Methods

This study was performed under the auspices of a Notice of Claimed Investigator Exemption for a New Drug, from the U. S. Food and Drug Administration, sponsored by the authors (Drs. Cody and Atlas). All components of this study were approved by the Committee on Human Rights in Research of New York Hospital-Cornell University Medical

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1. *Abbreviations used in this paper:* ANF, atrial natriuretic factor; hANP, human atrial natriuretic peptide; ir, immunoreactive; PAH, para-aminohippurate; TFA, trifluoroacetic acid; for other abbreviations see Glossary.

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College. All individuals participating in this study were admitted to the Adult Unit of the Clinical Research Center of New York Hospital-Cornell University Medical College. The study consisted of three interrelated protocols (Fig. 1), each of which is described in detail below.

Preparation of ANF for human study. At the time these studies were initiated, the exact sequence of the circulating form(s) of ANF was uncertain, and a synthetic 25-residue peptide (mol wt 2,508), based on the sequence of rat auriculin B (16) and corresponding to amino acids 102–126 of the ANF precursor, was studied. Recently it has been shown that a 28-residue peptide (mol wt 3,100), termed cardionatrin I (17) or ANF 99–126, is the principal form of ANF in rat blood (18, 19). The peptide used in these studies corresponds to residues 4–28 of the latter peptide and differs from the corresponding human sequence by a single amino acid substitution (Ile for Met at position 12); however, the 25- and 28-residue peptides have very similar biological potency both in vitro and in vivo (5). A single 100-mg lot of rat auriculin B was used in these studies. This peptide was synthesized manually, using conventional techniques, and was purified by gel filtration, ion-exchange chromatography, and reverse-phase high performance liquid chromatography. The peptide was dissolved in sterile 0.9% saline at a concentration of 100 μg/ml. The solution was resterilized by passage through a 0.22-μm filter (Millipore Corp., Bedford, MA) and dispensed in 0.7-ml and 5.0-ml aliquots into sealed sterile vials. At the time of dispensing, randomly selected vials were submitted for sterility and pyrogen testing. Prior to labeling of vials, the actual peptide content of the dispensed solution was determined by direct radioimmunoassay in quadruplicate. This was done in order to account for possible adsorption of peptide to the Millipore filter or to the walls of the vials. The results of this quantitation indicated that the vials contained ANF in a concentration of 81 μg/ml. All vials were stored frozen at −40°C from the time of dispensing until the time of preparation for administration, as we have found that this peptide is stable under these conditions. Vials were thawed prior to each individual study, and diluted in 0.9% sodium chloride to enable administration at a constant intravenous infusion of either 0.01, 0.03, or 0.10 μg/kg per min for a duration of 60 min. The concentration of peptide at the greatest concentration used was verified by radioimmunoassay of samples from the infusion line.

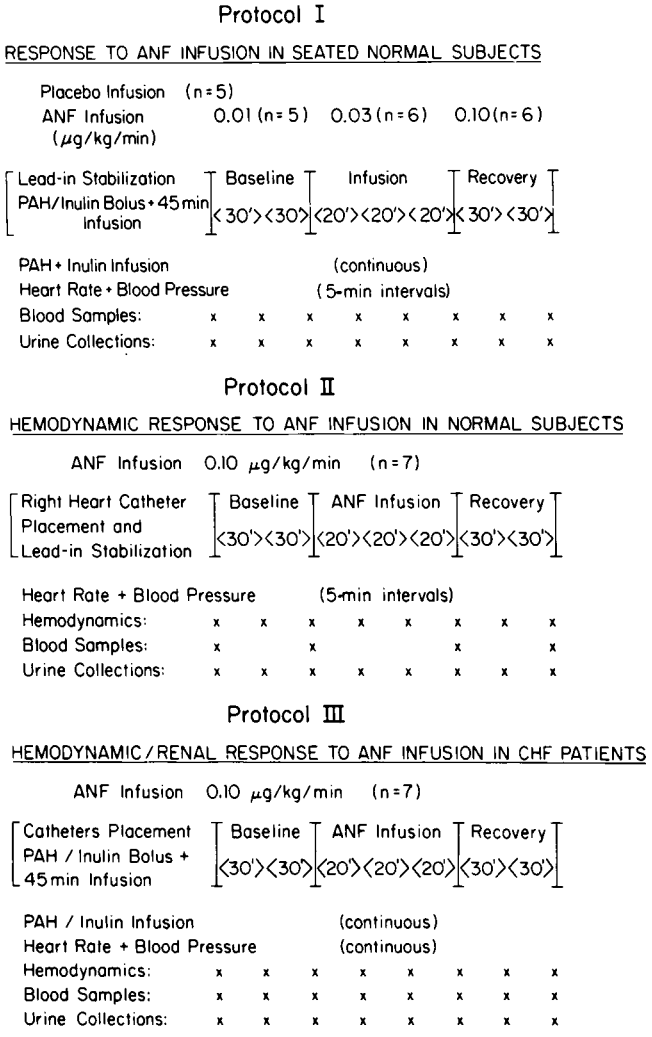

Figure 1. Schematic representation of the protocol design for normal subjects and patients with chronic congestive heart failure who comprised this study.

Renal and hormonal response to ANF infusion in normal subjects (protocol I). Renal, metabolic, and hormonal responses to the different doses of ANF infusion were studied in 22 normal male subjects (protocol I; Fig. 1). Subjects were considered normal on the basis of physical examination, biochemical screening, and electrocardiogram. They ranged from 19 to 46 yr of age, were taking no medications, and ingested their usual diet prior to admission. All subjects were studied in the morning, after overnight fast, in the seated position. Intravenous catheters were placed bilaterally, for administration of ANF and blood sampling, respectively. Urine samples were obtained by free voiding at timed intervals. After a stabilization period of 30–60 min after intravenous catheter placement, all individuals received an oral water load of 400 ml. Renal plasma flow and glomerular filtration rate were determined using the steady-state clearances of para-aminohippurate (PAH) and inulin respectively. PAH and inulin were given as a priming bolus, followed by a continuous infusion for 45 min to achieve steady-state plasma concentrations of 2.5 mg/dl (PAH) and 25 mg/dl (inulin) (20). After completion of a 45-min equilibration period, there were three 60-min phases of study: baseline, experimental infusion, and recovery. The baseline phase consisted of two 30-min urine collection periods, the experimental infusion phase consisted of three 20-min urine collection periods, and the recovery phase consisted of two 30-min urine collection periods. Urine output was replaced on a milliliter per milliliter basis throughout the study by administering oral water. The 22 normal subjects were divided into four groups. One group (n = 5) received a placebo infusion of 0.9% sodium chloride during the experimental infusion phase. The three remaining groups received ANF by continuous infusion at rates of 0.01 (n = 5), 0.03 (n = 6), or 0.10 (n = 6) μg/kg per min (4, 12, and 40 pmol/kg per min, respectively) during the experimental infusion phase. All infusions were given by a constant-rate infusion pump, with the final concentration of ANF adjusted for patient body weight. The volume administered per hour during the experimental infusion phase was 12.5 ml (placebo), 5 ml (0.01 μg/kg per min), 7.1 ml (0.03 μg/kg per min), and 12.5 ml (0.10 μg/kg per min). Equal volumes of 0.9% sodium chloride were given during the baseline and recovery phase for each individual. Heart rate and cuff blood pressure were recorded at 5-min intervals throughout the study. Upon completion of lead-in stabilization and each of the urine collection periods, blood samples were obtained for PAH and inulin concentrations, osmolality, plasma renin activity, aldosterone, and cortisol. Serum electrolytes, hematocrit, and serum protein (albumin and total) were obtained upon completion of lead-in stabilization at the end of each 60-min phase of the protocol. Blood samples were obtained at the end of the experimental infusion phase for plasma immunoreactive (ir)ANF. Urine collections were obtained at time 0, and at the end of each collection period, for volume, PAH and inulin concentrations, sodium, potassium, and osmolality. After the study, intravenous catheters were removed, and all individuals were observed overnight on the Clinical Research Center.

Hemodynamic response to ANF infusion in normal subjects (protocol II). To determine whether the renal effects of ANF administration were associated with changes in cardiac output or vascular tone, we evaluated the hemodynamic responses to ANF infusion in another group of seven healthy normal subjects, ranging from 19 to 52 yr of age (protocol II; Fig. 1). All subjects were studied in the morning, after overnight fast, in the supine position. Under local anesthesia, a balloon-tipped, flow-directed catheter was placed percutaneously from a basilic vein in the arm

to a final position in a branch of the pulmonary artery, confirmed by fluoroscopy. Cuff blood pressure and electrocardiographic heart rate were obtained at 5-min intervals. After catheter placement, there was a 2-h lead-in stabilization phase. This was followed by three phases that were identical in duration to those of protocol I. All subjects received a placebo infusion of 0.9% sodium chloride vehicle during the baseline and recovery phases, and ANF infusion at a rate of 0.10 μg/kg per min during the experimental phase. This infusion rate was chosen because it produced the most uniform effect on sodium and water excretion in protocol I. Recordings and urine collections were obtained upon completion of lead-in stabilization and at the end of each urine collection period. We recorded heart rate (HR, beats per minute), systolic (SBP) and diastolic (DBP) blood pressure (millimeters of mercury [mmHg]), right atrial pressure (RAP, mmHg), pulmonary artery pressure (PAP, mmHg), and pulmonary wedge pressure (PWP, mmHg). Mean arterial pressure (MAP, mmHg) was estimated from the DBP, plus one third of the difference between SBP and DBP. RAP, PAP, and PWP were obtained on a strip-chart recorder as both phasic and electronically dampened mean pressures, but for the purposes of this study, only the mean pressures are reported. Cardiac output was determined by the thermal dilution technique, in triplicate, using 10 ml of iced dextrose for each determination. Cardiac index (CI) was derived by correcting cardiac output for body surface area (liters per minute per square meter [liters/min per m^2]). Stroke volume index (SVI, ml/m^2) was obtained by dividing CI by the HR. Systemic (SVR) and pulmonary (PVR) vascular resistance were calculated using standard formulas (21) and expressed as dynes per second per centimeter^{-5} (dyn · s · cm^{-5}). Blood samples were obtained upon completion of each phase for hematocrit, serum electrolytes, osmolality, and protein. In addition, blood for plasma irANF concentration was obtained during the baseline phase (peripheral vein and right ventricle) and at the end of the ANF experimental infusion phase (peripheral vein). Timed, free-voiding urine collections were also obtained for volume, sodium, potassium, and osmolality. After completion of the study, the right heart catheter and intravenous infusion catheter were removed, and subjects were observed overnight on the Clinical Research Center.

Hemodynamic and renal response to ANF infusion in patients with congestive heart failure (protocol III). We then studied the hemodynamic and renal response to ANF infusion in a group of patients with chronic congestive heart failure (protocol III, Fig. 1). There were seven patients (six men, one woman) who ranged from 40 to 69 yr of age. This was a group of consecutive patients, drawn from a large population of chronic heart failure patients who are referred to the authors (Drs. Cody and Kubo). Subjectively, all patients had a history of heart failure, including one or more of the following symptoms: dyspnea on exertion, paroxysmal nocturnal dyspnea, ankle swelling, or effort related fatigue. Objectively, chronic left ventricular dysfunction and dilatation were documented by cardiac catheterization, echocardiography, or radionuclide cineangiography. Patients with valvular heart disease, myocardial infarction within the preceding 6 mo, or recent acute decompensation were excluded. All heart failure patients were admitted to the Clinical Research Center for a period of 2–7 d prior to ANF infusion, receiving their maintenance dose of digoxin on an evening schedule, so that no patients received digoxin on the morning of study. All diuretics and vasodilator therapy were discontinued prior to admission, and patients were placed on metabolic sodium balance diet; six received 100 meq sodium intake, and one received 10 meq sodium intake (patient 6 in Tables II, III, and IV). All patients were studied in the morning in the supine position, after overnight fast. A balloon-tipped flow directed catheter was passed percutaneously from either a basilic vein, or an internal jugular vein, to a branch of the pulmonary artery. A cannula was also placed in the brachial artery of all patients. Catheters were placed using local anesthesia, and no premedication was administered. Inasmuch as patients with congestive heart failure typically have very low urine flow rates, a urethral catheter was passed into the bladder of all patients. After catheter placement and a 1-h stabilization period, a 400-ml water load (5% dextrose) was administered intravenously. PAH and inulin were then administered in a manner identical to protocol I. The three phases of this protocol and timing of urine collections were identical to protocol I, except for omission of recovery-phase recordings in four of seven patients. The experimental infusion phase consisted of administration of ANF at a rate of 0.10 μg/kg per min for 60 min. Hemodynamic parameters were obtained as in protocol II, except that heart rate and blood pressure were measured continuously, with the latter obtained by direct arterial recording. Blood samples were obtained as outlined in protocol I, together with samples for plasma irANF concentration in the baseline state, (peripheral vein and right ventricle). Timed urine collections were also obtained for volume, PAH and inulin concentration, sodium, potassium, and osmolality. All seven patients completed and tolerated all phases of the protocol. One additional heart failure patient was initiated into this study; however, at the onset of the experimental infusion phase, he developed symptoms compatible with a vagal reaction. The ANF infusion was discontinued, the patient received intravenous administration of 0.9% sodium chloride, and he recovered in the ensuing hour, without subsequent adverse effects. All heart failure patients were returned to the Clinical Research Center on completion of this protocol.

Measurement of renal excretory and hemodynamic parameters. The urine volume was precisely measured using graduated cylinders. Urine sodium and potassium concentration (milliequivalents per liter) were determined by flame photometry, and urine osmolality (milliosmoles per liter) by freezing point depression. Urine volume was expressed as the urine flow rate ($\dot{V}$, milliliters per minute) for each collection period, as well as net change in urine flow (milliliters per 60 min) representing the urine volume of the experimental infusion phase minus that of the baseline phase. Osmolar clearance (CosM) and free water clearance (C_{H_2O}) were determined for each collection period, expressed as milliliters per minute. Sodium and potassium excretion rates ($U_{Na}V$, U_KV; microequivalents per minute), were calculated for each timed collection period and the net change in $U_{Na}V$ (microequivalents per 60 min) was calculated as the difference in urinary sodium excretion between the experimental infusion phase and the baseline phase. Fractional excretion of sodium (Fe_{Na}, percent) was determined at the completion of each of the baseline, ANF infusion, and recovery phases. PAH and inulin were analyzed by spectrophotometric techniques (20). PAH was analyzed by conjugation to *N*-naphthyl ethylenediamine, quantitated by absorbance at 540 nm. Inulin was analyzed by the acid resorcinol method, quantitated by absorbance at 490 nm. Renal blood flow (RBF, milliliters per minute) was calculated by dividing PAH clearance (i.e., effective renal plasma flow) by the term (1-hematocrit). Filtration fraction (FF, percent) was calculated as the ratio of inulin clearance (i.e., glomerular filtration rate, GFR) to PAH clearance.

Blood biochemical determinations and hormonal assays. Serum sodium, potassium, chloride, and bicarbonate (millimoles per liter), creatinine (milligrams per deciliter), and osmolality (milliosmoles per liter), as well as blood urea nitrogen (milligrams per deciliter), hematocrit (volume percent), serum albumin (grams per deciliter), and total protein (grams per deciliter) were determined by standard analytical techniques. Plasma renin activity (PRA) was determined by radioimmunoassay of generated angiotensin I, expressed as nanograms per milliliter per hour (22). Plasma aldosterone (PA, nanograms per deciliter), and plasma cortisol (PC, micrograms per deciliter) were also determined by radioimmunoassay as previously described (11, 23).

Radioimmunoassay of endogenous plasma irANF concentration. The range of endogenous plasma irANF concentration was determined in peripheral venous blood from 70 normal volunteers derived from a previously characterized defined population (24), who were studied in the seated position while on a regular diet; and 31 patients with congestive heart failure on standard digoxin and diuretic therapy. In seven supine normal subjects undergoing hemodynamic study (protocol II), simultaneous blood samples were obtained from a peripheral vein and from the right ventricle. Simultaneous peripheral vein and right ventricular samples were also obtained from the seven congestive heart failure patients in protocol III, and from 10 additional untreated congestive heart failure patients who did not receive the ANF infusion. All simultaneous blood samples for irANF were obtained at the end of the baseline phase, in the supine position. All blood samples were collected in prechilled Vacutainers (Becton, Dickinson & Co., Rutherford, NJ) containing potassium

EDTA which were then placed on ice; plasma was obtained by centrifugation at 4°C and was stored at −40°C until assayed.

Plasma irANF was measured by radioimmunoassay after extraction on C_{18} Sep-Pak cartridges (Waters Associates, Milford, MA), as recently described (25). Plasma samples (3 ml) were applied to individual prewashed cartridges, which were then washed with H_2O (3 ml), 0.1% trifluoroacetic acid (TFA, 3 ml) and 10% acetonitrile in 0.1% TFA (3 ml). Immunoreactive ANF (irANF) was then eluted with 80% acetonitrile in 0.1% TFA (two washes, 3 ml each), and the pooled extract was dried overnight in a Speed-Vac evaporator (Savant Instruments, Inc., Hicksville, NY). Recoveries of ANF standard and of radiolabeled ANF (see below) added to plasma were 74±9% (SE) and 78±7%, respectively (range 58–84%); recoveries in individual samples were determined by addition of ^{125}I-ANF (1,000 cpm) to the plasma prior to extraction.

Of the two radioimmunoassay procedures described previously (25), only the homologous assay was used for data reported in this study. This assay employed antiserum raised against human ANF 99–126 or alpha-human atrial natriuretic peptide (α-hANP) (Peninsula Laboratories, Inc., Belmont, CA), at a final dilution of 1:27,000, and α-hANP as standard and tracer. The homologous assay was also used to estimate plasma irANF levels during infusion of exogenous rat auriculin B, because the latter peptide has 100% crossreactivity with the antiserum to α-hANP. Plasma extracts were reconstituted in 250 μl of assay buffer (0.1 M sodium phosphate, pH 7.5, containing 0.3% bovine serum albumin (BSA) and 0.1% Triton X-100); duplicate 100-μl aliquots of unknowns or standard were incubated with diluted antiserum (100 μl) for 24 h at 4°C. Tracer (10,000 cpm in 100 μl of assay buffer) was then added and the reaction continued for 18 h at 4°C. Instead of the polyethylene glycol precipitation method used previously (25), bound and free peptide was separated by addition of 1 ml of ice-cold dextran-coated charcoal (1.5% charcoal/0.15% dextran T-70 in assay buffer); after centrifugation, both the pellet and supernatant were counted in a gamma counter. Results were calculated from standard curves of bound/free counts per minute vs. log ANF standard and were corrected for internal recovery. The sensitivity of the assay was 0.5 fmol per tube with an IC_{50} of 8.1±0.4 fmol. All values are expressed as femtomoles per milliliter. Preliminary studies show that the principal form of irANF measured by this assay in human plasma has a retention time on reverse-phase high-performance liquid chromatography identical to that of the 28-residue α-hANP (25).

Statistical analysis. Analysis of variance for repeated measures, with subsequent Dunnett *t* test, was used to determine the significance of changes during time-dependent multiple observations (26). One-way analysis of variance, with Bonferroni adjustment of the *P* value, was used for simultaneous comparisons of multiple groups. Paired or unpaired *t* tests were used to test the significance of single comparisons. All values are expressed as mean±standard error of the mean. Changes were considered significant, at $P < 0.05$.

Results

Endogeneous plasma irANF concentration. In seated normal subjects, plasma irANF concentration was 11±0.9 fmol/ml (range 1.1–27 fmol/ml). Plasma irANF was significantly higher (71±9.9 fmol/ml, range 2.1–236 fmol/ml) in 31 patients with congestive heart failure ($P < 0.01$, Fig. 2 *a*). In seven supine normal subjects, simultaneous samples for irANF averaged 15±2.8 and 19±5.9 fmol/ml, respectively, in peripheral venous and right ventricular plasma (Fig. 2 *b*). A right ventricular increment was not observed in one of the seven subjects, so that this difference did not achieve statistical significance. In 17 congestive heart failure patients, simultaneous samples for irANF concentration averaged 79±15 fmol/ml in peripheral venous plasma and 156±27 fmol/ml in right ventricular plasma (difference $P < 0.01$, Fig. 2 *b*).

Responses to ANF infusion in normal subjects (protocol I). In normal subjects, the greatest magnitude and consistency of response to ANF occurred with infusion of 0.10 μg/kg per min (40 pmol/kg per min). The renal responses to this infusion rate, compared with those of individuals receiving placebo infusion are shown in Fig. 3. Throughout the study, individuals receiving placebo demonstrated no significant change in renal excretory function or renal hemodynamic parameters. ANF infusion induced significant increases in $U_{Na}V$, $\dot{V}$, and both CosM and C_{H_2O}. A prompt onset of these responses was noted within the first collection period (E_1), with steady-state responses achieved during the remainder of the infusion phase (E_2, E_3). Comparing baseline (B_2) values with the steady-state response of the infusion phase (E_3), $U_{Na}V$ increased from 160±23 to 725±198 μeq/min ($P < 0.05$), with an increase of FE_{Na} from 1 to 4% ($P < 0.05$). $\dot{V}$ increased from 10±1.6 to 20±2.6 ml/min ($P < 0.05$), with

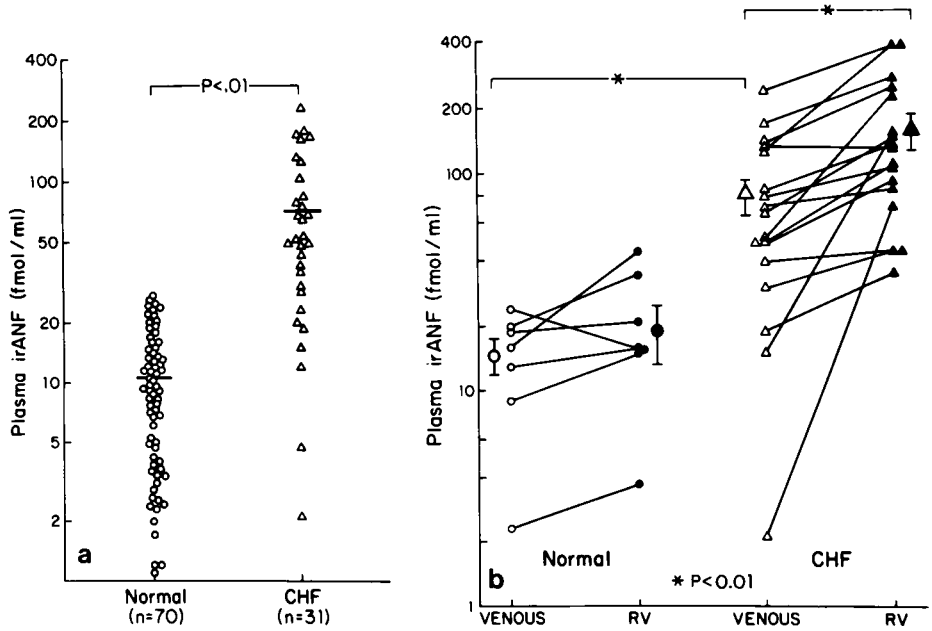

Figure 2. Plasma immunoreactive ANF (irANF) in normal subjects and patients with congestive heart failure (*CHF*). (*a*) Plasma irANF in normal subjects (○), and in patients with chronic congestive heart failure (△). (*b*) Plasma irANF simultaneously sampled from a peripheral vein and the right ventricle (*RV*) in normal subjects studied in protocol II (*circles*) and in chronic congestive heart failure patients studied in protocol III ($n = 7$) as well as 10 additional patients undergoing right heart catheterization under similar conditions (*triangles*). There was a significant step-up of irANF concentration at the right ventricle level in heart failure patients.

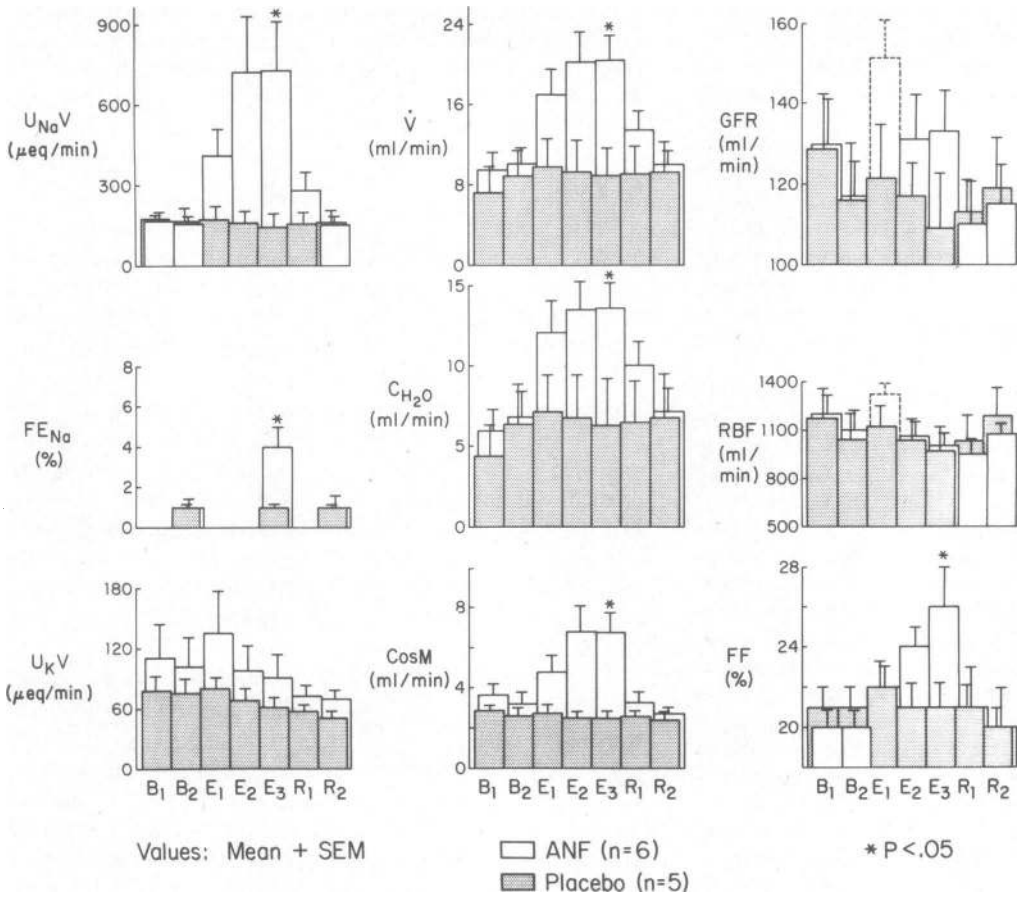

Figure 3. Renal excretory and hemodynamic response to ANF infusion in normal subjects. The stippled bars represent individuals receiving the placebo infusion, and the open bars represent individuals receiving ANF infusion at a rate of 0.10 μg/kg per min. Baseline phase (B_1, B_2), experimental infusion phase (E_1, E_2, E_3) and recovery (R_1, R_2) phase were each of 60 min duration. There was a rapid onset of ANF effect, with steady-state responses achieved during E_2 and E_3. Significant changes (*) in sodium and water excretion, as well as filtration fraction were observed during ANF administration. After discontinuation of ANF infusion, these parameters returned to basal values, indistinguishable from the placebo infusion values.

significant increases of both CosM from 3.2±0.6 to 6.8±1.2 ml/min, and C_{H_2O} from 6.8±1.6 to 13.6±1.6 ml/min (both $P < 0.05$). The rise in C_{H_2O} was proportional to the rise in V—i.e., when both terms were factored for GFR, there was a linear relationship between C_{H_2O} and $\dot{V}$ which was unaltered by ANF infusion (not shown). Changes in U_KV were not significant. In the first (non–steady-state) collection of the experimental phase, there was an initial large increase in inulin clearance which, owing to the abrupt rise in urine flow rate, may not be indicative of an increase in GFR alone (shown by the *dashed open bar*). Compared with the baseline value of 117±15 ml/min, the steady-state increase of GFR to 133±10 ml/min by the end of the ANF infusion did not achieve statistical significance. However, GFR did decrease to basal levels during the recovery phase. The decrease of RBF during the steady-state ANF infusion (1,100±125 to 998±77 ml/min) was not significant. The tendency for increased GFR, while RBF decreased, resulted in a significant increase of FF from 20±1 to 26±2% ($P < 0.05$). With discontinuation of the ANF infusion, there was a prompt reversal of the renal responses to ANF, with all significant changes returning to basal values, indistinguishable from the placebo group, by the end of the recovery phase.

As shown in Table I, baseline urine osmolality was reduced to 99±23 mosmol/kg by the water load. Also shown, there were no significant changes of serum electrolyte concentration, creatinine, BUN, serum or urine osmolality during ANF infusion.

The hormonal responses to ANF infusion (0.1 μg/kg per min) are shown in Fig. 4. Values are expressed as a percent of baseline (time 0) in order to compare directly the responses in the placebo and ANF infusion groups. Baseline plasma renin activity was 1.9±0.7 ng/ml per h for the placebo group, and 2.9±0.4 ng/ml per h for the ANF infusion group. By the end of ANF infusion, plasma renin activity decreased to 67% of baseline (i.e., from 2.9±0.4 to 1.9±0.2 ng/ml per h, $P < 0.02$). At the end of the recovery phase, plasma renin activity was not significantly different from the preinfusion baseline or the placebo group value. Plasma aldosterone demonstrated a similar response. Baseline plasma aldosterone was 5.4±0.8 ng/dl for the placebo group and 7.2±1.8 ng/dl for the ANF group. During the ANF infusion, plasma aldosterone decreased to 60% of baseline, i.e., from 7.2±1.8 to 4.4±1.3 ng/dl ($P < 0.05$). After discontinuation of ANF infusion, there was no significant difference

Table I. Serum Electrolyte and Metabolic Response to ANF: Normal Subjects (Protocol I)

	Baseline	ANF*	Recovery
Serum			
Sodium	138±1.3	139±0.9	136±0.9
Potassium	4.2±0.2	4.2±0.1	4.4±0.1
Chloride	103±1.1	102±1.3	103±0.9
Bicarbonate	25±1	27±1	27±0.8
Blood urea nitrogen	13±2	12±2	12±1
Serum creatinine	0.9±0.09	1.0±0.1	0.9±0.1
Serum osmolality	282±2	283±2	279±2
Urine osmolality	99±23	92±6	86±17

* Infusion rate: 0.10 μg/kg per min.

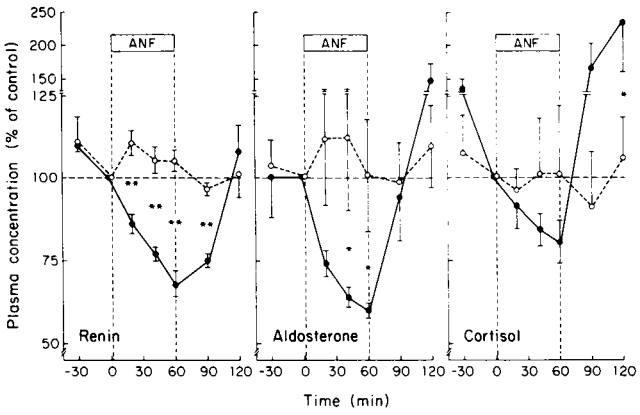

Figure 4. Hormonal responses to ANF infusion in normal subjects. Changes in plasma renin activity, plasma aldosterone, and plasma cortisol are compared in the placebo infusion group (○) and in subjects who received ANF at a rate of 0.10 μg/kg per min (●). Values are expressed as percent of the baseline value at time 0, which represents completion of the second 30-min baseline collection period (B_2). * $P < 0.05$; ** $P < 0.02$. Absolute values are provided in the text.

in plasma aldosterone levels for the two groups. There was however, an apparent overshoot of plasma aldosterone by the end of the recovery phase. Baseline plasma cortisol concentrations were 11±3 μg/dl (placebo group) and 8.5±1.8 μg/dl (ANF infusion group). During the ANF infusion, plasma cortisol tended to decrease to a value that was 80% of baseline (absolute value, 6.8±1.2 μg/dl). After discontinuation of ANF infusion, there was a rebound increase ($P < 0.05$) of plasma cortisol to a value of 200% compared with baseline (absolute value, 14.6±1.8 μg/dl).

Less consistent responses were noted with lower rates of ANF infusion, i.e., 0.01 and 0.03 μg/kg per min (4 and 12 pmol/kg per min, respectively). Fig. 5 summarizes the steady-state values of renal parameters and PRA for individuals in the placebo infusion group, and the three ANF infusion groups. Each parameter ($\dot{V}$, $U_{Na}V$, FF, and PRA) changed consistently and reversibly in subjects receiving the highest infusion rate (0.10 μg/kg per min) whereas none of these parameters was altered during placebo administration. The mean changes induced by infusion of 0.03 or 0.01 μg/kg per min were not significant. Reversible increases in $\dot{V}$, $U_{Na}V$, and FF and reversible decreases in PRA were, however, noted in at least three of six subjects receiving the 0.03 μg/kg per min and in one subject receiving 0.01 μg/kg per min. Decreases in PA were also observed in these subjects (not shown). Thus the threshold response to exogenous ANF infusion occurs at a rate of 0.03 μg/kg per min (12 pmol/kg per min) or less in normal subjects. The plasma irANF concentrations (fmol/ml) achieved at the end of the infusion for the four groups were: 19±3 (placebo), 49±11 (0.01 infusion), 238±70 (0.03 infusion), and 868±54 (0.10 infusion).

The responses of HR and blood pressure for the four groups of seated normal subjects are summarized in Fig. 6. A significant change in SBP was observed during the ANF infusion of 0.10 μg/kg per min, where the decrease to 104±5 at the end of the infusion, and continued reduction to 102±5 by the end of recovery, was significant ($P < 0.05$). There was no significant change in DBP for any of the groups throughout the study.

Hemodynamic responses to ANF infusion in normal subjects (protocol II). Inasmuch as the ANF infusion of 0.10 μg/kg per min resulted in consistent renal and metabolic changes, we investigated the hemodynamic characteristics of the response to this rate of ANF infusion in supine normal subjects (Fig. 7 *a*, and Table II). In response to ANF infusion, the only significant hemodynamic alteration was a reduction of PWP from 11±1 to 7±1 mmHg ($P < 0.05$), which persisted throughout the first stage of recovery. There was no significant change in arterial pressure, CI, or systemic vascular resistance. ANF infusion in these individuals was associated with a peak plasma ANF concentration of 710±118 fmol/ml, and an increase of $\dot{V}$ (from 6.4±0.7 to 13.8±2.0 ml/min, $P < 0.05$) and $U_{Na}V$ (from 217±69 to 504±193 μeq/min, $P < 0.05$). These responses were comparable, although somewhat less, to the changes elicited by this dose in seated subjects.

Hemodynamic and renal responses to ANF infusion in patients with congestive heart failure (protocol III). Based on the observed changes in normal subjects, ANF was administered to seven patients with chronic congestive heart failure at an infusion rate of 0.10 μg/kg per min, and complete hemodynamic, renal, and metabolic characterization was obtained. The hemodynamic responses to ANF administration are summarized in Fig. 7 *b* and Table II. Compared with baseline values, the following significant hemodynamic changes were observed: PWP decreased from 31±4 to 25±5 mmHg ($P < 0.05$); CI increased from 1.77±0.06 to 2.07±0.13 liters/min per m^2 ($P < 0.05$); and SVR decreased from 1,831±208 to 1,597±243 dyn·s·cm^{-5} ($P < 0.05$). Significant changes in blood pressure were not observed. The baseline renal functional parameters of heart failure patients were markedly abnormal compared with those of normal individuals (Table III). Baseline values were: $U_{Na}V$ 31±22 μeq/

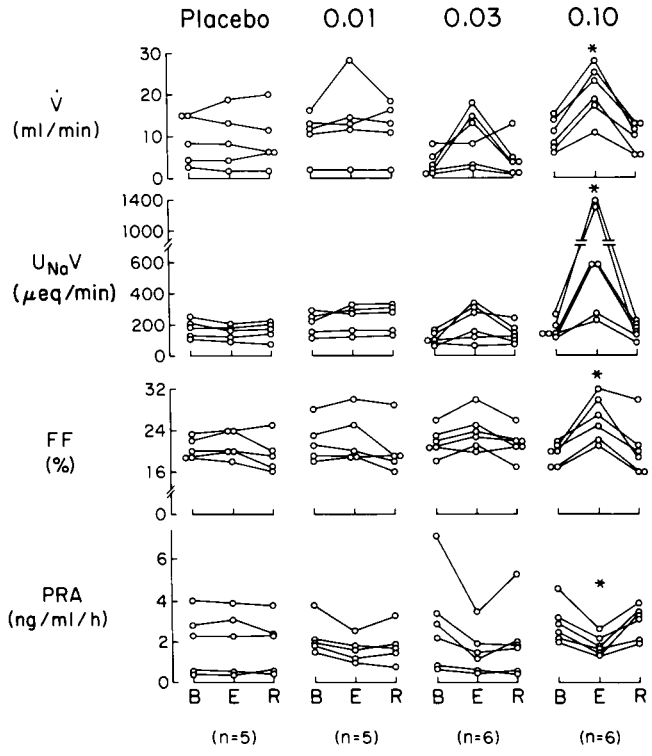

Figure 5. The response of urine flow rate ($\dot{V}$), sodium excretion rate ($U_{Na}V$), filtration fraction (FF), and plasma renin activity (PRA) for all normal subjects in the dose titration study (protocol I). Values obtained at the completion of the baseline (*B*), experimental infusion (*E*), and recovery (*R*) phases of this study are presented. * $P < 0.05$.

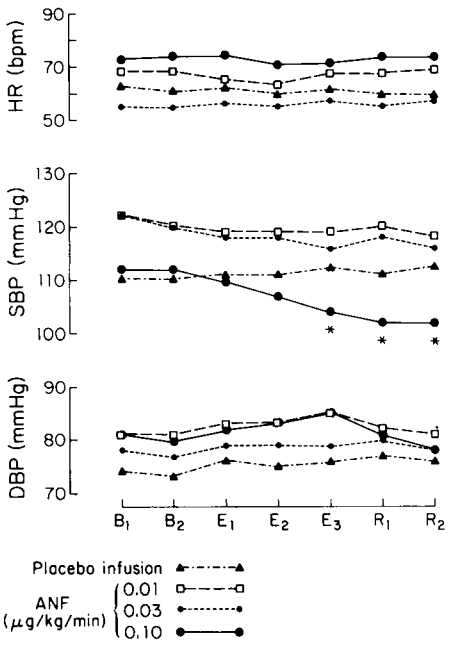

Figure 6. Mean values of heart rate (HR), systolic blood pressure (SBP), and diastolic blood pressure (DBP) for all seated normal subjects in protocol I. Administration of ANF at a rate of 0.10 μg/kg/min during the experimental phase (E_1–E_3) resulted in a significant reduction of SBP that persisted into the recovery phase (R_1 and R_2). * $P < 0.05$ compared with baseline.

min, Fe_{Na} 0.39±0.30%, U_KV 66±11 μeq/min, $\dot{V}$ 1.67±0.38 ml/min, C_{H_2O} −3.7±0.22 ml/min, and CosM 2.03±0.25 ml/min. GFR was 93±13 ml/min, RBF was 542±104 ml/min, and FF was elevated to 33±4%. Within the group of heart failure patients, there was considerable variability in the baseline status, and in the renal response to ANF infusion (Table III). The responses of $\dot{V}$ and $U_{Na}V$ were, on average, minimal and not significant. However, one patient (no. 4) had a modest diuresis and natriuresis. In response to ANF administration, PRA decreased from 8.6±5.2 to 6.1±3.4 ng/ml per h, PA decreased from 22.3±7.6 to 10.9±3.1 ng/dl, and PC decreased from 17.3±2.4 to 13.3±1.8 μg/dl. These changes were not significant by paired *t* test, although nonparametric analysis of the 50% reduction of plasma aldosterone was significant (Wilcoxon, $P < 0.05$). There was no significant change in serum electrolytes, creatinine, BUN, or serum or urine osmolality (not shown).

Comparison of net changes in renal excretory function. Fig. 8 summarizes the net change in $\dot{V}$ and $U_{Na}V$ during the experimental phase for all seated normal subjects and heart failure patients studied. In normal subjects, the renal excretory responses to ANF infusion were dependent on the dose of ANF administered. For individuals receiving the placebo infusion, the net change in $\dot{V}$ was 90±41 ml/60 min and in $U_{Na}V$ was −644±429 μeq/60 min. Infusion of ANF at a rate of 0.01 μg/kg per min resulted in an increase of $\dot{V}$ of 159±92 ml/60 min and $U_{Na}V$ of 540±1,325 μeq/60 min. Placebo and 0.01 responses were not significant compared with baseline. Infusion of ANF at a rate of 0.03 μg/kg per min resulted in a net increase of $\dot{V}$ of 291±101 ml/60 min, and $U_{Na}V$ of 6,362±2,511 μeq/60 min ($P < 0.05$ and <0.07, respectively). Infusion of ANF at a rate of 0.1 μg/kg per min increased net $\dot{V}$ by 572±90 ml/60 min and $U_{Na}V$ by 27,760±8,632 μeq/60 min (both $P < 0.01$). Similar increments were observed in the supine subjects studied under protocol II (249±82 ml/60 min and 12,639±4,063 μeq/60 min, both $P < 0.05$). In contrast, patients with congestive heart failure demonstrated minimal net changes in $\dot{V}$ (27±14 ml/60 min) and $U_{Na}V$ (1220±907 μeq/min) at the ANF infusion rate of 0.10 μg/kg per min. These responses were considerably less than those of normal subjects who received the 0.03 μg/kg per min infusion, and, except for one patient, were within the range observed with placebo infusion.

Effect of ANF infusion on the intravascular compartment. Table IV summarizes the responses of hematocrit and serum protein concentration during ANF infusion at 0.10 μg/kg per min. The table includes seated normal subjects (protocol I), su-

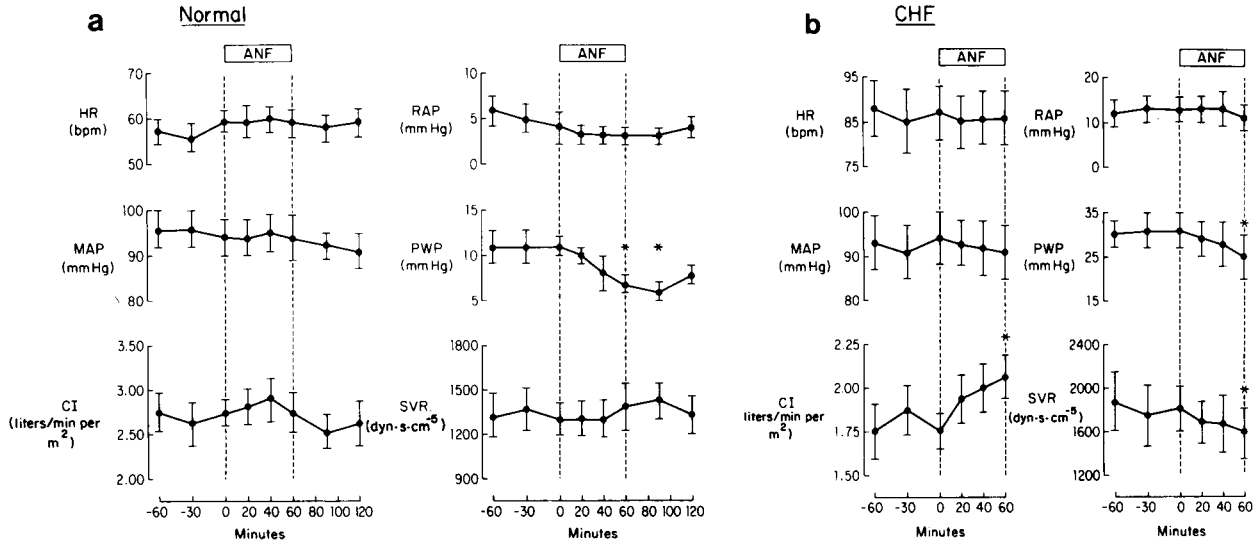

Figure 7. Hemodynamic responses to ANF infusion at a rate of 0.10 μg/kg per min. Onset of ANF infusion is indicated by time 0. (*a*) Hemodynamic responses in normal subjects; (*b*) hemodynamic responses in patients with congestive heart failure. For heart failure patients, only the baseline and ANF infusion phases are presented, because only three of the seven patients were formally monitored during the complete recovery period. Values are given as mean±SEM. * $P < 0.05$.

Table II. Peak Hemodynamic Response to ANF Infusion

Subject		HR	SBP	DBP	MAP	RAP	PAP	PWP	CI	SVI	SVR	PVR
		bpm	*mmHg*						*liters/min per m²*	*ml/m²*	*dyn·s·cm⁻⁵*	
Normal volunteers												
1	Base	63	116	70	85	1	11	6	2.65	42	1,246	73
	ANF	75	108	68	81	1	10	4	3.05	41	1,031	76
2	Base	58	126	88	101	8	18	14	2.74	47	1,274	55
	ANF	53	130	78	104	6	14	8	1.56	48	1,436	88
3	Base	63	130	96	107	10	22	15	2.55	40	1,593	115
	ANF	62	138	96	110	8	20	12	2.05	33	2,087	164
4	Base	65	100	64	76	2	18	10	3.28	50	839	91
	ANF	62	100	66	77	3	14	6	3.18	51	867	94
5	Base	57	128	84	99	8	17	11	2.13	37	1,754	116
	ANF	53	118	90	99	4	14	8	2.06	39	1,891	119
6	Base	62	114	82	93	1	13	8	2.83	46	1,244	67
	ANF	54	120	84	97	1	6	2	2.43	45	1,513	62
7	Base	45	130	76	94	1	13	10	2.98	66	1,162	37
	ANF	54	130	74	93	1	11	8	3.75	69	914	29
Base	$\bar{X}$	59	121	80	94	4	16	11	2.74	47	1,302	79
	SEM	3	4	4	4	2	2	1	0.14	4	112	11
ANF	$\bar{X}$	59	121	79	94	3	13	7*	2.73	47	1,391	90
	SEM	3	5	4	4	1	2	1	0.24	4	182	16
CHF patients												
1	Base	83	116	74	90	23	53	34	1.92	23	1,204	342
	ANF	78	115	70	84	20	46	30	2.24	29	988	247
2	Base	104	108	67	79	10	57	43	1.67	16	1,605	326
	ANF	109	110	68	79	9	55	42	1.84	17	1,481	275
3	Base	99	156	116	126	22	62	40	1.49	15	2,930	789
	ANF	96	168	116	126	23	58	38	1.48	15	2,922	567
4	Base	59	170	60	99	1	23	15	1.99	34	2,101	170
	ANF	60	155	56	88	1	14	4	1.99	33	1,872	213
5	Base	101	122	73	91	7	51	31	1.84	18	1,639	390
	ANF	100	140	72	89	5	47	20	2.49	25	1,211	389
6	Base	82	122	70	85	15	39	25	1.76	21	1,614	323
	ANF	80	124	68	83	12	37	21	2.22	28	1,300	275
7	Base	81	115	72	87	14	39	27	1.71	21	1,723	283
	ANF	77	110	66	87	10	31	21	2.21	29	1,406	183
Base	$\bar{X}$	87	130	76	94	13	46	31	1.77	21	1,831	351
	SEM	6	9	7	6	3	5	4	0.06	2	208	52
ANF	$\bar{X}$	86	132	74	91	11	41	25*	2.07*	25*	1,597*	310
	SEM	6	9	7	6	3	6	5	0.13	3	243	50

Infusion was at the rate of 0.10 μg/kg per min. * $P < 0.05$ base vs. ANF.

pine normal subjects (protocol II), and supine heart failure patients (protocol III). In normal subjects, administration of ANF resulted in a significant and reversible increase of hematocrit from 45.7±0.08 to 48.6±0.9 vol % (seated subjects), and 41.5±1.2 to 43.7±1.4 vol % (supine subjects), both $P < 0.05$ compared with their respective baseline values. Serum albumin (g/dl) also tended to increase in both groups although these changes were not statistically significant. Total protein (g/dl) increased reversibly from 6.8±0.1 to 7.3±0.3 in seated normal subjects, and from 6.7±0.2 to 7.1±0.2 in supine normal subjects (both $P < 0.05$ compared with respective baseline values). After discontinuation of ANF administration, there was a reduction of these values towards baseline. In the additional normal individuals who received ANF infusion of 0.01 or 0.03 μg/kg per min, hematocrit and serum protein concentration were not changed significantly. In contrast to the normal subjects, there was no significant change of hematocrit, albumin, or total protein during ANF administration in patients with congestive heart failure.

Discussion

These studies demonstrate that constant infusion of synthetic ANF in normal human subjects leads to sustained and fully reversible diuresis and natriuresis, associated under steady-state conditions, with increases in osmolar and free water clearance,

Table III. Renal and Hormonal Response to ANF: CHF Patients

Patient	Sodium intake		irANF	$U_{Na}V$	Fe_{Na}	U_KV	$\dot{V}$	C_{H_2O}	CosM	GFR	RBF	FF	PRA	PA	PC
	meq/24 h		*fmol/ml*	*μeq/min*	*%*	*μeq/min*	*ml/min*	*ml/min*	*ml/min*	*ml/min*	*ml/min*	*%*	*ng/ml per h*	*ng/dl*	*μg/dl*
1	100	BASE	84	1	0.01	91	0.75	−1.09	1.84	135	565	44	39	56	25
		ANF	—	2	0.01	101	0.95	−1.32	2.27	114	604	34	26	22	13
2	100	BASE	105	24	0.25	69	0.80	−0.85	1.65	72	376	31	1.5	36	26
		ANF	—	57	0.40	84	1.45	−1.59	3.04	108	649	26	1.5	11	20
3	100	BASE	132	2	0.03	30	0.53	−0.53	1.06	46	167	47	6.4	35	19
		ANF	—	5	0.09	27	0.65	−0.39	1.04	45	148	51	5.4	22	18
4	100	BASE	2.1	157	2.2	21	2.97	0.71	2.26	57	1,055	8	0.31	1.5	9.0
		ANF	—	286	3.4	31	5.20	2.17	3.03	64	844	12	0.39	1.3	6.1
5	100	BASE	48	27	0.17	80	2.15	−0.41	2.56	119	624	32	1.9	8.3	13
		ANF	—	22	0.17	57	1.85	0.01	1.84	97	450	37	1.6	5.4	11
6	10	BASE	67	4	0.03	101	2.80	−0.30	3.10	125	590	34	7.6	11	14
		ANF	—	4	0.04	59	2.20	0.39	1.81	87	374	37	6.0	8.1	14
7	100	BASE	48	3	0.02	68	1.67	−0.09	1.76	95	420	35	3.3	8.5	15
		ANF	—	39	0.24	110	3.15	0.87	2.28	119	623	30	2.1	6.7	11
Base		$\bar{X}$		31	0.39	66	1.67	−0.37	2.03	93	542	33	8.6	22.3	17.3
		SEM		22	0.30	11	0.38	0.22	0.25	13	104	4	5.2	7.6	2.4
ANF		$\bar{X}$		59	0.63	67	2.21	+0.48	2.19	91	527	32	6.1	10.9	13.3
		SEM		39	0.46	12	0.59	+0.26	0.27	10	85	5	3.4	3.1	1.8

renal hemodynamic alterations, suppression of plasma renin and aldosterone, reductions in systolic blood pressure and pulmonary wedge pressure, and evidence of hemoconcentration. With some notable exceptions, these findings are generally similar to those recently reported in normal subjects by Weidmann et al. (27), who evaluated responses to an infusion rate similar to the highest dose that we used. In the present study, the response to ANF in patients with congestive heart failure was considerably different, in that, as a group, there appears to be marked attenuation of the renal and hemoconcentrating responses to the peptide.

Our data define a broad range of immunoreactive ANF concentration in the plasma of normal subjects on random sodium intake and demonstrate a significant increase in circulating ANF levels in patients with congestive heart failure. The latter finding is consistent with earlier preliminary reports (14, 15). In our series, plasma irANF was, on average, sevenfold higher in heart failure, with 80% of the patients having frankly elevated levels. A subset of patients had levels in the normal range, but further study is needed to better define the determinants of the broad range of irANF levels in normal subjects as well as heart failure patients. The increased irANF concentration found in right ventricular compared with peripheral venous blood suggests that ANF is secreted by the heart, most likely via the coronary sinus (28). This gradient within the heart was statistically significant and of greater magnitude in the patients with congestive heart failure, indicating that hypersecretion of ANF is a major determinant of the increase in plasma ANF in heart failure. In view of the evidence that blood volume redistribution, or other maneuvers which acutely increase atrial stretch, provoke increases in ANF secretion (25, 29, 30), it seems probable that chronic increases in atrial wall tension contribute to the elevations in plasma ANF in patients with heart failure. This hypothesis is supported by the recent study of Bates and co-workers (31).

The finding that ANF infusion leads to significant reductions in plasma renin and aldosterone in seated normal subjects is consistent with earlier experiments showing that ANF decreases renin secretion rate (11, 32) and plasma renin and aldosterone (11) in intact animals and also inhibits aldosterone production in vitro (33–35). The failure to demonstrate suppression of renin and aldosterone in recent clinical studies (13, 14, 27) is probably attributable to the fact that measurements were performed under non-steady-state conditions and/or in supine subjects, in whom the renin-angiotensin-aldosterone axis is already relatively suppressed. It has been postulated that the ability of ANF to suppress renin secretion, whether or not direct effects on the juxtaglomerular cells are involved, may depend on an intact renal hemodynamic response to the peptide (36–38), and it has been shown that direct adrenal effects contribute to the aldosterone-lowering effect of ANF in vivo under certain circumstances (36–38). Our studies do not provide further definition of the mechanisms involved but are consistent with these observations. It is of interest, in this regard, that in heart failure patients, who were refractory to the renal effects of ANF, plasma renin levels were not significantly decreased, consistent with an inability to increase sodium delivery to the macula densa during ANF infusion. Nonetheless, plasma aldosterone fell, on average by 50%, a response which was probably of biological significance.

In contrast to results obtained in the intact dog (11), our data suggest that ANF infusion may tend to lower plasma cortisol in humans. ANF is known to inhibit steroidogenesis largely at the early steps of the biosynthetic pathway (34), and there is evidence of species differences in its specificity for inhibiting aldosterone production, in that ANF has been shown to also inhibit glucocorticoid production in bovine (35), but not rat (33), adrenal cells in vitro. More striking was our finding of a marked rebound in cortisol (and, to a lesser extent, aldosterone) after discontinuation of the ANF infusion. Further study is needed to define the mechanism of this effect.

In normal individuals, the sustained increases in sodium excretion and urine flow rate elicited by the highest rate of ANF

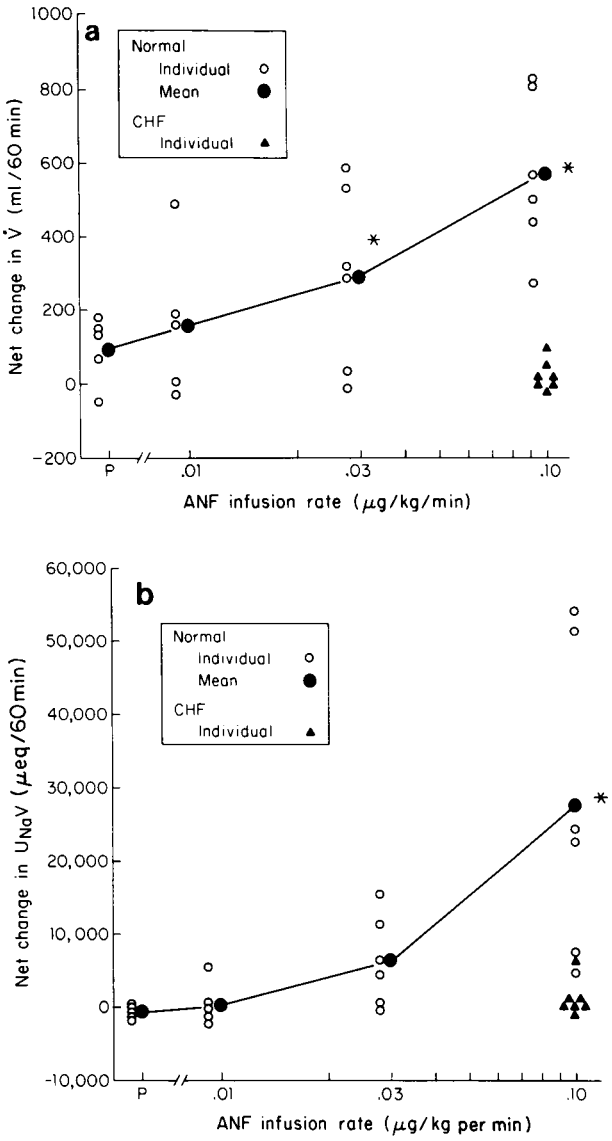

Figure 8. Net changes in urine volume (V̇) and sodium excretion ($U_{Na}V$) during the 60-min experimental infusion phase, related to ANF infusion rate. For the normal subjects, individual values (o) and mean values (●) are given for placebo (*P*) and ANF infusion groups. The asterisks indicate that total excretion during ANF infusion was significantly ($P < 0.05$) greater than total excretion during the respective baseline phase. (▲) Individual responses of congestive heart failure patients to ANF infusion of 0.10 μg/kg per min.

infusion were associated with a significant increase in filtration fraction, a pattern of renal hemodynamic response similar to that demonstrated in experimental animals (11, 32, 39). It has been proposed that the renal hemodynamic actions of ANF (e.g., increases in GFR and possibly blood flow redistribution, together with secondary impairment of tubular sodium reabsorption), may be largely responsible for the potent natriuretic effect of ANF (3, 10, 11, 39–41). One finding of the present study that was not anticipated by prior studies in animals was the rather marked and consistent increase in free water clearance observed in hydrated normal subjects. In the setting of a depressed baseline urine osmolality, this finding probably reflects increased free water formation in the thick ascending limb of Henle's loop, which would imply increased delivery of sodium to that nephron segment while sodium reabsorption is maintained; this hypothesis would be consistent with available micropuncture data (3, 39) and further suggests that a large portion of the increase in excreted sodium is derived from portions of the nephron proximal to the ascending limb (3). Because our subjects may have received a submaximal water load, however, we cannot entirely exclude the possibility that the increase in free water resulted from events in the collecting duct, even including inhibition of vasopressin release and/or antagonism of vasopressin action. In this regard, ANF has been shown to decrease plasma vasopressin in dehydrated or hemorrhaged animals (12) and to antagonize vasotocin-induced water transport by the toad urinary bladder (42). Further study will therefore be required to clarify the mechanism(s) of ANF-induced water diuresis.

In patients with heart failure, there was a marked diminution of the renal responses to exogenous ANF. Given that these patients generally had increased circulating levels of the endogenous hormone, the present studies raise the possibility that a decreased renal responsiveness to ANF could contribute significantly to the pathogenesis of sodium and water retention in this edematous disorder. In considering the mechanisms responsible for the attenuated response to ANF infusion in heart failure, it should be noted that, under basal conditions, many of the patients studied were retaining sodium while on a 100-meq sodium diet. This would be consistent with a previous study by our group (43) demonstrating that only 50% of such patients achieved sodium balance at this level of intake, while the remainder continued to avidly retain sodium. The responsible mechanism could not be identified, but this phenomenon was associated with different patterns of renin–angiotenin system activity. Indeed, the present data provide evidence for some degree of heterogeneity in ANF responsiveness among heart failure patients, in that at least one patient (patient 4, Table III) demonstrated a response to ANF administration that was not unlike that observed in normal subjects. This patient, who was in sodium balance at the time of the study, had the least severe heart failure, as judged by right atrial and pulmonary wedge pressures (Table II), lack of activation of the renin–aldosterone axis (Table III) and a normal level of plasma irANF (2.1 fmol/ml). Thus, our findings do not permit a clear cut distinction between the two possible explanations for refractoriness to ANF in heart failure—namely, either a generally decreased end-organ responsiveness associated with chronically elevated circulating levels (i.e., receptor "down-regulation"), or a more specific renal hyporesponsiveness due, for instance, to baseline renal hemodynamic abnormalities. The latter hypothesis would be consistent with the observation that the three patients in whom GFR tended to increase (patients 2, 4, and 7) also had a natriuresis, albeit attenuated, in response to ANF (Table III). Furthermore, the fact that ANF lowered plasma aldosterone in patients with CHF (Table III) speaks against a generalized down-regulation of its receptors.

Our data indicate that ANF infusion in normal subjects produces hemoconcentration of far greater magnitude than could be expected on the basis of renal fluid losses alone. An increase in hematocrit was also noted by deBold and co-workers in their initial report using bolus infusion of crude atrial extracts (2) and has been demonstrated during continuous infusion of synthetic ANF in dogs (11). In the present study, we observed a significant increase of hematocrit in normal subjects in both the seated and supine positions. This was associated with a significant increase of total protein and, to a lesser degree, serum albumin. Both hematocrit and protein decreased to near-baseline levels after

Table IV. Hematocrit and Serum Protein Responses to ANF Infusion

Subject	Hematocrit			Albumin			Total protein		
	Baseline	ANF	Recovery	Baseline	ANF	Recovery	Baseline	ANF	Recovery
	vol %	*vol %*	*vol %*	*g/dl*	*g/dl*	*g/dl*	*g/dl*	*g/dl*	*g/dl*
Normal individuals									
Seated ($n = 6$)									
1	48.3	49.4	49.4	—	—	—	—	—	—
2	47.4	50.9	48.9	4.6	4.9	4.7	7.1	7.8	7.3
3	44.4	46.3	43.3	4.3	4.4	4.2	6.4	6.6	6.0
4	45.1	49.7	47.6	3.9	3.9	3.6	6.5	6.6	6.3
5	42.9	45.4	43.3	4.4	4.7	4.4	6.9	7.6	6.7
6	45.8	50.1	46.7	4.3	4.8	4.4	6.9	7.9	7.1
$\bar{X}$	45.7	48.6*	46.5	4.3	4.5	4.3	6.8	7.3*	6.7
SEM	0.8	0.9	1.1	0.1	0.2	0.2	0.1	0.3	0.2
Supine ($n = 6$)									
1	41.1	42.9	43.2	4.0	4.0	3.9	6.7	7.4	7.2
2	40.8	44.5	44.4	3.7	4.3	3.8	6.9	7.6	7.0
3	36.5	37.5	36.2	3.5	3.5	3.6	6.7	7.0	6.7
4	45.8	49.4	49.3	3.6	3.9	3.5	6.3	6.6	6.0
5	42.9	44.1	43.3	4.3	4.4	4.3	6.8	6.8	6.8
6	42.1	43.6	42.5	4.0	4.4	4.1	7.0	7.3	7.2
$\bar{X}$	41.5	43.7*	43.2	3.9	4.1	3.9	6.7	7.1*	6.8
SEM	1.2	1.4	1.6	0.2	0.2	0.2	0.2	0.2	0.2
CHF Patients ($n = 7$)									
1	46.0	43.9	—	2.5	2.5	—	4.7	4.6	—
2	38	36.5	—	3.7	3.6	—	6.4	6.5	—
3	41.2	40.0	—	3.1	3.0	—	6.1	5.8	—
4	41.2	41.3	—	3.2	3.2	—	5.9	6.0	—
5	37.6	37.1	36.5	3.0	3.0	2.9	5.6	5.7	5.2
6	36.2	35.6	34.8	2.9	2.9	2.8	5.7	5.5	5.2
7	33.3	34.6	33.1	3.4	3.5	3.2	5.9	6.0	5.7
$\bar{X}$	39.1	38.4	—	3.1	3.1	—	5.8	5.7	—
SEM	1.6	1.3		0.1	0.1		0.2	0.2	

* $P < 0.05$ compared with baseline.

discontinuation of the ANF infusion. Cumulative renal sodium losses induced by the highest rate of ANF infusion (on average 28 meq) corresponded to ~200 ml of isotonic fluid. Such urinary losses, if derived from the extracellular space, would be expected to increase hematocrit and serum protein concentration by only ~1–2%, not the 5–7% observed in this study. This finding therefore indicates that ANF induces a fluid compartment shift from the vascular to the extravascular space, as has previously been postulated (11), and suggests that the peptide increases capillary hydrostatic pressure and/or permeability. With regard to the latter possibility, it is noteworthy that ANF receptors are present in cultured endothelial cells (44), and that endothelial binding sites are rather prominently labeled in autoradiographic studies in the intact animal (45). An interesting observation in our studies was the absence of change in hematocrit or protein concentration in the patients with congestive heart failure. This could not be attributed to differences in body position, as both seated and supine normal individuals demonstrated this hemoconcentrating effect. Although the reasons for this attenuated response remain to be defined, it is worth considering that increased interstitial fluid pressure in heart failure would likely oppose any substantial fluid shift induced by an increase in capillary permeability. The mechanism responsible for this fluid shift is an area clearly deserving of further investigation, because it suggests an additional mechanism through which ANF might contribute to intravascular volume regulation.

The systemic hemodynamic response to ANF infusion observed in these studies is complex, and cannot be totally attributed to intravascular volume contraction. Although ANF is known to relax precontracted vascular smooth muscle in vitro, the significant blood pressure reduction induced in normotensive and certain hypertensive animal models has been ascribed to reduction of cardiac output (46–48). In our supine normal subjects, ANF administration was associated with a significant reduction of pulmonary wedge pressure and to a lesser degree, right atrial pressure; however, there was no significant change in blood pressure, cardiac output, or calculated systemic vascular resistance. This is in contrast to the results of Weidmann et al. (27) who noted a significant decrease of systolic and diastolic blood pressure, when ANF was given as a combination of bolus and sustaining infusion. In our study, constant ANF infusion did decrease systolic blood pressure in the seated normal subjects.

In this group, preload reduction by ANF might be expected to cause a pronounced fall in cardiac output, so that systolic blood pressure would be more likely to decrease, an effect which might be exaggerated following bolus administration (13). The patients with congestive heart failure, whose baseline state is characterized by a markedly reduced cardiac index and increased systemic resistance, had a somewhat different profile of response. These individuals demonstrated a small, but significant reduction of pulmonary wedge pressure from very high basal levels, and a significant increase of cardiac index and reduction of systemic vascular resistance. The hemodynamic effects of ANF could be due to alteration in preload conditions of the heart, as a result of either a transmembrane fluid shift at the level of the capillary or venule, with a reduction of venous volume, or an increase of venous capacitance due to a direct venodilating effect. This is consistent with the reduction in cardiac filling pressures in supine normal subjects without apparent effect on cardiac output. A reduction in preload could explain the overall hemodynamic response to ANF in congestive heart failure. In these patients, where ventricular function is on the steep portion of the pressure-volume curve, preload reduction could decrease ventricular distention, with improvement of overall function. This improvement would translate into an increase of cardiac output, perhaps with an autoregulatory reduction of systemic vascular resistance. This would therefore not produce a net change in mean arterial blood pressure. This hemodynamic response to ANF would be analogous, in many respects, to the responses evoked by pharmacologic intervention with low-dose nitroglycerin or loop diuretics. In view of in vitro (8–10) and intact animal (48) data, we cannot exclude a direct vasodilator effect of ANF on arterial smooth muscle, particularly in heart failure patients where the basal state is characterized by excessive vasoconstriction. Further evidence for a direct vasodilator effect of ANF is the observed increase of forearm blood flow following administration of ANF into the brachial artery of normal subjects and patients with heart failure (49). Additional studies will be necessary to define further the vascular and cardiac effects of ANF infusion in heart failure.

In conclusion, the present study has identified three general categories of steady-state response to ANF in humans: (*a*) natriuresis and diuresis, associated with alteration of renal hemodynamics, manifest primarily by an increase of filtration fraction; (*b*) suppression of plasma renin and aldosterone levels; (*c*) hemodynamic effects consistent with preload reduction due either to a vasodilating effect, or to a transcapillary fluid shift, resulting in hemoconcentration. Although detectable responses were elicited by lower infusion rates in some normal individuals, further study is required to define the physiologic significance of these actions. The refractoriness to the renal and hemoconcentrating effects of ANF infusion in patients with congestive heart failure, where endogenous plasma irANF is increased, suggests that decreased responsiveness to the endogenous hormone may play a role in the pathogenesis of sodium and water retention in this disorder.

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