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Research Article

To investigate whether the response of atrial natriuretic factor (ANF) to volume expansion is impaired in the early stages of dilated cardiomyopathy, the effects of saline load (SL; 0.25 ml/kg.min for 120 min) were assessed in 12 patients with dilated cardiomyopathy and asymptomatic to mildly symptomatic heart failure (HF) and in nine normal subjects (N). SL increased plasma ANF levels in N (from 14.3 +/- 2 to 19.5 +/- 3 and 26 +/- 4 pg/ml, at 60 and 120 min, respectively, P less than 0.001), but not in HF (from 42.9 +/- 9 to 45.9 +/- 9 and 43.9 +/- 8 pg/ml). Left ventricular end-diastolic volume (LVEDV) and stroke volume were increased (P less than 0.001) by SL in N but not in HF. Urinary sodium excretion (UNaV) increased in N more than in HF during SL, whereas forearm vascular resistance (FVR) did not change in N and increased in HF (P less than 0.001). In five HF patients SL was performed during ANF infusion (50 ng/kg, 5 ng/kg.min) that increased ANF levels from 37.1 +/- 10 to 146 +/- 22 pg/ml. In this group, SL raised both LVEDV (P less than 0.01) and ANF (P less than 0.05), whereas FVR did not rise. In addition, the UNaV increase and renin and aldosterone suppressions by SL were more marked than […]

Failure of Atrial Natriuretic Factor to Increase with Saline Load in Patients with Dilated Cardiomyopathy and Mild Heart Failure

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Abstract

To investigate whether the response of atrial natriuretic factor (ANF) to volume expansion is impaired in the early stages of dilated cardiomyopathy, the effects of saline load (SL; 0.25 ml/kg · min for 120 min) were assessed in 12 patients with dilated cardiomyopathy and asymptomatic to mildly symptomatic heart failure (HF) and in nine normal subjects (N). SL increased plasma ANF levels in N (from 14.3±2 to 19.5±3 and 26±4 pg/ml, at 60 and 120 min, respectively, $P < 0.001$), but not in HF (from 42.9±9 to 45.9±9 and 43.9±8 pg/ml). Left ventricular end-diastolic volume (LVEDV) and stroke volume were increased ($P < 0.001$) by SL in N but not in HF. Urinary sodium excretion ($U_{Na}V$) increased in N more than in HF during SL, whereas forearm vascular resistance (FVR) did not change in N and increased in HF ($P < 0.001$). In five HF patients SL was performed during ANF infusion (50 ng/kg, 5 ng/kg · min) that increased ANF levels from 37.1±10 to 146±22 pg/ml. In this group, SL raised both LVEDV ($P < 0.01$) and ANF ($P < 0.05$), whereas FVR did not rise. In addition, the $U_{Na}V$ increase and renin and aldosterone suppressions by SL were more marked than those observed in HF under control conditions. Thus, in patients with dilated cardiomyopathy and mild cardiac dysfunction, plasma ANF levels are not increased by volume expansion as observed in N. The lack of ANF response is related to the impaired cardiac adaptations. The absence of an adequate increase of ANF levels may contribute to the abnormal responses of HF patients to saline load. (*J. Clin. Invest.* 1991. 88:1481–1489.) Key words: aldosterone • atrial peptides • cardiac function • heart failure • renin-angiotensin system

Introduction

Although left ventricular dysfunction represents the primary abnormality in congestive heart failure, it is now generally accepted that the clinical manifestations of this syndrome reflect a complex interaction between central cardiac and peripheral vascular functions. In particular, several neurohormonal mechanisms are often activated to maintain an adequate blood flow to peripheral tissues in spite of the reduced cardiac function. The observation that plasma norepinephrine concentrations (1), plasma renin activity (2, 3), and arginine vasopressin secretion (4) are often increased in congestive heart failure have led to a better comprehension of the pathophysiologic mechanisms, with a major impact on the therapeutic management of the disease.

Plasma levels of atrial natriuretic factor (ANF)[1] are also elevated in patients with heart failure (5–9) and show good correlation with atrial pressure levels (8, 10–12) and prognosis (13) in heart failure. In view of its natriuretic, vasorelaxant, and renin-inhibitory properties (14), ANF has been regarded as an ideal counterregulatory mechanism to the activation of vasoconstrictor and sodium-retaining hormones in heart failure. However, important physiologic aspects of ANF regulation in heart failure remain to be clarified.

A first question arises from the observation that sodium and water retention and high renin and aldosterone levels commonly occur in patients with heart failure despite high circulating levels of ANF. This may indicate that the ANF elevation is inadequate to the degree of heart failure and suggests that a relative deficiency of the atrial hormone contributes to the development of congestive heart failure. In this regard, studies in experimental animal models of severe chronic congestive heart failure demonstrated absence of ANF increase in response to volume expansion (15, 16). It is not clear, however, whether an impaired ability to further increase ANF levels in response to volume and/or pressure overload also occurs in the earlier and milder stages of the disease, and whether this abnormality is only a consequence of the maximal stimulation of ANF release, as it may occur in the more severe forms of congestive heart failure.

Secondly, it is important to define whether the impaired ANF response in heart failure may contribute to the cardiorenal and hormonal abnormalities associated with the development of heart failure. Such a modulatory role of high circulating ANF levels in heart failure is suggested by a number of studies, using different experimental approaches such as autoimmune animals (17), monoclonal antibodies (18), exogenous ANF infusion (19), and inhibition of ANF metabolism (20).

The present study was designed to further address these questions in humans. For this purpose, we examined the response of circulating ANF levels to progressive isotonic volume expansion in patients with dilated cardiomyopathy and chronic mild left ventricular dysfunction, and in control normal subjects. In these two groups, we also examined the hemodynamic, hormonal and renal adaptations to volume loading. Finally, to assess the contribution of the altered ANF response to the abnormal cardiorenal and endocrine responses to saline load in a subgroup of patients with heart failure, we performed the study during sustained infusion of ANF.

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1. *Abbreviation used in this paper:* ANF, atrial natriuretic factor.

Methods

Study subjects and patients. The study population included 9 normal volunteers and 17 patients with dilated cardiomyopathy and chronic, stable, mild heart failure. The experimental protocol was approved by the Ethical Committee of our institution and each subject gave written, informed consent before entering the study. The normal group comprised seven male and two female subjects, aged 30–54 yr. Normal status was established by history, physical examination, and laboratory analyses, which included a blood count, serum glucose and cholesterol concentrations, indexes of renal and hepatic function, an electrocardiogram, and M- and B-mode echocardiograms. The patients with heart failure included 12 men and 5 women, ranging in age from 30 to 60 yr. The individual characteristics of these subjects are presented in Table I. The cause of heart failure was idiopathic dilated cardiomyopathy in nine and coronary artery disease in eight subjects. Patients were considered to have an idiopathic dilated cardiomyopathy when no obvious underlying cause of heart failure could be discovered during routine evaluation. The diagnosis of coronary artery disease was based on the documentation of at least one myocardial infarction. Patients with angina pectoris, recent myocardial infarction within the previous 3 mo, hypertension, renal failure, or recent acute cardiac decompensation, as defined by the sudden accumulation of pulmonary congestion or peripheral edema, were excluded from the study.

The definition of mild heart failure was based on the following criteria: (*a*) no reduction or mild reduction in their functional capacity as assessed by New York Heart Association classification (class I or II); (*b*) mild to moderate limitation of the exercise capacity, as determined by cardiopulmonary exercise testing using a standard protocol (upright bicycling with a stepwise increase of 10 W/min; $n = 13$); the mean exercise duration was 9.8±0.6 min, and peak oxygen consumption averaged 17±1.3 ml/kg · min; (*c*) echocardiographic end-diastolic left ventricular dimension ≥ 55 mm; (*d*) left ventricular ejection fraction, as determined by radionuclide technique ≤ 50%. Right heart catheterization performed in seven of the patients with heart failure within 3 d of this study showed end-diastolic left ventricular pressure of 21±2 mmHg. Five patients had required treatment with digitalis and/or diuretics.

Experimental protocol. All drug therapy was discontinued at least 3 d before the study. Alcohol, caffeine, cigarettes, and physical exercise were all prohibited within 24 h of the study. After admission to the clinic ward, all subjects were maintained on a daily diet containing 100 meq of sodium, 50 meq of potassium, and 1,500 ml of water. Daily 24-h urine collections were analyzed for sodium, potassium, and creatinine excretion. When subjects had achieved metabolic balance, as assessed by the stability of urinary electrolyte excretion, the studies were undertaken with the subject in the postabsorptive state.

The study was begun at 7:30 a.m. with the subject in a comfortable sitting position after voiding. The temperature (22°C) as well as the lights of the study room were maintained constant. The patient was asked to drink 500 ml of water to ensure sufficient urinary flow. 30 min after insertion of two intravenous lines into superficial forearm veins, 20-min baseline clearance periods were performed. Throughout the study, at the end of each period the patients drank an amount of water equivalent to the sum of blood losses and urinary flow. As soon as balance was achieved (consistent urinary volumes in two consecutive clearance periods), two 30-min baseline periods were observed and the average of the urinary, hormonal, and hemodynamic measurements obtained during the two baseline periods was used for the analysis. Subsequently, an intravenous isotonic saline load (0.9% NaCl, 0.25 ml/kg · min) was started and maintained at constant rate for 2 h. Arterial blood pressure was measured at 10-min intervals by using standard sphygmomanometric technique, following the recommendations of the American Heart Association (21). Heart rate was continuously monitored by electrocardiographic lead II. Urinary samples for determination of volume, sodium, and potassium were obtained at the end of each clearance period. Venous blood samples for determination of hematocrit, plasma renin activity, aldosterone, norepinephrine, and immunoreactive atrial natriuretic factor levels were obtained at 30-min intervals.

Forearm blood flow and calculated vascular resistance were measured at 60-min intervals by strain-gauge plethysmography. M- and

Table I. Individual Characteristics of the Patients with Mild Heart Failure

Patient	Age/Sex	Diagnosis	NYHA class	LVEDD	LVEF	ETD	Peak Vo_2
	yr			*mm*	*%*	*min*	*ml/kg · min*
1	59/M	CAD	I	58	48	8	14
2	40/M	ICM	I	69	47	12	26
3	37/F	ICM	II	76	20	N/A	N/A
4	50/F	ICM	I	57	50	7	15
5	49/M	CAD	II	62	40	N/A	N/A
6	60/M	CAD	I	62	27	6.3	14
7	56/M	ICM	II	63	38	8	17
8	54/M	ICM	II	66	27	N/A	N/A
9	55/M	ICM	II	63	49	9.3	17
10	30/F	CAD	I	55	46	N/A	N/A
11	60/M	ICM	I	55	50	10	13
12	44/M	CAD	II	62	32	12	20
13*	30/F	ICM	II	64	37	8.5	18
14*	57/M	CAD	II	57	40	13	19
15*	41/F	ICM	II	63	44	8	16
16*	49/M	CAD	II	63	35	11	19
17*	56/M	CAD	I	60	46	12	20

Abbreviations: CAD, coronary artery disease; ETD, exercise test duration; ICM, idiopathic cardiomyopathy; LVEDD, echocardiographic left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction (radionuclide technique); NYHA, New York Heart Association; Peak Vo_2, total body oxygen consumption at peak exercise. * Patients undergoing the study with ANF infusion.

B-mode echocardiograms were also recorded at 60-min intervals for measurements of atrial and ventricular dimensions, calculation of left ventricular ejection fraction, and estimation of stroke volume and the derived parameters.

To evaluate the possible effects of higher plasma ANF levels on hormonal and cardiorenal responses, in five patients from the heart failure group, saline load (at the rate indicated above) was given during sustained, constant intravenous infusion of α-human-(99–126)-ANF (50 ng/kg bolus, followed by 5 ng/kg · min) started 60 min before the beginning of volume expansion. Preliminary experiments with a 3-h infusion of ANF in patients with heart failure (n = 5) showed that the 60-min period of ANF infusion is a sufficient time to achieve a stable increase of plasma levels, since no further increase was observed after 30 min (baseline, 59±10 pg/ml; 30 min, 182±20 pg/ml; 60 min, 185±22 pg/ml; 120 min, 166±29 pg/ml; 180 min, 171±30 pg/ml, all $P < 0.001$ vs. baseline; 60-, 120-, and 180-min measurements were not different from 30-min value). In these experiments as well, hemodynamic, renal, and hormonal responses remained unchanged after the 60-min measurement (data not shown).

Laboratory methods. All blood samples were collected on ice and spun immediately (within 10 min); the plasma was then separated and frozen until the time of assay, which did not exceed 5 wk.

Plasma renin activity was measured by radioimmunoassay according to the method described by Menard and Catt (22) (sensitivity, 50 pg per tube angiotensin I; intraassay and interassay variability coefficients, 6% and 10%, respectively). Plasma immunoreactive ANF levels were determined by radioimmunoassay as previously described by our laboratory (23), by using rabbit antiserum (RAS 8798, Peninsula Laboratories Europe, Merseyside, UK), iodinated human ANF-(99–126) (2,000 Ci/mmol, Amersham Berks, UK), and α-human ANF-(99–126) (Bissendorf GmbH Peptide Wedemark, FRG) as a standard. ANF was extracted from plasma with Sep-Pak C_{18} cartridges. The recoveries determined on each plasma sample by adding to it a minimal amount of radiolabeled ANF, ranged from 74% to 90%. Intraassay and interassay variation coefficients were 6.5% and 10.5%, respectively. The radioimmunoassay sensitivity was 1 fmol per tube. Plasma aldosterone concentrations were estimated by a radioimmunoassay technique using a commercial kit (Sorin, Saluggia, Italy). Plasma norepinephrine assay was performed with reverse-phase high-performance liquid chromatography with electrochemical detection, after extraction and concentration by adsorption onto activated alumina (24). Potassium and sodium levels in urine were measured by ion-selective electrodes (E2A Na/K system, Beckman Instruments, Inc., Arlington Heights, IL).

Echocardiographic measurements. Wide-angle, two-dimensional echoes were recorded using a phased-array sector scanner (model 77020 AC, Hewlett-Packard Co., Andover, MA).

All studies were videotaped on ¾-in videocassette recorders equipped with a back-spacer search module, which allows frame-by-frame bidirectional playback. The video frame rate of the system is ~ 60 frames per second.

All patients were studied in the sitting position using multiple views through the apical window. Two views were selected for measurements: apical-four chamber and apical-two chamber. The left ventricular long axis (L_{Max}) was measured at end-diastole as the longest major axis in either of the two apical views. The measurements of L_{Max} were rounded off to the closest whole number to ensure reproducibility. Left ventricular end-diastolic area (EDA) was measured using the largest of all the left ventricular minor axies measured. Left ventricular end-diastolic volume (EDV) was calculated according to the single-plane ellipse method as: EDV (ml) = $8/3\ EDA^2/(\pi \cdot L_{Max})$ (25). The same measurements were undertaken in end-systole in order to calculate end-systolic volume. Ejection fraction was measured using the averages of all the end-diastolic and end-systolic volumes (25). All studies were performed by the same investigator and read independently by two experts unaware of the protocol. The readings obtained showed correlations for both L_{Max} ($r = 0.97$, $P < 0.001$) and end-diastolic area ($r = 0.96$, $P < 0.001$). Excellent correlations were also obtained for the measurements of end-diastolic volume ($r = 0.97$, $P < 0.001$) and end-systolic volume ($r = 0.95$, $P < 0.001$) between the two observers. The variability of multiple measurements of volumes over 2 h did not exceed 3.8%. Stroke volume was derived as the difference between end-diastolic and end-systolic volumes, and cardiac output and total peripheral resistance were estimated by using standard formulas. In our laboratory, the echocardiographic measurements of stroke volume were significantly correlated with the measurements obtained by the thermodilution technique ($r = 0.88$, $P < 0.01$). Radionuclide assessment of ejection fraction was performed with the patient at rest in the supine position according to the methods previously reported from this laboratory (26). Individual echocardiographic measurements of ejection fraction were significantly correlated with the corresponding radionuclide ejection fraction values obtained in our patients ($r = 0.68$, $P < 0.01$).

Measurements of forearm blood flow. Forearm blood flow was measured with a mercury in sylastic strain-gauge plethysmograph using a venous occlusion technique as previously described (27). The strain gauge was placed ~ 5 cm below the antecubital crease. Forearm blood flow (ml/min · 100 g) was calculated from the rate of increase in forearm volume while venous return from the forearm was prevented by inflating the cuff at the upper arm. The pressure in the venous occlusion or congesting cuff at the upper arm was 40 mmHg. Circulation of the hand was arrested by inflating a cuff around the wrist. The wrist cuff was inflated before determining forearm blood flow and continuously throughout the measurements. Blood pressure was determined with mercury sphygmomanometric method in the same arm. Forearm vascular resistances (mmHg/ml/min · 100 g) were calculated by dividing mean blood pressure by forearm blood flow. Mean blood pressure was calculated by adding to diastolic blood pressure one-third of pulse pressure.

Statistical analysis. Data are presented as means±SEM. Distribution of the data was assessed by the Bartlett test. χ^2 analysis was used for comparison of descriptive parameters. Comparisons of the basal data of normals and heart failure patients was performed by unpaired t test or Wilcoxon rank test as appropriate. Between-groups comparisons of the responses to saline load were tested by two-factor analysis of variance (factoring for group and time) for repeated measures. Two-way analysis of variance with Dunnett's correction was used for determining significant differences within the same group. Pearson's correlation coefficients were used to examine relationships among variables.

Results

Characteristics of the study groups. The two groups of subjects were comparable with regard to clinical characteristics and renal function. In the heart failure group left ventricular end-diastolic diameter was significantly greater and ejection fraction smaller than in the normal subjects (Table II).

Fig. 1 shows individual and mean values for plasma ANF, renin activity, and aldosterone and norepinephrine concentrations. Individual hormonal values in patients with chronic heart failure ranged widely and were frequently elevated. However, only mean plasma ANF level was significantly elevated in the heart failure patients (41.2±6 vs. 14.5±2 pg/ml, $P < 0.05$).

Hormonal and renal responses to saline load in normal subjects (n = 9) and in patients with dilated cardiomyopathy (n = 12). In normal subjects saline load was associated with a progressive increase in plasma ANF levels ($F = 12.6$, $P < 0.001$) which became significant since the 60-min measurement (Fig. 2, *left panel*). Plasma renin activity ($F = 38.1$, $P < 0.001$) and aldosterone concentrations ($F = 24.8$, $P < 0.001$) were progressively reduced, whereas plasma norepinephrine concentrations (not shown in the figure) did not change significantly (from 489±60 to 565±70 and 572±69 pg/ml, respectively). Urinary sodium excretion rate ($F = 4.54$, $P < 0.05$) (Fig. 2), potassium excretion rate (42±5, 47±7, and 60±8 μeq/

Table II. Characteristics of the Study Groups

	HF (n = 17)	N (n = 9)
Age (*yr*)	48.7±2.4	46.3±3.3
Sex (male/female)	12:5	7:2
Weight (*kg*)	73.5±2	72.7±2
Height (*cm*)	166±1	168±2
Serum creatinine (*mg/dl*)	0.95±0.04	0.86±0.05
SBP/DBP (*mmHg*)	119±4/74±2	118±2/77±2
HR (*beats/min*)	68.1±2.0	62.3±4.0
$U_{Na}V$ (*meq/24 h*)	98±14	101±19
U_KV (*meq/24 h*)	48±4	54±5
LVEDD (*mm*)	62.1±1.3	49.7±1.6*
EF (%)	31.0±2.0	53.6±4.0*

Data are expressed as mean±SEM.
Abbreviations: DBP, diastolic blood pressure; EF, echocardiographic left ventricular ejection fraction; HF, heart failure subjects; HR, heart rate; LVEDD, echocardiographic left ventricular end-diastolic diameter; N, normal subjects; SBP, systolic blood pressure; U_KV, urinary potassium excretion rate; $U_{Na}V$, urinary sodium excretion rate.
* $P < 0.001$ vs. N.

min), and urinary volume (2.1±0.5, 3.1±0.9, and 4.3±1.2 ml/min) also increased significantly (both $P < 0.05$).

As shown in Fig. 2 (*right panel*), in the patients with heart failure plasma ANF levels did not change in response to saline loading ($F = 0.826$, NS). This response was significantly different from that observed in normals ($F = 15.7$, $P < 0.001$). Plasma renin activity and plasma aldosterone concentrations were suppressed by saline load also in the heart failure patients (Fig. 2, *right panel*). Although two-factor ANOVA did not show differences in the time/group interaction, a significant influence of group was found. Therefore, a comparison of percent reductions in renin and aldosterone was performed. This analysis showed that the percent reductions in renin (normal, −42.7±4 at 60 min, −63.2±3 at 120 min; heart failure, −37.2±3 at 60 min, −48.1±6 at 120 min, NS and $P < 0.05$, respectively) and aldosterone (normal, −33.5±4 and −51.9±6, respectively; heart failure, −19.9±5 and −29.6±6, both $P < 0.05$) were smaller in the heart failure group. Plasma norepinephrine concentrations did not change significantly (from 566±55 to 591±65 and 746±86 pg/ml) in the heart failure group.

Finally, urinary sodium excretion rate (Fig. 2) was significantly increased by saline load also in the heart failure group, although the rise was significantly smaller than that observed in normals ($F = 4.81$, $P < 0.05$). The responses of urinary volume (from 1.69±0.6 to 1.87±0.7 and 2.05±0.7 ml/min at 120 min, NS) and potassium excretion rate (from 61±11 to 64±10 and to 65±9 μeq/min) did not achieve statistical significance in the heart failure group.

Hemodynamic responses to saline load. Mean arterial pressure (normal, from 90.8±2 to 93.1±2 and 92.4±2 mmHg, NS; heart failure, from 85.3±2 to 86.7±3 and 90.2±3 mmHg, NS) and heart rate (normal, from 62.3±4 to 59.7±4 and 57.7±4 beats/min, NS; heart failure, from 68.0±3 to 65.7±3 and 63.7±3 beats/min, NS) did not change significantly during saline load.

Fig. 3 shows that the responses of cardiac and systemic hemodynamics to saline load were different in the two groups. In particular, in the normal group (*left panel*) stroke volume ($F = 5.93$, $P < 0.01$), left ventricular end-diastolic volume ($F = 12.4$, $P < 0.001$) and end-systolic volume (from 54±11 to 62.6±12 and to 67.2±14 ml at 60 and 120 min, $P < 0.01$) increased, while left ventricular ejection fraction and forearm vascular resistance did not change. In contrast, in the patients with heart failure (Fig. 3, *right panel*) left ventricular end-diastolic volume did not change, end-systolic volume (not shown in the figure) increased (from 138±25 to 152±19 and 157±20 ml, $P < 0.05$), ejection fraction ($F = 4.48$, $P < 0.05$) and stroke volume ($F = 9.50$, $P < 0.01$) fell, and forearm vascular resistance rose ($F = 7.69$, $P < 0.01$). Also the behavior of calculated total peripheral resistance differed in the two groups, since no

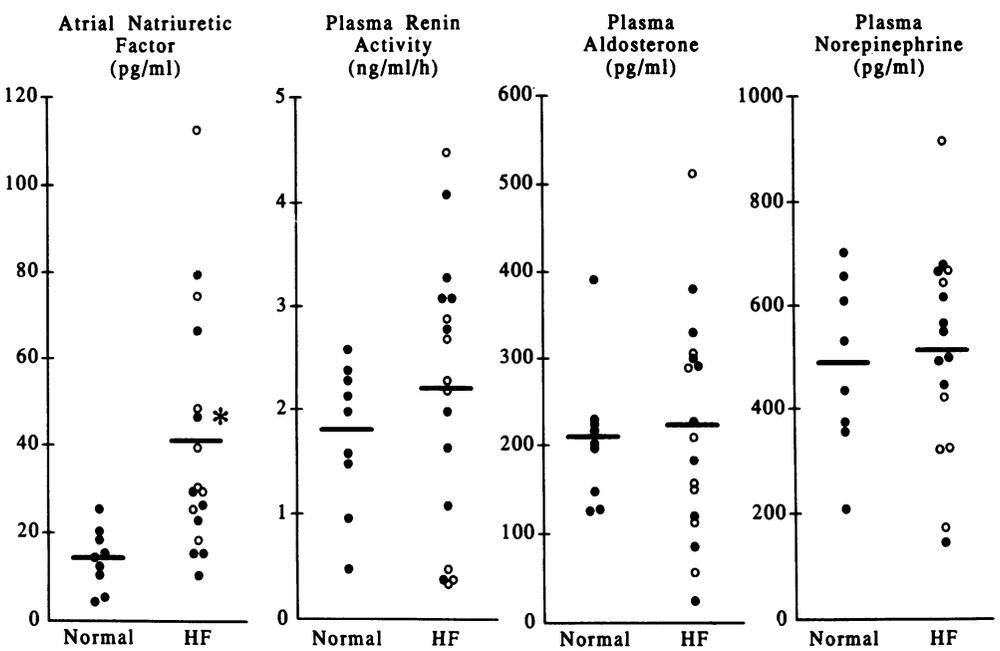

Figure 1. Individual and mean values of baseline hormonal levels in normal individuals and patients with dilated cardiomyopathy and mild heart failure (*HF*). Among the heart failure patients, those who had coronary artery disease are identified by open symbols, while the solid symbols indicate those with idiopathic cardiopathy. *$P < 0.05$ vs. normal.

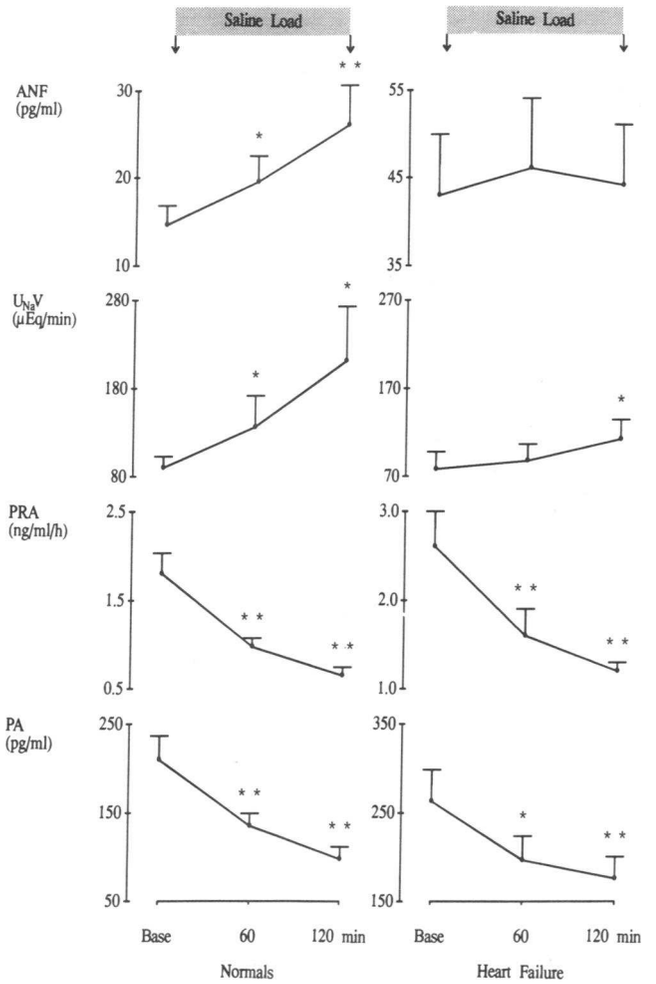

Figure 2. Effects of saline load on atrial natriuretic factor (*ANF*), urinary sodium excretion ($U_{Na}V$), plasma renin activity (*PRA*), and plasma aldosterone (*PA*) in the two groups. * or **$P < 0.05$ and $P < 0.01$, respectively as compared to baseline. Between-groups significant differences in ANF ($F = 15.7$, $P < 0.001$) and urinary sodium excretion ($F = 4.81$, $P < 0.05$) were found.

change was observed in normals (from 1,990±82 to 1,887±145 dyn·s·cm^{-5} at 120 min), while a significant increase was found in the patients with heart failure (from 1,993±248 to 2,350±352 dyn·s·cm^{-5} at 120 min, $P < 0.05$).

Fig. 4 shows that volume expansion induced comparable reductions of hematocrit in the two groups. Thus, a similar degree of plasma volume increase could be estimated (+11% and +16% at 60 and 120 min, respectively, in both groups) by applying the formula $P = 100/(100 - H_1) \cdot 100(H_1 - H_2)/H_2$, in which P is the percent change in plasma volume and H_1 and H_2 are the initial and the final packed cell volumes. In view of the hemodilution associated with saline loading, we also normalized the plasma ANF concentrations by hematocrit values at each time of the experiment. This analysis confirmed that the ANF/hematocrit ratio increased only in normals (from 0.35±0.05 to 0.49±0.09 and to 0.68±0.11, respectively, $P < 0.001$) but not in the heart failure group (from 1.04±0.3 to 1.15±0.3 and to 1.10±0.3, respectively, NS). Also the increases of atrial volumes, as calculated by echocardiography, were comparable. In contrast, the percent increase in left ventricular end-diastolic volume was significantly greater in normals than in heart failure patients at each time control. The changes in left ventricular end-diastolic volume were paralleled by consistent changes in ANF levels. The absolute values of left ventricular end-diastolic volume and plasma ANF levels during volume expansion were significantly correlated in normals ($r = 0.740$, $n = 36$, $P < 0.001$), but not in the patients with dilated cardiomyopathy ($r = 0.341$, $n = 27$, NS).

Effects of saline load in patients with heart failure during exogenous infusion of ANF (n = 5). The constant infusion of ANF (50 ng/kg bolus, 5 ng/kg·min) caused an increase of plasma ANF levels (from 37.1±10 to 146±22 pg/ml, $P < 0.01$).

The cardiorenal and hormonal effects of ANF alone and the subsequent effects of saline load during ANF infusion are presented in Table III. The only significant effects of ANF alone at this dose were reductions of forearm vascular and total peripheral resistance ($P < 0.001$ and 0.01, respectively) and of plasma renin activity ($P < 0.01$).

Saline loading during ANF infusion significantly increased left ventricular end-diastolic volume. In addition, plasma ANF levels further rose as compared to the values obtained after 1 h of ANF alone. This finding is supported by the analysis of the

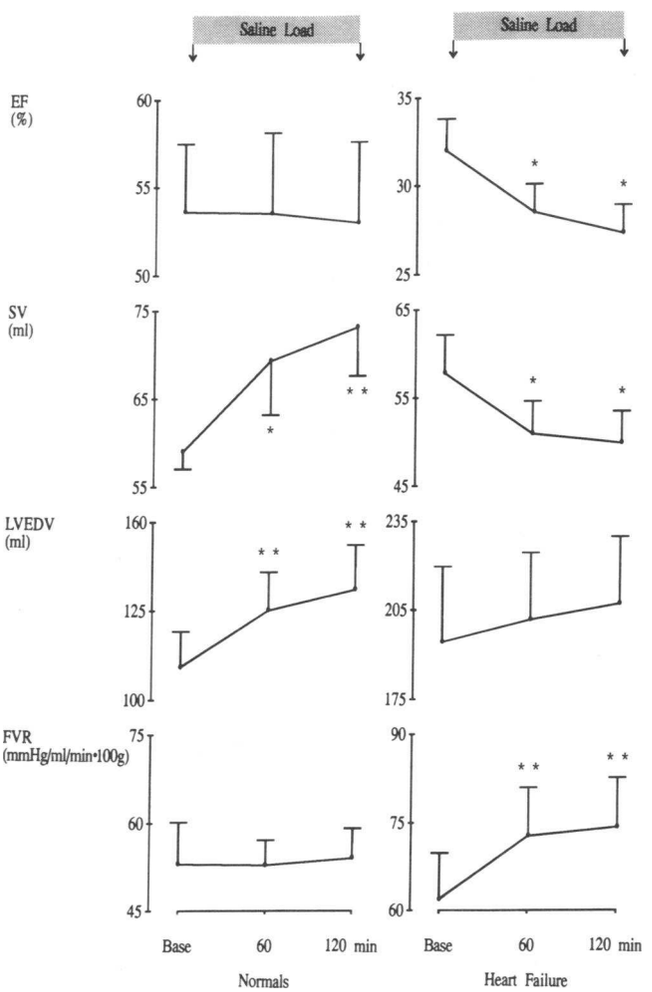

Figure 3. Effects of saline load on left ventricular ejection fraction (*EF*), stroke volume (*SV*), left ventricular end-diastolic volume (*LVEDV*), and forearm vascular resistance (*FVR*) in the two groups. Symbols as in Fig. 2.

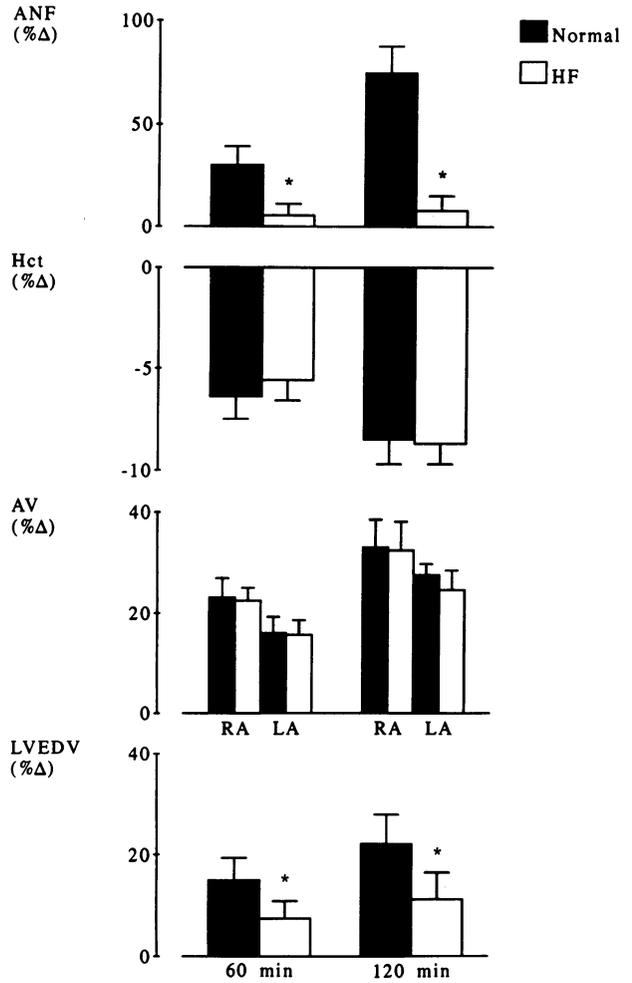

Figure 4. Percent changes (%Δ) of ANF, hematocrit (*Hct*) atrial volumes (*AV: RA,* right atrium; *LA,* left atrium) and left ventricular end-diastolic volume (*LVEDV*) at 60 and 120 min of saline load, in normals (*solid bars*) and heart failure patients (*open bars*) $*P < 0.05$ vs. normals.

ANF/hematocrit ratio which increased significantly under these circumstances (from 3.38±0.4 to 5.36±0.9 and to 5.11±0.6, respectively, $P < 0.05$). Volume expansion in these patients was also associated with significant reductions of plasma renin activity and aldosterone and with an increase of urinary sodium excretion rate. Table III also shows that in this group left ventricular ejection fraction did not fall and forearm vascular and total peripheral resistance did not increase as observed in the untreated heart failure group. The reduction of hematocrit was not different from that caused by saline load alone in the patients with heart failure.

Fig. 5 summarizes comparisons of the responses to saline load observed in the two groups of heart failure patients with and without concomitant ANF infusion. As shown in this figure, in the heart failure group starting with higher ANF levels the natriuretic response was significantly greater, plasma renin activity (only at 60 min) and aldosterone suppressions were significantly accentuated and, finally, the vasoconstrictive response in the forearm observed in the patients with heart failure was abolished.

Discussion

The experiments described in this article show that cardiorenal and hormonal adjustments to acute volume expansion are markedly impaired in patients with dilated cardiomyopathy and mild heart failure. A striking first abnormality is the inability to increase plasma ANF levels in response to an acute rise of cardiac preload. Whereas plasma ANF levels almost doubled in normals, there was no increase in the subjects with dilated cardiomyopathy. This finding confirms and extends previous observations obtained in animal models of acute and chronic congestive heart failure, showing impaired ability to raise ANF levels (15, 16, 28). In those studies, however, more severe forms of heart failure with extremely high baseline levels of ANF were investigated, and the absence of further increase in circulating ANF in response to acute volume expansion was interpreted as the consequence of a maximal stimulation of ANF release. In agreement with previous reports in patients with mild heart failure (New York Heart Association class I or II) (6, 10, 13, 29, 30), our patients had only a moderate (two- to threefold) elevation of mean baseline plasma ANF concentrations and in some cases the values were even within the normal range (Fig. 1). Despite this, the ability to increase plasma ANF levels in response to volume load was markedly and uniformly depressed in our patients. Therefore, in these patients mechanisms other than an exhaustion of ANF release secondary to maximal stimulation must be considered.

The inability to raise ANF levels in response to moderate volume expansion observed in our patients may appear in contrast with previous reports by Uretsky et al. (31) and by Rodeheffer et al. (32). However, in the study of Uretsky et al. (31) a more rapid volume expansion (11% in 30 min) was induced by infusion of mannitol, thus causing marked stimulation of arginine vasopressin which may exert a facilitating action in the volume-induced increase of ANF (33). Also, mannitol infusion was accompanied by an increase of plasma osmolality, which may represent an independent stimulus for ANF release (34, 35). In addition, those studies (31, 32) were performed in patients with more severe congestive heart failure and in the supine position, which may have caused extreme stimulation of ANF release. Finally, although in our study the patients with dilated cardiomyopathy showed smaller ANF responses than normals to volume expansion, even when normalized for hematocrit reductions, it is likely that hemodilution attenuated the increase in ANF concentrations in both groups. This might have prevented detection of small increases in ANF levels in the heart failure group.

In our study, volume expansion caused comparable atrial distension in the two groups, as indicated by the equivalent increases in atrial volumes during saline load. In addition, although invasive atrial pressure measurements were not included in our protocol, it is unlikely that a different response of atrial wall stretch in the two groups might have accounted for the different ANF responses. In fact, the level of atrial wall stretch achieved during volume expansion was presumably higher in the heart failure group than in normals, in that left ventricular dimensions increased much less in the patients with heart failure whereas increases of atrial dimensions were comparable. In spite of this, plasma ANF levels did not increase in the patients with heart failure. Taken together, these considerations indicate that, for similar increments of cardiac preload, circulating ANF levels increased only in normal subjects.

Table III. Steady State of ANF Infusion (50 ng/kg, 5 ng/kg · min) and of Saline Load during ANF Infusion in Five Patients with Dilated Cardiomyopathy and Mild Heart Failure

		ANF infusion		
			Saline load	
	B	B_1	60	120
			min	
ANF (*pg/ml*)	37±10	146±22*	213±51§	193±13§
PRA (*ng/ml · h*)	1.32±0.4	1.03±0.3*	0.48±0.15§	0.37±0.1‖
PA (*pg/ml*)	126±37	136±42	60±19§	27±7‖
PNE (*pg/ml*)	353±76	407±85	431±96	359±73
$U_{Na}V$ (*μeq/min*)	112±20	140±37	220±57§	302±126‖
EF (%)	29.2±3.0	31.0±2.7	31.4±3.0	30.4±4.0
SV (*ml*)	56.6±5.6	61.6±6.1	63.4±4.7	62.0±6.1
TPR ($dyn \cdot s \cdot cm^{-5}$)	2101±145	1908±73*	1998±79	1991±79
FVR (*mmHg/ml · min · 100 g*)	58.8±6.7	49.0±6.4‡	47.4±5.6	49.8±4.3
LVEDV (*ml*)	193±20	197±26	204±25§	213±26‖
Hct (%)	42±2	42±2	39±2§	39±2§

Data are expressed as mean±SEM.

Abbreviations: ANF, plasma atrial natriuretic factor concentrations; EF, echocardiographic left ventricular ejection fraction; FVR, forearm vascular resistance; Hct, hematocrit; LVEDV, left ventricular end-diastolic volume; PA, plasma aldosterone concentrations; PNE, plasma norepinephrine concentrations; PRA, plasma renin activity; SV, stroke volume; TPR, total peripheral resistance; $U_{Na}V$, urinary sodium excretion rate. * and ‡ = $P < 0.01$ and $P < 0.001$ vs. B. § and ‖ = $P < 0.05$ and $P < 0.01$ vs. B_1.

Although the mechanisms underlying the impaired ANF response in heart failure cannot at present be defined, our findings suggest that this inability is related to the abnormal adaptation of left ventricular dynamics observed in these patients. In fact, ANF increased only when left ventricular volume and stroke volume increased. Furthermore, a significant correlation between left ventricular volume values and plasma ANF levels achieved during volume expansion was found in normals but not in the patients with heart failure.

Thus, the inability to increase peripheral plasma ANF levels in patients with dilated cardiomyopathy might actually reflect the inadequate pumping function of the failing ventricle in response to an increased preload, as suggested by the decrease of ejection fraction. In this regard, the observation that the ANF response to volume expansion is preserved in a nondilated model of heart failure (36) is indirectly consistent with the hypothesis that ventricular dilatation may influence the ANF response. Finally, the observation in our experiments with ANF infusion that volume expansion restored a significant increase of left ventricular volume and was associated with further increases of ANF levels substantiates this view. This latter finding also indicates increased secretion of the atrial peptide, since under circumstances of improved cardiac output, metabolic clearance, if anything, should increase thus reducing ANF levels.

A mechanism that may have contributed to prevent the increase of ANF levels by volume expansion is suggested by the recent work of Hintze et al. (37) in the conscious dog showing that, beyond the release rate from atrial myocytes, the washout of ANF via changes in atrial blood flow is an important factor influencing changes in circulating ANF levels. An abnormal adaptation of coronary vascular resistance to volume expansion, as observed for peripheral resistance in our study, as well as the lack of increase of stroke volume may have curtailed the washout of ANF from the heart, thus becoming a limiting factor for the increases in plasma ANF levels. Finally, the observation that in the more severe or advanced stages of heart failure the circulating levels of ANF are much higher than those observed in the milder or initial forms (38) further suggests that mechanisms other than impairment of the biosynthetic properties or the secretory function are involved in the abnormal ANF response observed in our patients.

With regard to the source of ANF release in heart failure, recent studies suggest that ventricles contribute to ANF secretion in this condition (39, 40). Although our protocol was not specifically designed to investigate this aspect, the observation that the changes of ANF circulating levels paralleled those of ventricular dimensions throughout the studies might be interpreted as the consequence of changes of ventricular secretion in relation to changes of ventricular stretch. However, this may reflect as well the relationship between ANF levels and ventricular output. In addition, recent observations do not support contribution of ventricular secretion to the ANF response to acute cardiac pacing in a dog model of chronic congestive heart failure (41).

Our study illustrates several other abnormal responses to volume expansion in heart failure. In fact, although basal hormonal, renal, and hemodynamic profiles were comparable in the two groups of subjects, abnormal adaptations were unmasked during volume expansion in the patients with heart failure. First, the natriuretic and diuretic responses to volume load were significantly depressed in the patients with dilated heart as compared to normals. Secondly, the renin and aldosterone suppressions by saline/volume load in this group were relatively smaller. In this regard, it is of interest that the responsiveness of the renin-angiotensin-aldosterone system to acute saline load is relatively preserved in patients with mild degree of heart failure, in agreement with the results of previous stud-

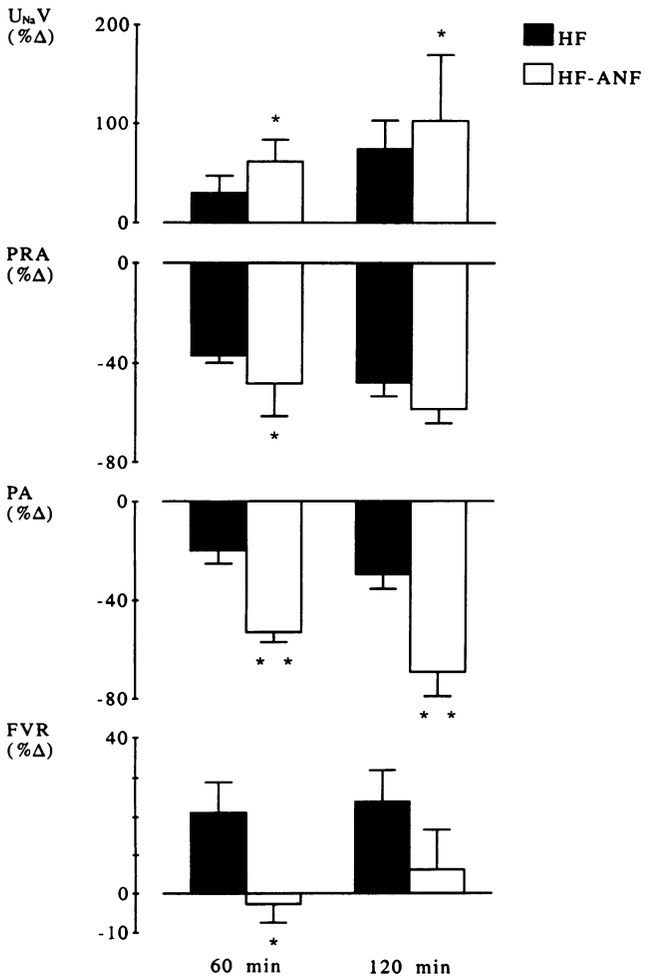

Figure 5. Percent changes (%Δ) of $U_{Na}V$, PRA, PA, and FVR (for abbreviations, see Figs. 2 and 3) at 60 and 120 min of saline load (*solid bars*) and to saline load during the steady-state phase of ANF infusion (*open bars*) in patients with dilated cardiomyopathy and mild cardiac dysfunction (*HF*). * or ** $P < 0.05$ and $P < 0.01$ vs. HF.

ies with chronic oral salt loading (42). Finally, in the heart failure group ejection fraction and stroke volume fell, and forearm vascular and calculated total peripheral resistance paradoxically increased during volume expansion. Therefore, the overall mechanisms of adaptation to increased preload that attempt to maintain circulatory homeostasis in patients with dilated cardiomyopathy are quite different from those operating in physiologic states. These differences observed in our study during volume expansion also emphasize the importance of dynamic evaluations in order to define the time of onset of the hormonal and cardiorenal abnormalities in patients with mild or initial heart failure.

To evaluate whether the reduced ability to respond to volume expansion with further increases in circulating ANF may contribute to the deteriorating natriuretic capacity, to the escape of the renin-angiotensin-aldosterone system and to the vasoconstriction observed in our patients with heart failure, we studied the effects of raising circulating ANF levels. For this purpose, we infused a dose of ANF resulting in a stable increase of plasma ANF levels within the pathophysiological range. The only significant responses produced by this dose of ANF were vasodilatation and slight reduction of plasma renin activity. The vasodilatory effect is consistent with previous reports showing peripheral vasodilatation in response to low dose ANF (43, 44) and may be the consequence of a direct vasorelaxant effect. However, these findings are also consistent with previous reports showing a reduced natriuretic action of ANF in patients with heart failure (7). The effect of ANF on forearm vascular resistance may also be the consequence of a potentiation exerted by the peptide of the tonic inhibitory influence of vagal afferents on the vasomotor center (45–47). This hypothesis is supported by the observation that the paradoxical forearm vasoconstrictive response induced by saline load in the heart failure group was completely prevented by pretreatment with ANF. These data indirectly confirm our previous observation that ANF sensitizes cardiopulmonary receptors with vagal afferents (48) which are altered in heart failure (49).

During the steady-state phase of ANF infusion, other abnormal responses evoked by saline load in the patients with heart failure were also significantly modified. In particular, while circulating levels of ANF were maintained elevated by exogenous infusion, the natriuretic and the renin-aldosterone-inhibitory effects caused by saline loading in the heart failure group were significantly augmented as compared to those observed with lower levels of ANF. The responses modified in the presence of ANF, i.e. increased natriuresis, more profound inhibition of renin and aldosterone, and prevention of forearm vasoconstriction, clearly recall three characteristic effects of the atrial peptide (10). Therefore, higher circulating levels of ANF significantly modify the impaired adaptations to volume expansion in the heart failure group.

In summary, the present results demonstrate that in patients with dilated cardiomyopathy and mild cardiac dysfunction, plasma ANF levels do not increase in response to sustained isotonic-volume load. It should be emphasized that our observations were obtained in mild heart failure and, therefore, they cannot be extended to more severe forms of heart failure. Our results also indicate that the abnormal cardiac adaptations to volume expansion may account for the inadequate ANF response. These two abnormalities may reflect the early exhaustion of the preload reserve mechanisms in dilated cardiomyopathy. In addition, the experiments with ANF infusion strongly suggest that the lack of an appropriate rise of ANF circulating levels is at least partially responsible for the reduced natriuretic response and renin-aldosterone inhibition, as well as for the forearm vasoconstrictor response to volume expansion in heart failure. Taken together, these findings indicate that the ANF response to acute increases of preload is absent in dilated cardiomyopathy and identify the ANF deficiency as a possible cause of the altered renal, hormonal and vascular responses observed in this condition.

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