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Relation of Neurohumoral Activation to Clinical Variables and Degree of Ventricular Dysfunction: A Report From the Registry of Studies of Left Ventricular Dysfunction

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Objectives. This study examined the relation between neurohumoral activation and severity of left ventricular dysfunction and congestive heart failure in a broad group of patients with depressed left ventricular function who were not recruited on the basis of eligibility for a therapeutic trial.

Background. Previous studies have established the presence of neurohumoral activation in patients with severe congestive heart failure. It is not known whether the activation of these neurohumoral mechanisms is related to an impairment in left ventricular function.

Methods. From the 6,273 patients recruited into the Studies of Left Ventricular Dysfunction Registry (SOLVD), a subgroup of 859 patients were randomly selected, and their plasma norepinephrine, plasma renin activity, arginine vasopressin and atrial natriuretic peptide levels were correlated with clinical findings, New York Heart Association functional class, left ventricular ejection fraction and drug use.

Results. There was a weak but significant correlation between ejection fraction and an increase in plasma norepinephrine (rho = −0.18, $p < 0.0001$), plasma renin activity (rho = −0.24, $p < 0.0001$) and arginine vasopressin (rho = −0.12, $p < 0.003$). The only exception was atrial natriuretic peptide, which showed the best correlation to ejection fraction (rho = −0.37, $p < 0.0001$). Deterioration in functional class was associated more with increases in atrial natriuretic peptide ($p = 0.0003$) and plasma renin activity ($p = 0.0003$) and less with an increase in plasma norepinephrine. Of the clinical variables, elevated jugular venous pressure and third heart sound (S_3) gallop were significantly associated with increased levels of plasma norepinephrine, plasma renin activity and atrial natriuretic peptide. We then compared the relation of neurohormones with clinical signs, functional status, ejection fraction and drug therapy and controlled for mutual interactive effects. After adjustment, a decrease in ejection fraction was still significantly related to an increase in plasma norepinephrine, plasma renin activity and atrial natriuretic peptide. In contrast, only a difference between functional classes I and III/IV was associated with an increase in plasma renin activity and atrial natriuretic peptide levels.

Conclusions. Neurohumoral activation in patients with heart failure is related to severity of left ventricular functional depression, and this relation is independent of functional class or concomitant drug therapy.

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Plasma norepinephrine (1,2), plasma atrial natriuretic peptide (3) and plasma renin activity are increased in patients with heart failure (4). Despite progress in understanding the pathophysiology of heart failure, the mechanisms underlying the activation of the neurohumoral systems in patients with long-standing congestive heart failure are not well understood. In particular, it is not clear whether severity of impairment in left ventricular function or degree of symptoms relate to the level of neurohumoral activation. When cardiac output decreases after myocardial damage, neurohormones are activated to preserve circulatory homeostasis. Thus, the severity of myocardial damage, impairment of left ventricular function and severity of symptoms may relate to the degree of neurohumoral activation. For example, an increase in arginine vasopressin levels in patients with congestive heart failure has been found to correlate directly with hemodynamic severity of heart failure (5). Further, these neurohumoral responses may also be modified by patient age and by etiology of the disease. In the experimental study by Watkins et al. (6), a reduction in cardiac output and decrease in blood pressure resulted in elevation of plasma renin activity and associated vasoconstriction and sodium retention. However, the circulating neurohumoral mechanisms returned to normal during the compensated state of heart failure as plasma volume and cardiac stroke volume increased. Previous clinical studies in patients with stable heart failure have demonstrated only a modest correlation between impaired left ventricular function and neurohumoral activation (7,8). However, none of the studies took into account the interrelation of factors, such as age, severity of symptoms or use of drugs.

The Studies of Left Ventricular Dysfunction (SOLVD) Registry included patients with a wide range of depressed left ventricular function to explore the relation between neurohumoral activation and severity of left ventricular dysfunction in congestive heart failure. In a randomly selected group of patients from this SOLVD Registry, we tested the association of plasma neurohormone levels with left ventricular ejection fraction, New York Heart Association functional class and other clinical variables after controlling for selected covariates. In particular, we examined those neurohormones that have been previously demonstrated to be increased in patients with congestive heart failure (1–5).

Methods

Study design. The SOLVD was a multicenter program of research in patients with left ventricular dysfunction and heart failure. It included two trials and a registry. The Prevention Trial showed that enalapril, a converting enzyme inhibitor, prevented the development of symptoms of heart failure in patients with a left ventricular ejection fraction ≤35% without therapy for heart failure (9). The Treatment Trial demonstrated that enalapril reduced mortality in patients with mild to moderate heart failure (mainly functional classes II and III with left ventricular ejection fraction ≤35%) with symptoms of heart failure requiring treatment (10). The registry was conducted in 18 of the 23 SOLVD centers. Patients with ejection fraction ≤0.45% or a radiologically confirmed discharge diagnosis of congestive heart failure during the period January 1, 1988 to February 28, 1989 were invited to participate. Patients with nonvalvular congenital heart disease, any noncardiac life-threatening disease, lack of reliable means of follow-up or failure to consent were excluded. Baseline information abstracted from the patients' charts included the following: demographic data, clinical history, physical examination, ejection fraction, etiology of the disease, chest X-ray film, medication used, electrocardiogram (ECG) and laboratory results.

From the 6,273 patients recruited into the SOLVD Registry, a subgroup of 898 patients were randomly selected on the basis of etiology to obtain more data on "rarer" etiologies (36% of registry patients with ischemic or hypertensive heart disease, 100% of patients with other etiologies). Details of registry methods and sampling procedures have been published elsewhere (11). In this subgroup a detailed history was obtained, and physical examination, two-dimensional echocardiography, ejection fraction, chest X-ray film, 12-lead ECG and a 6-min walk test (12) were conducted. Of these patients, 859 (96%) had at least one measurement of neurohormone levels; the present analyses were conducted using this sample. In addition, neurohormonal levels were obtained in 56 age- and gender-matched control subjects with no history or physical findings of heart disease or hypertension. None were taking medications known to influence neurohormone levels.

The study protocol was approved by the local hospital review boards and the National Institutes of Health. All control subjects and patients provided written informed consent.

Neurohormone measurements. An intravenous cannula (short 18- or 20-gauge plastic catheter connected to a three-way stopcock) was inserted in an arm vein. The catheter was flushed with heparinized saline solution, and patients were then allowed to rest supine in a quiet room for 30 min. Five milliliters of blood was placed into a prechilled tube containing reduced glutathione and ethylenebis (oxyethylenenitrilo) tetraacetic acid for measurement of plasma norepinephrine. The sample was centrifuged within 1 h at 4°C at 2,500 rpm for 12 min. Plasma was then stored at −20°C or colder. Five milliliters each for plasma renin activity, arginine vasopressin and atrial natriuretic peptide analysis were placed into prechilled evacuated tubes containing sodium ethylenediaminetetraacetic acid. The three tubes were mixed gently and were placed on ice and centrifuged within 1 h of collection at 4°C at 2,500 rpm for 12 min. The supernatant plasma was stored as for plasma norepinephrine.

Neurohormone assays. Samples were shipped on dry ice to the neurohormone core laboratory at the University of Texas Medical School at Houston. Plasma norepinephrine was measured by a radioenzymatic method (13). Plasma

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renin activity was measured using the modified radioimmunoassay technique of Sealey and Laragh (14). Plasma arginine vasopressin and atrial natriuretic peptide were measured by a simplified radioimmunoassay using commercially available antibodies (15). All samples were analyzed in a blinded manner without knowledge of the patients' characteristics.

Sensitivity and reproducibility of assays. The radioenzymatic assay for plasma norepinephrine has a sensitivity of 1 pg/ml with a sample/blank radioactivity count ratio >2. In addition, the interassay coefficient of variation was 6.1% (calculated from 20 different assays done consecutively on 20 different days). The radioimmunoassay for plasma renin activity is dependent on the sensitivity of the antibody used in the assay, which can reliably detect 2 pg of angiotensin I (sample/blank ratio 2). This assay can detect very low plasma renin activity (0.1 ng/ml per h). The interassay coefficient of variation for plasma renin activity was 12.6%. The radioimmunoassay for atrial natriuretic peptide has a sensitivity of 2 pg/ml, and the sensitivity for arginine vasopressin is 0.22 pg/ml. The interassay coefficients of variation for atrial natriuretic peptide and arginine vasopressin were 13.7% and 11.6%, respectively. In addition, previously assayed quality control samples of plasma norepinephrine were mailed every 2 months from the core laboratory to the SOLVD study centers. These samples were stored and returned in the same way as the patient samples. On return these quality control samples were reanalyzed to assess the stability of samples during storage and transportation. There were no significant differences in the plasma norepinephrine values (278 ± 28 vs. 269 ± 32 pg/ml, p = NS) in 123 samples analyzed in this manner.

Left ventricular dimensions and ejection fraction were measured by two-dimensional echocardiography using the multiple diameter method (16). Left ventricular dimensions were measured below the tip of the mitral leaflets at end-diastole (onset of QRS complex) and at end-systole (one frame before mitral valve opening). The average variability in ejection fraction measurement was <3%. Left atrial dimensions were taken at the point of maximal anterior root motion in the same plane as the aortic root. In most cases (78.4%) ejection fraction was measured on the same day that neurohormone levels were determined. All left ventricular measurements were made by the echocardiographic core laboratory at Baylor College of Medicine in Houston.

Statistical methods. The neurohormonal data were not normally distributed. Simple comparisons and correlations were therefore conducted using nonparametric methods (e.g., Spearman rho correlation [17]). The rest of the analyses were multivariate linear models conducted on the natural logarithms of the neurohormone values (18). This transformation was selected because it was found to make the distributions normal or near normal. All models were adjusted for clinic (17 dummy codes to define the 18 clinics) and etiology (one dummy code to determine ischemic or hypertensive heart disease vs. other etiology). This latter adjustment was to control for the differing proportions of etiologies represented, by design, in the sample. Eighteen clinical variables were tested against each of the neurohormones.* Ejection fraction and all other continuous variables were treated as such in the models. Two dummy variables categorized functional class at three levels: class I versus II versus III/IV. Concomitant drug use (angiotensin-converting enzyme or diuretic agents, or both) was also categorized at three levels: neither versus one versus both types of medication prescribed. All other categoric variables were considered at two levels. All p values are two-tailed.

Results

Baseline clinical data. A total of 6,273 patients were recruited into the registry on the basis of an ejection fraction ≤45% (87%) or radiologic evidence of congestive heart failure (13%). Of these, 69% had signs of congestive heart failure (defined as the presence of edema, third heart sound (S_3) gallop, elevated jugular venous pressure on examination or history of breathlessness on exertion). In the registry, as in the main SOLVD trial, ischemic heart disease was the dominant etiology of heart failure. However, in the subgroup of 859 patients participating in the present analysis, the frequency of etiology was different because of stratification that oversampled subjects with heart failure due to etiologies other than ischemic or hypertensive heart disease. The characteristics of patients entering the registry substudy are presented in Table 1. The age and gender distribution was comparable to that of the control group. The average age in the registry substudy group was 59 ± 12 years (mean ± SD, range 21 to 91); in the control group the average age was 56 ± 12 years (range 37 to 80). Men comprised 78% of the participants in the registry substudy, and underlying ischemic heart disease was present in 52%. In the control group 70% were men. In the registry substudy group the mean cardiothoracic ratio and left ventricular ejection fraction were 0.53 ± 0.07 and 30.0 ± 9.0%, respectively. Median values and interquartile ranges (25% to 75%) for all four neurohormones were significantly higher in patients entering the registry substudy compared with control subjects (plasma norepinephrine, 420 [312 to 592] vs. 316 [246 to 446] pg/ml, $p < 0.0001$; plasma renin activity, 1.3 [0.4 to 4.5] vs. 0.5 [0.3 to 0.8] ng/ml per h, $p < 0.0001$; atrial natriuretic peptide, 72 [42 to 130] vs. 48 [31 to 74] pg/ml, $p < 0.0001$; arginine vasopressin, 2.2 [1.7 to 2.9] vs. 1.8 [1.4 to 2.4] pg/ml, $p < 0.0007$, respectively).

Neurohormone levels and left ventricular function. Figure 1 shows the relation between ejection fraction and neuro-

*The 18 clinical variables tested were age, gender, race, ejection fraction, New York Heart Association functional class, etiology of heart failure, elevated jugular venous pressure, third heart sound (S_3) gallop, pulmonary edema, history of peripheral edema, history of breathlessness, signs of congestive heart failure, distance walked in 6-min walk test, cardiothoracic ratio on chest X-ray film, diuretic use, digoxin use, beta-adrenergic blocking agent and angiotensin-converting enzyme inhibitor use.

Table 1. Patient Characteristics

	Registry (Participants not in Substudy) (n = 5,414)	Registry Substudy (n = 859)
Age (yr)	63 ± 12	59 ± 12
Male (%)	73	78
Race (%)		
White	86	85
Black	11	11
Other	1	2
Etiology (%)		
Ischemic heart disease	71	52
Idiopathic cardiomyopathy	11	24
Hypertensive heart disease	8	4
Aortic valvular heart disease	4	7
Specific*	3	8
Mitral valvular heart disease	3	5
Myocarditis	0.1	0.5
Ejection Fraction (%)	31 ± 9	30 ± 9
NYHA functional class (%)		
I	NA	36.6
II	NA	47.4
III	NA	14.3
IV	NA	1.7
CT ratio	NA	0.53 ± 0.07
Diuretic agent use (%)	55	58
Digitalis use (%)	43	47
Beta-blocker use (%)	15	16
ACE inhibitor use (%)	33	33
Serum sodium (mEq/liter)	138 ± 4	139 ± 3
Serum potassium (mEq/liter)	4.3 ± 0.5	4.3 ± 0.5
Blood urea nitrogen (mg%)	22 ± 13	19 ± 9
Serum creatinine (mg%)	1.3 ± 0.5	1.2 ± 0.3

*Specific etiologic group included the following causes: alcoholic cardiomyopathy, chronic myocarditis, cocaine related, diabetic cardiomyopathy, hemochromatosis, hyperthyroidism, idiopathic hypertrophic obstructive cardiomyopathy, postpartum cardiomyopathy, sarcoid heart disease, rheumatic heart disease and acute or subacute bacterial endocarditis. Data presented are mean value ± SD or percent of patients. ACE = angiotensin-converting enzyme; CT ratio = cardiothoracic ratio; NA = not applicable; NYHA = New York Heart Association.

hormones. There was a weak but significant correlation between decrease in ejection fraction and increase in plasma norepinephrine (rho = −0.18, p < 0.0001), plasma renin activity (rho = −0.24, p < 0.0001) and arginine vasopressin (rho = −0.12, p < 0.003). The only exception was atrial natriuretic peptide, which showed the best correlation with ejection fraction (rho = −0.37, p < 0.0001). Because patients with a lower ejection fraction are more likely to be taking larger doses of diuretic agents and angiotensin-converting enzyme inhibitors, we adjusted for the effect of concomitant diuretic agent or angiotensin-converting enzyme inhibitor therapy, or both. Even after adjusting for the differences in drug treatment and functional class, ejection fraction was significantly related to log plasma norepinephrine (p < 0.0001), log plasma renin activity (p < 0.0001) and log atrial natriuretic peptide (p < 0.0001) but not to log arginine vasopressin (Table 2).

We also examined the relation between neurohormones and left atrial size and left ventricular systolic or diastolic dimensions. All four neurohormones statistically significantly correlated with either left atrial size or left ventricular systolic or diastolic dimensions (Table 3). However, except for atrial natriuretic peptide, correlations with other neurohormones were weak. Plasma atrial natriuretic peptide level most closely correlated with left atrial size (rho = 0.34, p < 0.0001) and left ventricular systolic volume (rho = 0.30, p < 0.0001). This is consistent with the observation that of the four neurohormones examined, atrial natriuretic peptide showed the best correlation with cardiothoracic ratio on chest X-ray film (rho = 0.30, p < 0.0001). This association between enlarged cardiac size and atrial natriuretic peptide levels is supported further by the fact that patients with elevated jugular venous pressure had significantly higher levels of atrial natriuretic peptide, as did those with pulmonary edema (p < 0.0001 for both analyses).

Neurohormone levels, functional class and 6-min walk test. Figure 2 shows the relation between functional class and the neurohormones. As with ejection fraction, as the functional status deteriorated in patients from functional class I to IV, there was an early increase in plasma atrial natriuretic peptide (p = 0.0003) and plasma renin activity (p = 0.0003) levels (class I vs. III/IV). In contrast, plasma arginine vasopressin levels did not change significantly with a deterioration in functional class. Plasma norepinephrine levels differed significantly only between those in functional class IV versus classes I to III (p < 0.002). However, it should be noted that only a small number of patients were in functional class IV (14 patients). Patients with a low ejection fraction and those in a higher functional class were likely to be taking larger doses of diuretic agents or angiotensin-converting enzyme inhibitors, or both. When we adjusted for the effect of diuretic agent or angiotensin-converting enzyme inhibitor therapy, or both, as well as ejection fraction, the relation between functional class and neurohormone levels was less striking and was observed only with atrial natriuretic peptide and plasma renin activity in patients in functional class I versus III/IV (atrial natriuretic peptide, p < 0.0001; plasma renin activity, p = 0.04) (Table 2). Because of the possible association between functional class and submaximal exercise capacity, we also examined the correlation between the distance covered during the 6-min walk test and plasma neurohormone levels. Although the levels of all four neurohormones correlated statistically to the distance walked, these correlations were very weak (Table 3).

Neurohormone levels and clinical variables. The relation between the four neurohormones and clinical variables is shown (Table 4). After adjusting for clinic and etiology, the clinical variables associated with log plasma norepinephrine were age, elevated jugular venous pressure and third heart sound (S_3) gallop. Significantly higher plasma norepinephrine levels were present in older subjects (p = 0.002) and in

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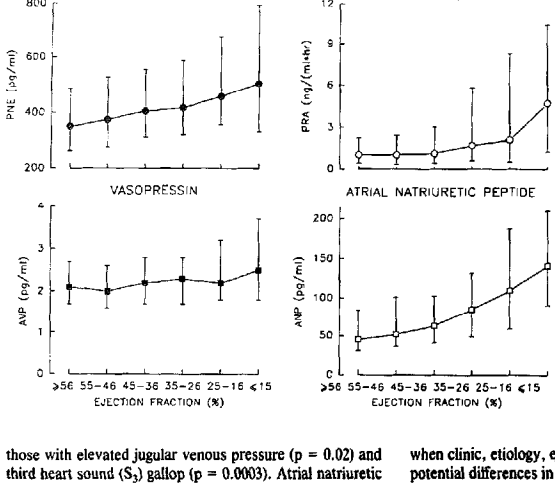

Figure 1. Relation between left ventricular ejection fraction and the four neurohormones. Left ventricular ejection fraction was grouped according to patients with ejection fraction ≥56% (90 patients) 55% to 46% (123 patients), 45% to 36% (138 patients), 35% to 26% (218 patients), 25% to 16% (166 patients) and <16% (28 patients). Note the increase in plasma norepinephrine (PNE), plasma renin activity (PRA) and atrial natriuretic peptide (ANP) with increasing left ventricular dysfunction. In contrast, arginine vasopressin (AVP) does not change. Median values and interquartile ranges (25% to 75%) for the four neurohormones are shown.

those with elevated jugular venous pressure ($p = 0.02$) and third heart sound (S_3) gallop ($p = 0.0003$). Atrial natriuretic peptide levels were also significantly higher in older patients ($p = 0.0003$). Patients with third heart sound (S_3) gallop had significantly higher atrial natriuretic peptide levels ($p = 0.0001$), as did those with pulmonary congestion on chest X-ray film ($p = 0.0001$) and elevated jugular venous pressure ($p = 0.0001$). Age, race, elevated jugular venous pressure and third heart sound (S_3) gallop were associated with plasma renin activity. In contrast to plasma norepinephrine and atrial natriuretic peptide, plasma renin activity was significantly lower in older patients ($p = 0.03$). There was a small but statistically significantly higher plasma renin activity in white patients than in black patients ($p = 0.04$), even when clinic, etiology, ejection fraction, functional class and potential differences in drug therapy were adjusted between the two groups. The increase in plasma renin activity level was also significantly related to elevated jugular venous pressure ($p = 0.0001$) and third heart sound (S_3) gallop ($p = 0.0001$). It is possible that in the more symptomatic patients, larger doses of diuretic agents or angiotensin-converting enzyme inhibitors may affect plasma renin activity levels. We therefore reexamined the relation between these clinical variables and plasma renin activity, after adjusting for the effect of drug therapy. Even with this adjustment a significant relation was found between increased plasma renin activity levels and third heart sound (S_3) gallop ($p = 0.0002$) and elevated jugular venous pressure ($p = 0.003$). In contrast

Table 2. Relation Between Neurohormones, Ejection Fraction and New York Heart Association Functional Class With and Without Adjustment for Significant Covariables*

	Ejection Fraction		Functional Class	
Neurohormone	Unadjusted	Adjusted for NYHA Functional Class and Diuretic Agent/ACE Inhibitor Use	Unadjusted	Adjusted for EF and Diuretic Agent/ACE Inhibitor Use
Log PNE	− ($p < 0.0001$)	− ($p < 0.0001$)	+ ($p = 0.01$)	NS
Log PRA	− ($p < 0.0001$)	− ($p < 0.0001$)	+ ($p = 0.0001$)	+ ($p = 0.04$ when comparing class I with III/IV; p = NS when comparing class I with II)
Log AVP	− ($p = 0.02$)	NS	NS	NS
Log ANP	− ($p < 0.0001$)	− ($p < 0.0001$)	+ ($p = 0.0001$)	+ ($p = 0.0001$ when comparing class I with III/IV; p = NS when comparing class I with II)

*All analyses adjusted for clinic and etiology of heart disease. The direction of relation of increase in neurohormonal levels to decrease in ejection fraction (EF) or deterioration (increase) in New York Heart Association (NYHA) functional class is provided only if statistically significant. ACE = angiotensin-converting enzyme; ANP = atrial natriuretic peptide; AVP = arginine vasopressin; PNE = plasma norepinephrine; PRA = plasma renin activity.

Table 3. Correlation of Plasma Neurohormones With Left Ventricular Dimensions and 6-min Walk Test

	PNE	PRA	AVP	ANP
LV systolic volume (cm)				
Rho	0.13	0.22	0.10	0.30
p value	0.0005	<0.0001	0.009	<0.0001
LV diastolic volume (cm)				
Rho	0.06	0.19	0.10	0.26
p value	NS	<0.0001	0.007	<0.0001
LA dimension (cm)				
Rho	0.04	0.05	0.08	0.34
p value	NS	NS	NS	<0.0001
CT ratio				
Rho	0.03	−0.02	0.10	0.30
p value	NS	NS	0.02	<0.0001
6-min walk distance (m)				
Rho	−0.10	−0.11	−0.12	−0.19
p value	0.009	0.002	0.002	<0.0001

ANP = atrial natriuretic peptide; AVP = arginine vasopressin; CT ratio = cardiothoracic ratio; LA = left atrium; LV = left ventricle; PNE = plasma norepinephrine; PRA = plasma renin activity; Rho = Spearman rank-order correlation coefficient.

to the other three neurohormones, arginine vasopressin was not significantly associated with age or clinical findings of congestive heart failure, except for elevated jugular venous pressure ($p = 0.03$) and pulmonary edema on chest X-ray film ($p = 0.003$).

Elevated jugular venous pressure or third heart sound (S_3) gallop are more likely to occur in patients with lower ejection fraction or in those in a higher functional class. Because there was a significant relation between plasma neurohormone levels and left ventricular ejection fraction and functional class, we determined whether the observed relation between neurohormone levels and various clinical features was dependent on the degree of underlying left ventricular dysfunction or functional class. For plasma norepinephrine and plasma renin activity, when the influence of ejection fraction and functional class was controlled, only the presence of third heart sound (S_3) gallop remained significantly related to plasma norepinephrine ($p = 0.03$) or plasma renin activity ($p = 0.04$) levels. For atrial natriuretic peptide and arginine vasopressin, when the influence of ejection fraction and functional class was controlled, only pulmonary edema on chest X-ray film remained related to atrial natriuretic peptide ($p = 0.009$) and arginine vasopressin ($p = 0.006$) levels.

Relation between drug therapy and plasma neurohormone levels. Treatment with diuretic agents and angiotensin-converting enzyme inhibitors is known to increase plasma renin activity. Therefore, we examined the effect of diuretic agents with and without angiotensin-converting enzyme inhibitor therapy on plasma neurohormone levels (Table 5). Significantly elevated levels of plasma norepinephrine ($p = 0.04$), plasma renin activity ($p < 0.0001$), atrial natriuretic peptide ($p < 0.0001$) and arginine vasopressin ($p = 0.0003$) were found in patients with than without diuretic agent therapy. Addition of angiotensin-converting enzyme inhibitors (enalapril, captopril or lisinopril) significantly increased plasma renin activity levels only, regardless of whether the patient had diuretic agent therapy or not ($p < 0.0001$).

It is possible that the more symptomatic patients may have received more diuretic agent or angiotensin-converting

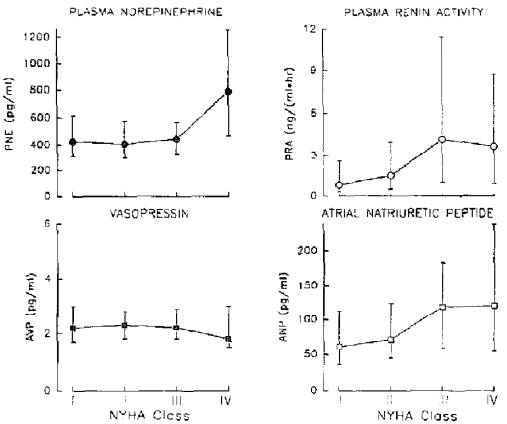

Figure 2. Relation between New York Heart Association (NYHA) functional class and the four neurohormones. Functional classes were grouped as follows: class I = 306 patients; class II = 397 patients; class III = 127 patients; class IV = 14 patients. Note that arginine vasopressin (AVP) does not increase with a deterioration in functional class. Plasma norepinephrine (PNE) increases significantly only when the patient's condition deteriorates to class IV. Both plasma renin activity (PRA) and atrial natriuretic peptide (ANP) show an early increase as functional class deteriorates and are not significantly different in class IV patients. Median values and interquartile ranges (25% to 75%) are shown.

Table 4. Descriptive Statistics of Norepinephrine, Plasma Renin Activity, Arginine Vasopressin and Atrial Natriuretic Peptide Concentrations (Median and Interquartile Ranges) by Clinical Variable

	Plasma Norepinephrine		Plasma Renin Activity		Arginine Vasopressin		Atrial Natriuretic Peptide	
	n	pg/ml	n	ng/ml per h	n	pg/ml	n	pg/ml
Age (yr)								
≤50	153	375 (286–559)	156	1.74 (0.5–6.7)	125	2.2 (1.7–3.0)	124	57 (35–128)
51–60	241	397 (301–557)	242	1.54 (0.4–3.8)	223	2.2 (1.7–2.8)	222	68 (42–122)
61–70	303	421 (321–608)	306	1.2 (0.4–4.9)	270	2.3 (1.8–3.0)	279	79 (45–143)
71–80	98	482 (367–612)	99	1.2 (0.4–3.8)	95	2.1 (1.7–2.9)	95	84 (51–146)
>80	16	525 (430–736)	16	0.8 (0.3–1.1)	16	2.2 (1.7–3.4)	16	112 (47–129)
Gender								
Female	179	426 (336–605)	183	1.3 (0.4–4.0)	167	2.1 (1.7–2.5)	167	67 (46–130)
Male	633	416 (308–583)	637	1.3 (0.4–4.7)	571	2.2 (1.7–3.0)	570	74 (42–132)
Race								
Black	96	411 (311–541)	97	0.6 (0.09–2.7)	82	2.6 (2.0–3.8)	82	78 (49–167)
White	684	422 (312–596)	691	1.5 (0.5–4.9)	628	2.2 (1.7–2.8)	628	71 (42–129)
Other	15	395 (308–494)	15	0.5 (0.09–2.3)	12	2.1 (1.5–3.2)	11	86 (42–182)
Etiology								
IHD	419	415 (310–595)	425	1.2 (0.4–3.8)	398	2.1 (1.7–2.7)	398	69 (42–122)
Idiopathic	192	428 (322–592)	194	1.8 (0.5–5.6)	162	2.2 (1.7–3.0)	162	75 (44–136)
Other	100	424 (314–548)	100	0.9 (0.3–4.3)	90	2.3 (1.8–3.0)	89	96 (52–145)
Specific	69	452 (305–626)	68	2.0 (0.4–6.8)	56	2.4 (2.0–3.8)	56	76 (34–139)
HHD	32	390 (256–538)	33	1.3 (0.4–3.4)	32	2.8 (2.1–3.4)	32	78 (52–132)
Elevated JVP								
Yes	61	465 (355–651)	61	4.9 (0.6–11.2)	52	2.6 (2.6–3.4)	51	137 (66–215)
No	742	415 (312–583)	750	1.3 (0.4–3.8)	677	2.2 (1.7–2.8)	677	69 (42–125)
S_3 gallop								
Yes	109	486 (363–626)	109	3.5 (1.1–9.4)	93	2.3 (1.9–3.0)	92	117 (60–184)
No	695	409 (309–586)	703	1.2 (0.4–3.6)	637	2.2 (1.7–2.8)	637	69 (41–123)
Pulmonary edema*								
Yes	195	433 (329–617)	197	1.6 (0.5–6.3)	167	2.5 (2.0–3.5)	167	101 (60–162)
No	542	420 (310–594)	552	1.3 (0.4–4.0)	509	2.1 (1.7–2.8)	508	67 (40–122)
CT ratio								
≤ 0.52	341	425 (317–587)	348	1.5 (0.6–4.0)	311	2.2 (1.7–2.8)	311	61 (38–100)
> 0.52	342	432 (313–626)	345	1.3 (0.4–4.7)	315	2.3 (1.8–3.1)	314	98 (53–167)
Digitalis use								
Yes	392	423 (318–596)	394	2.2 (0.6–6.9)	357	2.3 (1.8–3.2)	356	89 (52–152)
No	415	413 (307–575)	422	1.0 (0.3–2.4)	376	2.1 (1.7–2.7)	376	62 (38–111)
Beta-blocker use								
Yes	121	408 (306–559)	125	0.6 (0.2–1.9)	117	2.0 (1.7–2.6)	117	69 (41–118)
No	685	422 (313–594)	690	1.6 (0.5–5.2)	616	2.2 (1.7–2.9)	615	73 (44–133)

*Pulmonary congestion as determined by chest X-ray film. †Median value for cardiothoracic (CT) ratio was 0.52. The small differences in the number (n) of patients for each neurohormone is due to the fact that some of the samples received were technically inadequate for analysis. HHD = hypertensive heart disease; specifically including alcoholic and postpartum cardiomyopathies, other included myocarditis and valvular heart disease; IHD = ischemic heart disease; JVP = jugular venous pressure; S_3 gallop = third heart sound; other abbreviations as in Table 3.

enzyme inhibitor therapy, or both, and the observed relation between drugs and neurohormones may have been related to the lower ejection fraction or higher functional class in these patients. When the effects of ejection fraction and functional class as well as clinic and etiology were adjusted, diuretic agent or angiotensin-converting enzyme inhibitor therapy or both, significantly altered plasma renin activity ($p < 0.0001$) and atrial natriuretic peptide ($p = 0.02$) levels only. The median plasma renin activity level in patients without diuretic agents or angiotensin-converting enzyme inhibitors was 0.5 ng/ml per h (range 0.2 to 1.2) (217 patients). This increased to 1.35 ng/ml per h (range 0.4 to 2.7) (190 patients) with the addition of a diuretic agent and to 2.9 ng/ml per h (range 0.5 to 11.4) (63 patients) when an angiotensin-converting enzyme inhibitor was added. The highest levels, 3.8 ng/ml per h (range 1.15 to 10.3), were observed in patients receiving both diuretic agent and angiotensin-converting enzyme inhibitor (232 patients). A similar increase in median atrial natriuretic peptide levels was also noted with diuretic agent or angiotensin-converting enzyme inhibitor therapy, or both. The lowest levels (56.4 pg/ml, range 37.0 to 94.0) were seen in patients receiving neither angiotensin-converting enzyme inhibitors nor diuretic agents. With either angiotensin-converting enzyme inhibitor or diuretic agent only, the level of atrial natriuretic peptide increased to 70.6 pg/ml (range 39.2 to 121.3) and 85.6 pg/ml

Table 5. Descriptive Statistics of Norepinephrine, Plasma Renin Activity, Arginine Vasopressin and Atrial Natriuretic Peptide Concentrations (Median and Interquartile Ranges) With Diuretic Agent or Angiotensin-Converting Enzyme Inhibitor Use, or Both

		Plasma Norepinephrine		Plasma Renin Activity		Arginine Vasopressin		Atrial Natriuretic Peptide	
	Drug Use	n	pg/ml	n	ng/ml per h	n	pg/ml	n	pg/ml
Diuretic agent	No	210	419 (309–579)	217	0.5 (0.2–1.2)	200	2.1 (1.6–2.6)	200	56.4 (37–94)
ACE inhibitor	No								
Diuretic agent	Yes	190	425 (305–594)	190	1.35 (0.4–2.7)	172	2.4 (1.8–3.3)	172	85.6 (48.1–152.1)
ACE inhibitor	No								
Diuretic agent	No	64	392 (280–552)	63	2.9 (0.5–11.4)	56	2.2 (1.8–2.8)	50	70.6 (39.2–121.3)
ACE inhibitor	Yes								
Diuretic agent	Yes	230	435 (332–628)	232	3.8 (1.2–10.3)	214	2.2 (1.8–3.0)	213	87.6 (51.7–145.3)
ACE inhibitor	Yes								

ACE = angiotensin-converting enzyme; other abbreviations as in Tables 3 and 4.

(range 48.1 to 152.1), respectively. As with plasma renin activity, the highest levels of atrial natriuretic peptide were observed in patients receiving both diuretic agents and angiotensin-converting enzyme inhibitors (87.6 pg/ml, range 51.7 to 145.3). Of the other drugs, treatment with digitalis and beta-blockers also had a significant effect on plasma renin activity and atrial natriuretic peptide levels, after adjusting for ejection fraction, functional class, clinic and etiology. Digitalis use was significantly associated with increased plasma renin activity ($p < 0.0001$) and atrial natriuretic peptide ($p = 0.04$) levels. Beta-blocker use was associated with decreased plasma renin activity ($p < 0.0003$) and increased atrial natriuretic peptide ($p = 0.04$) levels.

Discussion

Neurohumoral activation is an important manifestation of heart failure. Previous studies have focused predominantly on patients with severe congestive heart failure. To date this is the largest study to examine neurohumoral activity in patients with a broad range of left ventricular dysfunction and congestive heart failure. Furthermore, patients in this study were not recruited on the basis of eligibility for a therapeutic trial and are thus likely to be more representative of the population of patients with left ventricular dysfunction. These results show that when compared with age- and gender-matched control subjects, all four neurohormones are elevated in patients with left ventricular dysfunction. The wide range of neurohormonal levels suggests highly variable neurohumoral activity in these patients. When patients develop congestive heart failure secondary to impairment of left ventricular function, there is an increase in neuroendocrine activity, even at the expense of increased blood volume and further reduction of myocardial function (19). Therefore, it is not surprising that we found a significant relation between elevated levels of plasma norepinephrine, plasma renin activity and atrial natriuretic peptide and third heart sound (S_3) gallop and elevated jugular venous pressure.

Plasma norepinephrine. All four neurohormones were statistically significantly but weakly correlated with left ventricular ejection fraction, with atrial natriuretic peptide showing the best correlation. This relation was demonstrable even after excluding the effect of drug therapy on various neurohormones. The absolute plasma norepinephrine level in patients with congestive heart failure is influenced by alterations in neuronal uptake and clearance and metabolism of norepinephrine released from the sympathetic nerve endings. However, measurement of efferent sympathetic nerve traffic by intraneuronal recording in the peroneal nerve in patients with heart failure demonstrates evidence of increased central sympathetic outflow (20). If the stimulus for activation of the sympathetic nervous system is poor pump function, then it is not unreasonable to expect a correlation between left ventricular ejection fraction and plasma norepinephrine levels. Previous clinical studies in patients with moderate to severe congestive heart failure have shown a weak correlation between the hemodynamic severity of congestive heart failure and plasma norepinephrine levels (8,21). This study also demonstrated a weak correlation between plasma norepinephrine and left ventricular ejection fraction in an expanded cohort of patients with a broader spectrum of left ventricular dysfunction.

Plasma renin activity. The neurohormones to show the strongest correlation with ejection fraction were plasma renin activity and atrial natriuretic peptide, which increased as ejection fraction declined. This relation was consistent in patients with and without diuretic agents or angiotensin-converting enzyme inhibitors, or both. Although several studies have reported stimulation of the renin-angiotensin system in congestive heart failure, there is no agreement as to its frequency or magnitude in patients with varying degrees of heart failure. Dzau et al. (22) reported that the system is markedly activated during acute decompensation but is near normal when patients have recovered from the acute episode. Therefore, the clinical status of the patient in congestive heart failure (compensated or decompensated) will affect the measured plasma renin activity levels. Further, diuretic agent use and sodium restriction affect the renin-angiotensin system and increase plasma renin activity (23,24). However, Anand et al. (25) showed that five of eight patients with advanced, untreated chronic congestive heart

failure, with salt and water retention, had elevated plasma renin activity levels. Similar findings have been reported by Brown et al. (26) in untreated patients with congestive heart failure. These data suggest that depending on the cohort of patients investigated and the clinical severity of congestive heart failure involved and treatments used, plasma renin activity levels can vary significantly. In this study, we investigated patients with varying severity of congestive heart failure who were taking a wide variety of drugs. Even when we controlled for the effect of diuretic agent or angiotensin-converting enzyme inhibitor therapy, or both, there was a significant relation between ejection fraction and plasma renin activity. This suggests that severity of left ventricular dysfunction, either directly (inadequate renal perfusion) or indirectly (renal sympathetic stimulation and renin release), or both, may modulate the plasma renin activity measured in patients with congestive heart failure.

Atrial natriuretic peptide. Atrial natriuretic peptide is synthesized in the myocardium and released mainly in response to increased atrial stretching (27). Plasma atrial natriuretic peptide levels increase with increased atrial pressure and atrial stretch in patients with worsening heart failure (28,29). Hara et al. (30) found that atrial natriuretic peptide levels in plasma correlate inversely with the level of ejection fraction and directly with the severity of heart failure. Similarly, Rouleau et al. (31) found an inverse correlation between atrial natriuretic peptide and cardiac index. In this study atrial natriuretic peptide was inversely correlated to ejection fraction. More important, it was the hormone that correlated most strongly with left atrial and left ventricular systolic and diastolic dimensions as well as with cardiothoracic ratio. However, these correlations were modest, which could have been due to the fact that the atrial dimensions measured by two-dimensional echocardiography may not correlate strongly with left atrial pressure as a result of stretching and alterations in atrial compliance in patients with chronic heart failure (32), the cardiothoracic ratio could also be altered by the presence of concomitant lung disease. Because patients with increased cardiac size are most likely to have elevated jugular venous pressure, pulmonary congestion on chest X-ray film and clinical evidence of congestive heart failure, it is not surprising that the elevated levels of atrial natriuretic peptide were also significantly related to these clinical findings.

Argine vasopressin. Of the four neurohormones examined, plasma arginine vasopressin levels showed the smallest increase in patients with congestive heart failure compared with normal subjects and showed the weakest association with a difference in ejection fraction or functional class. This may be due to the fact that in congestive heart failure there is an augmentation of the release of arginine vasopressin, which may not increase further as the clinical condition deteriorates. Although a previous study demonstrated an increase in arginine vasopressin levels in direct proportion to hemodynamic and clinical severity of heart failure (5), the present study failed to show a strong relation between arginine vasopressin and ejection fraction or functional class. In congestive heart failure the precise mechanism for the release of arginine vasopressin is unclear but is believed to be due to nonosmotic causes (33,34). Plasma arginine vasopressin and plasma renin activity (35) levels are frequently increased in parallel because of compromised end-organ perfusion. Furthermore, baroreceptor stimulation is a common stimulus for release of both arginine vasopressin and plasma renin activity, and increases in angiotensin II may directly stimulate the hypophyseal production of arginine vasopressin (36). Although we observed progressive increases in plasma renin activity with increasing left ventricular dysfunction, there was no increase in arginine vasopressin levels. This suggests that release of arginine vasopressin is probably not a primary mechanism for the maintenance of peripheral resistance in most patients with congestive heart failure, and the nonosmotic mechanisms of arginine vasopressin release may induce only a limited increase in plasma arginine vasopressin levels.

Effect of drug therapy. In previous studies in patients with severe congestive heart failure, hemodynamic indexes of cardiac function or left ventricular ejection fraction have been shown to bear little relation to the measures of functional status or exercise capacity (37,38). Therefore we also examined the relation between the neurohormones and functional class and the 6-min walk test. There was no association between arginine vasopressin and functional class, which is consistent with the possibility that arginine vasopressin may not be a primary neurohormone activated in heart failure. Plasma norepinephrine was significantly higher only in patients in functional class IV. This is similar to findings that patients with severe symptomatic heart failure have the highest plasma norepinephrine levels (39,40). However, it should be noted that there were only 14 patients in our study with functional class IV symptoms. Therefore, this finding must be interpreted with caution because the small number of patients in this group may have produced this relation. In contrast plasma renin activity and atrial natriuretic peptide showed an increase in patients with functional class II symptoms. The parallel increase in both hormones as the functional capacity decreases is not surprising because many of the physiologic effects of atrial natriuretic peptide oppose the effects of the renin-angiotensin system. Thus, the secretion of atrial peptides may increase to counteract many of the detrimental circulatory and renal effects of angiotensin II. Weak but statistically significant correlations were found between exercise capacity (6-min walk test) and all four neurohormones. This indicates that although patients with neurohumoral activation also have impairment of exercise capacity, it is likely that other mechanisms influence the patient's ability to exercise.

Functional capacity and neurohormones. When the effects of diuretic agents or angiotensin-converting enzyme inhibitors, or both, were adjusted using a multivariate regression model, patients with lower ejection fraction were found to have significantly higher plasma norepinephrine,

atrial natriuretic peptide and plasma renin activity levels, whereas those in a higher functional class had significantly higher levels of atrial natriuretic peptide and plasma renin activity. Similarly, other studies have shown high plasma norepinephrine (25) or plasma renin activity levels (25) in patients with untreated heart failure. When diuretic agents and angiotensin-converting enzyme inhibitors are added for the management of congestive heart failure, patients have a significant increase in plasma renin activity levels (24). The increase in plasma renin activity with angiotensin-converting enzyme inhibitor therapy is usually secondary to the decrease in angiotensin II levels in the circulation, which leads to a reflex increase in plasma renin activity levels because of the absence of negative feedback. Therefore, it is reasonable to assume that patients with low ejection fraction had increased neurohormonal levels that increased further with the addition of concomitant diuretic agent or angiotensin-converting enzyme inhibitor therapy, or both.

Conclusions. This study broadens our understanding of the neurohumoral activation that was obtained from the first SOLVD neurohormonal study, which showed that neuroendocrine activation occurs in asymptomatic patients with depressed left ventricular ejection fraction and is increased further with development of symptoms and addition of drug therapy (41). The results from this study suggest that in patients with congestive heart failure, the increase in plasma neurohormonal levels may be linked to a depression in left ventricular ejection fraction that is modulated by a complex interplay between symptoms, age of the patient and the medications used for treatment.

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