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Articles

Altered Tissue Degradation and Distribution of Atrial Natriuretic Peptide in Patients With Idiopathic Dilated Cardiomyopathy and Its Relationship With Clinical Severity of the Disease and Sodium Handling

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▶ Abstract

Background Atrial natriuretic peptide (ANP) has been suggested to play an important role in heart failure, preserving cardiorenal homeostasis through maintenance of the sodium balance and inhibition of the detrimental effects of the neurohormonal vasoconstrictor system. The current study was designed to investigate whether there is a disturbed renewal and distribution of ANP in patients with idiopathic dilated cardiomyopathy (IDC) with differing clinical severity of disease.

Methods and Results We used a tracer method to perform a cross-sectional study of 15 IDC patients with differing clinical severity (New York Heart Association functional class I to III), prospectively divided into two groups according to their functional class (group 1, classes I and II; group 2, classes II-III and III). Eleven normotensive, nonobese male volunteers also were studied as a control group. Main ANP kinetic parameters were derived from the disappearance curve of the labeled hormone after the bolus injection of [¹²⁵I]-labeled ANP. A high-performance liquid chromatography technique was used to separate the radiolabeled hormone in each plasma sample. Patients in group 1 showed higher ANP metabolic clearance rate (MCR) ($2731.9 \pm 726.2 \text{ mL} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$) than patients of group 2 ($1718.4 \pm 621.2 \text{ mL} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$) and control subjects ($1873.1 \pm 551.2 \text{ mL} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$). ANP disposal (MCR) positively correlated with biological hormonal effect (urinary sodium excretion) both in control subjects and in patients. In IDC patients of both groups, however, MCR values were always higher (approximately doubled) than the values found in control subjects at the corresponding sodium excretion. This finding indicates that a reduced ANP biological activity is associated with hormone degradation in patients. Moreover, patients of group 2 showed significantly higher ANP production rates ($395.6 \pm 183.8 \text{ ng} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$)

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than group 1 ($166.0 \pm 139.0 \text{ ng} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$) and control subjects ($130.7 \pm 105.4 \text{ ng} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$) despite a marked reduction in sodium excretion. Patients with IDC showed a progressive reduction in the total distribution volume (group 1, $19.8 \pm 5.8 \text{ L/m}^2$; group 2, $12.7 \pm 6.9 \text{ L/m}^2$; control subjects, $27.0 \pm 11.6 \text{ L/m}^2$) of the hormone; this probably was due to a reduction in exchanges of ANP with peripheral tissues.

Conclusions Our study demonstrates a markedly altered degradation and distribution of ANP in patients with IDC, even in those at the early stage of clinical disease (classes I and II, group 1) who have ANP plasma levels in the normal range.

Key Words: atrial natriuretic factor • cardiomyopathy • metabolism • peptides

► Introduction

In recent years, it has been postulated that patients with idiopathic dilated cardiomyopathy (IDC) may develop heart failure not only as a result of primary deficiency of cardiac contractility but also as a result of an alteration of peripheral mechanisms responsible for regulation of vascular contractility and of fluid and electrolyte homeostasis.^{1 2} Sodium and water retention, which are the principal clinical characteristics of congestive heart failure, are caused mainly by the activation of neurohormonal vasoconstrictor systems (including the renin-angiotensin-aldosterone system, the arginine-vasopressin system, and sympathetic nervous system).^{3 4 5}

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Since atrial natriuretic peptide (ANP) has natriuretic and vasodilator effects and inhibits the renin-angiotensin-aldosterone, arginine-vasopressin, and sympathetic nervous systems, it was suggested to play an important role in asymptomatic left ventricular dysfunction by preserving cardiorenal homeostasis through maintenance of the sodium balance and inhibition of the detrimental effects of the neurohormonal vasoconstrictor system.^{2 3 5}

At present, this hypothesis is supported only by indirect observations. In fact, the hypothetical role of ANP in heart failure has generally been investigated by measuring the circulating levels of the hormone.^{6 7 8 9 10 11 12} However, in a previous kinetic study (see Reference 22), the first one performed in humans under steady-state conditions using a tracer method, we demonstrated that ANP plasma levels could not be considered a reliable estimate of the amount of hormone produced by the heart and still less of the activity of the overall ANP system (as, on the contrary, it was assumed a priori in several physiological and clinical studies in which only the ANP circulating levels were measured) because (1) the plasma pool of the hormone is only 1/15 to 1/20 of the total body pool, and (2) the plasma half-life of ANP is very short (only a few minutes). Indeed, the plasma levels of ANP closely parallel the instantaneous secretion rate, and it is therefore likely that they may greatly fluctuate during a 24-hour period in response to different pathophysiological stimuli.

On the other hand, it is conceivable that the metabolic clearance rate (when measured under steady-state conditions) may represent a reliable estimate of the potential uptake and degradation rate of ANP by the periphery and, consequently, of the overall hormonal activity, because it reflects the functional state of the overall systems (such as receptors and degradation enzymes), which require hours or even days for their modulation. In previous studies on ANP kinetics, high doses of the peptide were administered, greatly increasing ANP circulating levels and thereby inducing a major perturbation of the hormone kinetics; consequently, these studies were performed under a non-steady-state condition.^{12 13 14 15 16 17 18 19 20 21} These techniques are not suitable for the detection of the fine interrelations between ANP and the other neurohormonal systems in basal and/or pathophysiological conditions.

In the present report, we used the previously described tracer method²² to perform a cross-sectional study of 15 IDC patients subdivided into two groups according to their differing clinical severity of disease (New York Heart

Association functional class) in order to investigate the relations between ANP distribution and removal and sodium handling. Eleven normotensive, nonobese male volunteers also were studied as a control group.

▶ Experimental Subjects

Patients

Fifteen patients (age, 23 to 59 years) with IDC and normal arterial blood pressure (mean systolic blood pressure, 124±13 mm Hg; mean diastolic blood pressure, 78±8 mm Hg) were enrolled in the study; their main clinical parameters are reported in Table 1. Patients were prospectively divided into two groups according to their functional class: the first group comprised 7 patients (patients 1 through 7) with less severe symptoms of disease, who were in class I or II, while the second one consisted of 8 patients (patients 8 through 15) with more severe symptoms, who were in classes II-III or III.

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View this table: [Table 1. Cardiac Index, Ejection Fraction, Pulmonary Wedge Pressure, Right Atrial Chamber Dimensions, Left Atrial Chamber Dimensions, Right Atrial Pressure, and NYHA Class of Patients](#)
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Patients of group 1 were treated only with a relative restriction of both physical activity and sodium intake (using a personalized, well-controlled diet); pharmacological treatment with vasodilators (generally an ACE inhibitor) and diuretics (generally a loop diuretic) was added for patients of group 2. Because it is well known that several drugs can affect ANP metabolism, we decided to stop all the drugs (that is, ACE inhibitors and diuretics) 3 days before admittance to the metabolic ward. For the same reasons, patients with congestive heart failure (such as some patients in classes III and IV) being treated with drugs with a relatively long half-life (such as digitalis drugs) were not included in the study. Moreover, patients with atrial fibrillation or other arrhythmias, which can affect the secretion and metabolism of ANP,²³ were also excluded from the study.

In all patients, a thorough clinical history and physical examination, two-dimensional color Doppler echocardiography, ²⁰¹Tl stress scan, coronary angiography, and left ventriculography were performed in order to exclude any ischemic, valvular, hypertensive, or other forms of secondary cardiomyopathy. All patients also underwent further biohumoral tests to exclude metabolic, rheumatologic, autoimmune, or viral disorders. The presence of arrhythmias was evaluated by 48-hour Holter monitoring. Myocardial contractility, dimensions, and function were assessed by two-dimensional echocardiography, radionuclide ventriculography, and hemodynamic study.

All patients had normal renal function as judged from their serum creatinine levels and creatinine clearance rates. In the patients of group 2, normal renal function was confirmed by measurement of the glomerular filtration rate and effective renal plasma flow using classic nuclear medicine techniques.

The patients were hospitalized in the metabolic ward of our institute for the time necessary to perform the kinetic studies. At entrance, all patients were following a strictly controlled sodium intake diet; the mean inpatient and between-patient variability (coefficient of variation, CV) in sodium intake (as evaluated by an expert dietician through daily interviews as well as by the clinicians, who measured daily urinary sodium excretion) was less than 10%, thus reflecting the variability in food composition and differences in the amount of food consumed daily by the patients. Body weight, plasma and urinary creatinine, and electrolytes were measured daily; to ensure that a sodium balance was being achieved, urine collections were promptly analyzed for volume and electrolyte excretion. The ANP turnover study was performed only after three consecutive 24-hour urinary collections demonstrating the achievement of a steady state of sodium excretion.

A relatively wide range of sodium intakes was investigated (see Table 2²²): 10 patients (patients 1 through 4, 8 through 12, and 15) were studied at a relatively restricted sodium intake; 2 patients (patients 5 and 13) were studied at a low sodium intake; 2 patients (patients 6 and 14) were studied twice at different sodium intakes: patient 6 was studied at a relatively high (study 6a) and, 1 month later, at a low sodium intake (study 6b); patient 14 was first studied at restricted sodium intake (study 14a) and, 1 month later, at a low sodium intake (study 14b); patient 7 was studied twice at the same sodium intake (110 mmol/d) with a 7-day interval between the two studies (studies 7a and 7b) to evaluate the reproducibility of the tracer method.

View this table: **Table 2.** Plasma Concentrations of ANP, PRA, and Aldosterone and 24-Hour Urinary Sodium Excretion in Control Subjects and Patients
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In 16 studies, a steady state of sodium excretion was achieved with an amount of sodium daily assumed equal to that excreted by the kidney; in only two patients with more severe disease (patients 8 and 9) was the sodium intake (on average, 120 mmol/d) higher than the ability of their kidneys to excrete sodium, and consequently sodium retention was observed.

▶ Control Group

Eleven normotensive, nonobese male volunteers (age, 26 to 54 years) from our medical and nursing staff served as the control group. These subjects were studied at variable sodium intakes using the same protocol previously described²²; in the present study, their hormonal and kinetic data are reported for comparison with those obtained in patients. Hemodynamic parameters in the control group were not evaluated for ethical reasons. All subjects were completely free from cardiac symptoms and myocardial function, as assessed by two-dimensional echocardiography, was completely normal.

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Informed consent was obtained from all subjects studied, and the protocol was approved by the local ethics committee. All participants received a daily dose of 20 drops of saturated Lugol solution from the day before until the day after the kinetic study.

▶ Methods

Preparation of the Tracer

Synthetic α -h₁₋₂₈ANP (Bachem Feinchemikalien AG) was iodinated with Na¹²⁵I or Na¹³¹I (both supplied by Sorin) by means of a lactoperoxidase technique the day before the kinetic study, as previously described.²² The labeling mixture then was purified using both ion exchange chromatography and high-performance liquid chromatography (HPLC) procedures.²² Because only the fraction containing the monoiodinated labeled peptide was used to prepare the tracer, the specific activity of [¹²⁵I]ANP ranged between 2000 and 2200 Ci/mmol (650 to 700 μ Ci/ μ g), that is, a value very close to the theoretical maximum specific activity for the monoradioiodinated hormone (2200 Ci/mmol).

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Experimental Protocol

On the day after labeling, (9 to 10 AM), the HPLC fraction containing the monoiodinated [¹²⁵I]ANP was loaded onto a DEAE Sephadex column and eluted with phosphate buffer, pH 7.4, 0.05 mol/L, in polypropylene tubes containing 0.5 mL of a 4% human albumin solution; the purified tracer then was sterilized with a 0.22- μ m filter (Millipore, Waters Associates) immediately before the injection.

Each subject was kept at rest in a clinostatic position for at least 30 minutes, and an automatic blood pressure recorder and electrodes were positioned for continuous ECG and blood pressure monitoring throughout the study.

A known amount (about 80 μCi) of freshly prepared tracer (corresponding to about 100 ng of [^{125}I]ANP) was intravenously bolus injected, and 13 to 15 venous blood samples then were collected before and during the 40 minutes after injection (at 1.5 and 2.15 minutes, then about every minute for 5 minutes, every 2 minutes from 8 to 16 minutes, and finally every 5 to 10 minutes). A volume of 0.9% NaCl solution equal to the volume (typically about 100 mL) of blood withdrawn was infused through the three-way stopcock during the study.

The blood samples collected were immediately put into ice-chilled disposable polypropylene tubes containing aprotinin (500 KIU/mL of plasma) and EDTA (1 mg/mL of plasma), and the plasma then was separated in a refrigerated centrifuge within 1 hour.

Determination of [^{125}I]ANP Recovery Using [^{131}I]ANP as Internal Standard

During the kinetic study, the exchange chromatographic fraction of [^{131}I]ANP, prepared the day before and stored at -20°C , was purified by HPLC as described for [^{125}I]ANP.²² To determine the recovery of labeled ANP throughout the extraction and purification procedures, a known amount of purified [^{131}I]ANP (about 3000 to 4000 cpm) was added to each plasma sample immediately after the blood separation by centrifugation, as previously described.²²

Extraction and Purification of Plasma-Labeled ANP and Metabolites by HPLC

Three-milliliter plasma samples were loaded onto Bond Elut C-18 cartridges (Analytical International) activated with 2 mL of methanol and washed with 4 mL of 1% TFA. After a 10-mL washout with 0.1% TFA, labeled peptides were eluted with 3 mL of a solution containing methanol/TFA 99:1. The collected effluent was evaporated using a vacuum centrifuge, and the samples were reconstituted with 60 μL of 0.1% acetic acid and after 0.45- μm filtration were subjected to HPLC.²²

Gamma Counting

The ^{125}I and ^{131}I activities were measured in a gamma counter (1282 CompuGamma CS, LKB Wallac) with an efficiency of 54% and 60%, respectively; the counting time was 20 minutes for each fraction, and the operating conditions were chosen to obtain a high (sample)²-to-background ratio. After background subtraction, the measured ^{125}I counts were corrected for ^{131}I spillover into the ^{125}I channel (which was 6% under the chosen conditions).

Assay Methods

For the ANP assay, blood samples (two 8- to 10-mL samples) were collected just before the injection of the tracer (-15 minutes and 0 time) into ice-chilled disposable polypropylene tubes containing aprotinin (500 KIU/mL of plasma) and EDTA (1 mg/mL of plasma). They were immediately separated by centrifugation and were then frozen and stored in various aliquots at -70°C until assayed.

Plasma ANP concentrations were measured by using a radioimmunoassay (RIA) kit (RIA for α -human ANP, Peninsula Laboratories, Inc), following the manufacturer's instructions with some modifications.^{24 25} The analytical performance and characteristics of this RIA were described in detail previously^{24 25}; in particular, the between-assay imprecision tested in several plasma pools with different ANP concentrations (range, 15 to 600 pg/mL) after extraction with Sep-Pak cartridges ranged between 15% and 45% CV. To increase assay precision, plasma samples were extracted in duplicate, and each plasma extract also was assayed in duplicate by RIA; moreover, the plasma samples with lower ANP concentrations were repeatedly measured in different runs of assays using different volumes of plasma extracts (from 2 to 4 mL). The recovery of the Sep-Pak C-18 extraction was calculated by adding, before extraction, a known amount of [^{131}I]ANP to each sample assayed; the values obtained by RIA were corrected by extraction recovery (mean recovery, 65%).

In three cases, the plasma ANP also was measured with a direct (without extraction) RIA kit (HANP-KIT-CISD, CIS diagnostic K.K., 26-19, Nishi-Ikeburo 5-Chome, Toshima-Ku) in the plasma samples collected during the

kinetic study to verify whether circulating levels of the peptide hormone varied throughout the study. This RIA was used because it required only 100 μL of plasma to perform the assay, thus allowing the determination (at least in duplicate) of ANP concentration in the residues (about 0.5 to 1 mL) of experimental samples (collected during the kinetic study) after HPLC analysis. Plasma ANP concentrations were measured with this RIA kit, following the manufacturer's instructions. The between-assay imprecision of the direct ANP assay was better (CV ranging from 8% to 16%) than that of RIA using a preliminary plasma extraction.

Other Assay Methods

Plasma renin activity (PRA) and plasma aldosterone were measured by RIA kits (supplied by Sorin Biomedica, Saluggia), following the manufacturer's instructions. Urine electrolytes were measured by using an automated system (ASTRA, Beckman).

Data Analysis

The mathematical analysis of the plasma disappearance curve of ANP was described in detail in a previous study.²² A brief summary of the formulas used to compute the kinetic parameters addressed in the present study is reported. The experimental data were optimally fitted for all subjects by a sum of two exponentials ($P(t)=A_1 e^{-\lambda_1 t} + A_2 e^{-\lambda_2 t}$, $\lambda_1 < \lambda_2 < 0$), using a weighted least-squares method. The area (AUC), first order moment (AUMC), and zero-time intercept ($P(0)$) were calculated²⁷ from the coefficients A_i (%dose/liter) and exponents λ_i (min^{-1}) as follows:

$$AUC = \int_0^{\infty} P(t) dt = -(A_1/\lambda_1 + A_2/\lambda_2)$$

$$AUMC = \int_0^{\infty} t P(t) dt = A_1/\lambda_1^2 + A_2/\lambda_2^2$$

$$P(0) = A_1 + A_2$$

The following kinetic parameters were computed without assuming a definite model structure (compartmental or noncompartmental): (1) metabolic clearance rate, $MCR=100/AUC(\text{L}/\text{min})$, and (2) initial distribution volume, $IDV=100/P(0)$ (liters); IDV, computed according to the isotopic dilution principle, is the volume (plasma equivalent) of the initial distribution space where the circulating ANP rapidly equilibrates after entering in the blood stream: it includes, besides plasma volume itself, the sites of initial (2 to 3 minutes) reversible and irreversible uptake of the hormone. It is likely that, for substances that are very rapidly removed such as ANP, the degradation occurring in the first minutes after tracer injection can induce an increase in the estimated value of IDV.

Two additional kinetic parameters were obtained by using the noncompartmental approach, that is, assuming that all ANP degradation took place in the sampling pool: (1) mean residence time in the body, $MRTB=AUMC/AUC$, and (2) total (plasma equivalent) distribution volume, $TDV=MCR \times MRTB=100 \times AUMC/(AUC)^2$ (liters).

It is worth noting that both IDV and TDV are "plasma equivalent" volumes and do not directly correspond to true physical (or physiological) volumes; these two distribution parameters may be found to be greater than actual body volumes (for example, plasma volume, extracellular volume, or cellular volume) due to the possibly increased ANP concentration in some tissue compartments as compared with that measured in plasma.

Peripheral (plasma equivalent) distribution volume (PDV) can be computed from the difference between total and initial distribution volume values, $PDV=TDV-IDV$ (liters).

When the renewal parameter MCR is multiplied by the plasma concentration of cold ANP, an estimate of the amount of hormone degraded per unit time (disposal rate) is obtained; under steady-state conditions, the disposal rate also estimates the amount of ANP produced per time unit (production rate, PR).

Statistical Analysis

The statistical analysis was carried out by a Macintosh IIsi personal computer using the STATVIEW 4.0 and SUPERANOVA programs (Abacus Concepts, Inc). The data for the three independent groups (groups 1 and 2 of patients and control subjects) were analyzed by ANOVA, and the significant differences between the pairs of means were tested by the Scheffé's test. The Scheffé's test was chosen for multiple comparisons because it is generally considered one of the most conservative tests and also because it is very robust to violations of the assumptions typically associated with multiple comparison procedures (including heterogeneous variances). The results are expressed as mean \pm SD.

► Results

Hormone Concentrations

The basal plasma levels of ANP and PRA found in our IDC patients and in the control subjects at the beginning of the kinetic study are reported in Table 2.

ANP levels were on average in the normal range in the patients of group 1 and were significantly increased (ANOVA, $P<.0004$) in the patients of group 2 (group 1 versus group 2, $P<.0025$; group 2 versus control subjects, $P<.0015$). ANP levels were weakly and negatively correlated with urinary sodium excretion values ($r=-.521$, $P=.0268$, $n=19$). Furthermore, significant positive linear regressions were found between ANP values and both pulmonary wedge pressure ($r=.735$, $P=.0018$, $n=15$) and left atrial chamber dimension ($r=.678$, $P=.0055$, $n=15$) values but not with right atrial chamber dimension values.

The time course of ANP circulating levels throughout the kinetic study, measured in three typical patients (two patients from group 1 and one patient from group 2) with greatly different ANP levels, was reported in Fig 1. Wide but not systematic fluctuations of ANP levels were observed when compared with basal values (before tracer injection).

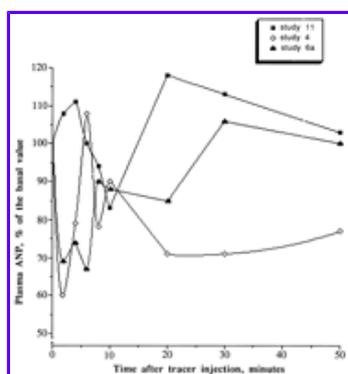


Figure 1. Plot shows time course of plasma atrial natriuretic peptide (ANP) concentrations in three patients in whom hormonal levels were assayed in several experimental plasma samples collected throughout the kinetic study by a direct (without extraction) radioimmunoassay method. Values are expressed as percent of the basal values (sample collected before tracer injection).

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PRA levels were significantly increased in patients with IDC (patients, 3.191 ± 3.970 ng/mL/h; control subjects,

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0.667±0.356; $P=.0463$) and tended to progressively increase with the clinical severity of the disease even if the mean values were not significantly different between the two groups of patients (2.009±1.641 ng/mL/h versus 4.373±5.259 ng/mL/h). Moreover, significant positive correlations were also found between PRA levels and pulmonary wedge pressure ($r=.610$, $P=.0157$, $n=15$), left atrial chamber dimension ($r=.527$, $P=.0437$, $n=15$), and right atrial chamber dimension ($r=.517$, $P=.0484$, $n=15$) values.

These data, taken as a whole, suggest that activation of both vasoactive hormonal systems increased with progression of clinical severity of the disease and with the deterioration of hemodynamics, even if the residual effects of drugs (for example, the effect of ACE inhibitors on PRA levels) cannot be excluded.

Plasma aldosterone did not significantly differ among the patient groups and control subjects and did not show any correlations with ANP or PRA values, nor with the clinical severity of the disease; this finding may be explained by the opposite effects of ANP and the renin-angiotensin system on aldosterone production and by the action of other important biological factors (including some neurotransmitters and electrolytes) that also can independently affect mineralocorticoid production by the adrenal gland.²⁶

Results of the Turnover Study

Six representative, individual disappearance curves obtained in control subjects and in the two groups of patients with IDC, studied at different sodium excretion rates, are reported in Fig 2. In all control subjects and patients, the experimental plasma disappearance curves of [¹²⁵I]ANP were satisfactorily fitted by a biexponential function.

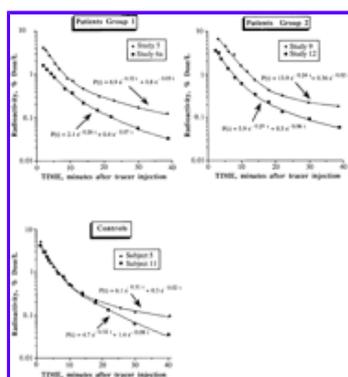


Figure 2. Representative disappearance curves of labeled atrial natriuretic peptide in normal subjects and in the two groups of patients with idiopathic dilated cardiomyopathy. For each studied group, the kinetic curves of two patients or subjects at different sodium intakes (low and relatively high) are represented. For each disappearance curve, the two exponential fitting functions are also reported, together with the corresponding values for the coefficients and exponents (min^{-1}).

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The main kinetic parameters calculated from the experimental plasma disappearance curves of [¹²⁵I]ANP in all control subjects and patients studied are reported in Table 3. The patients with IDC, taken as a whole, showed significantly lower values for TDV (patients, 16.3±7.2 L/m²; control subjects, 27.0±11.7 L/m²; $P=.0048$) and MRTB (patients, 7.4±2.1 minutes; control subjects, 18.2±19.2 minutes; $P=.0240$), and higher PR (patients, 280.8±197.4 ng/min/m²; control subjects, 130.7±105.4 ng/min/m²; $P=.0283$) than control subjects.

View this table: **Table 3.** Main Kinetic Parameters of the Subjects Studied

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When the patients were divided into two subgroups (Table 3), patients of group 1 showed higher (ANOVA, $P=.0040$) ANP MCR values than patients of group 2 ($P=.0082$) and control subjects ($P=.0197$). Patients of group 2 showed significantly lower (ANOVA, $P=.0146$) IDV values than those of group 1 ($P=.0276$) and control

subjects ($P=.0469$), as well as lower (ANOVA, $P=.0050$) TDV values than control subjects ($P=.0051$). In addition, patients of group 2 showed significantly higher (ANOVA, $P=.0008$) ANP PR values than those of group 1 ($P=.0085$) and control subjects ($P=.0015$).

Based on the biexponential shape of the time course of plasma disappearance curves, the body pool of the hormone can be considered a sum of two exchanging spaces: the first one (initial distribution space, IDV) exchanging almost instantaneously with plasma (into which the tracer was injected) and the second one exchanging more slowly with plasma. Patients with IDC showed a progressive reduction in TDV, mainly due to a significant (ANOVA, $P=.0067$) contraction of peripheral distribution spaces (PDV calculated as TDV-IDV) (Table 3). Indeed, in patients of group 1, IDV values were superimposable to those of control subjects (group 1, 11.3 ± 4.7 L/m²; control subjects, 10.7 ± 3.4 L/m²), and only the PDV tended to be reduced (group 1, 8 ± 3 L/m²; control subjects, 16 ± 9 L/m²; $P=.0556$); conversely, in patients of group 2, both IDV (7 ± 2 L/m², $P=.0469$) and PDV (6 ± 5 L/m², $P=.0110$) values were significantly reduced compared with control subjects.

As already found in control subjects,²² a positive regression between ANP MCR values and the daily excretion of sodium was also observed in the IDC patients of both groups (Fig 3). Different relations between MCR values and sodium excretion were, however, observed in patients and control subjects (slopes significantly different, $P<.01$; intercepts not significantly different). Indeed, throughout the range of sodium excretion studied, the MCR values of patients of both groups were always higher (approximately doubled) than the values found in control subjects at the corresponding sodium excretions (Fig 3). This finding can be better appreciated by observing the ratio between ANP MCR values and the corresponding urinary sodium excretions determined in each patient and control subject (Fig 4); a significant difference was found for this ratio between both group 1 (27.7 ± 11.4 [mL/min/m²]/[mmol/d]) and group 2 (24.2 ± 8.0 [mL/min/m²]/[mmol/d]) and control subjects (10.7 ± 0.9 [mL/min/m²]/[mmol/d]). These data indicate that in both groups of patients, the same sodium excretion was associated with an approximately doubled removal rate of the hormone compared with control subjects; this suggests that ANP peripheral utilization is less effective in excreting sodium in patients with IDC.

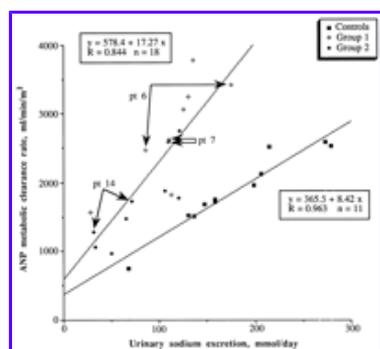


Figure 3. Linear regressions between daily urinary sodium excretion (mean of all 24-hour urinary collections, mmol/d) and atrial natriuretic peptide (ANP) metabolic clearance rate (mL/min/m²) values found in control subjects and patients. Arrows indicate values for the three patients studied twice.

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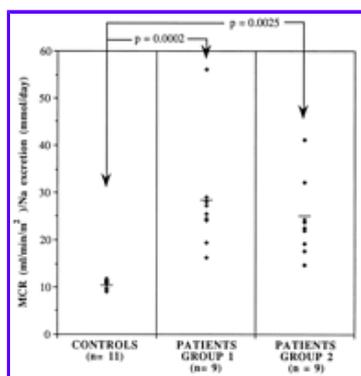


Figure 4. Plot shows ratio of atrial natriuretic peptide metabolic clearance rate ($\text{mL}/\text{min}/\text{m}^2$) and sodium excretion (mmol/d) values in the two groups of idiopathic dilated cardiomyopathy patients and in control subjects.

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Discussion

In studies of the possible pathophysiological role of ANP in heart failure, generally only the circulating levels of the hormone have been measured.^{6 7 8 9 10 11 12} These studies demonstrated that ANP assay actually represents a useful tool in the follow-up of patients with heart failure.²⁷ Indeed, our data confirm²⁷ that ANP levels can be considered a good index of clinical severity of disease (as indicated by the significant difference between ANP levels observed in the two groups of patients) and/or deterioration of hemodynamics (as indicated by the significant correlations with PWP and LACD).

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On the other hand, it has been shown that plasma ANP levels exhibit marked variability attributable to pulsatory pattern of secretion.²⁸ The time course of plasma ANP levels of the three patients (Fig 1 \clubsuit) in whom several experimental blood samples collected during the kinetic study were assayed also confirms a marked variability in circulating plasma ANP concentrations in humans at rest, mainly reflecting a very rapid pulsatory pattern of hormone secretion. The fact that plasma levels closely reflect pulsatory pattern of secretion is confirmed by our findings of very rapid decrease of labeled ANP (plasma half-life about 2 minutes for the predominant fast exponential component); this suggests some caution in interpreting isolated plasma values.²⁸ Furthermore, our findings indicate that isolated ANP levels cannot be considered per se a reliable index of hormonal effect, as suggested by the weak and negative correlation with urinary sodium excretion found in our IDC patients.

An evaluation of the main turnover parameters (such as MCR) might represent a significant improvement in the assessment of the functioning of the overall ANP system as compared with the measurement of ANP circulating levels only. Different items of information can be derived from the evaluation of the renewal and degradation pathways, which require hours or even days for their modulation. Indeed, our tracer method provided reproducible kinetics results (Table 3 \clubsuit), as demonstrated by the two kinetic studies performed on patient 7 (studies 7a and 7b) with a 7-day interval at the same sodium intake (Table 2 \clubsuit).

Some authors^{12 13 14 15 16 17 18 19 20 21} have infused pharmacological doses of the hormone to evaluate its biological effects and/or to estimate the main kinetic parameters of ANP in patients with heart failure. However, the infusion of pharmacological doses of the hormone, which per se produces some biological effects, clearly perturbs the steady-state condition, thereby affecting the estimation of turnover parameters. On the contrary, the tracer method used in the present study to evaluate ANP kinetics does not affect the steady-state condition.²² In addition, our kinetic approach, using a bolus injection of the tracer (instead of a constant infusion), makes it possible to calculate additional kinetics parameters (such distribution volumes, mean transit times, and mean residence times), providing more accurate and complete information on ANP kinetics²² (Table 3 \clubsuit).

Since there is experimental evidence that ANP is rapidly degraded by lysosomal hydrolases after its binding to the cell surface and subsequent internalization via receptor-mediated endocytosis,^{29 30} it is likely that degradation and biological hormonal effects may be strictly related. Moreover, as the chief function of the hormone is perceived to be an action on the kidney to facilitate the excretion of sodium and water,^{31 32} it is conceivable that ANP degradation rate can be related to renal sodium handling. Indeed, we found in a previous study²² and we now confirm in a larger series of normal subjects a close correlation between sodium excretion and ANP clearance (Fig 3 ).

The significant increase in ANP MCR (relative to sodium intake/excretion) observed in patients in the early phase of clinical disease, as compared with normal subjects, indicates the presence of a disturbed mechanism underlying hormone degradation and its biological effects (for example, sodium excretion). In this respect, the different relations between ANP MCR and daily sodium excretion found in patients versus control subjects, that is to say, the fact that an approximately doubled ANP MCR was required in patients to produce the same biological effect (that is, the same sodium excretion over a 24-hour period) as in control subjects (Fig 3  and Table 3 ) can be interpreted as a hormone degradation that is biologically "less effective" in excreting sodium. Indeed, because a reduced biological effect (for example, sodium excretion) is associated with an elevated ANP removal (Fig 4 ) one can suppose that the hormone may be degraded in organs and tissues, such as lung tissue and vascular cells, which are rich in degradative enzymes and receptors and are interposed between the secretion site(s) and the natriuretic target organ (for example, kidney).^{29 30 31 32} Note that this type of reduction of biological activity can be detected only by means of tracer kinetic studies under steady-state conditions. Besides peripheral degradation, tissue distribution also was found to be profoundly disturbed (for example, a reduction of exchanges with peripheral target tissues) in patients with IDC, both taken as a whole and when they were subdivided into two subgroups based on the clinical severity of the disease (those with minimal signs and symptoms of disease and minimal activation of neurohormonal vasoactive system and those with more severe symptoms of heart failure) (Table 2 .

Finally, our findings of a reduced MCR in the presence of a significant increase in ANP PR in patients with severe clinical disease (group 2), when compared with patients at an early stage of clinical disease (group 1), are consistent with the hypothesis of a resistance syndrome to the natriuretic effect of ANP in patients with IDC.

Conclusions

Our data show that subjects with idiopathic dilated cardiomyopathy, even with only mild hemodynamic abnormalities and little or no symptoms of heart failure, have abnormalities of ANP kinetics. These abnormalities are present even when plasma concentrations of ANP, PRA, and aldosterone may be different from those of control subjects.

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Footnotes

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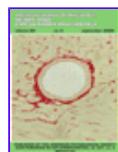
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