

Atrial Natriuretic Peptide Is Not Degraded by the Lungs in Humans

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ABSTRACT

In an attempt to identify and quantify the sites of atrial natriuretic peptide (ANP) degradation, particularly the lungs, a new tracer method to study ANP metabolism *in vivo* in humans was developed and applied to patients with left ventricular dysfunction. Thirteen male, normotensive, cardiac patients with different degrees of left ventricular myocardial involvement were enrolled in the study. The study protocol required constant infusion (3 patients) or bolus injection (10 patients) of ^{125}I -labeled ANP just upstream of the right atrium and blood sampling from different sites (pulmonary artery, aorta, inferior vena cava, and femoral vein) during the hemodynamic study. Data analysis was based on a kinetic model consisting of three blocks in series (right heart, lungs and left heart, and periphery) supplied by the same plasma flow (plasma cardiac output). Plasma levels of native ANP were measured with a sensitive and specific immunoradiometric assay method. ANP values measured in the aorta (163.9 ± 144.8 pg/mL, $n = 80$) were superimposable on those mea-

sured in the pulmonary artery (161.8 ± 136.5 pg/mL, $n = 80$). Negligible extraction of ^{125}I -labeled ANP was found in the lungs and left heart block (on average $0.08 \pm 3.92\%$), whereas the peripheral block extraction ($46.2 \pm 7.8\%$) accounted for almost total hormone removal from the blood (whole body extraction was $46.4 \pm 6.6\%$). ANP metabolic clearance rate (3.11 ± 1.48 , range 1.4–6.8 L/min) declined with the progression of left ventricular dysfunction (plasma cardiac output 3.46 ± 1.08 , range 1.2–5.7 L/min), and a close correlation between metabolic clearance rate and cardiac output was evident. Our data suggest that lungs do not extract, or extract only very small amounts, of labeled ANP administered iv to patients with different degrees of left ventricular myocardial involvement, and whole body extraction of labeled ANP remains relatively stable with the progression of disease, and the large reductions in clearance values observed in our patients can be ascribed mainly to the reductions in cardiac output. (*J Clin Endocrinol Metab* 83: 2898–2906, 1998)

HUMAN atrial natriuretic peptide (ANP) is a 28-amino acid polypeptide with potent natriuretic and vasodilatory effects, which is produced mainly by cardiomyocytes (1). ANP levels have been found to be elevated in diseases characterized by an expanded fluid volume, including cardiac and renal insufficiency. In particular, circulating ANP increases with the progression of clinical severity of cardiac insufficiency and with the deterioration of hemodynamics, so that a positive relation between mortality and ANP levels was found in severe heart failure (2, 3).

Extensive studies in both animals and humans have documented that the main pathway of ANP secretion into the circulatory blood flow is through the coronary sinus into the right atrium (1). However, the contribution of individual organs to ANP clearance *in vivo* in humans has not yet been defined. In particular, although the lungs have been implicated as a site of ANP degradation, it has not been well demonstrated whether circulating ANP is extracted and degraded (or not) by the lungs. The studies aimed at identifying and quantitating sites of ANP degradation (4–12) were based on the measurement of the arteriovenous (AV) difference of the hormone concentration, in most cases derived from the collection of a small number of plasma samples with ANP

concentration assessed by RIA. All of the studies reported values of circulating ANP in the aorta superimposable to those measured in the pulmonary artery (4, 6–12). Although Schutten *et al.* (6) concluded that ANP extraction does not occur across the lungs, others (4, 7–12) suggested that there may be partial extraction of ANP, the circulating levels in aorta being restored by the hormone newly secreted into the blood from the left heart. Clearly, the approach based on sampling upstream and downstream of district(s) (*i.e.* the pulmonary artery and aorta), in which production of the hormone may occur, as it has been hypothesized for left heart, is not suitable for evaluating the ANP extraction. To avoid the possible contribution to ANP production of left heart and to overcome the technical difficulties related to pulmonary veins sampling, some authors sampled pulmonary wedge blood; additional concerns, however, can be raised when computation of ANP removal by the lungs was based on AV difference in which the pulmonary wedge blood was used as an indirect estimate of ANP levels in pulmonary venous blood (7, 8).

Even if ANP secretion between the sampling sites can be excluded, estimation of the hormone extraction from AV difference measured in a few pairs of simultaneously collected blood samples can be considered reliable only when production rate remains fairly constant. In the case of ANP, the pulsatile secretion and the very rapid clearance (13–15) may generate oscillations in its plasma levels, mainly in the proximity of the secretion site (*i.e.* pulmonary artery), thus

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making an estimate of the extraction unreliable unless quite a long sampling schedule is adopted. Finally, the relatively poor accuracy and precision of native ANP measurement by RIA methods generally employed to measure the hormone (16, 17) may partially contribute to generate inaccurate values of hormone extractions.

All these drawbacks can be overcome by a tracer study in which labeled ANP is administered. Two different experimental protocols have been used by us. The first based on constant infusion of radiolabeled ANP into the right atrium followed by simultaneous blood collection from pulmonary artery, aorta, inferior vena cava, and femoral vein. The second using a bolus injection of tracer into the right atrium with blood sampling from two sites only (pulmonary artery and aorta). Data collected according to both protocols were analyzed using a circulatory model, which allows us to take account of the cardiac output simultaneously measured by thermodilution (18, 19). We report here the extraction of labeled ANP from lungs, heart, peripheral tissues, and whole body in 13 patients with different degrees of left ventricular myocardial involvement. The relationship between whole body extraction of the hormone, metabolic clearance rate (MCR), and the hemodynamic state is also discussed.

Subjects and Methods

Experimental subjects

Thirteen male, normotensive, hospitalized patients with different degrees of left ventricular dysfunction were enrolled in the study after a complete baseline cardiologic evaluation, including physical and X-ray examination, two-dimensional echocardiography, and radionuclide-angiography. All the patients had to submit to a complete hemodynamic study because of their cardiac disease. Their main clinical parameters are reported in Table 1. Because it is very difficult to apply this experimental protocol to normal subjects for evident ethical reasons, we studied patients with a wide range of myocardial involvement (from very mild to severe, *i.e.* from 51–12% left ventricular ejection fraction), clinical symptoms (New York Heart Association functional class from I to III–IV), and plasma ANP levels (*i.e.* from values in the normal range of 5–28 pg/mL to greatly increased values of >200 pg/mL; Table 1).

Because it is well known that several drugs can affect ANP metabolism (1), we decided to stop all the drugs at least 3 days before the kinetic study. Therefore, patients with overt congestive heart failure, who could not stop the drugs for ethical reasons, and patients treated with drugs with a relatively long half-life (such as digitalis drugs) were

not included in the study. We also excluded patients with atrial fibrillation or other arrhythmias, which could affect the secretion and metabolism of ANP (1).

All the patients received a daily dose of 20 drops of saturated Lugol solution from the day before until the day after the kinetic study.

The study protocol was approved by the local ethics committee and written consent was obtained from the patients before the study.

Hemodynamics

The study was carried out in the hemodynamic ward of our institute after selective left and right coronary angiograms were performed using the Judkins technique. A no. 7 French triple lumen flow-directed balloon-tipped catheter was inserted transcutaneously through the right femoral vein and advanced to obtain right atrial, pulmonary artery, and pulmonary capillary wedge pressures. Cardiac index was calculated as the ratio of the mean of at least five thermodilution cardiac output measurements to body surface area (m^2). After hemodynamic assessment, the flow-directed catheter was removed, and a no. 7 French multipurpose multiple side hole catheter was positioned in the pulmonary artery. A no. 5 French pigtail multiple side hole catheter was advanced into the ascending aorta through an introducer positioned in the right femoral artery for diagnostic study. Pulmonary artery pressure, aortic pressure, and one electrocardiographic lead were continuously monitored. Blood samples were simultaneously drawn from the pulmonary artery and ascending aorta. In the studies with labeled ANP infusion (patients 1, 2, and 3; Table 1) a third catheter was positioned in the inferior vena cava, 2–3 cm upstream from its entrance into the right atrium and downstream from the entrance of the superior hepatic veins into the vena cava. At the end of the experimental study, the diagnostic procedure was completed with biplane left ventriculograms.

Experimental protocol

Synthetic αh_{1-28} ANP was iodinated with $Na^{125}I$ or $Na^{131}I$ as previously described in detail elsewhere (14, 20). A drop of the injected tracer that remained in the syringe after the injection was collected and tested for purity by high performance liquid chromatography; in all kinetic studies, less than 1% impurity was found.

Studies using constant infusion of tracer. In three patients (patients 1, 2, and 3; Table 1) a known amount of the tracer ($\sim 80 \mu Ci$) was drawn into a syringe containing 50 mL Emagel solution (Hoechst, Frankfurt am Main, Germany) and constantly infused at a rate of 0.4 mL/min, by means of a mechanical high-precision pump via a catheter inserted percutaneously from the antecubital vein to near the junction of the superior vena cava with the right atrium; after equilibration (20–30 min) four to five blood samples (4–5 mL) were simultaneously drawn from the aorta, pulmonary artery, inferior vena cava, and femoral vein.

TABLE 1. Main clinical data of patients studied

Patient no.	Study protocol	Age (yr)	Body surface (m^2)	ANPpa (pg/mL)	ANPa (pg/mL)	NYHA class	Cardiac disease	PAS (mmHg)	PAP (mmHg)	Wedge (mmHg)	EF (%)	CI (L/min/ m^2)
1	Infusion	28	1.96	5.4	2.6	I	IDCM	85	15	12	38	3.7
2	Infusion	39	1.78	28	20	II	PIDCM	89	15	10	18	3.5
3	Infusion	25	1.72	14	13	I	IDCM	85	12	4	50	5.1
4	Bolus	53	2.14	132	81	III–IV	PIDCM	80	46	46	23	2.5
5	Bolus	60	1.50	127	120	III	IDCM	75	30	30	20	2.9
6	Bolus	55	2.09	18	32	II	PIDCM	100	26	26	30	3.0
7	Bolus	48	2.34	293	295	III–IV	IDCM	85	38	38	18	2.3
8	Bolus	63	1.83	67	83	II	IDCM	120	12	26	28	2.7
9	Bolus	47	1.70	230	214	III	IDCM	67	42	33	12	2.05
10	Bolus	59	1.76	29	31	I	IDCM	90	23	17	45	3.18
11	Bolus	58	1.90	196	215	III	IDCM	102	31	23	15	2.89
12	Bolus	49	1.59	251	124	III–IV	IDCM	77	42	33	20	1.51
13	Bolus	63	1.86	15	17	I	ICD	90	16	10	51	2.95

Abbreviations: ANPa, ANP concentration in aorta before tracer administration (basal condition); ANPpa, ANP concentration in pulmonary artery before tracer administration (basal condition); ICD, ischemic coronary disease; PIDCM, postischemic dilated cardiomyopathy; IDCM, idiopathic dilated cardiomyopathy; PAS, mean systemic arterial pressure; PAP, mean arterial pulmonary pressure; EF, ejection fraction; CI, cardiac index.

Bolus injection studies. In 10 patients (patients 4–13; Table 1) a known amount ($\sim 80 \mu\text{Ci}$) of freshly prepared tracer was iv bolus injected through a catheter (see above) into the right atrium; several blood samples were then simultaneously collected from the aorta as well as the pulmonary artery before and during the 30 min following injection. To ensure a reliable definition of the area under the concentration curve (AUC) of the tracer throughout the first 2 min after injection, integrated blood samples were continuously withdrawn using a computerized and programmable automatic collector, specifically developed for this purpose by the Electronics Unit of our Institute. The device consists of: a) a peristaltic pump; b) a micro-controller that operates two drivers for piloting two stepping motors; c) an electronic apparatus for piloting an electromagnet to change the row of tubes; d) an operating panel with a display to program and visualize the work-cycle; and e) an electronic apparatus with emergency push-button to stop the work-cycle, if necessary. Thirteen 10-sec integrated blood samples (1.2 mL each) were simultaneously collected from the aorta and pulmonary artery throughout the first 130 sec; the remaining part of the curve (from 130 sec up to 30 min) was described by at least five discrete 5-mL blood samples, typically taken at 3.5, 8, 15, 20, and 30 min. The larger volume of the five discrete samples was necessary to also allow a reliable measure of ANP concentration during the final part of the curve where the ANP activity is extremely low.

A volume of 0.9% NaCl solution, equal to that of blood withdrawn, was infused. The blood samples collected were immediately put into ice-chilled disposable polypropylene tubes, containing aprotinin (500 kallikrein inhibiting units (KIU)/mL of plasma) and EDTA (1 mg/mL of plasma), and the plasma was rapidly separated in a refrigerated centrifuge at 4°C. All experimental blood samples were extracted with Bond Elut C18 cartridges (Analytical International, Harbor City, CA) and then purified by means of an high performance liquid chromatography procedure as previously described in detail (14, 20).

Computation of ^{125}I -labeled ANP blood/plasma partition factor

Because labeled ANP concentration was measured in plasma, directly measured blood flow (cardiac output) was corrected for the ANP blood/plasma ratio. The partition of known amounts of labeled ANP in 3 mL of blood (withdrawn immediately before the tracer injection/infusion) between plasma and cells (red cells, white cells, platelets, etc.) was measured for each patient after the common procedures of centrifugation and separation utilized during the kinetic study for all plasma samples; on average this factor was $63.0 \pm 5.3\%$.

ANP assay

Plasma ANP was measured with a direct immunoradiometric assay (IRMA), as previously described in detail (21). The sensitivity of this IRMA was $0.21 \pm 0.09 \text{ pg/tube}$ (corresponding to $2.1 \pm 0.9 \text{ pg/mL}$ for an assay plasma volume of 0.1 mL). The interassay imprecision was 11.4% and 8.0% for two plasma pools with ANP concentrations of $22.6 \pm 2.6 \text{ pg/mL}$ and $178.6 \pm 14.3 \text{ pg/mL}$, respectively, whereas the intraassay imprecision profile ranged from 8–4% for the same concentration interval. To improve the assay precision, plasma samples with ANP levels less than 15 pg/mL were repeatedly assayed using a higher volume of plasma (0.3 mL instead of 0.1 mL) (21).

For basal ANP assay (Table 1), just before injection of the tracer blood samples were collected from the aorta and pulmonary artery. We also collected 80 blood samples (on average 6 for each patient) from the aorta or pulmonary artery during the kinetic study, which contained no more than 2% dose/liter of injected ^{125}I -labeled ANP.

All blood samples were collected in ice-chilled disposable polypropylene tubes, containing aprotinin (500 KIU/mL of plasma) and EDTA (1 mg/mL of plasma); plasma samples were immediately separated by centrifugation, and then frozen and stored in various aliquots at -20°C , and finally assayed with the IRMA (20).

The normal range for plasma ANP obtained in 70 healthy adults of both sexes (age 20–70 yr) was $17.2 \pm 8.4 \text{ pg/mL}$ (range 3–34 pg/mL).

Data analysis

The body ANP system is represented by a circulatory model composed of three blocks [right heart (RH), lungs and left heart (L + LH), and periphery (P)] connected in series (see Fig. 1). The associated flow F is cardiac output corrected for the ratio of blood to plasma ANP concentrations (F is referred to as plasma cardiac output in this article). From the point of view of hormone turnover, each block of the model is characterized by its extraction (E_{rh} , $E_{\text{L+LH}}$, E_{p}).

Extractions of individual blocks: infusion approach. This analysis is an extension of the analysis previously used (18) for studies performed with a simplified experimental protocol (just one blood sampling site). The extractions of the three blocks are computed by the standard formula [$E = (c_{\text{input}} - c_{\text{output}})/c_{\text{input}}$] applied to the steady state concentrations (Fig. 2) of tracer experimentally measured in pulmonary artery (c_{pulm}), aorta (c_{aorta}), and inferior vena cava (c_{ven}):

$$E_{\text{rh}} = [(c_{\text{ven}} + \text{IR}/F) - c_{\text{pulm}}]/(c_{\text{ven}} + \text{IR}/F) \quad (1)$$

$$E_{\text{L+LH}} = (c_{\text{pulm}} - c_{\text{aorta}})/c_{\text{pulm}} \quad (2)$$

$$E_{\text{p}} = (c_{\text{aorta}} - c_{\text{ven}})/c_{\text{aorta}} \quad (3)$$

The output concentration from the periphery c_{ven} is estimated by the concentration in the inferior vena cava; the input concentration to right heart is $c_{\text{ven}} + \text{IR}/F$ where IR/F is the concentration increment produced by infusion rate IR diluted in flow F .

Extractions of individual blocks: bolus approach. The experimental data are, in this case, the two concentration curves sampled in the pulmonary artery and aorta (Fig. 3). The extractions are computed starting from the areas under the two curves AUC_{pulm} and $\text{AUC}_{\text{aorta}}$. Because of frequent and integrated sampling during the first minutes, it was possible to split the pulmonary curve into the curve generated by first pass of bolus and the recirculating curve generated by the labeled ANP, which returns to the pulmonary artery after passing through peripheral organs. The onset of recirculation in the pulmonary artery could be detected as a change in the descending slope of the initial peak evident when data are plotted in log scale. The area relative to the first pass ($\text{AUC}_{\text{pulm,1p}}$) is computed by adding to the area of the initial peak the area of the tail computed by monoexponential extrapolation of the descending branch of the peak

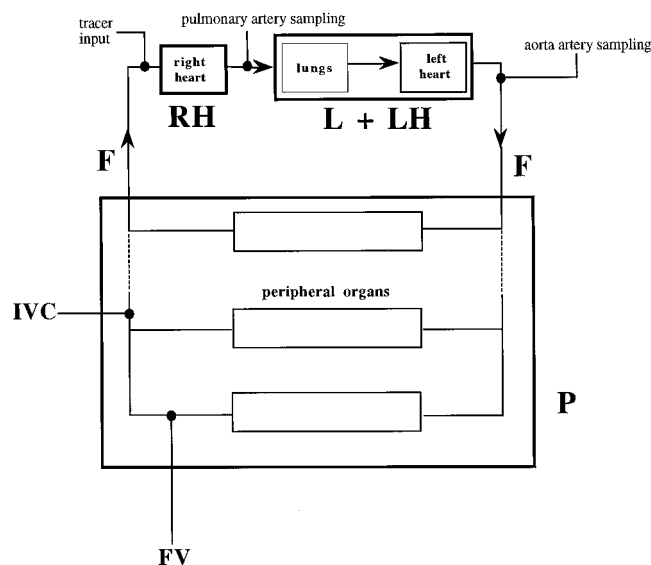


FIG. 1. Body ANP system schematically represented as three blocks (RH, right heart; L + LH, lungs and left heart; P, periphery) connected in series in a circular fashion. The same flow F circulates through blocks; F (plasma cardiac output) is cardiac output corrected by blood/plasma concentration ratio. Site of tracer administration, sites of sampling used in infusion protocol [pulmonary artery, aorta, inferior vena cava (IVC), and femoral vein (FV)], and sites of sampling used in bolus injection protocol (pulmonary artery and aorta) are indicated.

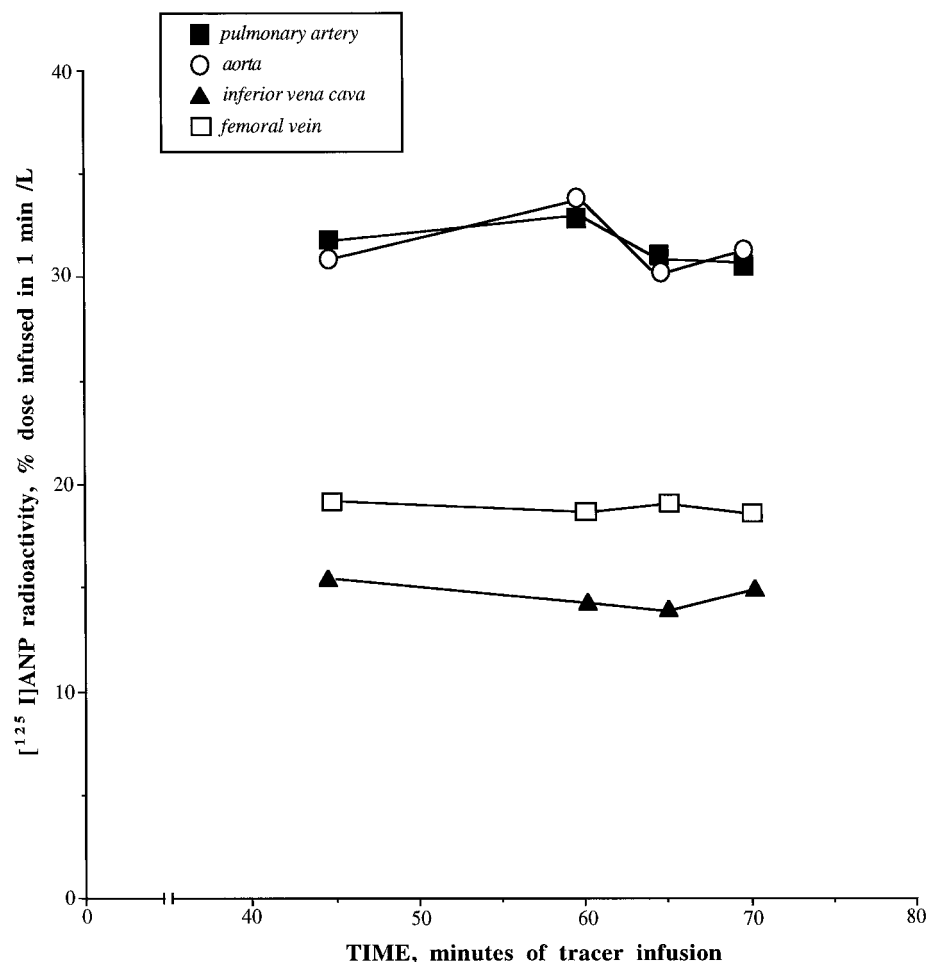


FIG. 2. Steady state plasma levels of ^{125}I -labeled ANP reached in pulmonary artery, aorta, inferior vena cava, and femoral vein after constant infusion of tracer (patient no. 3 in Table 2). Steady state plasma concentrations are normalized by infusion rate IR, *i.e.* expressed as: (% dose infused in 1 min)/liter; therefore MCR (in liter/min) is calculated as $100/(\text{normalized steady state concentration})$.

(Fig. 3). The area under the recirculating curve is calculated as difference $\text{AUC}_{\text{pulm,rc}} = (\text{AUC}_{\text{pulm}} - \text{AUC}_{\text{pulm,lp}})$.

The product of $\text{AUC}_{\text{pulm,lp}}$ times flow F is the total amount of labeled ANP that reaches the pulmonary artery; by comparing this amount to the injected dose D , extraction of RH block is computed:

$$E_{\text{rh}} = (D - F \text{AUC}_{\text{pulm,lp}}) / D \quad (4)$$

Extraction of the L+LH block is computed as:

$$E_{\text{L+LH}} = (\text{AUC}_{\text{pulm}} - \text{AUC}_{\text{aorta}}) / \text{AUC}_{\text{pulm}} \quad (5)$$

Extraction of the P block is computed as:

$$E_{\text{p}} = (\text{AUC}_{\text{aorta}} - \text{AUC}_{\text{pulm,rc}}) / \text{AUC}_{\text{aorta}} \quad (6)$$

Note the strict analogy of Eq 5 and Eq 6 with Eq 2 and Eq 3 where AUCs replace the steady state concentrations of tracer; to write Eq 6 in a simpler way, it was assumed $E_{\text{rh}} = 0$, in agreement with results of present studies in which no significant extraction of labeled ANP by the right heart has been observed (see *Results*).

MCR and whole body extraction: infusion approach. Overall degradation rate of the metabolic system is, as is usual, quantitated in terms of clearance rate MCR, computed as the ratio of infusion rate IR to steady state concentration in mixed venous plasma:

$$\text{MCR} = \text{IR} / c_{\text{ven}} \quad (7)$$

The availability of simultaneously measured cardiac output makes it possible to write the MCR in terms of flow and whole body extraction by the use of the circulatory model. The whole body is considered as a perfused organ whose extraction is:

$$E_{\text{wb}} = (c_{\text{pulm}} - c_{\text{ven}}) / c_{\text{pulm}} \quad (8)$$

For sake of simplicity it is assumed $E_{\text{rh}} = 0$ *i.e.* $c_{\text{pulm}} = c_{\text{ven}} + \text{IR}/F$, in agreement with the results of the present studies (see *Results*). The relationship between MCR and E_{wb} is obtained by stating that, under steady state conditions, the amount of labeled ANP degraded must be equal to the amount infused:

$$\text{IR} = (c_{\text{pulm}} - c_{\text{ven}})F \quad (9)$$

Dividing both sides of Eq 9 by c_{ven} we obtain:

$$\frac{\text{IR}}{c_{\text{ven}}} = \frac{(c_{\text{pulm}} - c_{\text{ven}})}{c_{\text{ven}}} F = \frac{(c_{\text{pulm}} - c_{\text{ven}})}{c_{\text{pulm}}} \frac{c_{\text{pulm}}}{c_{\text{ven}}} F$$

which can be written as:

$$\text{MCR} = F E_{\text{wb}} / (1 - E_{\text{wb}}) \quad (10)$$

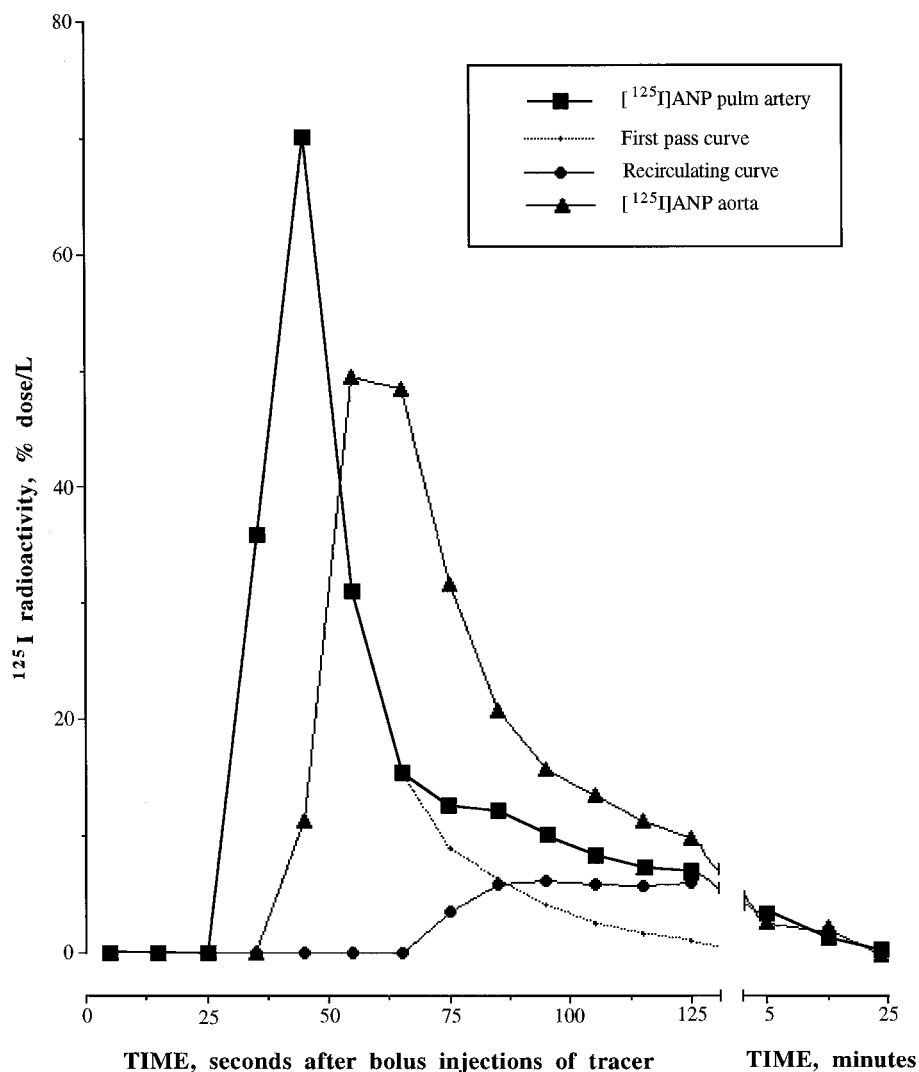
MCR and whole body extraction: bolus approach. MCR is computed, as is usual, from the ratio of injected dose D to the AUC in mixed venous plasma. In our study this curve is the recirculating curve sampled in the pulmonary artery, therefore:

$$\text{MCR} = D / \text{AUC}_{\text{pulm,rc}} \quad (11)$$

Using the experimental data produced by the bolus approach E_{wb} is defined by an equation analogous to Eq 8:

$$E_{\text{wb}} = (\text{AUC}_{\text{pulm}} - \text{AUC}_{\text{pulm,rc}}) / \text{AUC}_{\text{pulm}} \quad (12)$$

FIG. 3. Concentration curves of ^{125}I -labeled ANP sampled in pulmonary artery and aorta after bolus administration of tracer in right atrium; concentrations are in percent dose per liter and time is seconds after injection. Sharp initial peak produced by first pass of tracer (dotted line) is evident, followed by a slower curve caused by tracer that recycles. A similar pattern can also be seen in the aortic curve, with some spreading and a 10- to 20-sec delay compared with pulmonary curve. Note that sampling throughout first 130 sec after injection was performed by continuous withdrawal of blood through a catheter connected to a pump and an automatic collector. Concentration of each of these 13 integrated samples is average concentration in plasma during respective 10-sec interval. In addition to experimental points, tail of first-pass curve (computed by monoexponential extrapolation of steepest descent of peak of pulmonary curve) and recirculating curve (computed as difference between whole pulmonary curve and first-pass curve) are also shown. Note that all curves are delayed for approximately 30 sec because of the catheter used for sampling, as indicated by three samples free of radioactivity in pulmonary artery; delay, however, does not affect computations of kinetic parameters based on AUCs.



For the sake of simplicity, it is assumed $E_{rh} = 0$, that is $D = \text{AUC}_{\text{pulm},1p} F$; starting from the relation $\text{AUC}_{\text{pulm},1p} = \text{AUC}_{\text{pulm}} - \text{AUC}_{\text{pulm},rc}$ and multiplying both sides by F , we have:

$$D = (\text{AUC}_{\text{pulm}} - \text{AUC}_{\text{pulm},rc})F \quad (13)$$

Eq 13 is analogous to Eq 9 and states the balance between the injected dose and the amount degraded. Dividing by $\text{AUC}_{\text{pulm},rc}$ we obtain:

$$D/\text{AUC}_{\text{pulm},rc} = (\text{AUC}_{\text{pulm}} - \text{AUC}_{\text{pulm},rc})F/\text{AUC}_{\text{pulm},rc} \quad (14)$$

Eq 14, bearing in mind Eq 11, Eq 12, and the relationship $\text{AUC}_{\text{pulm},rc}/\text{AUC}_{\text{pulm}} = (1 - E_{wb})$, can be written in the form of Eq 10. In conclusion, MCR, either obtained from the infusion approach (Eq 7) or from the bolus approach (Eq 11), is equal to the product of plasma cardiac output F times a coefficient $[E_{wb}/(1 - E_{wb})]$, which depends on the whole body extraction. Note also that, by solving for E_{wb} , Eq 10 can be rewritten in the form:

$$E_{wb} = \text{MCR}/(F + \text{MCR}) \quad (15)$$

this last equation allows E_{wb} to be calculated from MCR if an estimate of F is available.

Statistical analysis

The unpaired t test was used for the comparisons between the two groups of patients; the nonparametric test, Mann-Whitney U test was

used when the variances of the two tested groups were statistically different by F test. The results are expressed as mean \pm SD if not otherwise stated.

Results

Native ANP levels in pulmonary artery and aorta

The ANP values measured by IRMA in the aorta (163.9 ± 144.8 pg/mL, $n = 80$) were, on average, superimposable on those measured in the pulmonary artery (161.8 ± 136.5 pg/mL, $n = 80$). A very close linear regression ($R = 0.968$, $y = -2.18 + 1.03 x$), not different from the identity line, was found between all the values of native ANP plasma concentrations simultaneously determined before and during the kinetic study in the pulmonary artery and in the aorta from all patients (Fig. 4). These data support the idea of no degradation in the lungs and left heart. However, if one takes into account the ratio of pairs of samples simultaneously drawn from the aorta and the pulmonary artery in each patient, an extraction value ranging between +57 and -79% (negative values are generated when the concentration in the pulmonary artery is lower than that in the aorta) was found. These findings indicate that computation of extraction based

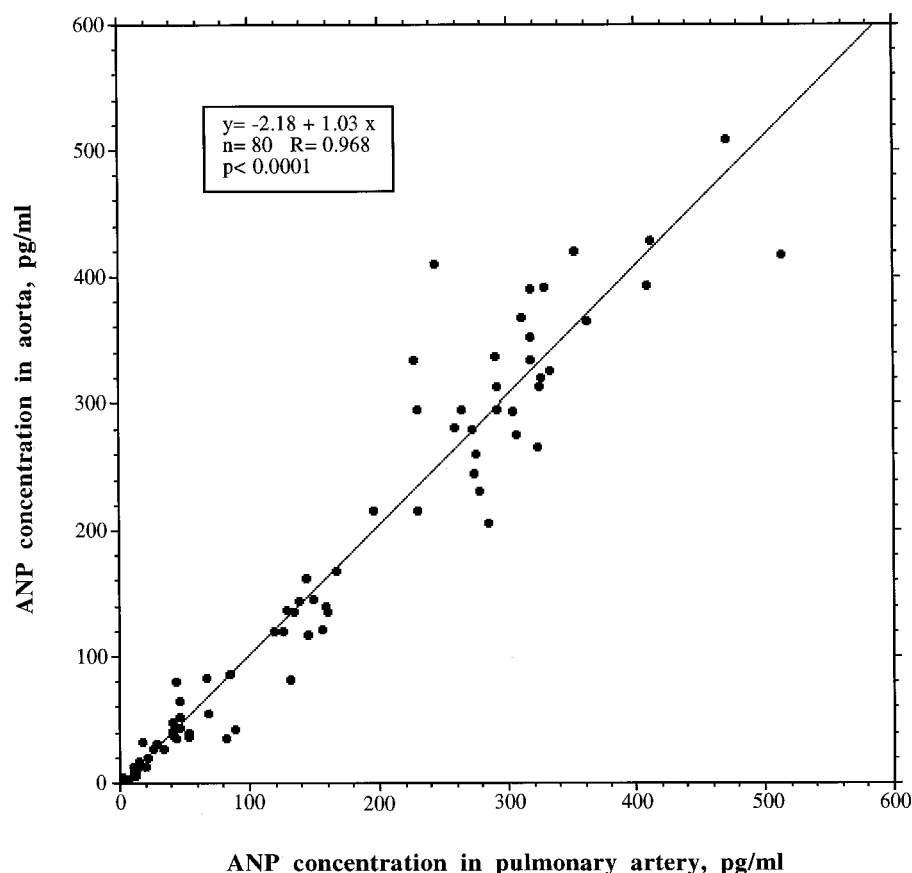


FIG. 4. Linear regression found between 80 values of native ANP plasma concentrations simultaneously sampled in pulmonary artery and in aorta, and measured with IRMA in all patients.

on the individual pairs of values is unreliable, probably because of pulsatory secretion that produces fluctuations in ANP levels particularly evident in the proximity of the secretion site.

Infusion studies

A typical set of experimental data obtained after equilibration of infused tracer is reported in Fig. 2. ^{125}I -Labeled ANP plasma concentrations, measured in the pulmonary artery, aorta, inferior vena cava, and femoral vein in the three studies (*i.e.* patients 1, 2, and 3) are reported in Table 2. That steady state for tracer was reached was confirmed by near constant levels of ^{125}I -labeled ANP found throughout the sampling period (20–25 min) (Fig. 2). In all three cases no differences between ^{125}I -labeled ANP concentrations in the pulmonary artery and in the aorta were detected, whereas markedly lower levels were observed in the inferior vena

cava (on average 48% lower in respect to pulmonary and aortic arteries). Plasma ^{125}I -labeled ANP was found to be slightly higher in the femoral vein than the inferior vena cava (Table 2), thus suggesting different hormonal extraction in peripheral districts.

Extractions of the three blocks (and of whole body), calculated for all patients according to the equations of the model, are reported in Table 2. No (or negligible) extraction of the hormone was found to occur in the right heart and lungs and left heart blocks in all studies (mean extraction 0.37%), so that the peripheral block extraction (51.6%, on average) accounts for almost all the hormone removal from blood (whole body extraction, E_{wb} , on average 51.9%).

Bolus injection studies

A typical time course of labeled ANP in pulmonary artery, in aorta, and in mixed venous plasma (recirculating curve in

TABLE 2. Infusion studies: mean steady state concentrations of labeled ANP normalized by the infusion rate (IR) and main kinetic parameters

Patient no.	Pulmonary artery (% IR/L)	Aorta (% IR/L)	Inferior vena cava (% IR/L)	Femoral vein (% IR/L)	E_{l+lh} (%)	E_p (%)	E_{wb} (%)	MCR (L/min)	F (L/min)
1	50.2	49.6	26.8	29.3	1.2	46.0	45.2	3.73	4.52
2	45.6	45.9	20.4	31.5	-0.5	55.5	56.1	4.90	3.84
3	31.5	31.4	14.7	18.8	0.4	53.2	54.4	6.80	5.70
Mean	42.4	42.3	20.6	26.5	0.37	51.6	51.9	5.14	4.69
SD	9.7	9.6	6.1	6.8	0.85	5.0	5.86	1.55	0.94

Abbreviations: E_{l+lh} , extraction of lungs and left heart block; E_p , periphery extraction; E_{wb} , whole body extraction; MCR, metabolic clearance rate; F, plasma cardiac output.

pulmonary artery) is shown in Fig. 3. Table 3 reports the AUCs in pulmonary artery (AUC_{pulm}) and in aorta (AUC_{aorta}). In addition, Table 3 reports the area relative to the first pass ($AUC_{\text{pulm,1p}}$) and the area relative to recycling ($AUC_{\text{pulm,rc}}$) of the pulmonary curve. The product of $AUC_{\text{pulm,1p}}$ first-pass radioactivity \times plasma cardiac output F (see Table 3) allows computation of the amount of labeled material that passes through the pulmonary artery (see *Data Analysis* and Fig. 3); this figure corresponds to the fraction of the dose that is recovered in the pulmonary artery, and allows a direct measurement of extraction coefficient of the RH block. The recovery of the ^{125}I -labeled ANP radioactivity in the pulmonary artery (*i.e.* the product of $AUC_{\text{pulm,1p}}$ and F , see Table 3) was on average $99.5 \pm 4.3\%$ of the dose injected into the right atrium, thus demonstrating both the validity of the continuous sampling protocol and the negligible ^{125}I -labeled ANP extraction from right heart. In addition, this experimental finding strongly supports the accuracy of the AUC relative to true mixed venous plasma, *i.e.* $AUC_{\text{pulm,rc}}$ and therefore the accuracy of MCR values (see *Data Analysis*, Eq 11).

From the values of AUC_{pulm} and AUC_{aorta} the extraction of the lungs and left heart block is computed; the virtually superimposable values for the two areas (on average 80.5 and 80.2% dose/liter min, respectively) indicate that extraction was undetectable (on average -0.01%). On the other hand, the values of $AUC_{\text{pulm,rc}}$ (43.9% dose/liter min on average) were found to be markedly lower than AUC_{aorta} (on average 80.2% dose/liter min), indicating that a large extraction from the periphery does occur (on average 44.5%), and is nearly superimposable on whole body extraction (44.8%).

Relationships between ANP clearance, whole body extraction, and hemodynamic state

Individual values for MCR and whole body extractions of labeled ANP are reported in Table 2 (infusion studies) and Table 3 (bolus injection studies) together with plasma cardiac outputs. The relationship between MCR values (measured by both approaches) and the corresponding plasma cardiac outputs is represented in Fig. 5; a significant positive linear regression can be observed, suggesting that MCR is markedly affected by alterations in hemodynamics. MCR [$3.11 \pm$

1.48 L/min, coefficient of variation (CV) = 47.7%] and plasma flow values F (3.46 ± 1.08 L/min, CV = 31.4%) varied among the 13 patients, both probably depending on the severity of cardiac disease. On the other hand, whole body extraction for all patients ($46.4 \pm 6.6\%$) is a more stable parameter (CV = 14.2%) and does not correlate with plasma flow ($R = 0.075$), suggesting that whole body extraction is a kinetic parameter less (or not at all) affected by the hemodynamic conditions.

Discussion

The present data demonstrate that human lungs (and heart) do not extract labeled ANP; either it is continuously infused or bolus injected. Indeed, we found superimposable labeled ANP steady state concentration values in the pulmonary artery (input of lungs) and aorta (output of lungs) during constant infusion of tracer in the right atrium, and superimposable AUC values of labeled ANP in the aorta and in pulmonary artery after bolus injection of tracer, indicating that tracer that enters lungs through the pulmonary artery is completely recovered in the aorta. It is also evident from all the present studies that ^{125}I -labeled ANP is extracted *in vivo* by peripheral organs, in agreement with previous data (1, 14, 20). This last observation gives further support to the hypothesis that labeled ANP shares similar biological activity with native hormone, as already reported by two studies demonstrating that labeled ANP is able to interact with specific receptors and can be degraded by specific enzymes in a similar manner to the native hormone (1, 21). Therefore, our data strongly suggest that native ANP is also not extracted and degraded by lungs or by the heart in humans. Previous studies, mainly based on the measurement of AV differences of native ANP, reported contrasting or inconclusive results of the role of the lungs and heart; lung extraction values ranging from 0–67% were found (4–12). Also, our data on native ANP measurement generate extraction values ranging from -79 to $+57\%$ if corresponding single pairs of samples simultaneously drawn from pulmonary artery and aorta are considered. On the other hand, our overall ANP values for pulmonary artery and aorta are, on average, superimposable, and are thus in agreement with similar data previously reported by others (4, 6–12).

TABLE 3. Bolus injection studies: AUCs and main kinetic parameters

Patient no.	AUC_{pulm} (% dose/liter min)	$AUC_{\text{pulm,1p}}$ (% dose/liter min)	$AUC_{\text{pulm,rc}}$ (% dose/liter min)	AUC_{aorta} (% dose/liter min)	$E_{\text{l+lh}}$ (%)	E_{p} (%)	E_{wb} (%)	MCR (L/min)	F (L/min)
4	67.2	28.5	38.7	69.1	-2.8	44.0	42.4	2.58	3.51
5	81.9	32.7	49.2	79.1	3.4	36.8	39.9	2.03	3.01
6	48.3	23.3	25.0	49.8	-3.1	49.3	48.2	4.00	4.25
7	63.6	30.7	32.9	67.3	-5.8	51.0	48.3	3.04	3.25
8	63.0	28.7	34.3	62.3	1.1	43.4	45.6	2.92	3.39
9	119.9	47.2	72.7	121.6	-1.4	40.5	39.4	1.38	2.13
10	69.7	29.1	40.6	65.3	6.3	37.3	41.8	2.46	3.41
11	75.6	27.9	47.7	71.5	5.4	33.0	36.9	2.10	3.57
12	148.3	85.9	62.4	143.8	3.0	57.9	57.9	1.60	1.20
13	67.5	32.0	35.5	71.7	-6.2	52.2	47.4	2.82	3.24
Mean	80.5	36.6	43.9	80.2	-0.01	44.5	44.8	2.493	3.096
SD	30.3	18.4	14.5	29.2	4.50	7.9	6.1	0.765	0.849

Abbreviations: AUC_{pulm} , area under whole pulmonary curve; $AUC_{\text{pulm,1p}}$, area under first pass pulmonary curve; $AUC_{\text{pulm,rc}}$, area under recirculating pulmonary curve; AUC_{aorta} , area under aorta curve; $E_{\text{l+lh}}$, extraction of lungs and left heart block; E_{p} , peripheral extraction; E_{wb} , whole body extraction; F , plasma cardiac output.

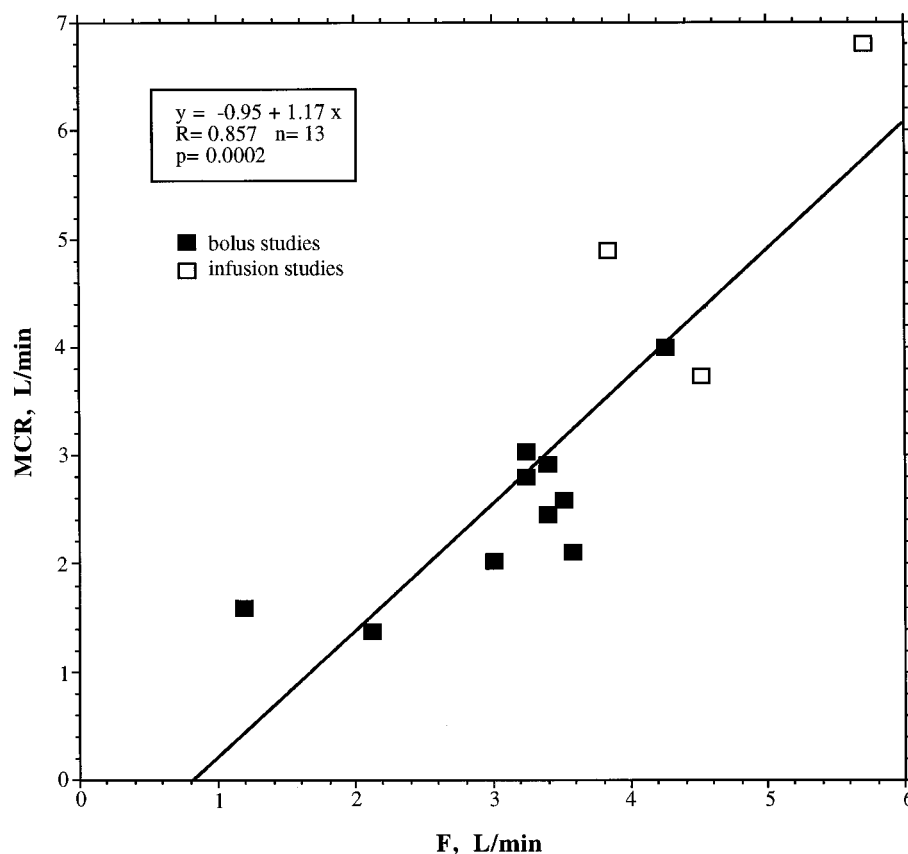


FIG. 5. Significant positive linear regression found between MCR values and plasma cardiac output (F) observed in 13 patients studied.

These controversial findings could probably be explained by fluctuations of the hormone concentrations in plasma generated by the pulsatory secretion pattern, particularly in the proximity of the secretory site, which limits the value of measuring instantaneous AV differences. By assuming identical behavior of labeled and native ANP, further support for this explanation is shown by the data in Fig. 3, reporting ANP plasma concentrations in the pulmonary artery, aorta, and periphery (recirculating curve) in response to a pulse administration of the hormone. It is evident that the ratio between ANP concentrations in a single pair of samples taken simultaneously in the aorta and in pulmonary artery can oscillate widely (with pulmonary ANP values higher than aorta values up to 50 sec and vice versa thereafter) because of the delay (and spreading) of the hormone peak in aorta after passing through the circulation of lungs.

Another important piece of experimental evidence emerging from data shown in Fig. 3 is that fluctuations observed at the peripheral level (recirculating curve) after a pulse of secretion into the right atrium are dampened out by peripheral extraction and by diffusion through the lungs and periphery. This last finding could help to explain why relatively stable concentrations of native ANP are observed in peripheral venous plasma notwithstanding a wide direct experimental evidence of a pulsatile secretion pattern of the hormone (1).

Our results are in contrast with the hypothesis, put forward by other authors (7–12), that significant extraction of endogenous ANP does occur in the lungs, but that the levels in the aorta, superimposable on those found in the pulmo-

nary artery, are restored thanks to a direct secretion of the hormone through the endocardium of the left heart. The above hypothesis seems to be highly unlikely, because it implies the presence of a peculiar feedback mechanism between the left heart and lungs by which the amount of hormone degraded in the lungs should be replaced by an identical amount secreted immediately into circulation by the left heart.

Our studies provide estimates of ANP clearance in cardiac patients obtained either by tracer bolus injection or constant infusion. The large variability in MCR values (range 1.38–6.8 L/min) observed in our series of patients seems to be related to the degree of heart failure. Some limitations to both methodological approaches, however, have to be considered. As far as the bolus protocol is concerned, its validity is clearly dependent on the accuracy in estimating the first-pass area ($AUC_{p_{pulm,1p}}$) and therefore the AUC in mixed venous plasma ($AUC_{p_{pulm,rc}}$). This depends on the estimation of the time of onset of the recycle and on the extrapolation of the first-pass peak. Direct evidence that the experimental approach we adopted was suitable to accurately split $AUC_{p_{pulm}}$ into $AUC_{p_{pulm,1p}}$ and $AUC_{p_{pulm,rc}}$ is given by the near 100% recovery of total injected dose, computed as $AUC_{p_{pulm,1p}}$ times plasma cardiac output, independently measured by thermodilution. It should be emphasized that the contribution of the extrapolated portion of the area to the total $AUC_{p_{pulm,1p}}$ is limited (*i.e.* no more than 10–15% of the first-pass area), thus minimally affecting MCR values (in our studies no more than 8%).

In infusion studies, a possible underestimation of the true

mixed venous blood returning to the right heart could induce an overestimation of MCR values, when they are computed from ^{125}I -labeled ANP concentrations measured in the inferior vena cava only. Indeed, a 27% higher ANP plasma concentrations in superior vena cava has been reported (22). However, because the blood flow in superior vena cava is about one third to one fourth that in inferior vena cava, different weights of the ANP concentration make the extent of the underestimation not significant (22) (*i.e.* <7% in our cases). Actually, we found an MCR mean value in infusion studies approximately 2-fold higher than that estimated by the bolus protocol (5.14 L/min *vs.* 2.49 L/min). In our opinion, a more likely explanation could be related to the lesser degree of myocardial dysfunction in patients submitted to infusion protocol, as also demonstrated by corresponding higher values of plasma cardiac output in those patients (4.7 L/min *vs.* 3.0 L/min, respectively).

The present tracer experiments in which, in addition to MCR, cardiac output was also measured, for the first time allow evaluation of interesting pathophysiological information, *i.e.* the effects of hemodynamics on ANP metabolism *in vivo* in humans. Indeed metabolic clearance of ANP can be viewed as dependent on two factors (see *Data Analysis*, Eq 10): the whole body extraction, which represents an intrinsic characteristic of overall hormonal degrading systems operating in the body, and the plasma cardiac output, which represents the contribution of hemodynamics. Our data demonstrate that ANP clearance values declined with the progression of left ventricular dysfunction because of decreased plasma cardiac output (Fig. 5), whereas whole body extraction remained relatively constant and was not correlated to plasma flow. This suggests that the overall degradative systems for ANP are unaffected during the evolution of heart failure. Figure 5 also shows that clearance values are similar to plasma cardiac output values or, in other words, the slope of the regression line between clearance and flow values is close to 1. According to Eq 15 (see *Data Analysis*) it can then be predicted that, when clearance value is similar to plasma cardiac output, the whole body extraction is approximately 50% as observed in our studies.

Because of the marked (on average 6-fold) increase in the circulating ANP in patients with different degrees of left ventricular dysfunction (ANP aortic levels in less severe disease, NYHA class I-II = 28.4 ± 26.1 pg/mL; in more severe disease, NYHA class III-IV, ANP = 174.8 ± 80.0 pg/mL, $P = 0.0043$, Mann-Whitney U test), the amount of hormone degraded significantly and progressively increased with the progression of disease despite the reduction of MCR probably associated to the concomitant contraction in blood flow. These results are in good agreement with our previous data reporting a marked and progressive alteration of ANP degradation and distribution in patients with left ventricular involvement (15, 23).

In conclusion, our data show that lungs and heart blocks do not extract any or extract only negligible amounts of labeled ANP administered *iv* to patients with different de-

grees of left ventricular dysfunction, suggesting that human lungs are also unable to degrade significant amounts of endogenously produced ANP. Our findings also indicate that ANP is largely (~50%) extracted during a single pass across the peripheral tissues independent from the compromised hemodynamics throughout the evolution of cardiac failure.

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