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Evidence That Atrial Natriuretic Peptide Tissue Extraction Is Not Changed by Large Increases in Its Plasma Levels Induced by Pacing in Humans

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ABSTRACT

Atrial natiurectic peptide (ANP) is a cardiac hormone with a very short plasma half-life, which plays an important role in a variety of clinical conditions associated with an increase in pressure and/or volume overload on the heart. The MCR of the hormone is considered to represent a stable parameter, reflecting the uptake and degradation rate of ANP by the periphery, only scarcely affected by rapid oscillations of circulating levels. To evaluate the extent to which MCR is affected by rapid and large variations of circulating levels of the hormone, we measured MCR in five patients with different degrees of myocardial function (from normal to severely impaired), in whom changes in ANP levels were induced by atrial and/or ventricular pacing. Cardiac output was simultaneously measured by thermodilution to calculate whole body extraction of ANP. During constant iv infusion of [^{125}I]ANP, the hormone MCR was determined both under basal conditions (at tracer equilibration, 20–30 min after the start of infusion) and during atrial and ventricular pacing. Pacing maneuvers, begun 50 min after the start of infusion, induced a marked and rapid increase in endogenous plasma ANP values in all patients (on the average, 3.7-fold compared to basal values; range, 1.8–5.68), whereas corresponding values of [^{125}I]ANP only minimally changed. The MCR of ANP (3.62 ± 1.06 L/min, mean ± SD) slightly decreased (by repeated measures ANOVA, $P = 0.0458$) during atrial and ventricular pacing procedures (3.35 ± 1.03 and 3.15 ± 0.74 L/min, respectively), reaching a mean value of 88.7 ± 9.0% compared to basal. The small decrease in MCR could be almost completely ascribed to hemodynamic factors; indeed, basal cardiac output (5.76 ± 1.70 L/min) was found, on the average, to be slightly decreased during atrial and ventricular pacing (5.28 ± 1.46 and 5.16 ± 1.33 L/min, respectively), and so whole body extraction of the hormone, measured before pacing (50.0 ± 12%), remains stable throughout the study period (50.4 ± 10.6% and 49.6 ± 10% during atrial and ventricular pacing, respectively). Our findings demonstrate that degradative mechanisms involved in ANP clearance are not saturable at least for acute elevations of ANP plasma levels up to 3–5 times the basal level. (*J Clin Endocrinol Metab* **82:** 884–888, 1997)

MAMMALIAN atrial cardiocytes synthesize, store, and release a polypeptide hormone with natriuretic and arterial smooth muscle relaxant properties, termed atrial natriuretic peptide or factor (ANP or ANF) (1, 2). The main hormonal actions of ANP are 1) the promotion of sodium and water excretion, 2) the reduction of systemic arterial pressure, and 3) the modulation of volume-regulating hormones (2). Owing to its physiological activity, it was established that ANP plays an important role in diseases characterized by an expanded fluid volume, including cardiac and renal insufficiency (2). In fact, circulating ANP levels were found to be proportional to the progression of the severity of cardiac insufficiency and to the deterioration of hemodynamics, and a positive relation has been reported between mortality and ANP levels in patients with congestive heart failure (3, 4).

The conclusions from the experimental and/or clinical studies on the pathophysiological role of ANP system are, in general, based on the circulating levels of the hormone; thus, it is assumed that plasma levels are an index of the hormonal production rate under the *a priori* hypothesis that degradation does not vary greatly in different conditions. From this clearly emerges the interest in measuring the MCR of ANP in various conditions to support these conclusions.

Moreover, plasma ANP levels are found to rapidly and widely fluctuate in response to different secretory stimuli, all of which are characterized by an increase in pressure or volume load of the heart (5, 6). In accordance with these findings, previous kinetic studies (5–7) have demonstrated that the plasma half-life of ANP is very short (only a few minutes), so that ANP concentrations are thought to closely parallel the instantaneous secretion rate, assuming that the degradation rate remains relatively constant.

To evaluate whether and to what extent the MCR of ANP is affected by rapid and large variations in its circulating levels, we measured the MCR by means of a tracer method in five patients with different degrees of myocardial function (from normal to severely impaired), in whom changes in ANP levels were induced by atrial and/or ventricular pacing.

Subjects and Methods

Experimental subjects

Five normotensive cardiac patients with different degrees of myocardial dysfunction were enrolled in the study. All patients were submitted to a baseline complete cardiological evaluation, including two-dimensional echocardiography and radionuclide-angiography. All patients had to undergo a hemodynamic and an electrophysiological (pacing) study to evaluate the myocardial function and because of some

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episodes of paroxysmal supraventricular and/or ventricular arrhythmias. Their main clinical and hemodynamic parameters are reported in Table 1. The patients were hospitalized in the metabolic ward of our institute; at the entrance, all patients were kept at relatively restricted sodium intake diet (100–120 mmol/day). All drugs were withdrawn at least 3 days before the study; patients treated with drugs with a relatively long half-life (such as digoxin or amiodarone) were not enrolled in the study. All patients received a daily dose of 20 drops of saturated Lugol solution from the day before until the day after the kinetic study.

Pacing technique

All kinetic studies were carried out at the hemodynamic unit of our institute in the morning after an overnight fast. Each patient was kept at rest in a supine position from 30 min before the start to 15 min after the end of the pacing study. A Swan-Ganz catheter was inserted transcutaneously through the left succlavia vein and advanced to obtain right atrial, pulmonary artery, and pulmonary capillary wedge pressures. The cardiac index was calculated as the ratio of the mean of at least five thermodilution cardiac output measurements to body surface area (square meters). Measurements of cardiac output and all the other hemodynamic parameters were carried out immediately before, during atrial and ventricular pacing (at the half-time of the pacing period), and after pacing. A flared pacing bipolar catheter was positioned into the right atrium via a percutaneous puncture of the right femoral vein. When a satisfactory pacing threshold had been achieved, the pacing rate was increased rapidly until atrioventricular block occurred. Then, after 5 min of rest, the catheter was positioned into the right ventricular apex; ventricular pacing was performed at 85% of the maximum heart rate, according to the patient's age and gender.

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

Tracer study

Synthetic human αANP-(1–28) (Bachem Feinchemikalien, Bubendorf, Switzerland) was iodinated with $Na^{125}I$ or $Na^{131}I$ (both supplied by Sorin, Saluggia Vercelli, Italy) and purified as previously described (5–7). The specific activity of [^{125}I]ANP ranged between 2000–2200 Ci/mmol (650–700 μCi/μg). A known amount of the tracer (100 μCi), put into a syringe containing 50 mL Emagel solution, was infused at constant rate from an antecubital vein by means of a mechanical high precision pump at a rate of 0.4 mL/min.

To measure the basal hormone clearance, the steady state level of labeled ANP (typically reached 20–30 min after the start of infusion) was determined by four blood samples (6 mL) obtained from an antecubital vein of the other arm (from 30–50 min after the start of infusion). The pacing procedure was begun 50 min after the start of infusion; three blood samples were withdrawn during and at the end of each of the two pacing (atrial or ventricular) periods. Infusion of tracer was stopped after three blood samples collected during the recovery period (10–15 min after the end of ventricular pacing and 90–100 min after the start of infusion). In one patient (patient 5; Table 1), six additional plasma samples were also collected after stopping the infusion (from 3–20 min after stopping of the infusion) to describe the disappearance curve of tracer from blood. The collected blood samples were immediately put into ice-chilled disposable polypropylene tubes, containing aprotinin (500 kallikrein inhibitor units/mL plasma) and ethylenediamine tetraacetate (1 mg/mL plasma), and the plasma was then separated in a refrigerated centrifuge at 4 C within 1 h. After each blood collection, the volume of blood withdrawn was replaced by an equal volume of saline, which also served for washing the catheter. Extraction, purification by high performance liquid chromatography, and measurement of labeled ANP in plasma samples were carried out as previously described (5–7).

Determination of [^{125}I]ANP blood/plasma partition factor

As the labeled ANP concentration was measured in plasma, blood flow (cardiac output) had to be converted into plasma flow through the ANP blood/plasma ratio. To determine this ratio, a known amount of labeled ANP was added to 3 mL blood obtained immediately before the tracer infusion. The partition of labeled ANP between plasma and cells (red cells, white cells, platelets, *etc.*) was measured after the common procedure of centrifugation and separation used in the kinetic study for all plasma samples. On the average, this factor was found to be 62.2 ± 2.7%.

ANP assay

Plasma ANP was measured with a direct (without plasma extraction) immunoradiometric assay (IRMA) method as previously described (8). The sensitivity of this IRMA method was 2.13 ± 0.91 pg/mL, and the between-assay imprecision was 11.4% (mean ± SD, 22.61 ± 2.57 pg/mL) for one pool and 8.0% (178.6 ± 14.3 pg/mL) for the other. For the ANP assay, two blood samples were obtained just before the start of the tracer study, and an aliquot of each blood sample was collected during the kinetic study into ice-chilled disposable polypropylene tubes containing plasma protease inhibitors (8). They were immediately separated by centrifugation, then frozen and stored at −20 C. To further improve the assay precision, plasma samples with the lowest ANP levels (*i.e.* <50 pg/mL) were assayed using larger volumes of plasma (300 μL) (8).

Data analysis

As usually performed in similar kinetic studies, plasma clearance MCR was computed from the ratio of the infusion rate (IR) to the steady state concentration of the tracer in peripheral venous blood: $MCR = IR/c_{ven}$ (9).

The availability of simultaneously measured cardiac output makes it possible to define ANP degradation not only in terms of plasma clearance, but also in terms of whole body extraction. The knowledge of cardiac output permits the employment of a more physiologically and anatomically meaningful model (circulatory model) that also incorporates the blood flow (at variance with the compartmental approach).

This model, schematically represented in Fig. 1, is composed of a single block that represents in an extremely simplified fashion the circulation of the perfused body and is characterized by ANP whole body extraction (E_{wb}; or, alternatively, by whole body transmission: $T_{wb} = 1 - E_{wb}$). A flow F circulates through the system. The concentrations c_{pulm} and c_{ven} are the steady state concentrations of labeled ANP measured, respectively, upstream (input) and downstream (output) of the extracting block; experimentally, these concentrations can be measured by

TABLE 1. Mean clinical and hemodynamic data of the patients studied

Patient no.	Age (yr)	Gender	BS (m^2)	UCD	NYHA class	CO (L/min)	CO_{ap} (L/min)	CO_{vp} (L/min)	MPAP (mm Hg)	PAWP (mm Hg)	MSP (mm Hg)	EF (%)
1	39	M	1.78	CAD	I	6.2	6.3	6.6	15	10	90	60
2	55	F	1.76	CAD	I	8.0	6.5	6.0	31	18	106	45
3	54	M	2.00	DCM	III	3.5	3.4	3.1	40	28	102	29
4	57	F	1.73	DCM	II–III	4.8	4.0	5.0	32	21	94	40
5	70	M	2.20	CAD	I	6.3	6.2	5.1	18	8	87	64
Mean	55.0		1.89			5.76	5.28	5.16	27.2	17.0	95.8	47.6
SD	11.0		0.20			1.70	1.46	1.33	10.4	8.2	8.0	14.4

BS, Body surface; UCD, underlying cardiac disease; CAD, coronary artery disease; DCM, dilated cardiomyopathy; CO, cardiac output; CO_{ap}, cardiac output during atrial pacing; CO_{vp}, cardiac output during ventricular pacing; MPAP, mean arterial pulmonary pressure; PAWP, pulmonary artery wedge pressure; MSP, mean arterial systemic pressure; EF, left ventricular ejection fraction, measured by radionuclide-angiography.

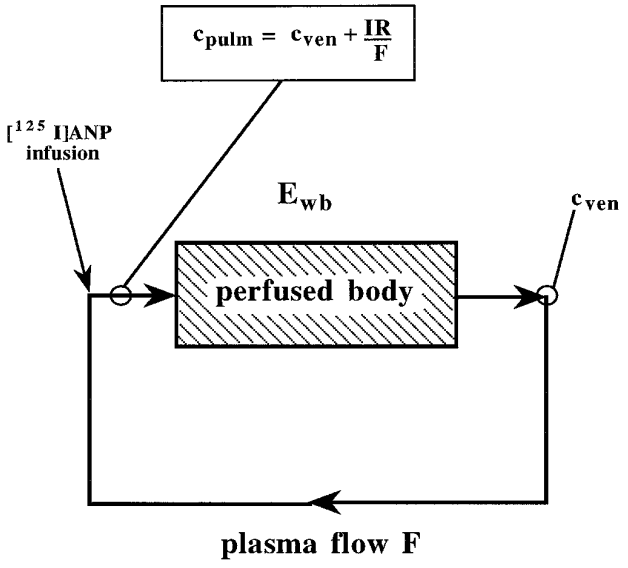

FIG. 1. Schematic representation of the simplified circulatory model used to derive the relation between extraction of the perfused body (E_{wb}), flow (F), and MCR. For details, see *Data analysis* section in *Materials and Methods*.

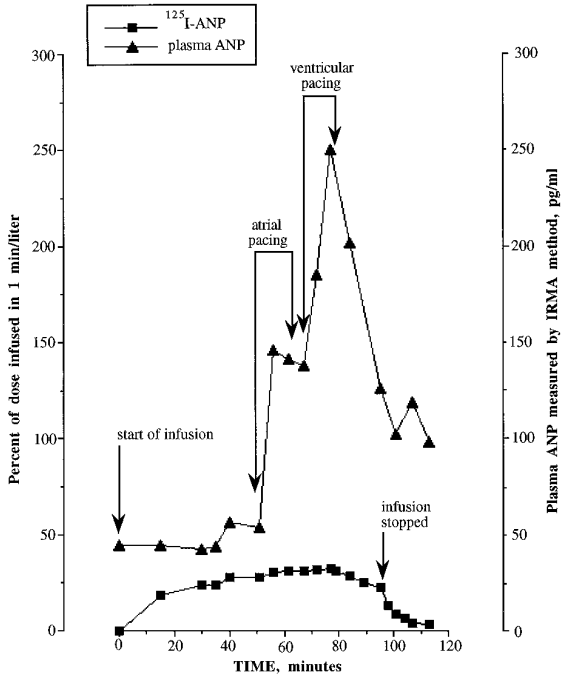

FIG. 2. [125I]ANP and native ANP concentrations measured in plasma during constant infusion of [125I]ANP. After an equilibration period (20–30 min), the patient (patient 5 in Tables 1 and 2) was submitted to atrial and ventricular pacing (indicated by the *arrows*) during the kinetic study. Plasma concentrations of labeled ANP are normalized by infusion rate (IR), *i.e.* expressed as a percentage of the dose infused in 1 min/L; the MCR is, therefore, calculated as 100/steady state tracer concentration. Tracer levels were also sampled after stopping the infusion, for an additional 20-min period, thus allowing for computation of the total area under the curve (AUC = 26.37% dose/L·min). The average MCR in the infusion period (95 min) was computed as 100/AUC = 3.79 L/min.

sampling the pulmonary artery (c_{pulm}) and a peripheral vein (c_{ven}). It is worth noting that as steady state plasma concentrations of labeled ANP (*i.e.* c_{pulm} and c_{ven}) are measured in plasma (as counts per min/mL plasma), the flow of the circulatory system must be plasma flow, which is computed multiplying cardiac output by the ANP blood/plasma partition factor (see above). The model is completely defined by the experimentally determined c_{ven}, F, and IR. The concentration c_{pulm} upstream of the extraction sites is computed as $c_{ven} + IR/F$, because before any extraction takes place, the increase in tracer concentration produced by infusion is equal to the infusion rate divided by flow.

Whole body extraction is by definition: $E_{wb} = (c_{pulm} - c_{ven})/c_{pulm}$ (Eq I) and substituting $c_{pulm} = c_{ven} + IR/F$, we obtain $E_{wb} = (IR/F)/(c_{ven} + IR/F)$ (Eq II). This last equation allows for the computation of whole body extraction starting from experimentally determined c_{ven}, F, and IR. It is important to derive how the value of clearance rate, MCR (computed as the ratio of IR to the peripheral vein concentration, c_{ven}), is related to E_{wb}. The relationship is obtained by writing that in steady state conditions for tracer, the amount of labeled ANP degraded per time unit ($c_{pulm} - c_{ven}$) F must be equal to the amount infused, IR: $IR = (c_{pulm} - c_{ven})F$ (Eq III). Dividing both sides of Eq III by c_{pulm}, we obtain: $IR/c_{pulm} = E_{wb}F$ or $MCR^* = E_{wb}F$ (Eq IV). Therefore, if the ratio IR/c_{pulm} is defined as clearance MCR* (the symbol MCR* is used for the ratio IR/c_{pulm} to distinguish it from $MCR = IR/c_{ven}$), we obtain the well known relationship stating that clearance is equal to flow × extraction. Note that this well known relationship holds for MCR* (and not for MCR), *i.e.* when clearance is calculated using the concentration measured in the pulmonary artery, where newly infused tracer is still unextracted.

However, the relation between MCR* and MCR is very simple; remembering that $c_{ven} = c_{pulm}T_{wb}$, we derive $MCR = MCR^*/T_{wb}$ and, finally, $MCR = (E_{wb}/T_{wb})F$ (Eq V). Equation V states that clearance MCR (computed using c_{ven}) is proportional to flow; the proportionality factor, however, is not extraction (as it is for MCR*; see Eq IV), but, rather, the ratio of extraction to transmission. This difference is due to the fact that clearance is computed using the concentration measured downstream of the extracting block (c_{ven}) instead of using the concentration upstream of the extracting block (c_{pulm}). From a theoretical point of view, it seems more correct to define clearance as MCR* instead of MCR. However, we will use MCR, as it is customary, and we note that, from a practical point of view, the values of MCR and MCR* are appreciably different only when the extraction is relatively high. When the E_{wb} is low, that is T_{wb} is near to 1, MCR and MCR* are practically the same. Note that this difference in the computation of clearance rate (MCR *vs.* MCR*) cannot be explained in terms of compartmental approach, which more or less explicitly assumes a uniform concentration in plasma compartment.

Results

A typical set of experimental data obtained from two patients with different degrees of myocardial involvement after equilibration of the infused tracer and during atrial and ventricular pacing is reported in Figs. 2 and 3. The achievement of steady state for tracer was confirmed by near-constant levels of labeled ANP (coefficient of variation = 8.7%) found 30–50 min after the start of infusion, before pacing. Pacing maneuvers induced a marked and rapid increase in endogenous plasma ANP values in all patients (on the average, 3.7-fold with respect to basal values; Table 2), whereas corresponding values of labeled ANP in plasma only minimally changed.

Individual values of MCR, native ANP plasma levels, and ANP whole body extraction (E_{wb}) measured before and during atrial and ventricular pacing in all studied patients are reported in Table 2. The MCR of ANP (3.6 L/min, on the average) only slightly decreased (by repeated measures ANOVA, $P = 0.0458$) during pacing procedures (3.4 and 3.2 L/min, on the average, during atrial and ventricular pacing, respectively), with a mean MCR value during ventricular pacing of 88.7% (SD = 9.0%) compared to that found under basal conditions.

The small decrease in MCR observed during the study could be almost completely ascribed to hemodynamic fac-

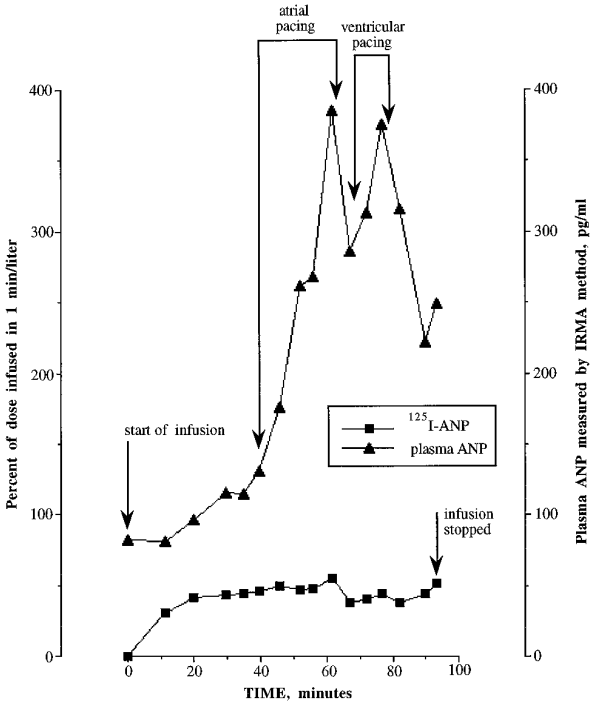

FIG. 3. [^{125}I]ANP and native ANP concentrations measured in plasma during the infusion of [^{125}I]ANP. During the kinetic study, the patient (patient 4 of Tables 1 and 2) was submitted to atrial and ventricular pacing (indicated by the *arrows*). Plasma concentrations of labeled ANP are normalized by the infusion rate (IR), *i.e.* expressed as a percentage of the dose infused in 1 min/L; the MCR is calculated as 100/steady state concentrations.

tors. In fact, cardiac output was, on the average, also found to slightly decrease during pacing and, as a consequence, whole body extraction of the hormone, measured before pacing (on the average, E_{wb} = 50.0%), remains stable throughout the study period (on the average, 50.4% and 49.6% after atrial and ventricular pacing, respectively; Table 2).

In patient 5 (see Fig. 2 and Table 2), a mean value (3.79 L/min) of ANP clearance rate was measured by dividing the total amount of dose administered during the 95-min infusion by the total area under the curve of tracer activity concentration; this value is very close to those measured before and during pacing, thus confirming the soundness of the experimental approach.

Discussion

The measurement of the rate of overall degradative systems for an endogenous substance (such as ANP) with a rapid biological response has great importance because of the potential linkage between its effect and its clearance rate. Indeed, degradation of the hormone can be directly associated with its biological effect or, alternatively, degradation can be involved in stopping the biological function of the hormone.

The present study was planned to evaluate whether rapid and large variations in plasma ANP concentrations in the range observed under normal and/or pathophysiological conditions are associated with concomitant changes in the plasma ANP clearance rate. The experimental approach chosen simulates well known physiological stimuli of ANP secretion, such as the increase in heart rate and/or atrial stretching. To this aim, during the hemodynamic study in cardiac patients, we resort to atrial and ventricular pacing that represents an appropriate stimulus to rapidly increase plasma ANP levels (10); the ANP clearance rate was simultaneously determined by the constant infusion of labeled ANP.

Under the assumption that clearance of labeled and unlabeled ANP is identical, the tracer approach shows three major advantages compared to the infusion of cold (unlabeled, native) hormone: 1) the possibility of easily identifying minimal changes in ANP clearance due to the accuracy of tracer measurement in plasma (5–7); 2) the lack of pharmacological doses of ANP, which can induce changes in the degradation rate of the hormone; and 3) the possibility of independently monitoring the degradation and secretion of ANP through the measurement of labeled and native ANP concentrations. In particular, as far as point 2 is concerned, from the knowledge of specific activity and from the measurement of the clearance rate of the injected labeled ANP, it is possible to calculate an increase in the native ANP concentration produced by tracer infusion of less than 0.5 pg/mL (*i.e.* <1% increment for concentrations in the normal range).

After infusing for a sufficient equilibration period, steady state concentrations of labeled hormone are produced; the maintenance of steady levels of labeled ANP during the constant infusion rate (experimentally controlled) of the tracer assures that degradation remains stable. The concomitant measurement of cardiac output combined with the use of a circulatory approach allows separate evaluation of the effect of hemodynamic changes on the ANP degradation rate and expression of ANP renewal not only as a clearance rate, but also as whole body extraction of the hormone.

The present data indicate that the rapid and large increase (>300%) induced in plasma ANP concentrations by pacing is associated with a relatively small reduction (~10%) in ANP clearance; this reduction can be (at least partially) be attributed to hemodynamic factors (*i.e.* to a reduction of cardiac output), whereas whole body extraction remains fairly stable.

ANP extraction (E_{wb}) of the perfused body was found to be, on the average, as high as 50%; this means that only about half of the ANP produced by the right heart is not extracted and recirculates after a single pass through whole body. The value of extraction reported here is very close to that previously reported for various organs such as kidney, liver, and limbs and calculated from the artero-venous difference in native hormone levels (11).

Our findings demonstrate that degradative mechanisms involved in ANP clearance are not saturable at least for acute elevations of plasma ANP levels up to 3–5 times the basal value. Plasma ANP concentrations depend on both the contribution of ANP release and the ANP degradation rate; our studies, showing short term native ANP plasma variations associated with unchanged clearance values of labeled infused ANP, suggest that, in general, the plasma variations may predominantly reflect the secretory pattern of cardiac

TABLE 2. MCR, whole body extraction (E_{wb}), and ANP concentrations order basal conditions and after pacing in the patients studied

Patient no.	MCR bas (L/min)	MCR ap (L/min)	MCR vp (L/min)	ANP bas (pg/mL)	Δ ANP (%)	E_{wb} (%)	E_{wb} ap (%)	E_{wb} vp (%)
1	4.40	4.20	4.00	21	333	53	52	49
2	2.77	2.67	2.61	37	306	36	40	41
3	4.71	4.53	3.74	230	183	68	68	66
4	2.26	2.08	2.25	81	470	43	46	42
5	3.97	3.25	3.15	44	568	50	46	50
Mean	3.62	3.35	3.15	82.6	372.0	50.0	50.4	49.6
SD	1.06	1.03	0.74	85.3	149.7	12.0	10.6	10.0

MCR bas, MCR value in the basal condition; MCR ap, MCR value during atrial pacing; MCR vp, MCR value during ventricular pacing; ANP bas, plasma ANP in the basal condition; Δ ANP, maximum percent increment in plasma ANP during pacing in respect to the basal value; E_{wb}: total (whole) body extraction in the basal condition; E_{wb} ap, total (whole) body extraction in the basal condition during atrial pacing; E_{wb} vp, total (whole) body extraction in the basal condition during ventricular pacing.

tissue. However, as significant interaction among natriuretic peptides (such as ANP, brain natriuretic peptide, and C-type natriuretic peptide) can occur *in vivo* at receptor and/or degradative enzyme sites (12–14), it cannot be excluded that in the presence of pathophysiological stimuli, changes in plasma levels of one of these hormones could be associated with an altered metabolism rather than an increase or decrease in cardiac secretion.

The fact that the ANP clearance mechanism(s) is very constant in the presence of rapid and large changes in plasma ANP levels could be considered an expected finding. However, it is worth remembering that data reported to date are conflicting. In fact, ANP administration of pharmacological doses has been alternatively reported to increase (15, 16) or reduce (17) the hormone clearance. At present, it is not clear whether these discrepancies are due to the different doses of administered hormone and/or to the techniques employed for clearance determination.

Finally, these findings reinforce the use of the ANP clearance rate as a useful index of whole body hormone utilization, as this is a remarkably stable parameter, relatively independent from fluctuations in plasma levels and strongly related to the efficiency of the overall systems involved in ANP extraction.

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