CLINICAL RESEARCH

Plasma α natriuretic peptide in cardiac impairment

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Abstract

Regional plasma α human atrial natriuretic peptide concentrations were measured, and their relation to intracardiac pressures assessed, in an unselected series of 45 patients undergoing diagnostic cardiac catheterisation. Arteriovenous gradients in plasma concentrations of α human atrial natriuretic peptide were consistent with its cardiac secretion and its clearance by the liver and kidneys. Plasma concentrations of the peptide in the pulmonary artery, aorta, and superior vena cava correlated closely with the mean right atrial and pulmonary arterial pressures, and similar, though weaker, positive relations were seen with the left ventricular end diastolic and pulmonary artery wedge pressures. Concentrations of both atrial natriuretic peptide and renin showed significant inverse relations with serum sodium concentrations.

Plasma concentrations of α human atrial natriuretic peptide are an additional objective indicator of the severity of haemodynamic compromise in patients with cardiac impairment.

Introduction

Atrial extracts and synthetic atrial peptides have potent diuretic and natriuretic effects in animals and man.[1-5] They also relax vascular smooth muscle,[6] inhibit vasoconstriction induced by angiotensin II and noradrenaline,[7] and reduce secretion of both renin[8] and aldosterone.[9,10] Increases in right atrial pressure enhance natriuretic activity in the perfusate of isolated rat heart-lung preparations.[11] Sustained supraventricular tachycardia in man[12,13] and experimental acute atrial distension in the dog[14] are associated with natriuresis. Atrial peptide deficiency has been described in one animal model of heart failure,[15] and preliminary reports suggest that plasma peptide concentrations are increased in clinical heart failure in man.[16,17] These observations suggest that atrial natriuretic peptides contribute to the regulation of circulating volume, are released secondary to increased atrial pressure, and may participate in the compensatory responses to cardiac impairment.

We used a sensitive radioimmunoassay to measure regional plasma concentrations of α human atrial natriuretic peptide and assessed their relation with concurrent intra-atrial pressures during diagnostic cardiac catheterisation in a series of patients with a wide range of cardiac lesions.

Patients and methods

We studied 45 unselected patients (23 men) (mean age 54·7 (range 17-78) years) who were undergoing diagnostic right and left heart catheterisation. The clinical presentations varied widely, and the indications for cardiac catheterisation included ischaemic heart disease, assessment of symptomatic valvular lesions, congestive heart failure of uncertain cause, and suspected failure of a valve prosthesis. Criteria for New York Heart Association clinical grades I, II, III, and IV were fulfilled by 7, 15, 16, and 7 patients respectively. Thirty three patients were in normal sinus rhythm when studied, and 12 had atrial fibrillation. Thirty four were taking drugs, including digoxin (15 patients), a loop diuretic (25), a thiazide diuretic (14), a converting enzyme inhibitor (six), and a β blocker (four). Seven patients were receiving drugs for hypertension. They all received an oral sedative (diazepam 10 mg) before catheterisation.

Right and left heart studies were performed with standard techniques through femoral arterial and venous entry sites. The first blood samples were taken from the aorta with a 7F Cordis pigtail catheter. Venous samples were successively taken (with a 7F Cournand catheter) from the renal vein, hepatic vein, inferior vena cava, superior vena cava, coronary sinus (when readily accessible), and pulmonary artery. The order and success of venous sampling varied depending on ease of access to the desired site. The right atrial pressure was recorded between sampling from the superior vena cava and pulmonary artery. The pulmonary arterial pressure was recorded immediately after taking a sample of pulmonary arterial blood and before recording pulmonary wedge pressure. The arterial and left ventricular

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pressures were recorded immediately after taking the arterial sample. Blood sampling took 10 to 20 minutes.

All pressures were recorded with 23Db Statham transducers (placed at mid-chest height) and a multichannel pen recorder (Mingograph, Elema Siemens). The results of routine plasma biochemistry screens performed within 24 hours before catheterisation were available in all cases. Plasma renin was measured using an established method.[18]

Blood (10 ml) was collected in chilled plastic tubes containing edetic acid as an anticoagulant and sufficient Trasylol to give a final concentration of 50 kIU/ml. Each sample was transported on ice and centrifuged at 1000 *g* for 10 minutes at 4°C; the plasma was then stored at −20°C. Atrial natriuretic peptide was extracted from 1-3 ml plasma on C18 reverse phase columns (Sep-pak, Waters Associates). Sep-paks were preactivated with 5 ml methanol and washed with 5 ml distilled water before application of acidified plasma (0·25 ml 2N hydrochloric acid/ml plasma). The cartridges were then washed three times with 5 ml 0·1% vol/vol trifluoroacetic acid, and the adsorbed peptide was eluted with 2 ml 60% acetonitrile/0·1% trifluoroacetic acid into plastic tubes.[19] The extracts were dried down under compressed air and reconstituted in 0·5 ml buffer (100 mM sodium phosphate, pH 7·4, containing 50 mM NaCl, 0·1% wt/vol bovine serum albumin, 0·1% wt/vol Triton X-100, and 50 kIU Trasylol/ml).[20]

Antibodies to human atrial natriuretic peptide were raised in New Zealand White rabbits by injection of immunogen (human atrial natriuretic peptide (Peninsula Laboratories) coupled to bovine thyroglobulin with a carbodi-imide coupling agent), mixed with complete Freund's adjuvant into the popliteal lymph nodes followed by intramuscular booster injections at intervals of five to six weeks. Animals were bled from the marginal ear vein, and the serum was stored frozen. The antibody used for the radioimmunoassay gave 50% binding of 2 pg iodine-125 human atrial natriuretic peptide (Amersham International) at a dilution of 1/33 000. Cross reaction of the antibody with a variety of synthetic atrial natriuretic peptide sequences (5-28 human atrial natriuretic peptide, 7-28 human atrial natriuretic peptide, atriopeptins I, II, and III, and rat atrial natriuretic peptide) was greater than 90%. No significant cross reactions with cardiodilatin, bradykinin, arginine vasopressin, angiotensins I and II, or adrenocorticotrophic hormone were seen.

For the assay, set up in duplicate, 100 µl reconstituted plasma extract, 100 µl antibody at a dilution of 1/10 000, and 2 pg labelled human atrial natriuretic peptide in 50 µl buffer were incubated at 4°C for 24 hours. Free and bound ligands were separated by adding 1 ml charcoal coated with dextran.[20] The mixture was immediately centrifuged for 10 minutes (1000 *g* at 4°C) and the free label counted. Non-specific binding of human atrial natriuretic peptide, estimated by adding excess synthetic human atrial natriuretic peptide (5 ng/tube), was consistently 3-4% both in standard solutions and in reconstituted plasma extracts; hence no correction for such binding was required.

A significantly lower percentage binding of labelled atrial natriuretic peptide was seen in tubes containing 0·8 pg synthetic peptide than in tubes containing no peptide ($p<0·001$, paired *t* test, n=8). Radiolabelled synthetic α human atrial natriuretic peptide (20 pg) added to 3 ml plasma before extraction was recovered at a mean rate of 91 (SEM 5·6)% (n=31). Recovery rates of cold synthetic atrial natriuretic peptide added to 3 ml plasma at concentrations of 100 pg/ml (32·46 pmol/l) and 500 pg/ml (162·28 pmol/l) were 86 (8·8)% (n=28) and 91 (8·0)% (n=25) respectively. The concentrations of peptide reported were not corrected for recovery. Interassay and intra-assay coefficients of variation were 7·8% (n=7) and 7·5% (n=12) respectively. Assay of fractions resulting from high pressure liquid chromatography on plasma extracts from three pools of plasma confirmed the presence of a single immunoreactive peak comigrating with synthetic 1-28 α human atrial natriuretic peptide.

Data were analysed with the *t* test for paired data to test for differences in regional plasma concentrations of α human atrial natriuretic peptide and Pearson's correlation coefficient to assess linear relations between haemodynamic variables and plasma peptide concentrations.

Results

Cardiac catheterisation confirmed a wide range of lesions of varying severity. Diagnoses included ischaemic heart disease (18 patients), pure and mixed valvular lesions with and without associated ischaemic heart disease (22), atrial septal defect (one), and dilated congestive cardiomyopathy (one); in five patients no lesion was found. Left ventricular contractile function, as assessed by angiography (in 36 patients) or echocardiography, or both, was normal in 21 patients and showed dysfunction ranging from minimal to severe among the remaining 24. Table I shows the mean intracardiac and arterial pressures and their ranges.

For various reasons, including difficulty with access to some venous sampling sites and inadvertent spillage or haemolysis of some samples, the number of results for most sites was less than 45 (table II). In four cases studies were restricted to measurement of right heart pressures with a Swan-Ganz catheter introduced into an antecubital vein, and samples were obtained from only the pulmonary artery and superior vena cava or systemic artery by femoral stab.

Plasma concentrations of α human atrial natriuretic peptide varied over a wide range at all sampling sites (table II). By far the highest concentrations occurred in the coronary sinus, where peptide concentrations were over five times higher than in the pulmonary artery (n=10, fig 1). Pulmonary arterial concentrations were significantly greater than systemic arterial concentrations (table II, $p<0·001$), which in turn, were significantly higher than those in the superior vena cava (table II, $p<0·001$). Concentrations of α human atrial natriuretic peptide in the pulmonary artery showed close linear correlation with concentrations in the aorta (r=0·95) and superior vena cava (r=0·91) ($p<0·001$).

TABLE I—*Intracardiac and arterial pressures and heart rate*

	Mean (SEM)	Range	No of patients
Blood pressure (mm Hg):			
Right atrial	6·0 (0·6)	0-16	45
Pulmonary artery	26·4 (1·8)	10-60	45
Pulmonary artery wedge	16·5 (1·3)	5-42	43
Left ventricular end diastolic	12·3 (1·1)	4-27	34
Mean arterial	91 (2·3)	60-150	45
Heart rate (beats/min)	82 (2·1)	52-120	43

TABLE II—*Regional plasma concentrations of α human atrial natriuretic peptide*

	α Human atrial natriuretic peptide concentration* (pg/ml)			
	Mean (SEM)	Range	Significance†	No of patients
Coronary sinus	1628 (311)	611-3240	$p<0·001$	10
Pulmonary artery	286 (32)	13-900		45
Arterial	239 (30)	16-850	$p<0·001$	40
Superior vena cava	194 (27)	13-793	$p<0·001$	42
Inferior vena cava	128 (17)	13-511	$p<0·001$	42
Hepatic vein	115 (17)	8-463	$p<0·001$	39
Renal vein	92 (17)	8-367	$p<0·001$	29

*Normal mean in peripheral venous blood=22·4 (17) pg/ml, range 5-55 pg/ml (n=24).
†Significance of difference in mean concentration from pulmonary arterial concentration (paired *t* test).

More appreciable arteriovenous gradients in plasma peptide concentrations were apparent for the liver and kidneys (table II, $p<0·001$, fig 1). This presumably contributed to the pronounced difference between superior and inferior vena caval α human atrial natriuretic peptide concentrations ($p<0·001$). The arteriovenous difference in peptide concentrations was consistently greater across the renal than the hepatic circulation ($p<0·001$).

Figure 2 shows the relations between pulmonary arterial α human atrial natriuretic peptide concentration and mean right atrial pressure, pulmonary arterial pressure, and pulmonary arterial wedge pressure. Pulmonary arterial, aortic, and superior vena caval plasma peptide concentrations correlated closely with concurrent mean right atrial pressure (r=0·83, 0·78, and 0·75 respectively; $p<0·001$) and pulmonary arterial pressure (r=0·72, 0·73, and 0·75 respectively; $p<0·001$). Similar, though weaker, positive relations were seen with pulmonary artery wedge pressure (r=0·69, 0·71, and 0·70 respectively; $p<0·001$) and left ventricular end diastolic pressure (r=0·33, 0·32, and 0·31 respectively; $p<0·05$). No relation was seen between plasma atrial natriuretic peptide concentration and mean aortic pressure (r=0·04, NS).

With several notable exceptions (patients with mitral valve lesions), atrial natriuretic peptide concentrations in the patients giving normal results of left ventricular angiography or echocardiography tended to cluster in the lower part of the range for the group (fig 2). Pulmonary arterial peptide concentrations in the 12 patients with atrial fibrillation were distributed evenly about the regression line relating right atrial pressure and pulmonary arterial α human atrial natriuretic peptide concentrations for the group (fig 2).

Active renin concentrations in the plasma were higher than normal (<50 µU/ml) in 14 cases, and the mean concentration for the group was appreciably increased (mean 201 (SEM 76) µU/ml, range 3-2419 (µU/ml). Serum sodium concentrations were inversely correlated with both atrial natriuretic peptide and renin plasma concentrations (fig 3). These two

circulating peptides showed a weak positive correlation with each other when data from the entire group were considered (r=0·25, NS). Those patients with evidence of normal left ventricular contractile function and plasma atrial natriuretic peptide concentrations in the lower portion of the range for the group (<150 pg/ml (48·69 pmol/l) n=11, fig 2) had renin concentrations within the normal range (<50 μU/ml). The four patients with the highest renin concentrations also had plasma atrial natriuretic peptide concentrations higher than the mean for the group as a whole (including the single highest atrial natriuretic peptide concentration).

A weak positive relation with serum creatinine concentration was seen for both plasma α human atrial natriuretic peptide (r=0·25, NS) and renin (r=0·31, p<0·05) concentrations. The age of the patient showed a weak positive correlation with plasma atrial natriuretic peptide concentration (r=0·30, p<0·05), but there was no discernible relation between plasma peptide concentration and heart rate.

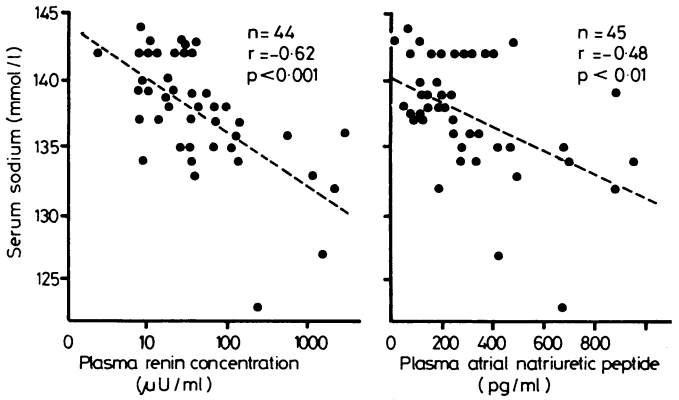

FIG 3—Inverse correlations of plasma renin concentrations (n=44) and atrial natriuretic peptide (n=45) with serum sodium concentrations.

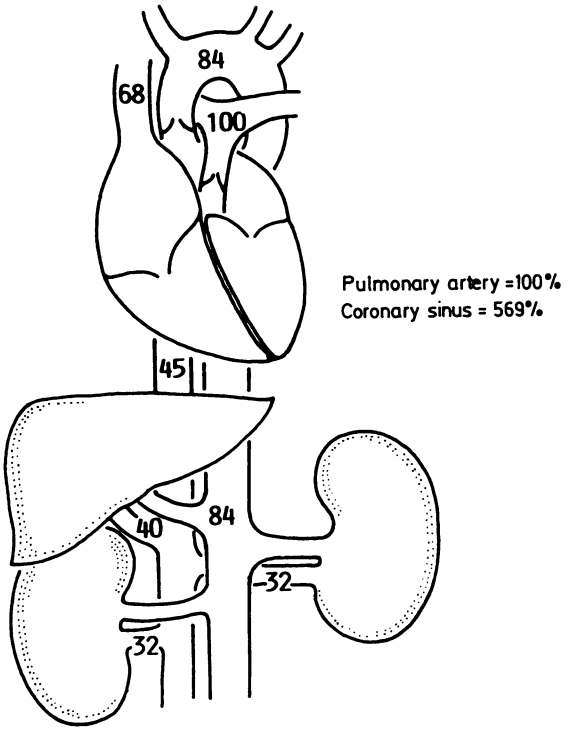

FIG 1—Mean regional plasma atrial natriuretic peptide concentrations shown as a percentage of matched pulmonary arterial concentrations.

Discussion

Our findings confirm that α human atrial natriuretic peptide is a circulating hormone secreted by the heart. Plasma atrial natriuretic peptide concentrations in coronary sinus blood were far greater than concentrations recorded at all other sites. The next highest concentrations of peptide were found in the pulmonary artery, which contained true mixed venous blood, including the admixture of coronary sinus flow, that had not yet crossed the vascular bed of any organ. Concentrations at this site were likely to reflect closely the cardiac secretion of α human atrial natriuretic peptide. Small decreases in peptide concentrations in blood that had traversed the head and arms suggested minimal net clearance of the hormone by the tissues concerned. Somewhat greater changes occurred across the pulmonary circulation. More striking, however, was the clearance of the peptide by the kidneys and liver. The peptide exerts powerful renal effects,[5] and may well be taken up by renal receptors[21] and be subject to renal metabolism or urinary excretion, or both. Our findings also suggest that hepatic metabolism of the peptide may occur.

Mean right atrial and pulmonary arterial pressures correlated most closely with pulmonary arterial plasma concentrations of α human atrial natriuretic peptide. Weaker positive relations were seen with pulmonary arterial wedge and left ventricular end diastolic pressures. Although caution must always be exercised in extrapolating statistical correlation to physiological cause and effect, these results are consistent with the hypothesis that atrial

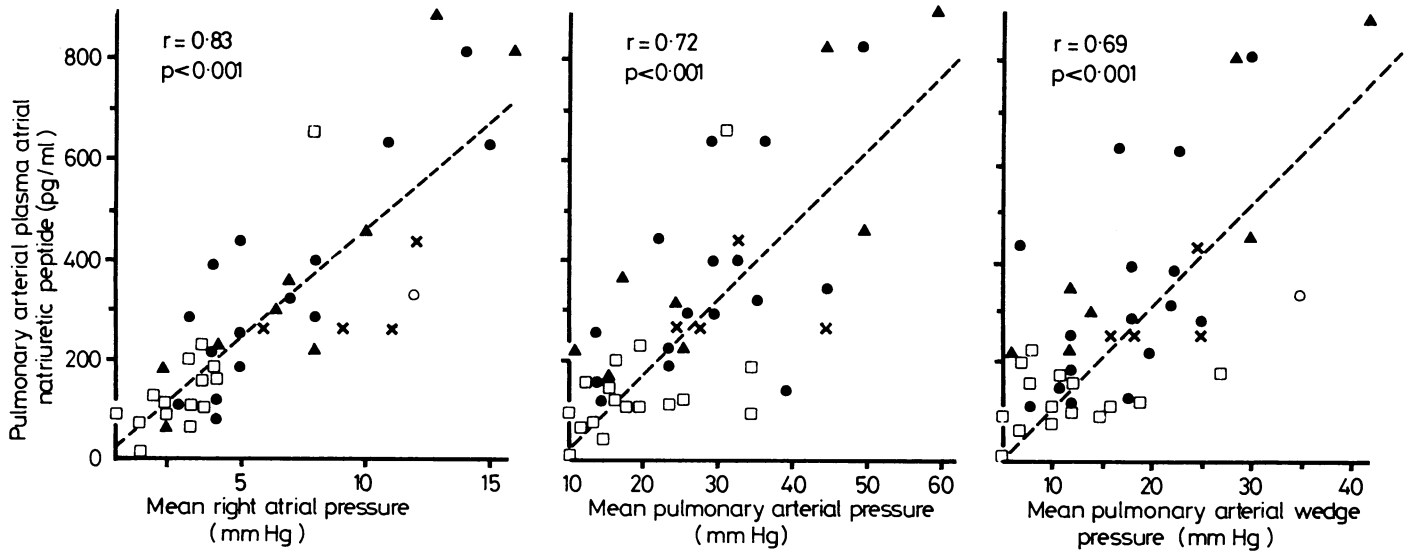

FIG 2—Correlation of mean right atrial, pulmonary arterial, and pulmonary wedge pressures with pulmonary arterial α human atrial natriuretic peptide concentrations in patients in sinus rhythm with normal left ventricular function (□), in sinus rhythm with impaired left ventricular function (●), with atrial fibrillation and normal left ventricular function (×), and with atrial fibrillation and impaired left ventricular function (▲).

pressure or distension, or both, have a primary role in the regulation of atrial natriuretic peptide secretion. In the present study most patients with increased right atrial pressure also had poor left ventricular function. Evidence of normal left ventricular contractility was associated with lower atrial natriuretic peptide concentrations. These concentrations, however, were appreciably increased in several patients with mitral valve lesions and increased atrial pressures but normal left ventricular angiographic appearances or end diastolic pressure, or both. This finding suggests that increased intra-atrial pressure is the stimulus for atrial natriuretic peptide release rather than impaired left ventricular function on its own. The closer correlation of peptide concentrations with right rather than left atrial pressures (as represented by mean pulmonary wedge and left ventricular end diastolic pressures) may reflect a more important role for the right sided chamber in the regulation of circulating atrial natriuretic peptide. This is consistent with the finding of higher concentrations of peptide in right rather than left atrial tissue.[20]

A striking increase in plasma atrial natriuretic peptide concentrations during paroxysmal supraventricular tachycardia has been reported.[22,23] In our patients with atrial fibrillation, however, the data points fell evenly about the regression line relating peptide concentrations to right atrial pressure for the group as a whole. This suggests that mean atrial pressure is the primary stimulus for atrial natriuretic peptide release regardless of atrial rhythm.

Hyponatraemia is a recognised feature of hyper-reninaemic states,[24] and a similar inverse relation between concentrations of plasma atrial natriuretic peptide and serum sodium occurred in our study (fig 3). Increased atrial natriuretic peptide concentrations may contribute to hyponatraemia by encouraging renal loss of sodium. Increased concentrations of both plasma peptide and renin in some patients with more severe cardiac impairment contrasts with our findings in normal volunteers, where stimuli such as shifts in dietary sodium intake and administration of intravenous saline infusions result in reciprocal changes in renin and atrial natriuretic peptide.[25-27] The current findings presumably reflect distortion of the normal relation between intracardiac pressures, circulating volume, and renal perfusion. Increased intra-atrial pressure may enhance secretion of atrial natriuretic peptide while reduced renal perfusion and increased activity of the sympathetic nervous system result in a concurrent increase in plasma renin concentrations.

The kidney is clearly important in the clearance of atrial natriuretic peptide from the plasma, and renal function may decline with cardiac failure. No detailed tests of renal function were performed in this study, but the relation between plasma creatinine and atrial natriuretic peptide concentrations is weak, suggesting that increased peptide concentrations in heart failure reflect increased cardiac secretion rather than reduced renal clearance.

The possible primary importance of intra-atrial pressure and the effects of cardiac rhythm on atrial natriuretic peptide secretion during cardiac impairment require evaluation by measurement of plasma peptide concentrations during changes in atrial pressures and cardiac rate and rhythm in appropriate clinical and controlled experiments. The present data are from a heterogeneous group in terms of age, sex, and diagnosis, and these variables may exert effects that confound interpretation of plasma α human atrial natriuretic peptide concentrations.

Increased concentrations of α human atrial natriuretic peptide in cardiac impairment may thus represent a compensatory increased secretion of a hormone with actions that tend to reduce intracardiac pressures by maintaining renal excretion of sodium and water in the face of reduced renal function and activation of the renin-angiotensin system often associated with congestive heart failure.[24,28] Plasma atrial natriuretic peptide concentrations might serve as a marker of the adequacy of treatment of cardiac impairment in a fashion analogous in the part played by plasma urea in the assessment of renal function. Further studies are required to establish whether cardiac decompensation, occurring despite increased concentrations of atrial natriuretic peptide, reflects a relative deficiency of peptide secretion, failure of end organ response to atrial natriuretic peptide, or the ascendancy of counteracting mechanisms, including progressive contractile impairment or valvular dysfunction and activation of the renin-angiotensin system, over the effects of atrial natriuretic peptide.

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100 YEARS AGO

To the long list of preventable causes of accidents which can be more or less attributed to want of thought and foresight, and the exercise of proper care, is that reprehensible practice which obtains among a class of excursionists and holiday-seekers, of flinging from the carriage-window of the train in which they are travelling a bottle or jar, for which, having been emptied of its contents, they have no longer any use. An illustration of the danger at all times attendant on such a reckless act occurred on Saturday last, when an empty bottle was flung by a careless passenger from the Manchester express train for Crewe, striking a platelayer named Dawson on the forehead, and inflicting a severe wound, which has since rendered his detention in the hospital necessary. (*British Medical Journal* 1886;ii:466.)