The atrial natriuretic factor

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SUMMARY In less than three years since the rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats was reported the factor responsible for the diuretic, natriuretic, and vasodilating activity of the atrial homogenates was isolated, its chemical structure elucidated, and its total synthesis achieved. Also the cDNA and the gene encoding for the atrial natriuretic factor in mice, rats, and man have been cloned and the chromosomal site identified. The major effects of this hormone are vasodilatation, prevention and inhibition of the contraction induced by noradrenaline and angiotensin II, diuresis, and natriuresis associated in most instances with a pronounced increase in glomerular filtration rate and filtration fraction, inhibition of aldosterone secretion, and considerable stimulation of particulate guanylate cyclase activity. High density specific binding sites have been demonstrated in the zona glomerulosa of the adrenal cortex, in the renal glomeruli, and in the collecting ducts, and in the brain areas involved in the regulation of blood pressure and of sodium and water (AV3V region, subfornical organ, nucleus tractus solitarius, area postrema).

In proposing a new concept for the organisation of modern medical research and in setting up the Clinical Research Institute of Montreal, I have been greatly inspired by the outstanding group of American physicians at the turn of the century, such as Welch, Barker, and Longcope at Hopkins; Rufus Cole at the Rockefeller Institute; William Pepper and AN Richards in Philadelphia; and Folin and Peabody in Boston, among many others, but I owe to Sir Thomas Lewis the inspiration in articulating my own observations and transferring them into what is now the Clinical Research Institute of Montreal.^{1 2} The philosophy and thoughts of Sir Thomas Lewis have been expressed in a collection of addresses published under the title of *Research in Medicine* by HK Lewis of London in 1938. This small book is now a collector's item and contains four major addresses of Sir Thomas Lewis on clinical research, all of them published in the *British Medical Journal* in 1930, 1932, 1933, and 1935.

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Atrial natriuretic factor illustrates the great benefits of institutes in which daily interaction between researchers with various types of expertise is possible. This I believe accounts for the rapid progress in our understanding of the atrial natriuretic factor. In barely three years since its isolation it has been sequenced in our laboratories in June 1983; totally synthesised by Nutt and co-workers in August 1983; the cDNA and of the gene encoding for the atrial natriuretic factor have been cloned; its chromosomal location has been identified; its effects in experimental hypertension, on vascular smooth muscle, on renal function, aldosterone and vasopressin secretion have been investigated; receptors have been found in various tissues including the brain, the adrenal cortex, the kidneys, and ciliary bodies; and the messenger of its cellular activity has been identified.³

In 1981 de Bold *et al* reported the crucial observation of a striking diuretic and natriuretic activity when homogenates from rat atria were administered intravenously to control rats.⁴ Because of my lifelong interest in the factors regulating sodium as they relate to hypertension and because of Cantin's work in the past 15 years on atrial cardiocytes, our group

immediately realised the importance of de Bold's observation—hence the major effort we have made in this field.

Historical data

Kisch first described the presence of granules in the atrial cardiocytes of guinea pigs.⁵ They are adjacent to one or occasionally to both poles of the nucleus of the cardiocyte and are interspersed among the elements of a voluminous Golgi complex (fig 1). Jamieson and Palade demonstrated that such granules are present in cardiocytes of the atria of all mammals, including man,⁶ and Cantin and his group showed that these granules incorporated both [³H]—leucine and [³H]—fucose and that protein synthesis took place in the Golgi complex.^{7,8} In 1976, Hatt's group demonstrated that the degree of granularity in these atrial cardiocytes varied with the water and sodium intake⁹ (table 1).

Fig 1 (a) Electronmicrograph of a granular cardiocyte showing the myofibrils, a well developed Golgi apparatus (G), and the granules (g). (b) Electronmicrograph of an atrial cardiocyte of a rat on a sodium free diet for 30 days showing a great increase in granularity.

Table 1 Specific granules expressed as a percentage of cell volume in atrial cardiocytes of rats⁹

Group	Right atrium	Left atrium
(1) Controls on standard diet	2.92 (p < 0.01)	1.11
(2) Total water restriction for 5 days	4.14*	1.77
(3) Total sodium restriction for 3 weeks	3.63	3.75‡
(4) Sodium load (1 g/dl sodium chloride as drinking fluid for 3 weeks)	1.40*	1.59
(5) Same as group 4 plus DOCA implant (25 mg for 3 weeks)	0.76†	1.19

*p < 0.05; †p < 0.005; ‡p < 0.01 compared with control group.
DOCA, desoxycorticosterone acetate.

Isolation and structure

Several active short peptides (21–33 amino acids) were isolated by different groups (fig 2).^{10–20} All of them contained the same sequence of 21 amino acids (Ser 103–Arg 125) with a cysteine-cysteine disulphide bridge. The differences in the active peptides isolated are caused by variations in the extraction and purification procedures and the choice of protease and peptidase inhibitors. Isolation and sequencing of larger peptides (up to 106 amino acids) showed that they all came from a common precursor.^{10–14} Synthetic deoxyoligonucleotides based on the amino acid sequence were used to clone the cDNA. This, in turn showed the structure of the pre-propeptide, which in rats is 152 amino acids long with two Arg-Arg residues of the carboxyl terminal, whereas the human pre-propeptide is made up of 151 amino acids without the two basic residues at the carboxyl terminal (fig 3).

The genes encoding for the atrial natriuretic factor in the rat and man have been cloned.^{21–33} The human gene has a span of 2 kb and consists of three coding blocks (exons) and two short introns of 122 and 1050 base pairs. The first exon codes for the signal peptide of 25 amino acids and the first 16 amino acids of the pro-hormone. The second exon codes for most of the pro-hormone from amino acids 41 to 150, including the atrial natriuretic factor, and the third exon encodes the last amino acid 151 Tyr or in the case of the rats, the last three amino acids Tyr 150, Arg 151, and Arg 152, which do not appear to be important for the biological activity of the atrial natriuretic factor.

Yang-Feng *et al*, in collaboration with Drouin and Nemer from the Clinical Research Institute of Montreal, demonstrated that the atrial natriuretic factor gene is located on the distal short arm of chromosome 1 in man and on chromosome 4 in mice.³⁴

Circulating form of atrial natriuretic factor

In man the 28 amino acid sequence Ser 99-Tyr 126 was isolated from atrial homogenates by Kangawa and Matsuo³⁵ and by Thibault *et al.*^{35a} It differs from the rat atrial natriuretic factor by having methionine instead of isoleucine in position 110 (fig 4). Although it has not yet been sequenced from human plasma, on reverse phase high performance liquid chromatography the peptide isolated behaves identically with the 28 amino acid sequence of Ser 99-Tyr 126.³⁵

The circulating form of the atrial natriuretic factor was isolated from plasma of morphine treated rats (morphine (10 mg/rat) increased circulating atrial natriuretic factor 40–50 times)³⁶ and from the perfused rat heart by the Langendorf technique after extraction with Vycor glass beads, immunoaffinity chromatography on Sepharose 4-B anti-atrial natriuretic factor, and reverse phase high performance liquid chromatography. It has been sequenced and shown to be the 28 amino acid peptide Ser 99-Tyr 126.^{37,38} Schwartz *et al* reported similar findings.³⁹

Structure activity relation

The structure activity relation of various atrial natriuretic factor peptides was studied by comparison with the synthetic atrial natriuretic factor made up of 26 amino acids (Arg 101-Tyr 126). The shorter peptides were obtained by sequential cleavage at the amino terminal by Edman degradation (amino acids 101, 102, 103, 104, to 105) or by the use of various carboxypeptidases at the carboxyl terminal (amino acids 122, 123, 124, 125, to 126).^{40,40a,41} The resulting shorter forms produced were all identified by amino acid analysis and sequencing. The biological activity of these shorter forms was compared with the 26 amino acids from Arg 101 to Tyr 126 in several systems: in vivo rat assay for diuresis and natriuresis; in vitro relaxation of strips of aorta and chick rectum, inhibition of aldosterone secretion from beef and rat zona glomerulosa cell cultures, specific binding to mesenteric artery smooth muscle membranes and to beef zona glomerulosa cell membranes.^{40–42} The 26 amino acid sequence Arg 101-Tyr 126 was found to be the most potent in these assays. The removal of Phe 124, Arg

Fig 2 Isolated forms of low molecular weight atrial natriuretic factor with diuretic, natriuretic, and vasorelaxant properties. Numbers above the amino acids are the international classification. The short active forms were all isolated from the carboxyl terminal. The names given to some low molecular weight peptides are shown. Atriopeptin I, which is made up of 21 amino acids, is not shown because of its very low potency. The isolation of human atrial natriuretic factor by Kangawa *et al*²² was soon confirmed by the group at the Clinical Research Institute of Montreal.^{35a} (Reproduced with the permission of Endocrine Reviews.)

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125, and Tyr 126 considerably decreased the biological activities of the atrial natriuretic factor, whereas the removal of amino acids 101, 102, 103, 104 had much less effect (table 2).

The 26 amino acid peptide Arg 101-Tyr 126 was found to be as potent and as active as the 28 amino acid peptide Ser 99-Tyr 126 in all assays used.^{40 40a}

The 26 amino acid peptide (Arg 101-Tyr 126) was sequenced in our laboratories in June 1983 and it was synthesised in August 1983 by Nutt *et al.*^{42a} This factor has been used in all the studies performed by our group and many others have used it too.

Effects of atrial natriuretic factor on the heart and haemodynamic function

Bolus injections of synthetic atriopeptin III (24 amino acids, Ser 103-Tyr 126) significantly reduced cardiac output in anaesthetised dogs and rats.^{43 44} Similar results were obtained with atriopeptin II (23 amino acids Ser 103-Arg 125).⁴⁵ On the other hand, in open chest anaesthetised dogs infusions of the atrial natriuretic factor (26 amino acids Arg 101-Tyr 126) did not significantly alter cardiac output or contractility (left ventricular dp/dt max).⁴⁶ The atrial

Fig 3 Amino acid sequence of the atrial natriuretic factor (ANF) pre-propeptide in man and in rats. (Reproduced with permission of Endocrine Reviews and of Zivin *et al.*²¹ and Nemer *et al.*³⁰.)

natriuretic factor has no inotropic effect (R Tarazi, personal communication).

The atrial natriuretic factor content of atrial tissue is significantly lower in the B10 14-6 strain of hamsters with a hereditary form of cardiomyopathy and severe congestive heart failure than in control age matched animals.^{47 47a} The most striking finding is the significant increase in mean circulating plasma immunoreactive atrial natriuretic factor at all stages

Fig 4 Amino acid sequence of human atrial natriuretic factor which is the same as that of the rat, except that in the latter the amino acid in position 110 is an isoleucine instead of a methionine. (Reproduced with the permission of Endocrine Reviews.)

of congestive heart failure from 25.6 (1.4) pg/ml in control hamsters to 236 (57) pg/ml. Blood pressure is always lower in these hamsters than in the controls. These findings suggest that the decreased amounts of atrial natriuretic factor found in atrial tissue are the result of depletion and that sodium retention and oedema would probably occur earlier if the atrial natriuretic factor had not already been secreted in large amounts. Infusions of the atrial natriuretic factor through Alzet minipumps at 30 pmol/kg/min for seven days to hamsters with congestive heart failure significantly reduced heart weight.

Significantly increased blood concentrations of immunoreactive atrial natriuretic factor have been reported in patients with paroxysmal tachycardia⁴⁸⁻⁵⁰ and in patients with congestive heart failure.^{51 52} In patients with various valvar or cardiomyopathic diseases there was a strongly significant correlation between plasma atrial natriuretic factor concentrations and left ventricular filling pressure,^{51 52} which strongly supported experimental data that suggested that atrial stretch is probably the major stimulus for the release of atrial natriuretic factor.^{53 54} Plasma immunoreactive atrial natriuretic factor concentrations were significantly higher in almost all patients with valvar disease, especially of the mitral valve.

In a study of 110 consecutive patients undergoing diagnostic coronary angiography for coronary heart disease mean (SD) plasma concentrations of immunoreactive atrial natriuretic factor were significantly

Table 2 Relative potency of rat atrial natriuretic factor peptides

Atrial natriuretic factor	Natriuresis	Relaxation		Binding to bovine adrenal capsules	Inhibition of aldosterone secretion
		Chick rectum	Rabbit aorta		
Gly96-Tyr126 (31 amino acids)	+++	—	++	++++	++++
N terminal					
Arg 101-Tyr 126	++++	++++	++++	++++	++++
Arg 102-Tyr 126	++++	+++	++++	++++	++++
Ser 103-Tyr 126	++++	++	+++	+++	++++
(Atriopeptin III-auriculin)					
Ser 104-Tyr 126	++++	++++	+++	+++	+++
Cys 105-Tyr 126	++++	++++	++++	+++	+++
COOH terminal					
Arg 101-Arg 125	++++	++++	+++	++++	++++
Arg 101-Phe 125	+++	—	++	++	—
Arg 101-Ser 123	++	+++	++	++	++
Arg 101-Cys 121	+	++	++	+	+
Atriopeptin I Ser 103-Ser 123	++	+	++	+	+
Atriopeptin II Ser 103-Arg 125	+++	+	++	+++	+++

++++, very active; +++, moderately active; ++, slightly active; +, minimally active.
Data from García *et al.*,^{40 40a} Thibault *et al.*,⁴¹ and De Lean *et al.*⁴²

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higher in patients with a reduction $\geq 70\%$ in coronary lumen diameter than in patients with atypical chest pains and normal coronary anatomy (121 (55) pg/ml vs 102 (37), $p < 0.01$).⁵⁵ Patients with a left ventricular end diastolic pressure > 14 mm Hg or with an ejection fraction of < 0.5 have significantly higher plasma concentrations of immunoreactive-atrial natriuretic factor (table 3).

Table 3 Mean (SD) plasma concentrations of immunoreactive atrial natriuretic hormone (IR-ANF)* in 110 consecutive patients with coronary heart disease⁵⁵

Group	IR-ANF (pg/ml)	p
Values in patients with normal coronary arteries	102 (37)	< 0.01
Values in patients with 70% reduction in coronary diameter	131 (55)	
Left ventricular end diastolic pressure:		< 0.0001
< 14 mm Hg (No 53)	109 (35)	
> 14 mm Hg (No 45)	148 (60)	< 0.001
Ejection fraction:		
> 0.5 (No = 81)	119 (44)	< 0.001
< 0.5 (No = 17)	1645 (70)	

*Direct RIA.

Effects of atrial natriuretic factor on the kidney

The major effect of the atrial natriuretic factor is a rapid (within 3–5 min), massive, and short (< 20 min) diuresis (5–20 fold) and natriuresis (5 to 50 fold) in rats, dogs, monkeys, and man.^{56–62} It also increases, to varying degrees, excretion of potassium, calcium, magnesium, and phosphate. The natriuretic response was 5–7-fold greater when the atrial natriuretic factor was administered during a pressor infusion of angiotensin II⁶³ and was greatly decreased in rats with atrial appendectomy.^{64 65} All the atrial natriuretic factor peptides isolated from Leu 94-Ser 103 to Tyr 126 have the same effects on the kidney, albeit in different degrees. In most studies in rats and man intravenous atrial natriuretic factor considerably increases glomerular filtration rate, filtration fraction, urine volume, and sodium excretion (table 4). Renin secretion is usually inhibited.^{58 66 67} In some studies and at lower doses,^{57 68–70} the atrial natriuretic factor does not have any effect on the glomerular filtration rate. Considerable diuresis and natriuresis were obtained without any change in glomerular filtration rate when infusions of atrial natriuretic factor were given to dogs under severe sodium restriction⁷¹ and to rats on a low protein (8%) diet.⁷²

From all studies done so far, it appears that the major factor involved in the diuresis and natriuresis is the increase in glomerular filtration rate and filtration fraction. Nevertheless, there must be a

tubular effect. The evidence available so far does not accord with a proximal tubular effect. Autoradiography did not show specific binding of ¹²⁵I-atrial natriuretic factor in the proximal tubules⁷³; no receptors were demonstrated by specific binding studies in the proximal tubules⁷⁴; and when atrial natriuretic factor was added to pure preparations of dog proximal tubules there was no change in cyclic guanosine monophosphate or in particulate adenylate cyclase activity.⁷⁵ Atrial natriuretic factor had no effect on glutamine, lactate, glucose, and oxygen uptake or on glucose or lactate production^{76 77}; on the other hand, Kohashi *et al* have recently shown that succinate stimulated oxygen consumption was inhibited when rat's kidney slices were incubated with the atrial natriuretic factor Ser 103-Tyr 126.⁷⁸ Atrial natriuretic factor had no detectable effect on the transepithelial potential and on volume absorption in the perfused isolated rabbit proximal tubules, either when the atrial natriuretic factor was added to the bath or was infused in the luminal perfusate.⁷⁹

High density binding sites for ¹²⁵I labelled atrial natriuretic factor were found in the glomeruli by radioautography,^{80 81} and Bianchi *et al* have recently demonstrated that most atrial natriuretic factor binding sites are found on the podocytes of the epithelial cells of the glomeruli⁸⁰ (fig 5). Silver staining of the glomeruli was abolished when an excess of cold atrial natriuretic factor was given simultaneously with the labelled atrial natriuretic factor. In contrast 60% of the binding sites for ¹²⁵I-angiotensin II are located over mesangial cells.

Studies of free water clearance, stop-flow experiments, and micropuncture reported by Sonnenberg *et al*,⁸² Schnernmann's group,⁸³ and Yakimura *et al*⁷⁰

Table 4 Renal response of dog to atrial natriuretic factor (ANF) (Arg 101-Tyr 126) infused into the renal artery⁵⁸

Variable	Control	ANF (0.3 µg/kg/min (45 min))
Mean arterial pressure (mm Hg)	123 (9)	118 (9)*
Renal blood flow (ml/min)	126 (8)	117 (8)†
Glomerular filtration rate (ml/min)	23.1 (3.5)	30.7 (1.9)†
Renal vascular resistance (mm Hg/ml/min)	0.99 (0.1)	1.04 (0.13)
Filtration fraction	0.19 (0.04)	0.27 (0.03)†
Fraction excreted of sodium filtered (%)	0.6 (0.2)	5.8 (0.8)†
Fraction excreted of lithium filtered (%)	32.2 (7.1)	60.3 (5.7)†
Fraction excreted of phosphate filtered (%)	8.7 (3.4)	41.6 (11.7)†
Renin secretion rate (ng/min)	296 (85)	17 (11)†

* $p < 0.05$; † $p < 0.01$, compared with control values. Values shown are mean (SD). Modified from Burnett *et al*.⁵⁸

suggested an effect on the distal tubules or early collecting ducts. Autography showed the greatest number of silver grains in the inner medulla of the rat kidney in the collecting ducts; and far fewer were seen in the interstitial cells⁸¹ (table 5). The localisation of atrial natriuretic factor in the intercalated cells of the collecting ducts in rat kidney has been shown by immunohistochemical methods.^{84 85} The absence of any change in oxygen or in glucose use at the level of the collecting ducts suggests that the atrial natriuretic factor reduces sodium transport in the distal tubules by an unfamiliar mechanism (table 6).

Table 5 Ultrastructural localisation of silver grains in the renal medulla after injection of ¹²⁵I-labelled atrial natriuretic factor

Structure	Outer medulla		Inner medulla	
	No of grains	%	No of grains	%
Vasa recta	266	70	49	15
Thin loop of Henle	16	4	66	21
Thick loop of Henle	44	12	—	—
Interstitial cell	—	—	62	19
Collecting duct	52	14	143	45
Total	380	100	320	100

Table 6 Sites of action of added atrial natriuretic factor in various dog kidney preparations

Pure preparations	Radioligand binding	Adenylate cyclase inhibition	Particulate guanylate cyclase activation	Cyclic guanylate monophosphate levels (increase over basal (fold))	O ₂ consumption	Glucose utilisation	Rate of glycolysis
Proximal tubule	0	0	0	0	0	0	—
Loop of Henle	+	++	+	2	0	0	—
Collecting duct	+	+++	+	3	—	—	0
Glomerulus	+++	+++	+++	50	—	—	—

Fig 5 (a) Radioautograph of a semi-fine section of rat glomerulus post-stained with toluidine blue after injection of ¹²⁵I-labelled atrial natriuretic factor. The silver grains follow the contour of the glomerular capillaries and are located mostly over the epithelial cells. (b) Part of the glomerulus seen by electronmicroscopy after injection of ¹²⁵I-atrial natriuretic factor. Silver grains are localised over the podocytes (arrows) of epithelial visceral cells (P). M, mesangial cells; C, capillary lumen.

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In pure preparations of glomeruli over 80% of guanylate cyclase was found in the particulate form; the remainder was in the fractions containing either collecting ducts or thick loops of Henle. There was no detectable particulate guanylate cyclase activity in the proximal tubules.⁷⁵

Effects of the atrial natriuretic factor on vascular smooth muscle and in experimental hypertension

The atrial natriuretic factor was shown by Currie *et al.*,¹⁸ by Garcia *et al.*,⁸⁶ and by several other groups⁸⁷⁻⁹⁰ to have a direct vasorelaxing effect on vascular smooth muscle, especially on the renal artery but also in the aorta and the mesenteric arteries. The addition of atrial natriuretic factor to a bath containing strips of renal artery or of aorta from rat or rabbit inhibits and prevents the contractile effects of added noradrenaline or angiotensin II.

Schiffrin *et al.*⁹¹ and Winquist^{90,92} studied the differences in the regional distribution of atrial natriuretic factor receptors in the rabbit vasculature. High affinity receptors were present in arteries and veins from different regions but there were more in arteries that responded well to low concentrations of atrial natriuretic factor, such as the renal artery, and less in tissues that were unresponsive to atrial natriuretic factor, such as the arteries of the ear and the femoral artery. The vasorelaxant selectivity of the atrial natriuretic factor, first suggested by Garcia *et al.*,⁸⁶ has been confirmed by Cohen and Schenck⁹³ and by Faison *et al.*⁹⁴ in isolated rabbit arteries. The aorta and the renal, carotid, and mesenteric arteries are very responsive; the pulmonary, femoral, and iliac arteries less so; and the saphenous, basilar, and ear arteries are comparatively unresponsive. According to Winquist,⁹² the vascular myogenic tone of the rabbit facial vein is probably the most sensitive bioassay for the atrial natriuretic factor. The arcuate arteries of the rat kidney were very sensitive to the vasorelaxing effects of the atrial natriuretic factor after activation with 40 mmol/l potassium salt solution, whereas small arteries from other territories (mesenteric, femoral, cerebral, and coronary) showed no response to the atrial natriuretic factor.⁹⁵

Garcia *et al.* made detailed studies of the effects of bolus injections or of continuous intravenous infusions of atrial natriuretic factor in rats with various types of hypertension.⁹⁶⁻⁹⁸ Bolus injections of atrial natriuretic factor (1 µg) to both two kidney-one clip and one kidney-one clip hypertensive rats reduced blood pressure to normal for 35 min to > 60 min. When the atrial natriuretic factor was infused intravenously at the rate of 1 µg/h continuously for 6 days to two kidney-one clip hypertensive rats, blood

pressure came down to normal (< 140 mm Hg systolic pressure) after the second day and remained so throughout the administration of atrial natriuretic factor (fig 6). At the end of this six day infusion period, both plasma renin activity and heart weight were significantly increased in the untreated two kidney-one clip hypertensive rats, whereas there was no change in plasma renin activity or in heart weight in those receiving atrial natriuretic factor infusions. Longer infusions (12 days) at lower doses (100 ng/h) produced the same results.

Continuous infusions of atrial natriuretic factor (100 ng/h) for six days in one kidney-clip hypertensive rats resulted in a fall in systolic pressure from a mean of 193 mm Hg systolic in the control period to 145 mm Hg after the third day of administration. The fall in blood pressure in rats receiving atrial natriuretic factor was accompanied by a significantly greater diuresis and natriuresis than that in similar untreated rats that had had sham operations or removal of one kidney.

Infusion of the atrial natriuretic factor at 100 ng/h for six days in spontaneously hypertensive rats (mean systolic pressure 177 mm Hg) reduced systolic blood pressure to 133 and 143 mm Hg on the fifth and sixth days of infusion respectively (fig 7).⁹⁸

Dahl's salt sensitive hypertensive rats had mean (SD) concentrations of plasma immunoreactive-atrial natriuretic factor that were three times higher

Fig 6 Effect of a chronic intravenous infusion of synthetic atrial natriuretic factor (ANF) 26 AA (Arg 101-Tyr 126) at 1 µg/h for 6 days by minipump in three groups of rats, one sham-operated, the second with two kidney-1 clip (2-K, 1-C) hypertension, and the third group similar to the second group but receiving the ANF infusion. (Reproduced with the permission of Proc Soc Exp Biol Med and of the authors.⁹⁷)

Fig 7 Effect of a chronic intravenous infusion of synthetic atrial natriuretic factor (ANF) 26 amino acid peptide (Arg 101-Tyr 126) at 100 ng/h through mini-pumps to Wistar-Kyoto control rats with or without ANF and to spontaneously hypertensive rats with or without ANF. (Reproduced with the permission of Proc Soc Exp Biol Med and of the authors.⁹⁸)

than those of the salt resistant rats (15 (54) pg/ml versus 52 (7), $p < 0.02$)⁹⁹ (J Gutkowska *et al*, personal communication), but according to Hirata *et al*¹⁰⁰ these animals show a considerably reduced natriuretic response to exogenously administered atrial natriuretic factor, possibly because of a reduced renal papillary plasma flow.⁹⁹ Isihara *et al* reported that intravenous injection of atrial natriuretic factor to deoxycorticosterone acetate-salt hypertensive rats reduced blood pressure considerably.⁶⁹ The atrial natriuretic factor inhibits the hypertension induced by chronic pressor infusions of noradrenaline in conscious rats whether it is administered before or during the hypertensive state induced by noradrenaline.¹⁰¹ These results indicate that the study of atrial natriuretic factor administration in hypertensive patients may be promising.

Effects of atrial natriuretic factor on adrenal cortex and brain

The atrial natriuretic factor significantly inhibits release of aldosterone both in cultures of beef and rat zona glomerulosa cells and in vivo in rats and man.¹⁰²⁻¹⁰⁸ There is an inhibitory effect not only on the basal secretion of aldosterone but also even more strongly in vitro when aldosterone release is stimulated by angiotensin II, corticotrophin, prostaglandin E₁, or forskolin (table 7).

Table 7 Inhibition by atrial natriuretic factor (ANF) (14 nmol/l) of aldosterone production in cultured bovine zona glomerulosa cells

	Control	ANF (pmol/well)	% inhibition
Basal	1.0 (0.1) (3)*	0.8 (0.2) (3)	20†
AI (10 nmol/l)	6.4 (0.5) (4)	3.7 (0.3) (4)	52†
ACTH (10 nmol/l)	8.8 (0.4) (2)	5.3 (0.7) (2)	44†
PGE ₁ (1 µmol/l)	12.4 (0.6) (2)	4.0 (0.2) (2)	72†
Forskolin (1 nmol/l)	11.5 (0.7) (2)	7.0 (0.4) (2)	43†

Values shown are mean (SEM).

*Number of replicate experiments.

† $p > 0.05$ vs control by two-way anova.

‡ $p < 0.01$ vs control by two-way anova.

ACTH, adrenocorticotrophin; PGE₁, prostaglandin E₁.

Binding of ¹²⁵I-labelled atrial natriuretic factor in the brains of rats and guinea pigs has been shown by means of an in vitro receptor radioautographic technique.¹⁰⁸ The distribution of binding sites for ¹²⁵I-atrial natriuretic factor in the brain was unique, with high density binding sites in the median eminence, the subfornical organ, area postrema, and the nucleus tractus solitarius, all areas known to be involved in the regulation of blood pressure and in renin dependent hypertension and in the regulation of water and sodium. Neurons that were immunoreactive to atrial natriuretic factor Ser103-Tyr126 were detected in the brain of rats, especially in the anteroventral region of the third ventricle (periventricular nucleus of the hypothalamus and pre-optic area).¹¹⁰⁻¹¹¹ Detectable concentrations of immunoreactive atrial natriuretic factor were found in homogenates of rat hypothalamus,¹¹²⁻⁸⁵ in the posterior pituitary,¹¹³ in the ganglia nodosa of the vagus, and in the cervical sympathetic ganglion in the rat.¹¹⁴ In addition, high density binding sites were seen in the "pigmented" epithelium of the ciliary processes of the eyes both in rats and in rabbits.¹¹⁵

Plasma concentrations measured by radio-immunoassay in experimental and human hypertension

The mean (SD) concentration of immunoreactive atrial natriuretic factor in the plasma of conscious rats with catheters implanted in the jugular vein for 24 hours was 94 (17) pg/ml.³ Administration of morphine, ether, and ketamine to rats increased plasma concentrations of immunoreactive atrial natriuretic factor by 50, nine, and two times respectively.³⁶

Studies of spontaneously hypertensive rats at 16 weeks of age (systolic pressure 195 mm Hg) showed that the mean (SEM) plasma concentration of immunoreactive atrial natriuretic factor was 275 (24) pg/ml compared with 140 (22) pg/ml in

The atrial natriuretic factor

Wistar/Kyoto rats with systolic pressures of 116 mmHg.¹¹⁶ Although there was no difference in immunoreactive atrial natriuretic factor content of the right atrium, the immunoreactive atrial natriuretic factor content of the left atrial tissue was significantly lower in spontaneously hypertensive rats than in Wistar/Kyoto rats. This may be related to the significant increase in left atrial pressure found in the spontaneously hypertensive rats by Noresson *et al*¹¹⁷ and would be consistent with many experimental studies suggesting that the atrial stretch or pressure is a major stimulant of the atrial natriuretic factor release.

Plasma concentrations of immunoreactive atrial natriuretic factor in patients with mild and moderate essential hypertension were measured by radio-immunoassay after purification on Sep-Pak cartridges (Millipore, Milford, MA, USA). The mean (SEM) plasma concentration of immunoreactive atrial natriuretic factor was 11.9 (1.0) pg/ml in 67 normotensive control subjects (mean age 34.7 and blood pressure 117/70 mm Hg), and 13.5 (1.5) pg/ml in 44 hypertensive patients (mean age 47 and blood pressure 158/94 mm Hg) (P Laroche, JR Cusson, J Gutkowska, *et al*, personal communication). This difference was not statistically significant. These results were confirmed recently by the group of Burnett *et al*.^{117a} They contrast with the slight but significant increase in plasma immunoreactive atrial natriuretic concentrations reported by Arendt *et al*,¹¹⁸ by Sugawara *et al*,¹¹⁹ and Sagnella *et al*¹²⁰; the latter two groups used the same plasma purification procedure by Sep-Pak cartridges. These results may differ because of the nature of hypertension, its severity, and the presence of some degree of congestive heart failure in the groups studied. All our patients had a thorough evaluation, including renal arteriography, and had mild or moderate essential hypertension.

Nevertheless, intravenous bolus injections of 3, 12.5, and 25 µg of human atrial natriuretic factor had no effect on blood pressure in healthy subjects despite peak plasma concentrations of up to 1100 pg/ml (JR Cusson, P du Souich, LA Rochelle, *et al*, personal communication). Nor did intravenous infusions of human atrial natriuretic factor of 3.2 µg/min for 30 min producing mean plasma concentrations of 230–280 pg/ml have any effect on blood pressure and systolic and diastolic pressures fell by 7 and 10 mm Hg respectively when 6.25 µg/min of atrial natriuretic factor was infused for 45 minutes (mean (SEM) plasma concentration 625 (87) pg/ml).¹²¹ These findings suggest that the atrial natriuretic factor does not reduce blood pressure unless plasma concentrations exceed 600 pg/ml.

On the other hand, several reports indicate

significant increases in right atrial pressure, pulmonary wedge pressure (reflecting left atrial pressure), and cardiothoracic volume in patients with essential hypertension^{122–124b} (table 8); these are all factors known to increase the release of the atrial natriuretic factor. These observations added to the fact that all reported measurements of plasma concentration of immunoreactive atrial natriuretic factor (after purification on Sep-Pak cartridges) were below 100 pg/ml in patients with mild and moderate essential hypertension strongly suggest that the atria were less responsive to increased right and left atrial pressures and cardiothoracic volume and hence released less atrial natriuretic factor (table 8).

Table 8 Central haemodynamics and hypertension

(1)	Increase of left atrial pressure in spontaneous hypertension ¹¹⁷
(2)	In patients with essential hypertension:
(a)	Increased right atrial pressure Increased pulmonary wedge pressure Increased cardiothoracic volume (CTV) Increased CTV: total blood volume ¹²³
(b)	Raised pulmonary systolic and diastolic arterial pressure

Such atrial hyporesponsiveness would be compatible with (a) Guyton's hypothesis¹²⁵ that the hypertensive kidney cannot excrete sodium loads, hence the increase in blood pressure to re-establish the sodium equilibrium, that is the "pressure natriuresis" demonstrated in rats by Tobian *et al*^{126–128}; (b) the inappropriately high aldosterone secretion rate, plasma concentration, and excretion rate during periods of high sodium intake in patients with essential hypertension,^{129 130} which could be caused by decreased inhibition of aldosterone release owing to a deficient atrial natriuretic response; and (c) a decreased degree of vasorelaxation with predominant vasoconstriction resulting from an insufficient release of atrial natriuretic factor and an increased intracellular sodium concentration. This would lead to the increased vascular reactivity and resistance that are fundamental to the hypertensive process¹³¹ and would accompany the high concentrations of plasma Na⁺/K⁺ ATPase activity inhibitor reported in patients with essential hypertension.¹³²

Since the atrial natriuretic factor is a peptide and as such is degraded by the gastric juice when given orally, its application in clinical states (essential hypertension, hypertensive crises, congestive heart failure and other oedematous states, primary and secondary hyperaldosteronism) depends on the synthesis of analogues, probably non-peptidic, which will be absorbed and will be biologically active when given by mouth.

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Addendum

Normal plasma concentrations of immunoreactive atrial natriuretic factor have recently been reported in patients with untreated essential hypertension without left ventricular hypertrophy.

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