EDITORIAL

THE HEART AS AN ENDOCRINE ORGAN

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INTRODUCTION

Atrial Natriuretic Factor (ANF) is a circulating peptide hormone synthesised primarily in the cardiac atria of mammals to regulate blood pressure and extravascular fluid volume. The peptide is part of a larger family of structurally related hormones which are also found in many other tissues. **In addition** to its peripheral site of action, **ANF is synthesised in the brain where it is believed to be involved in the central regulation of cardiovascular homeostasis, neuroendocrine functions and in influencing drinking behaviour.** Evidence so far suggests that there is a considerable plasticity in the regulation of the production and release of cardiac natriuretic peptides and, similarly, of their receptors under various conditions. This review attempts to summarise our understanding of this important peptide in terms of their general biological and functional significance. In this context, it will focus primarily on the structure, synthesis and secretion of ANF from the heart as well as the possible roles played by the peptide in general circulation under physiological and pathophysiological conditions. More extensive and detailed reviews covering different aspects of ANF can be found elsewhere[1-3].

The discovery of ANF in the heart was the culmination of many years of morphologic and biochemical analyses. In 1956, Kisch first described membrane-bound granules in the atrial myocytes of guinea pigs, and by 1964 similar granules had been described in a number of mammalian species[4,5]. These organelles appeared to be similar to those found in peptide hormone producing cells of other endocrine tissues[6]. Changes in dietary sodium and water have been shown to alter the granularity of rat atrial myocytes, suggesting that these granules may be involved in the control of and/or, subjected to the influence of extracellular fluid volume[6]. However, the content and function of these granules remained unclear until 1981 when de Bold et al[7] demonstrated that a granule-enriched extract of rat atrial tissue induced a marked natriuresis and a reduction in systemic atrial pressure in living rats. **They postulated the existence of a cardiac hormone or hormones capable of modulating electrolyte balance in the granules, which was therefore named atrial natriuretic factor.** Subsequently, the active peptides were purified and sequenced using cDNA libraries prepared from mRNA of atrial myocytes[8,9]. **A number of natriuretic peptides, conserved across mammalian species and distributed throughout the body, has been identified. The physiological significance of these peptides is elusive and their actions are more diverse than expected.**

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STRUCTURE OF PRE-PROANF GENE AND ITS PEPTIDE

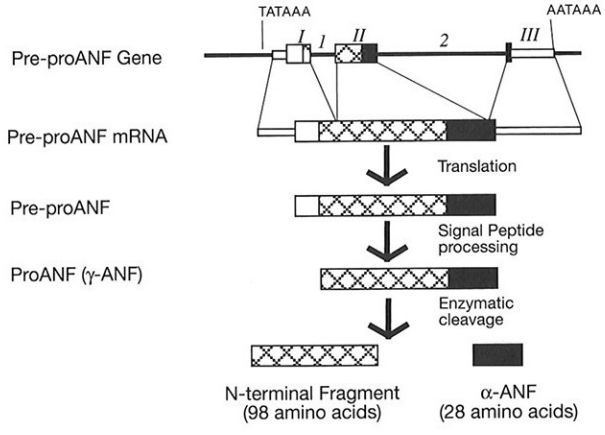
a) Gene for ANF

The nucleotide sequence for pre-proANF has been determined from various species including the genomic sequence from humans[10-12], rats[13,14], and cattle[15] and the cDNA sequence from humans[16], rats[17], dogs[18], and rabbits[18]. In rat, the cDNA sequence consists of about 850 bp which contain 5'- and 3'- untranslated regions and a region of 456 bp that encodes the pre-proANF of 152 amino acids (151 in human) (Fig 1). The gene consists of a short first intron (about 100 bp) and a long second intron (391 bp in rat, 527 bp in mouse, 1093 bp in human) that separate the three exons[10-13,15]. The first exon encodes the 5' untranslated sequence, a signal peptide and a small portion of the mature precursor (proANF) up to amino acid 16. The bulk of the coding region for the precursor is contained within the second exon and is followed by the large second intron. This separates the penultimate arginine residue of mature ANF from the carboxyl terminal tyrosine residue, which is encoded by the third exon along with the 3' untranslated region (Fig 1). This tyrosine residue is followed by codons for *Arg-Arg*-stop in the rodent genes. However, in all species the isolated peptide terminates with *Tyr* suggesting that the two arginines are cleaved off during post-translational processing[15,18].

b) Structure of Pre-proANF

Transcription of the ANF gene yields an mRNA species that

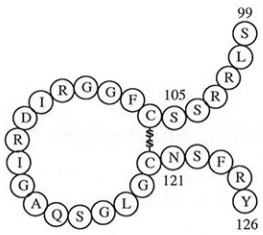
Fig 1 – A schematic representation of the rat pre-proANF gene and corresponding mRNA, as revealed by cDNA sequences. The steps involved in translation and subsequent protein processing are outlined. *I, II* and *III* denote the 1st, 2nd and 3rd exons; while *1* and *2* denote the 1st and 2nd introns. [Modified from Lewicki & Scarborough, 1990]



encodes a 152-amino-acid pre-proANF in rats[8]. The pre-proANF is then rapidly converted to a 126-amino-acid precursor peptide proANF (proANF$_{1-126}$, γ-ANF) by cleavage of the signal peptide (24 amino acid) at its amino-terminal end[19]. The pro-ANF has been shown to be the principle storage form of ANF and the major constituent of atrial granules[17,20]. When appropriate signals for hormone release are given, proANF is further split into an NH_2-terminal fragment, ANF$_{1-99}$, and the biologically active hormone, amino acid residues 99-126 (α-ANF$_{1-28}$)[21,22]. α-ANF is composed of a disulfide bonded 17 amino acid ring structure flanked by a 7 amino acid N-terminus and 5 amino acid C-terminus (Fig 2). Studies of structure-activity relationships have shown that the disruption of the ring structure leads to a loss of biological activity[23-26]. Systematic removal of N-terminal residues up to residue 7, preceding the disulphide bond, does not significantly affect vasorelaxant or natriuretic activity[21,26,27]. However, removing more than one residue from the C-terminal markedly reduces the natriuretic and vasorelaxant potency of ANF analogs. This structure-function effect is consistent with the notion of various ANF congeners interacting with a multiple receptor system[26-28] to induce graded degree of biological responses in different tissues.

The amino acid sequence of proANF molecules is highly homologous (~75%) across mammalian species[15,18]. Twenty-seven of 28 amino acids of the mature α-ANF are identical in all the species studies. The single amino acid difference is at position 12, which is ***Met*** for human, dog, porcine, and bovine; and ***Ile*** for rat, mouse, and rabbit. This degree of homology across mammalian species perhaps underlines its biological importance in evolution.

Fig 2 – The sequence and structure of α-ANF. The numbers indicate the position of α-ANF within the sequence of proANF$_{1-126}$ (γ–ANF, after removal of a signal sequence of 24 amino acid).



A *(Ala)*	Alanine	M *(Met)*	Methionine
C *(Cys)*	Cysteine	N *(Asn)*	Asparagine
D *(Asp)*	Aspartic acid	P *(Pro)*	Proline
E *(Glu)*	Glutamic acid	Q *(Gln)*	Glutamine
F *(Phe)*	Phenylalanine	R *(Arg)*	Arginine
G *(Gly)*	Glycine	S *(Ser)*	Serine
H *(His)*	Histidine	T *(Thr)*	Threonine
I *(He)*	Isoleucine	V *(Val)*	Valine
K *(Lys)*	Lysine	W *(Trp)*	Tryptophan
L *(Leu)*	Leucine	Y *(Tyr)*	Tyrosine

TISSUE DISTRIBUTION

ANF was first identified in cardiac atria. The generation of ANF-specific antisera and ANF mRNA-specific oligo-nucleotide probes has led to the detection of multiple sites of ANF synthesis in a variety of extra-atrial tissues. The ANF transcripts in all of the extracardiac sites are identical to the atrial gene transcripts in overall size. However the abundance of extra-cardiac ANF transcription is considerably less than that of the adult ventricle[29-31].

a) ANF expression in the heart

The major site of ANF gene expression in mammals is the atria with proANF mRNA constituting approximately 1-3% of the total mRNA[32]. The next **highest concentration of proANF mRNA is found in the ventricles** where the level of expression is 100-fold less than that of the atria. However, **ventricular synthesis is markedly elevated during fetal development and by haemodynamic overload, such as occurs in heart failure and hypertension[33,34].**

b) Distribution of ANF in other peripheral tissues

After the heart, the brain is the next major site of ANF expression. The detailed neuroanatomical distribution of ANF-like substances has been reported in the brain of rats[35-37], dogs[38], and frogs[39] using specific RIA and immuno-histochemical techniques. Although ANF is distributed widely in the CNS, the anterolateral border of the third ventricle (AV3V) regions and the hypothalamus are the most prominent sites. It suggests an **important physiological role of the natriuretic peptide system in the central control of cardio-vascular homeostasis and the water electrolyte system[40].**

ProANF mRNA or proANF-like immunoreactivity has also been described in the following tissues: aortic arch cells[31], lung[29,30], adrenals[41], kidney[42], gastrointestinal tract[43,44], thymus[45-47], spleen[48,49] and placenta[50]. ANF levels in these tissues are, however, considerably lower than that in the adult atrium, and are therefore unlikely to contribute substantially to plasma ANF levels, at least under physiological conditions[51,52].

REGULATION OF ANF GENE EXPRESSION

Recent advances in molecular biology have broadened the understanding of the regulation of eukaryotic gene including that of ANF. There is now good evidence suggesting that the **expression of the pre-proANF gene is regulated by diverse factors, including hormones, neurotransmitters, and mechanical factors such as atrial stretch and changes in intravascular volume.**

a) Regulatory sequence of ANF gene

The ANF gene, like most eukaryotic genes, carries a *TATAA* box sequence, about 30 bases upstream of the major transcription cap site, which is likely to dictate the transcription initiation site. At the 3' flanking end there is a *AATAAA* polyadenylation signal about 250 bp downstream from the termination codon[12,15]. The regions of maximum homology within the mouse, rat, and human ANF gene are confined to the DNA sequence located between -1100 bp and the transcription initiation site[10,12,53,54], possibly reflecting the importance of this region in the regulation of transcription. In rat, 2.4 kb of the 5'-flanking region from the ANF gene appears to contain all the regulatory elements required to direct high level of atrial expression of ANF[53]. Regulatory sequences between -693 and -137 bp and -1600 to -1000 bp have recently been reported[55,56] and indicate that regulation of the ANF gene may involve sequences that are proximal as well as distal to the promoter apparatus. Removal of these regions reduced ANF promoter activity by 20- to 30- fold. Other regions reported to regulate ANF expression include the region between -323 to -636 bp which contains a cAMP-responsive element-like recognition site, an AP-1 binding site as well as AP-2-, Egr-1-, and CARG-binding sites[55,57]. A steroid-responsive element has also been detected between residues -1000 and -700 bp that is necessary for glucocorticoid responsiveness[56]. Sequence analysis of the elements showed only partial homology to consensus glucocorticoid-responsive element (GRE).

The human ANF gene appears to contain most of its regulatory elements within the first 500 bp upstream from the transcription initiation site[58,59]. Deletion analysis of this region has revealed sequences homologous to the regulatory sequences in the rat gene[59]. These sequences appear to regulate atrial transcription. However, the corresponding region of the rat gene was unable to direct atrial transcription in primary culture of rat neonatal cardiocytes[60]. Therefore, unlike those sequences which encode the hormone, the

regulatory regions of ANF gene have evolved considerably between these species.

In vivo studies with transgenic mice carrying a construct containing 2.4 kb of 5' flanking sequence of rat ANF gene in front of the bacterial report gene CAT have demonstrated a low level of hypothalamic CAT activity[61], corresponding to the low level of mRNA for ANF detected in rat hypothalamus[30,62], suggesting that these 2.4 kb contain *cis*-acting elements are also important for transcription of ANF gene in the brain.

b) Factors affecting ANF gene expression

i) Mechanical Stretch

ANF gene expression is activated in atrial and ventricular myocytes, in response to volume overload and pressure, resulting in a limited increase of ANF mRNA in the atria and abundant expression of ANF mRNA in the ventricle[63].

The molecular basis for this induction of dominant gene expression by mechanical stretch is poorly understood. There has been some evidence that the proto-oncogenes c-*fos* and c-*jun* are elevated in cardiac hypertrophy[64-66]. Elevation of c-*fos* levels precedes increases in ANF expression[67], suggesting that the up-regulation of ANF gene expression during hypertrophy may involve proto-oncogenes. The common mechanism by which c-*fos* directs transcriptional events involves the formation of a heterodimer with c-*jun*, called AP-1, which can then bind to specific sequences of DNA[68]. Analysis of the sequences of ANF genes has indicated that there are three potential AP-1 binding sites in ANF 5' flanking region. The c-*jun*/c-*fos* complex has been shown to interact with this region and selective mutation of these sites suppressed basal activity of the ANF promoter[55,57,65,66,69].

Among the various stimuli that activate c-*fos*/c-*jun* complex formation and thus, binding to and regulation of certain AP-1-containing genes are those that stimulate signal transduction pathway involving protein kinase C (PKC)[70]. It has been also shown that the activation of PKC induces secretion of the ANF as well as expression of the ANF gene in the heart[71,72]. It thus suggests that PKC-regulated transcriptional factors may be involved in mediating the activation of ANF gene. In addition, α_1-adrenergic agonists that produce some of the feature of hypertrophy have been known to stimulate ANF gene expression, indicating that an α_1-adrenergic mediated mechanism, possibly involving PKC activation, may be a key component for ANF gene regulation by mechanical stress in the cardiomyocytes[57,73-75]. Several candidate *cis*-acting elements for α_1-adrenergic mediated expression were determined within the 316 bp (-638/323) of ANF sequence (CRE, AP-2, AP-1, Egr-1, CArG)[57]. Recent work done by Knowlton (1995) and his colleague demonstrated that α_{1A} but not α_{1B}-adrenergic receptor subtype mediates transcriptional induction of ANF gene expression, an increase in myocardial cell size as well as stimulation of phosphoinositide hydrolysis. Finally, activated myocardial α-thrombin receptors have been shown to induce hypertrophy and increase atrial natriuretic factor gene expression through both protein kinase C and protein tyrosine kinases pathways[76]. Despite the above findings, direct evidence for a role of PKC in the activation of the ANF gene in response to mechanical stretch is still needed.

ii) Glucocorticoids

Several lines of evidence indicate that glucocorticoids stimulate ANF gene expression. *In vivo* experiments show that glucocorticoids act directly to increase circulating ANF levels in the rat[77,78] as well as to enhance atrial and ventricular ANF mRNA levels[77-79]. This is consistent with the observations in primary cultures of atrial[56,80] and ventricular cells[56,81] with the latter being more sensitive to the steroid. This effect of glucocorticoid is blocked by its specific antagonist RU38486[82]. Glucocorticoids have also been shown to have profound effects on rat hypothalamic cells in culture[83]. Although treatment with dexamethasone alone does not modify the basal level of proANF mRNA in the culture, it significantly potentiates the stimulatory effect of forskolin[83] which elevates the cAMP level in the neurons. Huang et al have also reported that dexamethasone modulates proANF mRNA expression from cultured hypothalamic neurons by switching the adrenoceptor responsiveness of the cells from α_2- to that of β-adrenoceptor[84]. The notion of a direct modulation of transcription of ANF gene by glucocorticoids was strengthened by the identification of GRE mapped within the 2.4 Kb sequence upstream of the transcription initiation site in the rat[53] and in the second intron of the human ANF gene[11,14]. However, sequence analysis of these areas revealed multiple fragments with only partial homology to the glucocorticoid recognition sequence. None of the sequences conserved the hexanucleotide *TGTTCT* that is critical for steroid receptor interaction. This discrepancy has been partly clarified by the demonstration that purified glucocorticoid receptors are able to bind to a proximal *cis*-acting region upstream from the transcription site in the rat ANF gene[56]. This supports the *in vivo* observations and suggests a primary role for glucocorticoids in the modulation of ANF gene transcription.

iii) Other trophic factors

The recently identified vasoactive peptide endothelin has also been shown to increase the abundance of cardiac ANF mRNA[85]. Shubeita and colleagues have reported that endothelin-1 induces a pattern of early gene expression in cardiocytes that includes the proto-oncogene, c-*fos*, and the immediate early response gene, egr-1 as well as ANF mRNA[86].

BIOSYNTHESIS AND STORAGE FORMS OF ANF

a) Post-translational processing in the heart

In the atria, ANF is stored as a prohormone in specific electron-dense myocyte granules[87]. The pro-ANF is cleaved into its mature form of 28 amino acids, α-ANF, leaving a 98 amino acid N-terminal fragment at the point of release into circulation[21,22]. The enzyme responsible for cleaving the prohormone and the precise site of proANF processing has been a matter of considerable debate[88-96]. It is possible that **several sites of processing exist within the atria,** mediated by various enzymes under different regulatory control. The most recent evidence points away from storage granules to the membrane of the secretory myocytes or another unspecified atrial cells.

There are differences in the transcriptional regulation of ANF between the atrium and the ventricle as well as in the translation and the secretion of the peptide. The ventricles are virtually devoid of secretory granules, and appear to secrete α-ANF constitutively[91]. This is unlike the high degree of storage and bolus release that occurs in atrial cells and implies a lack of translational regulation of ventricular ANF. However, the form of α-ANF secreted by the ventricles is identical to that of the atria.

b) Post-translational processing in the CNS

The CNS and the heart share identical precursor molecules as demonstrated by comparison of cDNA from both tissues[30].

However, the processing and the storage forms are strikingly different. ANF is present in rat brain extracts at relatively low levels compared to the heart (about 50 ng in a rat brain, as opposed to 20 μg in a single atrium)[97]. In contrast to the form of ANF stored in the atrium, gel filtration studies have revealed that the predominant species of ANF in rat brain is α-ANF[98,99]. Further studies with RP-HPLC demonstrated that there are two major molecular forms of α-ANF_{4-28} and α-ANF_{5-28} in both rat[99] and pig brains[100]. Human and monkey brains also contain mainly low molecular weight forms of ANF[101]. There is now good evidence suggesting that the α-ANF_{5-28} is equipotent to α-ANF_{1-28} in terms of receptor binding and biological activity[102]. Using antiserum directed toward the ring portion of α-ANF, Lim et al have found that *Arg* residue at position 4 of α-ANF_{4-28} is removed from the N-terminal during or immediately prior to its release from the hypothalamic neurons in culture as α-ANF_{4-28} is detected in the cell extracts whilst α-ANF_{5-28} is present in culture media by RP-HPLC[103]. Recently, it has also been demonstrated that α-ANF_{5-28} is the major molecular species in hypophysial portal blood of the rat[104]. All these results indicate that the post-translational processing of the precursor molecules in the brain differ significantly from that in the atrium. A single basic arginine residue preceding α-ANF in the precursor (position 98 of γ-ANF) becomes a critical enzymatic cleavaging site of the molecule in the heart. On the other hand, two amino acid residues, *Arg-Arg*, at position 3 and 4 of α-ANF (positions 101 and 102 of γ-ANF) represent important sites for cleavage in the brain, giving rise to smaller congeners of α-ANF_{4-28} and α-ANF_{5-28}.

RELEASE OF ANF

a) Heart

It is generally believed that the major stimulus for ANF release from the heart is atrial distension or mechanical stretch. *In vitro* and *in vivo* evidences suggest that increases in right atrial volume are essential for ANF secretion, secondary to changes to intravascular volume status[1,105]. **The mechanochemical transducer is most likely located within the atrial myocytes, but its nature is unclear.**

Although atrial stretch has been considered as a potent stimulus for the release of ANF, **one recent and controversial report claimed that** in Langendorff-perfused, paced rat hearts, **stimulation of cardiac sympathetic nerves in a stretch-independent manner potently enhances ANF secretion[106].**

Humoral factors, particularly catecholamines and angiotensin II, have also been suggested as regulators of cardiac ANF release. Circulating catecholamines and angiotensin II stimulate ANF release mainly act indirectly through their haemodynamic effects. However, **norepinephrine has also been shown to modulate ANF secretion directly through both α and β adrenergic receptors,** but these effects are small compared to the effect of rapid changes in atrial wall tension. In experiments (both *in vivo* and *in vitro*) designed to minimise the haemodynamic effects of adrenergic agents, α_1-receptor agonists appear to stimulate ANF secretion through phosphoinositol breakdown and stimulation of PKC. The β-adrenergic agonists, which increase cAMP levels and stimulate protein kinase A pathway or PKA, can modestly increase ANF release under normotensive condition but in the non-beating heart, it produces inhibition[107-112]. **Increases in intracellular calcium have also been proposed as an important signal for ANF release** in a manner independent of the phosphoinositol system[113-115], possibly through its chronotropic and inotropic action on the heart.

Other secretagogues, including vasopressin and endothelin[116], have now also been included in the list. Systemic administration of endothelin antiserum has been shown to decrease basal and volume-stimulated plasma concentration of ANF, suggesting that circulating endothelin may be a physiological modulator of both basal and stimulated ANF release[117].

b) Central nervous system

For comparison to that of the heart, the regulation of ANF release from the hypothalamus is included here as **the peptide is also known to play an important role in modulating cardiovascular system at the central level.** Increasing evidence now suggests that in addition to neurotransmitters, humoral factors are essential in the modulation of ANF in the CNS. Several chemicals such as **high concentrations of K^+[118,119], calcium or sodium ionophores[120] as well as norepinephrine[119] have been reported to stimulate ANF release** from hypothalamic explants. Experiments on cultured hypothalamic neurons demonstrate that, unlike the heart, ANF release is mediated in a Ca^{2+}-dependent manner through membrane depolarisation[103,119,121] and not by changes in osmolarity or sodium concentration[121]. **The secretion of hypothalamic ANF is apparently modulated by both the cAMP dependent second messenger system and the glucocorticoids.** Huang et al have demonstrated that forskolin treatment increases the release and synthesis of ANF in dissociated hypothalamic cells cultured in serum-free media. Although dexamethasone suppresses[122] or has no effect on basal ANF release[83], glucocorticoid significantly enhances the effect of forskolin-stimulated ANF synthesis and release from hypothalamic cell cultures[83]. The administration of **norepinephrine increases hypothalamic ANF release** from the cultures through its action on α_2-adrenoceptors. Glucocorticoids have been shown to alter the activity of norepinephrine stimulated hypothalamic ANF neurons by switching their responsiveness from α_2- to that of β-adrenoceptors[84,123]. **Ascorbic acid has also been shown to enhance ANF release** without altering intracellular cAMP levels but by potentiating the stimulatory effect of forskolin[124]. More recently, Lee et al have demonstrated that **ANF neurons of the hypothalamus are dopamine-sensitive and the catecholamine may exert a direct stimulatory or inhibitory effect on the neurons** mediated through D_1 or D_2 receptors respectively, in a manner related to the differential dopamine binding sensitivity of the two receptor subtypes[125,126].

RECEPTORS OF ANF

By using radioligand binding and autoradiographic techniques, **specific ANF receptors have been identified in numerous tissues, including endothelial and smooth muscle cells of the vasculatures[127].** Cells of the **renal glomerulus** and **renal papilla,** as well as those found in the **lung, adrenal, pituitary and various regions of the brain have also been shown to possess biologically active receptors for ANF[128].**

Molecular cloning studies have identified two distinct classes of ANF-specific receptors in target tissues. One of them is biologically active and exists as two subtypes, termed natriuretic peptide receptor A and B. The type A receptor has a strong binding affinity to ANF and a lesser extent to its related member, brain natriuretic peptide or BNP, while the type B receptor recognises c-type natriuretic peptide or CNP as its primary ligand. This class of receptors (120-140 kDa)

presents as single-chain polypeptides and amino acid sequences deduced from their cDNA sequences suggest the existence of an extracellular ligand-binding domain, an intracellular guanylyl cyclase (GC) catalytic domain at their C-terminal and a protein kinase-like domain near their amino terminus. The latter is believed to serve a regulatory function[129]. Ligand occupancy of the extracellular domain leads to activation of cyclase activity and accumulation of cellular cGMP.

A second class of receptors, named type C, binds with relatively high affinity to each of the natriuretic peptides as well as to a number of structurally related homologues. It consists of a smaller protein (60-70 KDa) that forms homodimers, with a single extracellular domain and a short cytoplasmic tail which lacks the GC domain[130]. A widely held view is that these receptors are biologically silent and play a clearance role for the peptide[131]. However, recent evidence *in vivo* and *in vitro* suggests that the clearance receptors may be linked to some specific physiological activity[132,133]. The discrepancies in these experiments may be explained by the observations that this subtype of the non-GC-linked receptors may play some cellular functions different from those designated for that of type-A or type-B.

PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL ROLES OF ANF

An acute systemic administration of ANF has a wide range of biological actions[2,134,135]. **In the cardiovascular system, ANF decreases blood pressure, cardiac output, total peripheral resistance, and blood volume. Short-term administration or bolus injection of ANF produces rapid and dose-dependent decreases in arterial blood pressure in the absence of a reflex tachycardia.** It is also common to observe a prolonged hypotensive response persisting after administration of the peptide has stopped. The above hypotensive effects occur in both conscious and anaesthetised normotensive or genetically hypertensive animals. **The fall in cardiac output by short term infusion of ANF involves both direct and indirect action. Direct regulation occurs via decreased contractility, whereas a reduction in the central venous or right atrial pressure indirectly affects cardiac output by decreasing cardiac input.** The effects of ANF on total peripheral resistance were not consistent. Differences in resting vascular and autonomic tone in the various experimental models may contribute to this discrepancy. Decreases in plasma volume after ANF administration are due at least in part to a facilitation of transudation of plasma water to the interstitium.

ANF affects renal function at multiple levels. Acute administration of exogenous ANF results in natriuresis and diuresis. These effects are due to a combination of increased glomerular filtration rate through elevated capillary ultrafiltration, modulation of sodium transport in the medullary collecting duct, coordination of afferent and efferent arteriole tone, and decreased inner medullary hypertonicity[136].

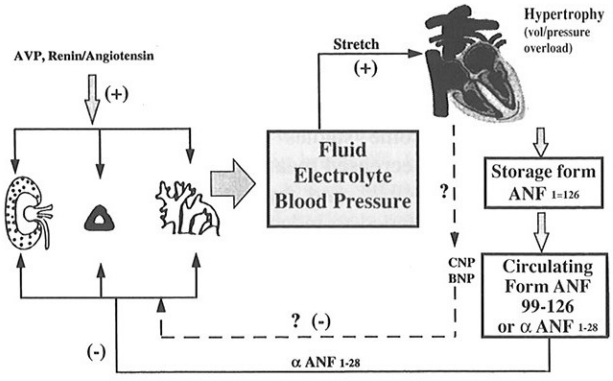
In the endocrine system, ANF is a potent inhibitor of the renin-angiotensin-aldosterone system at both the hormonal secretions and target organ levels. Specifically, short-term **infusion of ANF markedly lowers renin secretion rate from juxtaglomerular cells and decreases plasma renin concentration[2].** ANF has also been shown to act on other endocrine tissue. **It has been shown to inhibit angiotensin II (AII) induced vasoconstriction and pressor responses, vasopressin and corticotropin release from the pituitary gland, and aldosterone production and secretion from the adrenal cortex[137].**

Although the above acute effects of ANF are well studied, the effect of chronic elevation of ANF level on cardiovascular and renal function has been difficult to assess. Techniques to increase blood or tissue ANF concentrations are impeded by the temporal limitations of long-term infusion models and the extremely short half-life (20s to 20min) of the molecules *in vivo*. Although some studies suggested that **long-term infusions of ANF decreased mean arterial pressure in both normotensive[139,140,142,144] and hypertensive animals[138,141]**, other studies have failed to induce sustained hypotension in either normotensive[138] or hypertensive animals[143] by long-term ANF infusions. The hypotensive effect of ANF demonstrated by some authors has been shown to be accompanied by variable changes in cardiac output, total peripheral resistance, intravascular volume, and renal function. Interestingly, **transgenic mice which overproduce ANF are chronic hypotensive** but no obvious natriuretic or diuretic phenotype was observed[137] suggesting these mice may adequately compensate for the renal effects but not the haemodynamic effects of the hormone.

Human studies have also suggested that ANF plays an important role in a broad range of physiological and pathophysiological conditions. **In the cardiovascular system, ANF affects circadian rhythm of blood pressure by modulating renal function and vascular tone and by counteracting the action of renin-angiotensin-aldosterone system. The latter humoral factors are involved primarily in long-term blood pressure control[145].** Activity of the cardiac ANF system are known to be altered considerably in relation to the severity of heart failure. In this context, ANF and N-terminal proANF levels in the circulation may serve as useful indicators for prognostic value, even at an early stage of heart failure[146]. **In renal system, ANF acts on specific receptors in the kidney inducing hyperfiltration, inhibition of sodium transport and suppression of renin release, resulting in marked natriuresis and diuresis[147]. Besides, ANF also lowers blood pressure by inhibiting mineralocorticoids, particularly aldosterone biosynthesis and facilitates transudation of plasma water to interstitium. In healthy humans ANF levels are elevated in response to intravenous loading with saline, acute dietary sodium overload or immersion of the body into water[147]. In nephrotic patients, ANF enhances diuresis and natriuresis.** However, different degrees of natriuretic response are found in the patients when compared to healthy volunteers. **In renal failure, enhanced release of ANF is detected consequent to volume overload, diminished glomerular filtration rate and hypertension. Elevated plasma concentrations of ANF in end-stage renal disease are normalised following successful renal transplantation, suggesting that renal function is an important determinant of plasma ANF concentration.** Furthermore, fluctuations in plasma ANF-level during acute renal failure are closely related to rapid changes of blood volume pertinent to these patients[148].

In summary, **evidence so far clearly suggests that ANF is involved in the pathophysiology of cardiac failure, hypertension or other pressure and volume overload states (Fig 3). Furthermore, its effectiveness as a pharmacological agent in altering cardiovascular and renal functions under these adverse conditions makes it an exciting candidate for treating patients with excessive cardiac overload.** However, the constraint of its administration to intravenous route and its relative short half-life in circulation has placed a major restriction to wider

Fig 3 – A schematic illustration of the physiology of the cardiac ANF system.



application of the peptide to date. So far, the overall benefit from short-term, parenteral administration of ANF in a number of diseases has been variable and has often been limited by the development of hypotension. Findings from studies employing ANF antagonists, enzyme inhibitors and c-type receptor ligands have provided additional insights into the physiological and pharmacological actions of ANF. Further studies with these agents may yield new therapeutic approaches to the treatment of common cardiovascular disease.

ANF RELATED PEPTIDES

Several other compounds that are closely related to ANF have been independently identified in several laboratories based on molecular and biochemical similarities. They form a natriuretic peptide family consisting of ANF, Brain Natriuretic Peptide (BNP), and C-type Natriuretic Peptide (CNP)[149,150]. Despite the fact that these three types of natriuretic peptides are highly homologous, they have various biological effects. The differences in their cDNA structure, tissue distribution and receptor binding affinities suggest that they are functionally different and may play various roles in the regulation of volume and pressure homeostasis.

a) Brain Natriuretic Peptide (BNP)

BNP was first identified in the porcine brain as either a 26 or 32 amino acid peptide[100]. Like ANF, porcine BNP contains a 17 residue disulfide bonded ring which differs by only four amino acids from human ANF. Greater divergence from ANF sequences is exhibited in the N- and C-terminal residues[151]. BNP is more abundant in the cardiac ventricles than in the brain[152] and is also secreted into the circulation from the heart, suggesting a more important role to act as a circulating hormone rather than as a neuropeptide in the CNS.

Subsequent molecular analysis has identified the equivalent hormone in humans[153], rats[154], and dogs[155]. Like ANF, BNPs are derived from a common precursor, termed pre-proBNP, which contains between 121 and 134 amino acids depending upon species. The amino acids sequences of these BNPs are less conserved across species than are those of the ANFs, even in the COOH-terminal amino acid sequences essential for the biological activity. In the rat, the circulating form of the peptide is a 45 amino acid hormone, BNP-45[156], compared with the human BNP-32[157] and porcine peptides BNP-26, BNP-29 and BNP-32[151,157]. These data thus show that the proteolytic processing of the hormone is apparently different in each species, although whether the differences may subtly affect the biological function of these molecules is unknown.

BNP mimics both peripheral and central actions of ANF in experimental animals, including natriuresis, diuresis, hypotensive effect, relaxation of smooth muscle[100,158-160], and generation of cGMP in various cultured tissues[161,162]. **In humans, BNP infusion improves left ventricular function by inducing vasodilatation and a marked natriuresis. Plasma BNP concentrations were elevated in patients with acute myocardial infarction, supraventricular tachycardia or chronic renal failure. These findings indicate the significance of BNP as a new cardiac hormone** and suggest that, like ANF, BNP regulates blood pressure and intravascular volume.

A 35-amino-acid bovine peptide, aldosterone secretion inhibitory factor (ASIF) and chicken "ANF" also appear to belong to the BNP group of natriuretic peptides and represent a third example of species-specific processing of BNP compounds. ASIF contains sequences identical to that found in porcine BNP-32, but contains three additional amino acids residues[163]. It has been identified in chromaffin granules of bovine adrenal cells, a location suggesting that an intra-adrenal paracrine mechanism may be important for regulating aldosterone secretion. Chicken ANF ($cANF_{1-29}$) isolated from the atrium and ventricles is also more closely related to porcine BNP than to mammalian ANF[164] and is assumed to be a member of the BNP family[165].

b) Type-C Natriuretic Peptide (CNP)

The newest member of the natriuretic family is CNP, recently identified in the porcine brain[149]. CNP has the characteristic of 17 amino acid ring structure, but lacks the C-terminal that is required for biological activity of ANF and BNP. Preliminary data of a cDNA encoding porcine CNP demonstrates that there is a termination codon which directly follows cysteine. Therefore, the absence of the carboxyl-terminal residues in CNP does not appear to reflect post-translational processing, but rather results from a unique gene transcript. CNP was first isolated as a 22 amino acid peptide with a sequence identical in the pig, rat and human[166], but has now been shown to be present mainly as CNP-53 in the brain[167,168].

Although various members of the natriuretic peptide family share similar biological properties, there are clearly major differences within the group. **CNP is pharmacologically different from ANF and BNP, and acts primarily on smooth muscle with only mild diuresis and natriuresis[169,170]. In addition, CNP is a potent venodilator, while ANF is largely ineffective in this regard[171].** It has been shown that CNP's vasoactivity occurs through the type B receptor as distinct from that of ANF or BNP[149]. **The kidney is relatively deficient in CNP-sensitive type B receptors, which probably explains the limited natriuretic activity of CNP[172].** The dichotomy insensitivity to ANF *vs* CNP in the venous strips suggests a similar unequal distribution of the type A *vs* B receptors in this tissue, in this case favouring the latter. Another important difference between the various peptides is their tissue distribution. Unlike ANF and BNP, **CNP has not been found at high concentrations in the circulation[170]. It is, however, easily detected in selected tissues** such as vascular endothelium, brain (CNP is presents in the brain at 27- and 69-times the amounts of ANF and BNP respectively)[167], kidney, intestine, and heart. In the rat, CNP mRNA and CNP appear to be confined primarily to the brain[169,173]. **CNP in these locations may function as a local autocrine or paracrine regulator of activity in neighbouring cells** with physiological functions quite distinct from those of the hormonal peptides mentioned

above. **Such autocrine or paracrine regulation would, for example, be much more efficient in responding to local signals such as mechanical stress or regional ischaemia and effecting changes that are confined to a specific vascular bed.**

CONCLUSION

The elucidation of the heart ANF system playing an important endocrine role as discussed above has added a new dimension to the complexity of the physiology and pathophysiology of the cardiovascular system. In this context, it is interesting to learn that **circulating ANF of cardiac origin indeed acts as a central factor to integrate a broad range of interaction between the cardiovascular system and those involved in maintaining water/electrolyte homeostasis.** Thus, it is not unreasonable to anticipate that **aberrant production or clearance of biologically active ANF, or disrupted expression of functional receptors in target tissues will indirectly and adversely affect the function of the cardiovascular system.** The possibility that these deviating conditions may to some extent contribute to the severity of cardiovascular dysfunction now needs to be considered. More importantly, **findings generating from basic research on the structure-function relationship of the peptides and their interaction with various subtypes of receptors have opened up new opportunities for novel pharmacological intervention** through employing synthetic ANF analogues which possess greater specificity and effectiveness. **Adoption of this new approach will foster the development of novel drugs which act by complementing the therapeutic effect of conventional pharmacological products** unrelated to the ANF system. **This combined approach may raise a new hope for better management of patients with cardiovascular diseases, particularly those incapacitated by severe and chronic blood pressure, or fluid overload problems.**

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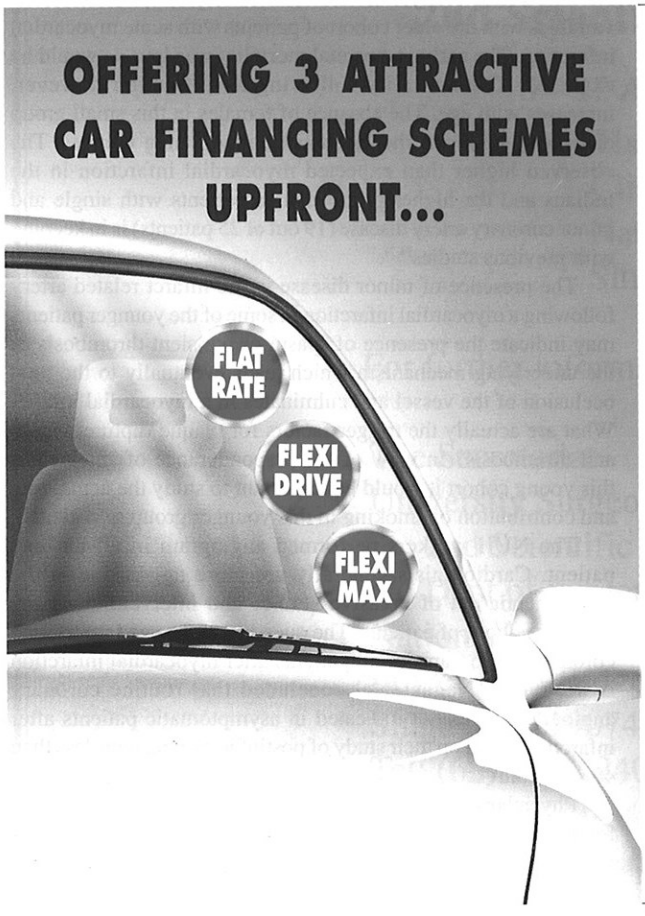

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