Modulation of the natriuretic peptide system
in heart failure: from bench to bedside?

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ABSTRACT
Since the discovery of atrial natriuretic peptide, the unravelling of the natriuretic peptide system has been a story of scientific success. However, bridging the gap between bench and bedside has proved difficult, and as yet has not provided any major clinical progress. In this review we will first give a detailed outline of the key elements constituting the natriuretic peptide system. Secondly, we will briefly explain the underlying rationale, basic concepts and evidence behind currently pursued strategies to potentiate the natriuretic peptide system. Thirdly, we will highlight some of the problems that have so far hindered successful translation of these theoretically viable treatment options into tangible clinical progress.

INTRODUCTION
Up-regulation of the natriuretic peptide (NP) system, most evidently in chronic heart failure (CHF), is not only a hallmark of many cardiovascular disease states, but also a useful diagnostic tool [1,2], prognosticator [3] and potentially a guide to optimize therapy [4,5]. The early recognition of the NP system as a potential target for therapeutic intervention was an important driving force behind the concentrated research efforts. This enthusiasm has recently been dampened by the somewhat disappointing results of clinical studies.

NP RESEARCH – AN HISTORICAL PERSPECTIVE
Historical descriptions of a phenomenon related to the endocrine heart were made more than 2000 years ago. Flavius, a Roman historian, called the workers diving to build the maritime harbour of Caesarea ‘urinatores’. Due to intra-thoracic pressure changes during immersion, the increased diuresis forced these divers to urinate frequently.
Thus the later discovery of the so-called Henry–Gauer reflex [6] was anticipated much earlier. The Henry–Gauer hypothesis postulates that changes in left atrial pressure induce changes in the release of arginine-vasopressin, which subsequently modulates the renal output of fluid [7,8]. However, results of the past decades indicate that this hypothesis is too simplistic in explaining the complexity of extracellular fluid volume [9–11]. The identification of atrial granules, using electron microscopy [12], possibly marks the real start of NP research. This morphological publication [12] appeared simultaneously, but independently, from the physiological studies by Henry and Gauer [6], demonstrating that diuresis is induced by atrial distension, a phenomenon long known and described earlier by Karel Frederick Wenkebach in his work about cardiac arrhythmias and their clinical significance [13]. Initially progress was slow, but following de Bold and co-workers’ seminal observation [14] that infusion of atrial,

Key words: heart failure, natriuretic peptide, vascular resistance.
Abbreviations: ACE, angiotensin-converting enzyme; ADM, adrenomedullin; Ang II, angiotensin II; BP, blood pressure; CHF, chronic heart failure; CO, cardiac output; CVP, central venous pressure; ET, endothelin; GC, guanylate cyclase; NEP 24.11, neutral endopeptidase 24.11; NOS, NO synthase; NP, natriuretic peptide; ANP, atrial NP; BNP, brain NP; CNP, c-type NP; NPRA/B/C, NP receptor type A/B/C; OMA, omapatrilat; PCWP, pulmonary capillary wedge pressure; RAAS, renin–angiotensin–aldosterone system; SNS, sympathetic nervous system; VPI, vasopeptidase inhibition; VSMC, vascular smooth muscle cell.
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Note the common structural motif is a 17-amino-acid loop formed by an intra-molecular disulphide linkage. The amino acid sequence within the ring is relatively preserved within a species; in humans only 5 amino acids within the ring differ between ANP, urodilatin, BNP and CNP.

but not ventricular, extracts into rats caused copious natriuresis, diuresis and hypotension, research gathered momentum and was advanced rapidly by the application of the emerging molecular biological methodologies. The bioactive substances causing this natriuresis were soon purified and their amino acid sequence identified by several researchers. In 1983 it was Flynn et al. [15] who first purified atrial NP (ANP) from rat atria to homogeneity and produced the first amino acid sequence of the molecule. One year later Kanagawa and Matsuo [16] reported the complete amino acid sequence of human α-atrial natriuretic polypeptide (Figure 1). The structure of the prohormone revealed that ANP comprised the C-terminal 28 amino acids of the precursor. It is sometimes written ANP(1–28) and often written ANP(99–126). In 1984, the mRNAs of the cardiac peptides of several species were analysed [17–20], and the gene structure of human ANP was also identified and sequenced [21–23].

**BIOCHEMISTRY AND MOLECULAR BIOLOGY OF NPs**

The NP family consists of several ‘mature’ NPs that share a common structural motif, consisting of a 17-amino-acid loop formed by an intracellular disulphide linkage (Figure 1). Furthermore, some authors also consider the N-terminal cleavage products, arising during processing from the pre-prohormones via prohormones to the final mature hormones, as essential parts of the NP system [24]. In humans, three ‘mature’ NPs, ANP, brain NP (BNP) and c-type NP (CNP), have been isolated. A further NP, dendroaspidis NP (‘DNP’), has been isolated from the venom of the green mamba [25] and ‘dendroaspidis NP-like immunoreactivity’ has been shown in patients with CHF [26]. The significance of these findings is currently under debate [27] and is the subject of ongoing research [28,29].

**Processing of NPs: from gene to peptide**

The structure of the ANP gene, in humans located on the long arm of chromosome 1 (p36.2), is shown in Figure 2. The ANP and BNP genes each comprise three exons separated by two introns (the BNP gene is also located on chromosome 1 in close proximity to the ANP gene, and the CNP gene is located on chromosome 2 and comprises two exons and one intron).

Exon 1 encodes the signal sequence, which is cleaved from the pre-prohormone (151 amino acids) in the endoplasmic reticulum to form a prohormone of 126 amino acid residues, which is the storage form of ANP, and the first 16 amino acid residues of the prohormone. Exon 2 encodes the majority of the remainder of the prohormone (i.e. aa 17–125 in humans), whereas exon 3 encodes only the tyrosine residue, i.e. amino acid 126 in humans and the three C-terminal amino acids in rat, mouse, rabbit and cow. As mentioned above, in human,
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Figure 2 Processing of the product of the ANP gene
The gene comprises three exons and two introns. Exon 2 codes for the majority of the pro-hormone (i.e. amino acids 17–125 in humans). Note the different post-translational processing of the cardiac and renal peptides from the same gene.

ANP is produced via a 151-amino-acid pre-prohormone, which after cleavage of a 25-amino-acid hydrophobic signal sequence is stored in atrial granules, predominantly as pro-hormone 1–126 (Figure 2 shows the gene structure and post-translational processing from pre-proANP to proANP, ANP and urodilatin). The proteolytic cleavage of proANP(1–126) occurs normally at Arg98–Ser99 in the C-terminal region during secretion to yield mature ANP(99–126). This cleavage step is performed by corin, a cardiac serine protease, and this protease may therefore be seen as the ‘proANP-converting enzyme’ [30,31].

ANP: a cardiac hormone
In the very early stages of ANP research, it was speculated that ANP may be stored in atrial cardiocytes, but produced elsewhere. This theory was based on the finding of axonal NP transport within the brain and the finding that especially in the brain, but also in peripheral tissue, there could be a mismatch between the presence of NPs and the presence of their corresponding receptors. However, it soon became clear that ANP was produced and stored, not only in the same region, but also in identical cells. Histological and immunocytochemical studies, as well as in situ hybridization analysis, confirmed expression of the ANP gene in human [32] and ovine fetal heart [33]. Moreover, whereas ANP [34] and ANP mRNA [35] are present throughout the heart in the early embryological stages of development, the ventricular representation regresses markedly towards the later developmental phases [35] (for review see [36]). Mature ANP is a 28-amino-acid peptide hormone with a molecular mass of 3080.5 kDa (C127H203N45O39S3). The structural motif is a 17-amino-acid loop, formed by an intramolecular disulphide linkage. The amino acid sequence within the ring is highly conserved within a species (in humans only 6 aa within the ring differ between ANP, BNP and CNP), whereas the N- and C-terminal tails vary markedly. Whereas the sequential integrity of the last four C-terminal amino acids is important for high NP receptor type A (NPR-A)-binding affinity, the additional presence of an aspartic acid residue in position 13 and a phenylalanine residue in position 26 appear to be crucial for activation of guanylate cyclase (GC) and cGMP production following receptor binding [37,38]. It is noteworthy that there are several reduced-size ‘mini-ANPs’ available that vary in their affinity to the NPR-A, as well as their ability to generate cGMP. Furthermore, due to variation in post-translational processing, several different ‘N-terminal ANPs’ exist [24]. The fate and biological activity of these cleavage products of the N-terminal tail, occasionally called ‘long-acting ANP’ [LANP, i.e. proANP(1–30)], ‘vessel dilator’ [i.e. proANP(31–67)] and ‘kaluretic peptide’ [i.e. proANP(79–98)], are highly controversial. Although they were previously believed to have little or no biological effects, some evidence suggest that they have some natriuretic, kaluretic and vasoactive potency [39], but that the mode of action is different from mature ANP [40]. For example, kaluretic peptide and long-acting ANP are believed to exert their biological action via inhibition of a Na+–ATPase [41] by increasing prostaglandin E2 [42].

ANP secretion
Following processing from pre-prohormone to prohormone (the storage form), cleavage and secretion of mature ANP is predominantly in response to increased transmural atrial pressure or stretch [43], which in an intact physiological organism is mainly a consequence of volume expansion. However, several other stimuli are capable of inducing ANP release (for review see [44]).
Figure 3  Representation of NPRs
Note all three receptors have an extracellular ligand-binding domain, as well as a transmembrane segment. NPRA and NPRB also contain a protein kinase homology region, which appears to exert some regulatory function on the GC domain.

These include vasopressin-, adreno-, endothelin (ET)-, angiotensin II (Ang II)-, enkephalin- and morphine-receptor stimulation. Evidence also suggests that ET, tumour necrosis factor-α and other cytokines may play a regulatory role in its production and release [45–48].

Urodilatin
Urodilatin is the name given to a naturally occurring paracrine-acting ANP homologue first reported by Schulz-Kappe et al. in 1988 [49]. The amino acid sequence reveals that it is an N-terminal extension of ANP. It comprises residues 95–126 of proANP, and therefore it is very likely that both urodilatin and ANP are derived from a common precursor, ANP(1–126). Urodilatin is believed to be a kidney-derived peptide and immunohistochemical studies identified high concentrations in the cortical tubules and around collecting ducts [50–52], contrasting with the predominant cortical location of renal ANP-binding sites [53]. Although the overall biological actions of urodilatin closely resemble those of ANP [54], it is believed that urodilatin is more important than ANP in regulating renal sodium excretion [55–57]. Like ANP, urodilatin is cleared by the NPRC, but in contrast is not degraded by neutral endopeptidase 24.11 (NEP 24.11) [58].

BNP
BNP was first isolated from pig brain as either a 26- or a 32-amino-acid peptide [59,60]. Human BNP was isolated in 1989 and found to comprise 32 residues, amino acids 77–108 of the BNP precursor peptide. Although these peptides, derived from the C-terminus of the proBNP molecule, show great similarity to the structure of ANP it is noteworthy that, in contrast with ANP, the amino acid sequence of BNP exhibits marked species variation. The same is true for the prohormones. Furthermore, the post-translational processing of BNP appears to differ from ANP with respect to the fact that conversion of BNP precursors occurs intracellularly, rather than during secretion. BNP binds to the same receptors as ANP (Figure 3) and is believed to exert its biological actions, namely natriuresis, diuresis and vasorelaxation, via the GC-coupled NPRA. BNP is predominantly synthesized in the ventricles and when tissue mass is taken into account, the total content of BNP mRNA is approx. 3-fold greater in the ventricles than in the atria. In marked contrast, ANP mRNA content in the ventricles is only 7% of that in the atria [61]. Interestingly, whereas 60% of circulating BNP is ventricular in origin, the mature BNP tissue concentration in the ventricles is only 1% of that in the atria. This observation of a large amount of production and secretion, but a small amount of storage of BNP in the ventricles (in health) somewhat parallels the previous finding that ventricular cardiocytes secrete ANP more rapidly after its synthesis via the constitutive pathway than atrial myocytes [62]. Due to the greater augmentation of gene expression, the more sustained production and secretion process, and the longer plasma half-life, the latter is thought to reflect resistance to NEP 24.11 [63].
BNP has certain practical advantages as a diagnostic tool over ANP. In disease states that cause hypertrophy, myocardial necrosis or heart failure there is greater induction and more rapid turnover of BNP mRNA and a much greater increase in circulating levels over basal levels compared with ANP [64]. For these reasons it has been suggested that BNP may act as an evolutionarily younger ANP-support peptide against ventricular pressure/volume overload, compensating for an insufficient ANP–NPRA system. However, this theory is not completely supported by evidence from knockout mouse models where interruption of the ANP–NPRA system was not persistently accompanied by up-regulation of BNP production. BNP appears slightly less potent in generating cGMP in human arterial and venous tissue than ANP [65], has similar potency in ET-preconstricted conductance and resistance coronary arteries [66], but has been shown to have less powerful local vasorelaxant effects in the resistance vasculature of the human forearm in health [67,68], although it was found to be roughly equipotent to ANP in the forearm resistance vasculature in CHF patients [68]. Hunt et al. [69] studied the biological effects of ANP and BNP infusions in normal subjects. Equimolar infusions caused a similar rise in ANP and BNP levels. Whereas the increase in cGMP was 4-fold higher with ANP than with BNP, natriuresis, contraction in plasma volume and inhibition of plasma aldosterone were comparable. Interestingly, while the pressor response to Ang II was unaffected by ANP or BNP, ANP but not BNP significantly inhibited the plasma aldosterone response to Ang II [69]. In a series of experiments, the same group further investigated the interactions between several NPs in health [70,71] and disease [72–74]. They found similar interactions between ANP followed by ANP–BNP infusion and BNP followed by BNP–ANP infusion with regard to metabolic clearance rate and disappearance rate from plasma (in health), suggesting that additive effects in regard to cGMP stimulation and blood pressure (BP) lowering effects resulted from dissociation of pre-bound hormones, presumably from biological or clearance receptors (for a review of the complex interactions between ANP, BNP and CNP see [75]). Intriguingly, no additive effect on the renin–angiotensin–aldosterone system (RAAS) and sympathetic nervous system (SNS) (heart rate and plasma catecholamines) was seen at the infused doses (leading to low pathophysiological concentrations) [70,71]. In a study in mildly hypertensive subjects both peptides (given intravenously) suppressed the RAAS to a similar degree [74], whereas in another study intra-renal BNP infusion did not induce changes in renal blood flow, secretion of active renin or creatinine extraction [76].

Interestingly, a recent study [77] using a canine model of pacing-induced acute heart failure showed that BNP infusion improved central haemodynamics, whereas there was resistance to the effects of ANP. The authors speculated that these findings would support the existence of a so far undetected BNP-selective NP receptor [77]. This theory is supported by a previous study by Goy et al. [78]. Using a NPRA-knockout mouse model, these authors [78] found that testis and adrenal gland retained statistically significant high-affinity responses to BNP, which could not be accounted for by NPRA, suggesting the presence of a novel receptor in these tissues that prefers BNP to ANP. However, at pharmacological doses the overall haemodynamic profiles of ANP and BNP given to patients with CHF appear to be comparable.

CNP

CNP, originally isolated from porcine brain [79], exists in two forms: as the 22-amino-acid form CNP-22, highly homologous to ANP but lacking the C-terminal tail, and as CNP-53, in which the N-terminal sequence is extended by 31 amino acids [80]. Unlike ANP and BNP, both of which originate from the heart, humoral CNP is believed to be of endothelial origin. However, the highest tissue levels in humans are found throughout the brain (10 times higher than the concentrations of ANP or BNP); consequently, CNP is believed to be a major non-conventional neurotransmitter. Its actions include inhibition of vasopressin and adrenocorticotropic hormone secretion, as well as modulating central BP regulation [81]. There is no entirely convincing evidence for CNP synthesis within the heart. Nonetheless, findings that specific CNP receptors (NPRA) and CNP gene transcripts exist within the vascular wall, the kidney [82] and within the central and peripheral nervous system strongly indicate a possible regulatory role for CNP in cardiovascular homoeostasis (for review see [83]). However, in contrast with ANP and BNP, CNP actions have an autocrine/paracrine, rather than an endocrine, mechanism. CNP is vasorelaxant in dogs [84] and humans [85], and when tested in isolated canine vessels [86] was relatively more potent as a venodilator than ANP. Furthermore, in canine arterial vessel preparations there were differential responses to ANP and CNP. CNP was more active in saphenous rings and less active in renal artery rings [86]. Similar findings were reported in the pulmonary circulation of newborn lambs, where ANP caused greater relaxation of pulmonary arteries than veins and CNP was more potent in relaxing pulmonary veins than arteries [87]. CNP also possesses coronary vasodilator properties. The mechanisms of this vasodilatation appear complex and include at least the particulate GC system (in dog coronaries) [88], as well as smooth muscle membrane hyperpolarization through potassium channel activation (in pig coronaries) [89]. In isolated canine femoral veins, the soluble and particulate GC system, as well as activation of large-conduction calcium-activated potassium channels, contributes to mediating vasodilatation [90]. In human preparations,
CNP has both veno- and arterial dilator actions [91–93]. Experiments in vitro, using human vascular tissue, showed CNP to augment cGMP production weakly (less than ANP) and equally in human saphenous veins, gastro-epiploic and internal mammary arteries [94]. Intra-arterial infusions of CNP in humans also induced much less arterial dilatation than ANP [93]. In our own study, comparing the effects of intra-arterial infusion of CNP and ANP on forearm capacitance in patients with CHF, CNP was much less potent than ANP [95]. Importantly, in contrast with ANP [96], the forearm arterial vasodilatation following local CNP infusion appears independent of nitric oxide (NO) synthase (NOS), but at least, partially dependent on hyperpolarization [85].

Other cardiovascular actions of CNP include inhibition of Ang II-stimulated ET-1 release in porcine endothelial cells [97], where CNP was found to be more potent than either ANP or BNP. In addition, CNP was found to inhibit vascular angiotensin-converting enzyme (ACE) activity [98]. CNP-22 inhibits vascular smooth muscle cell (VSMC) growth in tissue culture [99] and inhibits intimal thickening after vascular injury [100], raising the possibility that CNP may have an important anti-mitogenic role in the prevention of atheroma. Taken together, these findings support the existence of a vascular NP system [101] in which CNP participates as an endothelium-derived autocrine/paracrine regulator of vascular tone and remodelling.

**NP receptors, intracellular signalling and metabolism**

Circulating ANP is rapidly removed from the circulation by two mechanisms. First by binding to the abundantly expressed NPR$_C$ receptor (also called ‘clearance receptor’); secondly, via enzymic degradation through a zinc-dependent metalloprotease, termed NEP 24.11. This enzyme was previously known as ‘enkephalinase’, because of its role in morphine and enkephalin metabolism. NEP 24.11 is an non-specific enzyme that is also involved in the breakdown of a variety of vasoactive peptides, including the vasoconstrictors Ang II and ET-1, as well as several vasodilators including NPs, bradykinin, substance P and adrenomedullin (ADM) [102]. In sheep, the contribution of each ANP-eliminating factor is roughly equal [103]. In humans, the significance of both NP-eliminating systems is less well defined, but the contribution of NEP 24.11 is possibly less.

The plasma half-life of mature ANP is 150–200 s and several minutes for the various N-terminal proANP cleavage products. In contrast with CNP, where plasma levels appear to be consistently higher in venous than arterial blood, ANP has consistently higher arterial than venous levels (at a ratio of around 2:1) in all studied vascular beds so far. ANP is believed to exert its biological effects predominantly via binding to the NPR$_A$-type or NPR$_A$ (for review see [104]). Of all the known NPs, ANP exhibits the highest affinity to this receptor, found on the luminal surface of endothelial cells and the endothelial surface of VSMCs. The affinity for NPR$_A$ binding is ANP >> BNP >> CNP (Figure 3). The same trend is demonstrated with respect to cGMP production, following ligand–receptor binding. Although ANP appears to be the natural ligand for the NPR$_A$, CNP is the natural ligand for the NPR$_B$. So far no natural/primary BNP receptor has been found. Whereas the acute haemodynamic effects of BNP are weaker, its secretion process is more sustainable than that of ANP. The structures of NPR$_A$ and NPR$_B$ show close resemblance, whereas that of NPR$_C$ differs (Figure 3). NPR$_A$ and NPR$_B$ are membrane-bound GC-coupled receptors with molecular masses of 130–180 kDa [105–108]. The structure and roles of these GC receptors in BP regulation, cardiac and renal physiology has been reviewed elsewhere [109]. NPR$_C$, a homodimer of 64–66 kDa [110], is lacking a GC domain, but contains a 39-amino-acid intracellular tail that contains a G-protein-activating sequence. It appears to mediate signal transduction (for review see [111]) either through inhibition of adenylate cyclase (possibly via G$_i_2$) [112–117] or activation of phospholipase C (possibly via the $\beta\gamma$-subunit of G$_i$ and G$_s$) [111,118]. Following receptor–ligand (ANP/BNP/CNP to NPR$_{A,B}$) binding there is activation of the GC subunit. This in turn appears to be regulated by the attached protein kinase homology region, because if deleted, ligand binding and GC activity are uncoupled from each other [119]. Increased intracellular cGMP levels will ultimately result in VSMC relaxation. Furthermore, ANP is believed to affect metabolism of phosphatidylinositol bisphosphate to inositol 1,4,5-trisphosphate and diacylglycerol, a recognized important signal transduction pathway for hormones mobilizing intracellular calcium, in distinct partially opposing ways. Resink et al. [112] initially observed this process, and Hirata et al. [121] demonstrated that the truncated ANP analogue (amino acids 103–123) produced the same effect, thereby dissociating the action from NPR$_{A,B}$. This ANP–NPR$_C$-mediated stimulation of phospholipase C was observed in quiescent cells. Conversely, hormone-stimulated phospholipase C activity was inhibited by either ANP or other stimulants of GC activity [122]. Whereas stimulation of phospholipase C activity by ANP has only been observed in vascular tissue [120], an inhibitory effect has been observed in vascular tissue and the kidney [123,124]. Although the ultimate significance of ANP actions on phospholipase C activity are still not completely known, it is clear from the aforementioned that the proportional presentation of NPR$_{A,B}$ and NPR$_C$ within a vascular bed will affect the biological response to ANP exposure and may therefore differ in health and disease.
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Table 1  Phenotypes of genetically modified mice

<table>
<thead>
<tr>
<th>Gene</th>
<th>Human chromosomal location</th>
<th>Principal tissue distribution</th>
<th>Phenotype of knockout mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPRA</td>
<td>1q21</td>
<td>Vasculature, kidneys, adrenal gland</td>
<td>Hypertension, cardiac hypertrophy + dilatation, reduced testosterone levels and reduced live span</td>
</tr>
<tr>
<td>NPRB</td>
<td>9p21</td>
<td>Brain, ciliary body, vasculature</td>
<td>Not reported</td>
</tr>
<tr>
<td>NRTC</td>
<td>5p14</td>
<td>Endothelium, VSMCs, kidney, lungs, endocrine glands</td>
<td>Increased half-life of exogenous ANP, mild reduction in BP, increase in urinary output and bone turnover, deafness</td>
</tr>
<tr>
<td>ANP</td>
<td>1p36.2</td>
<td>Cardiac atria and ventricles</td>
<td>Salt-sensitive hypertension</td>
</tr>
<tr>
<td>BNP</td>
<td>1p36</td>
<td>Cardiac ventricles, brain</td>
<td>Patchy ventricular myocardial fibrosis in absence of hypertrophy</td>
</tr>
<tr>
<td>CNP</td>
<td>2</td>
<td>Brain, endothelium, ovary, uterus, testes</td>
<td>Not reported</td>
</tr>
</tbody>
</table>

PHYSIOLOGICAL ASPECTS IN HEALTH (ANP)

ANP is an almost ubiquitous hormone which has been detected in mammals, many reptiles, some single-cell organisms [125] and in several chlorophyll-containing plants, where it enhances solute flow [126]. NPs, and ANP in particular, appear to have a regulatory and integrating role in organo- and embryogenesis [36], i.e. they seem to play an active role in differentiation and regulation of multiple organ systems, including the skeletal system (stimulating osteoclasts and increasing bone turnover [127,128]), immune defence [129,130] (important role in host defence [131,132], histamine release [133] and activation of natural killer cells [134]), reproduction (testosterone production) and the central nervous system, where they act as a non-conventional neurotransmitter [135,136]. Furthermore, ANP lowers intra-ocular pressure in both normal and glaucomatous patients, unrelated to BP [137,138]. The renal actions, including a decrease in tubular Na+/water re-absorption, gave the hormone its name.

Cardiovascular effects

The cardiac actions of ANP appear to be ‘NO-equivalent’, including increased lucitropy [139,140] and facilitation of vagal effects. As far as integration of the cardiovascular system is concerned, ANP is well established in its role as a hormonal regulator of BP, sodium and fluid homoeostasis [141–143]. Recent research has highlighted its anti-proliferative potency [144–149], a property shared by BNP and CNP [150–152], emphasizing a potentially important role in cardiovascular remodelling. In CHF, the NP system appears to be the body’s endogenous defence against the deleterious effects of a chronically activated RAAS system [153–156]. The vasorelaxant effects of ANP (BNP and CNP) on resistance vessels are well documented in vitro [157] and in vivo [67,91,158,159], and it was initially believed that this effect explained the rapid fall of BP following intravenous ANP infusion. However, several animal studies [160,161] showed that the fall in BP and cardiac output (CO) was almost invariably associated with a fall in central venous pressure (CVP) and in fact an increase in total peripheral resistance. Groban et al. [162] demonstrated in humans that low-dose systemic ANP infusion reduced CVP without affecting BP. It is now generally accepted that the fall in CO/BP following systemic infusion of ANP is a consequence of reduced preload [163], and the mechanisms involved may include volume contraction as a consequence of diuresis and increased capillary filtration [74,164,165], and direct venodilatation [141]. While the former is well documented, evidence for the latter is sparse and ambivalent. Indeed ANP has no effect on dorsal hand veins [91,166], and little [167], or no [168], venodilatating potency on saphenous veins. However, Olson et al. [169], using an in vivo trout model, showed that ANP actively regulates venous capacitance (i.e. the entirety of small veins and venules), a finding we could confirm in the human capacitance vasculature [170].

The development of genetically modified animals, which have either augmented NPR populations or peptide production, or knockout of either peptide production, or NPRA or NPRC, have markedly improved our understanding of the role of NPs in cardiovascular control. Discussion of the rapidly expanding literature in this field can be found elsewhere [171,172] and is beyond the scope of the present review. However, the most pertinent findings of this research have been summarized in Table 1.

Finally, genetic targeting approaches to analysing hypertension have highlighted that molecular variants of the ANP gene may represent an independent risk factor for cerebrovascular accidents in animal models [173] and humans [174] (for reviews see [175,176]). Interestingly, these genetic variations exert their biological effects without altering the amino acid sequence of the mature ANP(99–126).
THERAPEUTIC PRINCIPLES AND RATIONALE

The NP system is the main endogenous defence system to counter-regulate the deleterious chronic activation of vasopressin, the RAAS and the SNS, which are found in several cardiovascular diseases [156]. However, it appears that in advanced disease states the beneficial effects of the NPs are masked by the opposing actions of increased sympathetic drive, increased circulating and local cytokines, and vasoconstricting factors. Thus reversing this imbalance in favour of the NP system, reducing vascular tone, inhibiting cardiovascular remodelling and decreasing neurohumoral activation, has been considered a promising therapeutic approach in the treatment of CHF.

Enhancement of the NP system can principally be achieved via three mechanisms, either in isolation or combination: (1) administration of NPs, peptide analogues or mimetics; (2) increasing synthesis and release of endogenous NPs; (3) prolonging lifetime and preventing breakdown of endogenous NPs.

Preventing the breakdown of endogenous NPs appeared to be the most promising approach, and can generally be achieved either by agents targeted to block the clearance receptor or by blocking enzymic degradation of endogenous NPs via blockade of NEP 24.11.

NP administration

ANP

A large number of studies have assessed the efficacy [177–179] and mechanisms of action [67,91,162,164,178–185] of ANP infusion in health and CHF. These studies varied widely in design (acute heart failure versus CHF, continuous versus repeated infusion, etc.) and patient selection. Importantly, some studies used shorter ANP analogues (‘mini-ANPs’) with a reduced biological profile. Not surprisingly, these studies have produced variable results, in particular regarding renal responsiveness following ANP administration. While some investigators found little [186–188] or no [179] effect on sodium and water excretion in patients with CHF, others observed marked responsiveness [189]. The causes of these discrepancies may be attributed to variations in study design. Since Ang II enhances cGMP degradation [190], it has been suggested [191] that concomitant ACE inhibition or Ang II1-receptor blockade may actually enhance the biological effects of NPs, potentially explaining preserved renal responsiveness in some of the more recent studies [177]. Interestingly, ACE inhibition appears to blunt renal ANP actions in healthy controls [192], while it was shown to have little effect on ANP-induced natriuresis, diuresis and creatinine clearance in CHF patients [188]. In a more recent study, Ang II1-receptor blockade, using irbesartan, has been shown to increase ANP levels (15.7 % over a 30-day treatment period), despite a drop in BP and a decrease in atrial and ventricular diameter [193].

However, a unifying feature of all the studies using ANP infusion is the almost invariable reduction in cardiac filling pressures. Elevating NPs, either by infusion [165,177] or by augmenting endogenous NPs [178], has been shown to increase stroke volume [165] and cardiac index [177] in patients with heart failure despite a fall in CVP [165,177,178], a finding apparently in conflict with the Starling mechanism. This is almost certainly due to relief of constraint by the stretched pericardium (pericardial constraint) and the volume-pressure-loaded right ventricle (diastolic ventricular interaction), resulting in an increase in true left ventricular pre-load, despite a fall in left ventricular end diastolic pressure, as previously shown by our group [194]. The fact that ANP infusion is able to reduce cardiac filling pressures, too early to be solely accounted for by a fluid shift into the interstitial space, and in absence of significant changes in diuresis and pulmonary and peripheral vascular resistance, has re-emphasized the role of ANP on venous tone. Indeed, we have recently shown that ANP modulates venous tone in health [170] and CHF [95,96] over a wide spectrum of physiological and pathophysiological ANP levels. This rendered further support to the intriguing finding by Serizawa et al. [195] showing that ANP acts as a vasodilator mainly on veins, rather than arteries, in patients with high pulmonary capillary wedge pressure (PCWP) and plasma noradrenaline concentrations.

Urodilatin

The rationale behind the use of the renal ANP homologue urodilatin instead of ANP is manifold. It has been suggested that urodilatin, rather than ANP, regulates renal sodium excretion [55,57] and urodilatin excretion has been found to be increased in CHF [196]. Some studies also found urodilatin to cause a less marked hypotensive effect when compared with ANP at equimolar doses [197]. Furthermore, there is some evidence that exogenously infused urodilatin interacts with the same receptors as ANP, but is, presumably due to its N-terminal extension, almost inert to enzymic degradation by NEP 24.11 [58]. A study by Bonatti et al. [65] studying the potency of ANP, BNP, CNP and urodilatin to stimulate cGMP production in rings of saphenous veins and internal mammary arteries showed that, although urodilatin had a slightly weaker overall effect, the ratio of its veno-arterial potency was higher than ANP and BNP. This may imply a better tolerability in patients with low output failure. Riegger et al. [198] offered several arguments for the therapeutic role of urodilatin in CHF. Further studies by Kentsch et al. [199] and Elsner et al. [200] also underscored a potential use of urodilatin in CHF. In their studies, urodilatin improved cardiac index and stroke volume. Urodilatin was infused...
intravenously for 10 h at a dose of 15 ng/kg per min in 12 patients suffering from CHF (New York Heart Association class II/III). In this randomized double-blind placebo-controlled trial [200], urodilatin was shown to significantly decrease systolic BP and CVP. However, diastolic BP and heart rate were unaffected. No relevant side effects were observed, showing that urodilatin was well tolerated in this prolonged infusion. So far, Clinical Phase I and II studies using urodilatin in the treatment of congestive heart failure, renal failure and bronchial asthma have been performed. The renal urodilatin system, its implications and indications have been reviewed elsewhere [201].

BNP
Intravenous infusion of nesiritide, a human recombinant B-type NP, has been investigated in more than 1700 patients with acute decompensated heart failure [202], and, as such, is the clinically most advanced NP in the drug development process. Indeed, it is the first new parenteral agent (Natrecor\textsuperscript{TM}) to be approved by the U.S. Food and Drug Administration for treating heart failure in more than a decade [203]. Nesiritide causes rapid dose-dependent vasodilatation that is sustained for the duration of treatment and it appears to have balanced arterial and venous effects, as demonstrated by decreases in systemic vascular resistance, systemic arterial pressure and mean pulmonary arterial pressure [204,205]. Vasodilatation occurs without a change in heart rate and is associated with increased stroke volume index and cardiac index [206]. A landmark study investigated the clinical use of nesiritide in patients with decompensated CHF [207]. The study enrolled patients in either an efficacy trial (n = 127, double-blind parallel group design) or a comparative trial (n = 305, open-label parallel group, 7 day follow-up). Nesiritide infusion significantly reduced PCWP, dyspnoea and fatigue, and improved global clinical status in the efficacy trial at 6 h. The improvements in global clinical status, dyspnoea and fatigue were sustained with nesiritide treatment for up to 7 days in the comparative arm and were similar to those seen with standard intravenous therapy. A healthcare cost analysis in 261 patients with CHF from an open-label randomized study comparing nesiritide with standard care suggested that treatment of decompensated CHF using nesiritide, instead of dobutamine, may reduce re-admission rate, healthcare costs and mortality [204]. The latter is most likely attributable to the better safety profile of nesiritide, which in contrast with the pro-arrhythmic and chronotropic effects of dobutamine, actually reduces ventricular ectopy or has a neutral effect [208,209].

Similar to the use of ANP, reports about the renal effects of BNP in heart failure are also variable. While Marcus et al. [210] and Yoshimura et al. [211] found that nesiritide or BNP respectively increased natriuresis and diuresis, Abraham et al. [212] reported that the magnitude of this response seemed to be blunted in 6 out of 10 patients.

Despite impressive haemodynamic and neurohumoral responses, which are generally very similar for the use of various NPs, some caveats remain; the short biological half-life, the need for parenteral administration and the high production costs are likely to limit the widespread use of NPs in the foreseeable future, at least in public healthcare systems such as the National Health Service.

Vasopeptidase inhibition (VPI)
A vasopeptidase inhibitor is the name given to a class of drugs, including omapatrilat (OMA), samapatrilat and fasidotrilat, which simultaneously inhibit both ACE and NEP 24.11 (for reviews see [213–215]). NEP 24.11 is a widely distributed ectodermal enzyme present, not only in endothelial cells, but also in cardiac myocytes, fibroblasts, smooth muscle cells, adrenal glands, brain, lung and renal tissue. Because NEP 24.11 is involved in the breakdown of both vasodilators, as well as vasoconstrictors, the balance of the effects of NEP 24.11 inhibition on total peripheral resistance will depend on whether the predominant substrate degraded consists of vasoconstrictors or vasodilators [213]. This of course may vary between vascular beds and may be more dependent on tissue levels than on circulating plasma levels of these vasoactive substances. For example, in the human forearm NEP 24.11 inhibition using candroxatrilat caused resistance vessel constriction [216]. Furthermore, the vascular responsiveness to NEP 24.11 inhibition may differ in the pulmonary and peripheral circulations, independent of or over and above the degradation ratio between vasoconstrictors and vasodilators. In CHF, NEP 24.11 inhibition reduces PCWP without significantly affecting afterload [213]. Inhibition of breakdown of endogenous vasoconstrictor peptides (i.e. Ang II and ET-1) can, at least partly, offset the beneficial effect of NP augmentation. Therefore combined inhibition of ACE activity to reduce Ang II and of NEP 24.11 to enhance endogenous NPs should theoretically provide additional benefits compared with mono-therapy. Some promising small animal experiments showed improved haemodynamics following VPI, over and above that of selective ACE inhibition or NEP 24.11 inhibition [217–220]. Similar findings have recently been reported in large animal models of experimental CHF [221]. Chen et al. [221] compared VPI with OMA and acute ACE inhibition with fosinoprilat in a canine model of pacing-induced mild heart failure [221]. Using intrarenal administration of a NP-receptor blocker, they demonstrated that the beneficial renal effects of OMA were mediated via augmentation of endogenous NPs. The same group recently extended these findings by comparing the effects of OMA, with and without a diuretic, with those of ACE inhibiton plus a diuretic in
the same animal model of CHF. Again OMA, with or without a diuretic, resulted in a more favourable cardio-
renal and humoral response than ACE inhibitor plus a diuretic. OMA given alone did not cause activation of the RAAS [222] (for review see [223]). Administration of NEP 24.11 inhibitors has also been shown to reduce bradykinin breakdown [224,225]. Since bradykinin is also a substrate for ACE, inhibition of both ACE and NEP 24.11 is likely to lead to an even greater enhancement of endogenous bradykinin activity [226]. While bradykinin clearly mediates some of the beneficial vascular effects of ACE and NEP 24.11 inhibition, these beneficial effects need to be weighed against the potential for an increased side-effect profile (i.e. angio-oedema), arising from dual blockade of bradykinin breakdown during VPI (for review see [227]). However, angio-oedema seems to be a lesser problem in the treatment of CHF compared with hypertension.

Early human studies in advanced CHF were promising. In a study by McClean and co-workers [228] in 48 patients, 3 months' treatment with OMA improved cardiac function and in turn clinical status. In a study comparing the effects of 40 mg of OMA with 20 mg of lisinopril on exercise tolerance in 573 patients with New York Heart Association classes II–IV, treatment with OMA led to a better clinical status and lower incidence of combined mortality and morbidity (admission and discontinuation of study treatment for worsening heart failure), while there was no significant difference in exercise tolerance, the pre-specified primary endpoint. Both agents increased exercise tolerance to a similar degree [229]. A further multicentre study, enrolling 369 patients with symptomatic heart failure, evaluated the haemodynamic and neurohormonal effects, safety and tolerability of increasing doses of OMA after a single oral dose and after 12 weeks of once-daily oral therapy [230]. Higher doses were associated with greater increases in vasodilatation and NPs, in addition to ACE inhibition. Furthermore, higher doses (20 mg and 40 mg) resulted in greater falls from baseline in PCWP and systolic BP than did 2.5 mg, while the incidence of adverse experiences and patient withdrawal were similar in all groups. First presentation of the only adequately powered mortality/morbidity trial [Omapatrilat Versus Enalapril Randomised Trial of Utility in Reducing Events (‘OVERTURE’)] at the American College of Cardiology in March 2002 received little enthusiasm [231]. The results indicated a non-significant reduction of 6% in the primary combined endpoint of all-cause death and cardiovascular hospitalizations with OMA, making the drug equivalent, but not superior, to ACE-inhibitor treatment alone. Following a re-evaluation, using criteria for non-inferiority, based on the Studies of Left Ventricular Dysfunction (‘SOLVD’) treatment trial, the final publication of OVERTURE, however, suggested a significant (hazard ratio of 0.89, \( P = 0.012 \)) incremental benefit on cardiovascular events with OMA of around 10% [232]. It has been argued that in patients with advanced CHF (and a systolic BP < 110 mmHg), the BP lowering effect of OMA may partly outweigh the beneficial neurohormonal profile. Consequently, it has been suggested that VPI may be most beneficial in early heart failure when NPs are activated in the absence of significant RAAS activation [215]. It remains to be investigated if VPI can delay disease progression from (relatively) asymptomatic to symptomatic forms of heart failure.

**Combined NP administration and NEP 24.11 inhibition/VPI**

Very recent work from the Christchurch Cardio-
endocrine Research Group and the Mayo Clinic has attempted to further augment the NP system in animal models of heart failure by combining inhibition of endogenous NP breakdown and exogenous NP admin-
istration, a concept first investigated by Seymour and co-workers [233,234]. Rademaker et al. [235] evaluated the combination of a 3-h infusion of ADM and an endopeptidase inhibitor (SCH32615) in an ovine model of pacing-induced CHF. ADM induced directionally similar, but greater, changes in all haemodynamic variables compared with SCH32615. Co-administration of ADM and SCH32615 produced haemodynamic effects greater than those achieved during ADM infusion alone. The authors [235] concluded that co-treatment with ADM and an endopeptidase inhibitor has beneficial renal and haemodynamic effects in heart failure beyond those of either agent separately. Chen and co-workers extended their previous studies using subcutaneous administration of BNP [236], by comparing this treatment with VPI (using OMA) and with a combination of both treatments in a canine model of pacing-induced heart failure [237]. Similar to the study by Rademaker et al. [235], combination therapy yielded greater renal, humoral and haemodynamic (increase in CO and reduction in filling pressures) effects compared with either treatment alone. While these studies are encouraging, it remains to be seen whether if oral VPI, combined with subcutaneous BNP administration, is able to provide longer-term benefits in patients with CHF.

**PROBLEMS AND PITFALLS: NP RESISTANCE**

There is ample evidence of reduced vascular NP respons-
iveness (resistance) in CHF [238,239]. The mechanisms underlying this NP resistance are potentially complex and are not completely understood. Theoretically, all of the following described below may contribute.

**NPR\(_{A+B}\) down-regulation**

Down-regulation of the biologically active binding sites for NPs, i.e. NPR\(_A\) and NPR\(_B\), has been reported.
Decreased ANP binding in a rat model of CHF was reported by Tsunoda et al. [240] in 1988, and was later suggested in humans, following the observation that the good correlation between the arterio-venous decrease in ANP levels and the arterio-venous increase in cGMP levels seen across the lower limb of patients with mild CHF was lost in patients with advanced disease stages [241]. This, albeit indirect, evidence was partially in keeping with an earlier study by Hirooka et al. [238] that showed a decreased forearm blood flow response in CHF patients compared with normal controls following intra-arterial ANP infusion. However, the reduced haemodynamic response in this study was not paralleled by reduced cGMP production; indeed, absolute cGMP levels were higher in the CHF group.

Foetal gene activation/endogenous β-ANP
One should principally differentiate between the response to endogenous NPs and exogenous NPs. Wei et al. [242] showed that β-ANP, an anti-parallel dimer of α-ANP with diminished cGMP generating potency, is the principal form of circulating ANP in patients with severe CHF. The fact that following ANP administration patients with advanced heart failure consistently exhibit a marked reduction in CVP and PCWP may be seen as supportive of the concept that NP resistance in CHF is more likely the consequence of an impaired endogenous ligand, rather than receptor down-regulation, at least in the capacitance vasculature and pulmonary circulation.

Clearance receptor (NPRC) up-regulation
An additional, potentially alternative, concept to explain NP resistance is that of NPRC up-regulation. Matsukawa et al. [243] showed that the NPRC locally modulates the physiological effects of vascular NP responses. A preliminary report suggested increased NPRC gene expression in failing hearts in humans [244]. This was supported by the finding of up-regulated NPRC expression in platelets of CHF patients [245]. However, the latter line of evidence is particularly controversial because platelets, in contrast with VSMCs and endothelial cells, only express NPRC and because previous investigations have contrastingly reported NPR down-regulation on platelets [246].

Receptor desensitization
NPRs are densely phosphorylated transmembranous GC-coupled receptors [247,248] (Figure 4). Ligand binding leads to dephosphorylation, which in turn appears to lead to desensitization of the receptor towards the ligand [249,250]. This mechanism per se could explain reduced vascular responsiveness, despite sustained or even up-regulated ligand/receptor availability. However, the ultimate significance of this process has not been determined since rephosphorylation of GC-A has not been accomplished.

Endothelial dysfunction – soluble and particulate GC cross-talk
There is accumulating evidence of intense cross-talk between both GC pathways (soluble and particulate),
especially in disease states such as CHF. Hussain et al. [251] found a co-operative interaction between NO and ANP. In their experiments [251], aortas from endothelial NOS-knockout mice were less sensitive to ANP than aortas from wild-type animals. The potency of ANP in wild-type animals was also decreased after pretreatment with NOS or soluble GC inhibitors. Wennberg et al. [252], using the non-peptide ANP receptor antagonist HS-142-1, revealed an interaction between NO and the NP system by demonstrating that co-inhibition with $N^G$-monomethyl-l-arginine (l-NMMA') and HS-142-1 significantly inhibited acetylcholine-induced vasorelaxation in isolated coronaries of dogs to a magnitude greater than either inhibitor alone, whereas vasorelaxation to acetylcholine was unaffected by pre-incubation with HS-142-1 alone. These findings are of particular interest because endothelial dysfunction may well (explain and) contribute to reduced vascular ANP responsiveness in disease states, such as hypertension and heart failure. Indeed, it could also explain the well-documented fall in cardiac preload, despite resistance in the arterial circulation, as venous endothelial function can be preserved, albeit with marked impairment of arterial endothelial function, as we have previously shown [253]. Furthermore, in disease states, signalling-pathway abnormalities downstream from NP–NPR interactions and activation of either soluble or particulate GC may also play a role in diminished NP responsiveness.

NEP 24.11 up-regulation

Alternatively, increased NEP 24.11 activity in CHF could also contribute to reduced NP responsiveness. Indeed, a recent study found increased NEP 24.11 activity in kidneys of different models of severe heart failure in the rat [254].

Area of uncertainty: cGMP, second messenger of vascular ANP actions?

While it is generally accepted that the renal actions of ANP, comprising pressure diuresis and increased Na$^+$ excretion, are cGMP mediated, there is some doubt that the same is true for vascular ANP responsiveness. von Geldern et al. [255] showed that in a murine model in vivo ANP-receptor blockade using A74186 resulted in a lack in cGMP increase, which was paralleled by a lack of Na$^+$ excretion and diuresis, while vascular responsiveness remained unaffected; in other words A74186 did not antagonize the hypotensive or vasorelaxant effects of ANP. These authors concluded that cGMP, although it may mediate the renal responses to ANP, is not responsible for the vascular and haemodynamic effects that result from the action of the hormone. Using a multitude of ANP analogues the same authors also found that the amino acids Asp$^{13}$ and Phe$^{26}$ were important for cGMP production following ligand–receptor binding [255,256], while they were not essential for ANP–NPR$\alpha$ binding [255]. Furthermore, a study by Elsner and co-workers [257], using the lipophilic cGMP analogue 8-bromo-cGMP in a conscious dog pacing-induced heart failure model, provided evidence that the renal effects of ANP can be attenuated in CHF, while vascular effects remained preserved. This was seen as being in agreement with the hypothesis that an intracellular defect beyond cGMP might be involved in the phenomenon of NP tolerance/resistance in CHF. However, it could also be seen as indirect evidence that the renal and vascular ANP effects are mediated via differing pathways. Furthermore, as outlined above there is strong evidence of cross-talk between the soluble and particulate GC pathways, and this might be of particular importance in disease states where one or both of these pathways become insufficient. It remains to be shown if this cross-talk is a clinically relevant compensatory or maladaptive mechanism.

‘ACE escape’

Finally, the phenomena of aldosterone escape [258] and Ang II reactivation [259], sometimes known as ‘ACE escape’, can theoretically contribute to the decreased renal and vascular response to ANP in CHF. While escape from the sodium-retaining effects of aldosterone is associated with significant increases in the circulatory levels of ANP [260], failure of mineralcorticoid escape in patients with hydropic diseases, such as cirrhosis [261], but potentially also CHF, may be due to the renal resistance to ANP, with insufficient increase of urinary cGMP excretion. The presence of this phenomenon may be seen as evidence of a shifted neurohormonal balance following ACE inhibitor treatment.

CONCLUSION AND PERSPECTIVE

NP research has come a long way since de Bold's seminal observations in the early 1980s [14,15]. While NP administration in advanced heart failure is effective but expensive, and restricted to continuous intravenous use, the first outcome study using VPI in CHF was somewhat inconclusive. Because the NPs oppose the actions of the RAAS and SNS, it remains important to further pursue their therapeutic potential in cardiovascular disease. However, in parallel, it appears mandatory to investigate the mechanisms that underlie reduced NP responsiveness in CHF.

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