Review

Atrial natriuretic peptide mimetics and vasopeptidase inhibitors

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Abstract

There is now substantial evidence supporting a role of the natriuretic peptides as a major defence mechanism against excess salt and water retention and high blood pressure. Because of this there has been considerable interest in the therapeutic potential of the natriuretic peptide system. Several approaches have been explored including the use of native peptides, the development of natriuretic peptides mimetics and targeting of endogenous clearance of natriuretic peptides. While ANP and BNP administration may be valuable in some circumstances, however, the limitations of the use of peptides especially for long-term treatment are well apparent. In view of this, considerable effort has been devoted to the development of orally active agents to enhance endogenous natriuretic peptides through inhibition of breakdown by neutral endopeptidase. This research has now led to the vasopeptidase inhibitors — dual inhibitors of both endopeptidase and angiotensin converting enzyme. These agents clearly provide a novel approach to enhance endogenous natriuretic peptide function on a background of reduced angiotensin II activity and may lead to an important advance in the treatment of hypertension and of conditions associated with overt salt and water overload.

Keywords: Antihypertensive/diuretic agents; Hormones; Receptors; Vasoactive agents; Vasoconstriction/dilation

1. Introduction

The major cardiovascular and renal actions of atrial natriuretic peptide (ANP) and the fact that the atria are strategically located to sense changes in intravascular volume clearly point to ANP as an important factor in the overall control of sodium balance and blood pressure [1–3]. The importance of ANP in the control of sodium excretion is strengthened by more recent experiments in transgenic mice in that mice with ANP gene deletion and those with natriuretic peptide type A receptor knock-out displayed higher blood pressures and substantial impairment in the renal response to volume overload [4–6]. The markedly raised plasma levels of ANP in humans with conditions associated with impaired renal sodium excretion and overt volume expansion also points to the potential importance of ANP as a compensatory mechanism in the presence of volume overload [7].

However, it is now apparent that ANP is part of a larger family of homologous natriuretic peptides including brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP) [8]. These peptides are synthesised as high molecular weight precursors that undergo processing to the mature forms which are located within the C-terminal region of the corresponding precursors. Despite some differences in sequence, a key feature of these peptides is the presence of a highly conserved 17-amino acid long S–S ring core (Fig. 1).

BNP, originally isolated from porcine brain, and subsequently, also isolated from the human heart, has a similar pattern of renal and cardiovascular actions to ANP [9]. Even though there are differences in pro-hormone processing, storage and release between ANP and BNP [8], circulating and cardiac levels of BNP are also raised in patients with cardiovascular and/or renal disease [7].

CNP displays some sequence homology with ANP, but lacks the C-terminal extension segment. Despite its name, the major systemic effects of CNP are vasodilatory rather than natriuretic. However, CNP is also made within the brain and there is evidence that it may contribute to the control of salt and water balance through central mechanisms [10]. Within the circulation, CNP is also produced
by endothelial cells [10] and therefore may have a local role in the regulation of vascular tone. In fact, recent work suggests that CNP may be co-released with nitric oxide and may be important in modulating both vascular tone and the development of atheromatous vascular disease [11,12].

In addition to these, a 32-amino acid extended form of ANP characterised by the presence of an additional four-amino acid sequence at the N-terminal site of ANP has also been isolated [13]. This peptide, termed urodilatin, differs from ANP in that it appears to be made mainly within the kidney [14]. Peptide infusion in humans has similar natriuretic effects as with infusion of ANP [15]; however, urodilatin has not been found in blood and does not appear to be part of the circulating natriuretic peptide system.

More recently, several natriuretic peptides have been identified in other animals; remarkably, one of these (DNP) isolated from the venom of the green mamba [16] is 38 amino acids long, and despite a much longer C-terminal extension, it has strong homology with the human ANP especially within the disulphide ring. DNP has similar renal and cardiovascular actions to ANP [17]. DNP-like immunoreactive material has now been identified in dog [17] and human heart [18] but the function of DNP-like peptide in mammals is unclear — it may act as further back-up mechanism for ANP and BNP but this remains to be established.

In view of the potent vasodilatory and natriuretic properties of ANP (Table 1) and of the realisation of the potential pathophysiological significance of ANP and BNP in hypertension and in conditions associated with abnormalities in sodium balance there has been considerable interest in the development of ANP mimetics and of orally active inhibitors of the endogenous clearance of natriuretic peptides as therapeutic agents. The present review examines the potential therapeutic value of targeting the natriuretic peptide system in cardiovascular disease with particular emphasis in the treatment of hypertension and of conditions associated with overt expansion of the extracellular fluid volume such as congestive heart failure and renal failure. More specifically the review will focus on the potential therapeutic value of ANP and BNP, on the neutral endopeptidase (NEP) inhibitors and on the newly introduced vasopeptidase inhibitors — dual inhibitors of ACE and NEP.

2. Receptor mechanisms and metabolism of natriuretic peptides

Soon after the identification of ANP as a novel peptide it was found that the peptide stimulated the production of cyclic GMP in several target tissues (e.g., renal, vascular and adrenal). Subsequently it was discovered that ANP was a potent activator of the particulate guanylate cyclase, thereby highlighting cyclic GMP as the second messenger for the actions of ANP. That this was the case was confirmed by using molecular cloning methods to derive

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![Amino acid sequences of the 28 amino acids atrial natriuretic peptide (α-ANP). Bold amino acid sequences represent regions of strong conservation amongst the different types of natriuretic peptides known to date.](image)

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Table 1

Major cardiovascular and renal effects of ANP

<table>
<thead>
<tr>
<th>Effect</th>
<th>Mechanism of action involves changes in:</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Systemic</em></td>
<td></td>
</tr>
<tr>
<td>Reduction in blood pressure</td>
<td>Vasodilation; blood volume reduction</td>
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<tr>
<td>Reduction in central venous pressure</td>
<td>Reduction in venous return</td>
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<tr>
<td>Stable heart rate</td>
<td>Reduction in sympathetic drive</td>
</tr>
<tr>
<td><em>Blood/plasma</em></td>
<td></td>
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<tr>
<td>Reduction in plasma renin</td>
<td>Renal secretion</td>
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<tr>
<td>Reduction in aldosterone</td>
<td>Adrenal synthesis</td>
</tr>
<tr>
<td>Increased haematocrit</td>
<td>Fluid shift from intravascular space</td>
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<tr>
<td><em>Renal</em></td>
<td></td>
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<tr>
<td>Diuresis/natriuresis</td>
<td>Renal haemodynamics/increased GFR</td>
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<td></td>
<td>Reduction in cyclic GMP-dependent distal sodium reabsorption</td>
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the sequence of the receptor and this demonstrated that guanylate cyclase was an intrinsic part of the ANP receptor [19]. However, it is now apparent that there are at least three major types of receptors (Fig. 2) with different affinities for the different forms of naturally occurring natriuretic peptides [20,21]. The full sequence of these receptors has been identified from molecular cloning methods and sequence analysis of the different receptors has provided an insight into the structural features important for functional activity.

The three natriuretic peptide receptors identified in mammalian tissues have been designated as NPR-A, NPR-B and NPR-C. Natriuretic peptide receptors A and B are linked to the production of cyclic GMP and mediate many of the cardiovascular and renal effects of the natriuretic peptides. The A receptor binds both ANP and BNP, although it is more selective for ANP. By contrast CNP appears to be the natural ligand for the B receptor. These two receptors are structurally similar, with approximately 44% homology in the ligand-binding extracellular domain. In both receptors, the predicted catalytic domain of the guanylate cyclase is located within the intracellular region, this region of the receptor also contains a putative kinase-like domain upstream of the guanylate cyclase domain [22]. The protein kinase-like domain seems to function as a regulatory element. When the kinase-like domain was removed by deletion mutagenesis, the resulting ANP receptor retained guanylate cyclase activity, but this activity was independent of ANP and its stimulation by ATP was markedly reduced [22].

The third receptor NPR-C, is also a membrane bound protein. This receptor has strong sequence homology to the other two natriuretic peptide receptors in the extracellular domain, but by contrast it lacks the intracellular catalytic domains of guanylate cyclase. All three natriuretic peptides bind to this receptor with similar affinity. NPR-C is widely expressed in a variety of tissues, however, the pattern of NPR-C expression is not identical to that of NPR-A. While both receptors have been identified in the kidney, adrenal, lung, and heart, colocalisation of both is only prominent in renal glomeruli. Some studies have suggested that the NPR-C may have a role in signal transduction as ANP led to inhibition of cyclic AMP production in isolated rat platelets [23]. Other investigations have demonstrated that atrial peptides have stimulatory effects on phosphoinositide hydrolysis in cultured bovine aortic smooth muscle cells. Both of these effects appear to be mediated through a guanine nucleotide regulatory protein [24].

While research continues on the possible links between the NPR-C receptor and G-proteins linked cyclic AMP production and on the cellular mechanisms for the antigrrowth properties of natriuretic peptides, it is now generally accepted that the NPR-C receptor seems to be of major importance in the metabolic clearance of natriuretic peptides. This is supported by several observations. Firstly, there is evidence of an enhanced renal response as a result of NPR-C blockade as suggested by original experiments of Maack et al. [25] who first described a role for NPR-C in hormonal clearance and buffering. These infused an ANP analog that was selective for the NPR-C receptor in the isolated, perfused rat kidney with the native rat ANP and found a trend toward both increased potency and an increased maximal response for urinary sodium excretion. Secondly, more recent experiments in isolated cells have demonstrated that ANP bound to NPR-C is rapidly internalised and subsequently degraded in a lysosomal compartment prior to receptor recycling to the cell surface [26]. Since under normal conditions there is a large reserve capacity (99%) of unoccupied NPR-C, this receptor provides an efficient mechanism for the clearance of circulating natriuretic peptides and for local modulation of the physiological effects of natriuretic peptides [27].

Both ANP and BNP are rapidly removed from the circulation. In humans, infusion studies with ANP have demonstrated a biexponential decay curve with a fast (half-life, 1–4 min) and a slower elimination behaviour (half-life, 5–15 min) [28]. In humans however, there are differences between BNP and ANP as the half life of the infused hBNP-32 is about 7-fold that of ANP [29]. This lower clearance may due to the lower affinity of BNP for the NPR-C and also for the guanylate cyclase linked NPR-A. Several tissues are involved in the elimination of endogenous natriuretic peptides. The lungs, liver, and kidneys are important in the elimination of natriuretic peptides. In addition to the clearance through binding to the specific NPR-C receptor another major disposal mechanism is enzymatic inactivation by neutral endopeptidase (EC 24.11) [30]. Neutral endopeptidase (NEP) is a metal-
lopeptidase widely distributed and also present within renal tubular cells and vascular cells. NEP cleaves the Cys–Phe bond in ANP thereby leading to a relatively inactive peptide. By contrast, BNP is cleaved at different sites within the peptide but, in sheep at least, NEP inhibition increased both ANP and BNP to a similar extent [31]. The relative proportion of each of these two clearance pathways to the elimination of natriuretic peptides in humans is not known. However, from investigations in animals, each system probably accounts for approximately half of the turnover of ANP and BNP [32].

3. Reduced size ANP analogs and designer natriuretic peptides

Natriuretic peptides are relatively large peptides and given the limitations in the use of peptides, from a therapeutic point of view there is clearly scope to develop lower molecular weight analogs — even better, there is scope to develop orally acting agents with ANP mimetic activity or substances which selectively enhance endogenous levels and activity of natriuretic peptides.

Accordingly there has been an intense effort on understanding structure-function relationships of natriuretic peptides, and of ANP in particular, in an attempt to reduce the size of the peptide and yet maintain its full biological activity [33]. Biological activity of ANP is not markedly dependent on the presence or actual type of amino acid around the N-terminal segment. Rat ANP tolerates removal of up to four amino acids before the disulphide bridge without much change in natriuretic or vasorelaxant activity. By contrast, biological activity is markedly dependent on the length and type of amino acid of the C-terminal segment. Sequential deletion of each of the amino acids in this region leads to gradual and substantial reduction in natriuretic effects, vasorelaxant activity and receptor binding affinity. Moreover, activity is also markedly dependent on the specific amino acid in this region suggesting that this region is important for receptor binding and stimulation.

The disulphide bridge is required for expression of full activity, as it stabilises the biologically active conformation of the peptide, but is not an absolute requirement for biological activity since linear peptides still retain residual activity. Further investigations have identified structural characteristics within the ring that discriminate between the NPR-A and the NPR-C type receptors and has led to the development of reduced size ANP analogues (mini-ANP), and ‘designer’ natriuretic peptides (vasonatrin and NPR-A specific ANP analogues).

3.1. Mini ANP

Schiller et al. [34] prepared ANP analogs with deletion of the residues in the C-terminal region of the ring structure through successive elimination of selected amino acids. The results demonstrated that successive elimination of these amino acids led to substantial and progressive reductions in receptor binding affinity and loss in potency of biological activity. These observations clearly suggest that the binding of ANP to its biologically active receptor is highly sensitive to the conformation of ANP and making it difficult to devise reduced size analogues. However, Li et al. [35] using a novel approach described a reduced size ANP analog of 15 amino acids which displayed a relatively high potency for the type A receptor. This was achieved by the identification of a functional epitope for receptor binding which was placed onto a smaller ring (Cys<sup>7</sup>–Cys<sup>13</sup>). Biological activity was then restored by optimising the remaining non-critical residues by means of phage display. Despite the substantial reduction in size, the 15-residue ANP analogue (mini-ANP) displayed a high binding affinity for the ANP-A receptor (EC<sub>50</sub> 0.48 nM) and biological activity (EC<sub>50</sub> 5.9 nM) and on a comparative basis, the mini ANP was about 7 times less potent than the native ANP.

3.2. Vasonatrin

The differences in profile of action of ANP and CNP have been exploited in an apparently novel way in the synthesis of vasonatrin. Wei et al. [36] worked on the hypothesis that addition of the C-terminal segment from ANP to CNP would lead to a unique peptide with combined venodilation and natriuresis. This chimeric peptide was tested both in vitro and in vivo and as expected proved to have natriuretic and venorelaxant activity that was intermediate in potency to that observed with ANP or CNP alone. Interestingly, vasonatrin displayed more potent arterial venodilation than either of the original peptides.

The potent venodilating and natriuretic actions of vasonatrin mimic the actions of both diuretics and nitrates suggesting a unique therapeutic potential for hypertension and for congestive heart failure. However, its action may be limited by its unusually marked arterial vasorelaxing actions. The mechanisms for the greater vasorelaxation of veins and arteries of vasonatrin compared with the parent peptides are as yet unclear but may be related to differences in degradation. Another possibility is that the chimeric peptide may interact with the clearance receptor and so displaces both endogenous ANP and CNP or stimulate an as yet unknown receptor that is not activated by ANP or CNP. Despite these unresolved issues, this work clearly forms a basis for the development of new analogs with specific actions on specific target organs and tissues.

3.3. NPR-A specific natriuretic peptides

More recent investigations on the structure–function relationship of natriuretic peptides has focused on the
identification of natriuretic peptide analogs more selective for the A-type receptor compared with the C-type receptor. The rationale for this is that these analogs would be expected to have decreased metabolic clearance, a longer half-life and increased potency. Moreover, there is also the potential for enhanced responsiveness in the kidney, because coexpression of NPR-C and NPR-A in the kidney may attenuate the response to peptide binding to both receptors.

One group [37] used the novel approach of mutant ANP phage display libraries to develop human ANP analogs which displayed much higher affinity for the NPR-A compared with the NPR-C receptor. However, further investigation of the in vivo effect of this peptide were hampered by the species specificity as the human recombinant peptide had a much reduced affinity for rat NPR-A. To overcome this problem these authors subsequently used a similar approach to develop an ANP analog with a high affinity for the rat NPR-A receptor compared with rat NPR-C receptor. This analog retained full potency compared with the parent rat ANP in both the cyclic GMP stimulation assays and in the relaxation of pre-constricted aortic rings [38]. Consistent with the earlier functional work, structural features determining receptor selectivity were located within the disulphide bridge. More interestingly, however, the new peptide when infused in the intact rat displayed more marked depressor and natriuretic effects compared with the native rat ANP.

The differences in these effects did not appear to be due to differences in neutral endopeptidase-mediated breakdown and hence were probably due to differences in selectivity for the NPR-A and the NPR-C receptors. These results therefore provide further evidence for a specific role for NPR-C in the clearance of endogenous ANP and thereby in the local attenuation of the effect of ANP. The importance of these investigations into the structure–function relationship of natriuretic peptides is underscored by the prospect that natriuretic peptide analogues more selective for the NPR-A receptor may well have improved activity over native ANP for the treatment of acute renal failure and acute heart failure.

4. ANP and BNP as therapeutic agents

While work is continuing to develop the ‘designer peptides’ along the lines described above for use in humans, numerous studies have investigate the potential therapeutic value of infusion of native ANP and BNP in several conditions and in particular in patients with essential hypertension, renal failure or congestive heart failure.

4.1. ANP for essential hypertension

Several early studies investigated the effects of exogenous ANP infusion in essential hypertension [39–43]. In general, significant natriuretic effects with variable and small reductions in blood pressure were observed. Other studies addressed the effects of low-dose and short-term infusion (up to 3 h) to achieve plasma levels of only around 2-fold over basal levels. There were significant natriuretic and diuretic effects and haemoconcentration suggesting reductions in intravascular volume in some cases. The effects on plasma renin and aldosterone were small (or not significant) [40,42,43]. Despite these effects, however, there were no consistent reductions in blood pressure. Further work [41], however, demonstrated that effective hypotensive actions of low-dose administration of ANP is likely to require more prolonged and sustained infusion of ANP (more than 12 h), thereby limiting its potential value when compared with existing antihypertensive agents.

4.2. ANP for renal failure

The renal effects of ANP, and in particular, increased glomerular filtration rate, inhibition of renal tubular re-absorption of sodium and chloride, and redistribution of blood flow to the renal outer medullary region pointed to beneficial value in the treatment of acute renal failure in critically ill subjects. This led to investigate the effects of ANP in patients with renal failure using anaritide a 25-amino acid synthetic form of ANP. Allgren et al. [44] reported a multicenter, randomised, double-blind, placebo-controlled clinical trial of anaritide infusion (0.2 μg/kg per min) over a 24-h period in critically ill patients with acute tubular necrosis. The primary end point was dialysis-free survival for 21 days after treatment. Other end points included the need for dialysis, changes in the serum creatinine concentration, and mortality. The results showed that the administration of anaritide did not improve the overall rate of dialysis-free survival in critically ill patients with acute tubular necrosis. A separate investigation [45] examined safety and efficacy of this peptide compared with placebo in patients with oliguric acute renal failure in a multicenter, randomised, double-blind, placebo-controlled trial. Although a trend was present, there was no statistically significant beneficial effect of ANP in dialysis-free survival or reduction in dialysis. Another study in people with chronic renal failure evaluated the efficacy of anaritide in patients with radiocast-contrast-induced nephropathy (RCIN) a common cause of hospital-acquired renal failure associated with a high mortality rate. The results showed that the infusion of anaritide before and during a radiocast contrast study did not reduce the incidence of RCIN in patients with preexisting chronic renal failure. This is consistent with the other two studies in patients with chronic renal failure and questions the value of ANP infusion in these situations.

4.3. ANP and BNP for congestive heart failure

Several groups investigated the effects of ANP in
patients with congestive heart failure (CHF) [47–53]. The initial studies examined the effects of high doses ANP, given either as bolus or as infusion (>5 μg/min). These observed an attenuation of natriuretic and diuretic responses in patients with CHF compared with healthy volunteers. This blunted effect could be due to concurrent activation of counter-regulatory systems or to the haemodynamic effect of reductions in blood pressure on renal function. Nonetheless, and despite these blunted responses it was also apparent that ANP infusion in patients with CHF decreased intracardiac pressures and increased cardiac output, thereby suggesting potential beneficial effects. Similarly, Goy et al. [50] using an infusion rate of 0.5–2 μg/min also confirmed beneficial effects in patients with CHF. At these doses there were no effects on blood pressure, heart rate or systemic vascular resistance consistent with a reduction in preload. However, increased sodium and water excretion were not maintained over a prolonged 20 h infusion of ANP.

By contrast, other investigations have demonstrated more sustained beneficial haemodynamic effects during infusion of human BNP in subjects with CHF [54–56]. These effects included reductions in cardiac filling pressures, and increased cardiac index. During infusion of BNP, there were also significant increases in urinary sodium excretion but, the absolute increase and the fractional excretion of sodium in response to BNP was lower in the CHF patients compared with normal controls [56]. Further studies on the value of BNP in CHF has now been carried out with nesiritide, a purified human BNP produced by recombinant DNA technology [57,58]. These studies have shown beneficial haemodynamic, neurohormonal and renal effects after bolus administration and 6-h infusion of nesiritide in CHF patients. Nesiritide was associated with significant decreases in pulmonary capillary wedge pressures and systemic vascular resistance with increases in cardiac index. This occurred in the absence of increases in heart rate. Treated patients also experienced improved symptoms of dyspnoea and fatigue.

Although it has generally been assumed that improved haemodynamic function will result in the resolution of symptoms in patients with decompensated congestive heart failure, most studies of new drugs for this purpose have focused on haemodynamic, rather than symptomatic endpoints. To determine the clinical value of nesiritide, a study group [58] undertook an efficacy and a comparative trial involving a total of 432 patients who were hospitalised because of decompensated congestive heart failure. In the efficacy trial, a double-blind, placebo-controlled design was used to determine the short-term efficacy of nesiritide with regard to haemodynamic measures and symptoms. The comparative trial compared nesiritide with standard intravenous agents, which served as active controls, in terms of clinical efficacy and adverse events.

In the efficacy trial, nesiritide (infusion at rates of 0.015 and 0.030 μg/kg per min for 6 h) decreased pulmonary-capillary wedge pressure by 6.0 and 9.6 mmHg, respectively (as compared with an increase of 2.0 mmHg with placebo, \(P<0.001\)). This was associated with an improvement in global clinical status. In the comparative trial, the improvements in global clinical status, dyspnoea and fatigue were sustained with nesiritide therapy for up to 7 days. The improvements were similar to those observed with standard intravenous therapy for severe congestive heart failure consisting primarily of dobutamine or milrinone. The authors concluded that the salutary clinical and haemodynamic profile of nesiritide would be a valuable addition to the initial treatment of patients admitted to the hospital for decompensated congestive heart failure.

Despite these encouraging results, however, there is some concern on the side effects of nesiritide [59]. The most common of these was dose-related hypotension. Also, other studies found that nesiritide was associated with sodium and water retention and decreased urine output in some patients with CHF. Although this may have been due to an imbalance in diuretic use, nesiritide-treated patients also experienced three cases of renal failure [59]. These issues clearly need to be addressed and additional studies need to be undertaken to further evaluate the benefits of BNP in patients with congestive heart failure.

5. Pharmacological manipulation of endogenous natriuretic peptides

Although the use of natriuretic peptides given as ANP or BNP may be of value in some circumstances as for example in severe congestive heart failure on a short-term basis, it is nevertheless also evident that the use of peptides has limited application. Hence, because of the problems associated with application of peptides (e.g., parenteral administration, rapid elimination, high commercial costs, etc.), especially in conditions likely to need long-term treatment considerable efforts have been made in the development of orally active inhibitors to block breakdown by neutral endopeptidase (NEP).

NEP is widely distributed; the kidney is a rich source of the enzyme but the enzyme is also found within the CNS where it appears to be involved in the inactivation of neuropeptides [60]. This enzyme also breaks down enkephalins and inhibitors of this enzyme were originally developed more than two decades ago as ‘enkephalinase’ inhibitors [61]. Once it was apparent that neutral endopeptidase also hydrolysed natriuretic peptides, these ‘enkephalinase’ inhibitors were also examined in terms of their renal and cardiovascular effects in experimental models of hypertension or heart failure.

When tested in animals endopeptidase inhibitors were associated with increases in ANP and displayed marked natriuretic effects suggesting a potentiation of the renal effects of natriuretic peptides. The effects on blood pressure in the SHR was more variable in that some but not every study demonstrated reductions in blood pressure, but
some studied demonstrated significant blood pressure reductions in the DOCA-salt hypertensive rat, a model of volume expanded hypertension [62–66].

Subsequent work in normal humans confirmed the effects found in animal experiments as several studies using mainly the NEP inhibitor candoxatril showed an increase in plasma ANP, an increase in renal sodium and water excretion and an associated increases in both plasma and urinary cyclic GMP [67–72]. These observations clearly reproduce those observed after infusion of low doses of exogenous ANP, thereby suggesting that the actions of these inhibitors may be mediated through increased levels of ANP and possibly also of BNP. Related work explored the potential therapeutic value of NEP inhibitors in several conditions and in hypertension and congestive heart failure in particular.

However, and despite the apparent enhancement of ANP activity, the majority of studies found no substantial reductions in blood pressure both in normotensives and in people with hypertension [67–73]. Similar observations were also made when the NEP inhibitor candoxatril was given on a longer-term basis term (200 mg twice daily for 28 days) in individuals with essential hypertension [74].

One possible reason for the limited effectiveness of NEP inhibitors as antihypertensive agents seems to be related to the non-specificity of this enzyme. NEP hydrolyses not only vasodepressor peptides (e.g., natriuretic peptides and bradykinins) but a also variety of vasopressor peptides including angiotensin II and endothelin. In fact, subsequent studies demonstrated that administration of NEP inhibitors was associated with enhancement of vasopressor peptides and of angiotensin II in particular [72,75,76]. These observations suggested that NEP inhibitors might be more effective in hypertensive conditions associated with low plasma renin as in hypertensives of African origin who are known to have low plasma renin activity [77].

Related studies have also explored the potential benefit of NEP inhibitors in conditions associated with overt expansion of the extracellular fluid volume and with markedly raised circulating levels of ANP as in chronic renal failure [78] or congestive heart failure [79–83]. Inhibition of NEP also stimulated renal sodium excretion in patients with chronic renal failure. In patients with mild chronic heart failure, candoxatril given acutely demonstrated some beneficial haemodynamic effects with no change in blood pressure or heart rate.

The spectrum of action of NEP inhibition in patients with congestive heart failure (e.g., natriuresis and diuresis in the absence of substantial activation of the renin–angiotensin system, tachycardia or hypokalaemia) provide potentially important advantages over some of the existing diuretics; however there is clearly a need for examination not only of long-term effects but also on quality of life scores and toxicity. The importance of this is underscored by the results of a recent trial of ecadotril (sinorphan, the S-enantiomer of the racemate acetorphan). In this multinational study [84], 279 patients with chronic heart failure were randomised into five equal groups to receive placebo or one of four doses (50, 100, 200, or 400 mg twice daily) of the NEP inhibitor ecadotril over a 13-week period. All patients enrolled were receiving inhibitors of angiotensin-converting enzyme and conventional diuretic therapy. This study however did not suggest that ecadotril could be promoted for the treatment of congestive heart failure. Firstly, patient-reported symptoms and quality-of-life scores failed to reveal any important, overall symptomatic benefit. Secondly, there was adverse event profile and in particular the highest dose of ecadotril, there was a relatively high frequency of aplastic anaemia. This led to cessation of clinical development of higher doses and reassessment of the possible risk/benefit ratio for lower doses of ecadotril.

6. Vasopeptidase inhibitors

While NEP inhibitors increase activity of endogenous natriuretic peptides, the concomitant enhancement of the effects of vasopressor peptides, angiotensin II in particular, is likely to offset their antihypertensive effects. Because of this, there has been considerable interest in combining NEP inhibitors with angiotensin converting enzyme inhibitors (ACE) inhibitors on the expectation that this combination would highlight the effects of natriuretic peptides in the presence of reductions in angiotensin II.

The benefits of ACE inhibitors in antagonising the deleterious effects of angiotensin II (Table 2) are now well established for both hypertension and for congestive heart failure. Therefore, inhibition of ACE activity to reduce angiotensin II and neutral endopeptidase to enhance endogenous natriuretic peptides, theoretically at least, should provide a powerful combination in the treatment of hypertension or congestive heart failure by potentiating the effects of raised endogenous natriuretic peptides. That this

| Table 2 Systemic, renal and other effects of angiotensin II (AT₁ receptor) |
|-----------------|-----------------|
| **Systemic**    |                 |
| Vasoconstriction| ↑               |
| Aldosterone secretion | ↑       |
| **Renal**       |                 |
| Increased sodium reabsorption |         |
| Mesangial cell contraction |         |
| Glomerular efferent vasoconstriction |         |
| Increase in glomerular capillary hydraulic pressure |         |
| Redistribution of renal blood flow |         |
| Feedback inhibition of renin release |         |
| **Other effects** |                 |
| Extracellular matrix proteins | ↑       |
| Plasminogen activator inhibitor-1 | ↑       |
| Transforming growth factor-β | ↑       |
| Macrophage activation | ↑       |
might be the case was supported by a series of experiments in animals [85–89]. A combination of ACE and NEP inhibitors effectively reduced blood pressure in both the SHR and in rats with volume expanded forms of hypertension and displayed beneficial haemodynamic effects in dogs with experimental heart failure.

The potential beneficial effects of dual ACE and NEP was subsequently demonstrated in humans. In one study, Stergiou et al. [90] found that in hypertensive patients, candesartan when added to lisinopril induced substantial reduction in both systolic and diastolic blood pressure (−19.1 ± 4.2 and −7.6 ± 3.0 for supine blood pressures compared with placebo). Similar reductions were observed for standing blood pressure. A separate study [91] with a different NEP inhibitor (sinorphan) also reported a synergistic effect in terms of reduction in blood pressure when this NEP inhibitor was combined with the ACE inhibitor captopril.

A further development was the realisation in the late 1990s about the possibility of encapsulating both NEP and ACE inhibitory properties within the same molecule. Both enzymes are ectopeptidases and both are zinc dependent. Also, both enzymes hydrolyse some peptides such as enkephalins, bradykinins or substance P at the same amide bond [30,60]. Although many compounds are highly selective inhibitors of either NEP or ACE activity some mercaptoalkyl inhibitors of these enzyme displayed partial inhibition of both enzyme. Further systematic modification of the NEP inhibitor thiorphan led to the development of glycoprilat and alatrioprilat as dual inhibitors of NEP and ACE with inhibitory constants in the nanomolar range. When tested in rats these were effective in blocking the pressor effects of angiotensin II and also increased urinary sodium and urinary cyclic GMP in the absence of a significant kaliuretic effect [92].

Several vasopeptidase inhibitors have been developed and tested for their renal and cardiovascular effects after oral administration in animal models of hypertension and heart failure [93–97]. These substances are potent inhibitors of both ACE and NEP in vitro with inhibitory constants in the low nanomolar range and equivalent to those of single ACE inhibitors. In the intact animal administration of vasopeptidase inhibitors leads to increases in circulating ANP (and BNP in some cases), urinary cyclic GMP and urinary sodium excretion without a marked effect on potassium excretion in association with substantial reductions in blood pressure. The effects on blood pressure appear to be independent of plasma renin status. In animal experiments, the effects of the vasopeptidase inhibitor omapatrilat on blood pressure were tested in three experimental models characterised by plasma renin levels: normal (SHR), reduced (DOCA-salt rats) and high (SHR on low sodium diet) [98]. After oral administration of omapatrilat there were substantial and prolonged reductions in blood pressure in all three models suggesting that its blood pressure lowering effects are not dependent on the level of the renin–angiotensin system.

Significant reductions in blood pressure have also been reported in the stroke-prone SHR [99]. Omapatrilat also improved survival in cardiomyopathic hamsters [100] and in sheep with heart failure there were significant acute and sustained improvements in cardiac haemodynamics and renal function [101].

These haemodynamic effects of vasopeptidase inhibitors are consistent with those expected after activation of the natriuretic peptide system on a background of reduced activity of angiotensin II. However, consistently raised plasma levels of ANP have not been found in every investigation and while this may be due to pharmacokinetic issues or a consequence of the effects of concomitant reduction in angiotensin II [102], there is additional evidence suggesting that enhanced natriuretic peptide activity may be an important factor in the cardiac haemodynamic actions of NEP inhibitors. For example, in dogs with heart failure, the cardiac haemodynamic effects of omapatrilat were substantially reduced by the natriuretic peptide receptor antagonist (HS-124) [103]. However, to some extent, the effects of neutral endopeptidase inhibitors may also involve bradykinin. Apart from natriuretic peptides and angiotensin II, neutral endopeptidase also inactivates bradykinin (Fig. 3). Reductions in bradykinin breakdown have been demonstrated after administration of NEP inhibitors [93,104]. Since bradykinin, of course, is also a substrate for ACE, inhibition of both ACE and NEP therefore is likely to lead to an even greater enhancement of endogenous bradykinin. But while bradykinin is a potent vasodilator and has natriuretic properties, the contribution of bradykinin in the cardiovascular and renal actions of NEP inhibitors remains to be resolved.

Several vasopeptidase inhibitors including sampatrilat, MDL 100,240 and omapatrilat have now been tested in humans. Administration of MDL 100,240 in normal humans [105] was associated with a fall in blood pressure.
Table 3  
Potential benefits of vasopeptidase inhibitors

<table>
<thead>
<tr>
<th>Substance</th>
<th>Comments/references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduction in blood pressure through increase in natriuretic peptide activity and decrease in angiotensin II</td>
<td></td>
</tr>
<tr>
<td>Facilitation of natriuresis/diuresis by increasing intrarenal vasodilator and natriuretic peptides</td>
<td></td>
</tr>
<tr>
<td>Prevention of compensatory rise in PRA and inhibition of aldosterone secretion</td>
<td></td>
</tr>
<tr>
<td>Enhanced diuretic effects without kaliuresis?</td>
<td></td>
</tr>
<tr>
<td>Broad spectrum of action in hypertension</td>
<td></td>
</tr>
<tr>
<td>Preservation of renal blood flow and GFR</td>
<td></td>
</tr>
<tr>
<td>Reduction in preload and afterload in congestive heart failure</td>
<td></td>
</tr>
</tbody>
</table>

Together with the preservation of renal haemodynamics and increased sodium and urinary volume, atrial natriuretic peptide and cyclic GMP excretion.

Walls et al. [106] compared the effect of sampatrilat, lisonopril and placebo in patients with hypertension in a randomised double-blind design for 10 days. Whereas there was a reduction in blood pressure after the first dose of lisonopril, there was no significant fall in blood pressure after the initial dose of sampatrilat. However, after 10 days of treatment, sampatrilat lowered systolic clinical and ambulatory blood pressure to a similar extent to lisonopril but there was a limited effect on diastolic blood pressure. Sampatrilat increased urinary cyclic GMP suggesting an enhancement of natriuretic peptide activity; however, its effect on plasma ACE was much less than that observed with lisonopril and this may possibly account for the limited antihypertensive effect of sampatrilat. Another study evaluated the antihypertensive effects of sampatrilat compared with lisinopril in black hypertensives, a group of patients known to respond poorly to monotherapy with ACE inhibitors [107]. Sampatrilat (50–100 mg/day) and lisonopril (10–20 mg/day) were given over a 56-day period in a double-blind, randomised, parallel group study design. In this study, sampatrilat displayed a sustained decrease in mean ambulatory blood pressure (−7.8±1.5 and −5.2±0.9 mmHg diastolic, respectively) which was greater than that obtained with lisinopril.

Investigations in humans confirmed that omapatrilat is a potent and long-lasting inhibitor of both NEP and ACE and in individuals with essential hypertension, omapatrilat produced dose-related (10–80 mg/day) substantial reductions in average blood pressure. Compared with placebo, it produced greater reductions in systolic blood pressure than diastolic blood pressure — at 80 mg/daily decreases of systolic blood pressure of 20–26 mmHg and of diastolic 14 and 17 mmHg were reported supporting a once-daily dosing [108,109]. Comparable reductions in blood pressure after omapatrilat have also been found in African-Americans as in Caucasians [110]. This is well in agreement with animal studies suggesting that the antihypertensive effects are not dependent on renin status given the low plasma renin activity of people of African origin.

Omapatrilat has also been evaluated in patients with congestive heart failure [111–113]. Beneficial haemody-

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Table 4  
Selected peptides and enzyme inhibitors targeting the natriuretic peptide system

<table>
<thead>
<tr>
<th>Substance</th>
<th>Comments/references</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Natriuretic peptides</strong></td>
<td>Tested mainly by intravenous route either as a bolus dose or as a continuous infusion. Limited therapeutic application for long term treatment</td>
</tr>
<tr>
<td>ANP</td>
<td>A 28-amino acid α-ANP. Investigated for possible use in hypertension, renal failure and congestive heart failure</td>
</tr>
<tr>
<td>BNP</td>
<td>A 32-amino acid human BNP. Investigated for possible use in severe congestive heart failure</td>
</tr>
<tr>
<td>Anaritide</td>
<td>A 25-amino acid synthetic ANP. Investigated in patients with renal failure [44–46]</td>
</tr>
<tr>
<td>Nesiritide</td>
<td>A 32-amino acid human BNP made by recombinant technology. Encouraging clinical trials in patients with severe congestive heart failure [57–59]</td>
</tr>
<tr>
<td>Mini ANP</td>
<td>Reduced size, 15-amino acid ANP analog. Experimental investigations in animals [35]</td>
</tr>
<tr>
<td>Vasonatrin</td>
<td>A chimeric CNP-ANP peptide. C-terminal tail from ANP has been added onto CNP. Displays natriuretic and venorelaxant activities. Experimental investigations in animals [36]</td>
</tr>
<tr>
<td><strong>NEP inhibitors</strong></td>
<td>Orally active substances which increase plasma ANP/BNP by inhibiting NEP</td>
</tr>
<tr>
<td>Acetorphan</td>
<td>Originally developed as an enkephalinase inhibitor. Increases plasma ANP and is natriuretic in normal humans [61]</td>
</tr>
<tr>
<td>Sinorphlan</td>
<td>Tested for hypertension and heart failure. Adverse side-effect profile reported in congestive heart failure [91]</td>
</tr>
<tr>
<td>Candoxatril</td>
<td>Most extensively investigated NEP inhibitor. Increases plasma ANP, is natriuretic and diuretic. Limited hypotensive effects in essential hypertension [74]. Some beneficial cardiac haemodynamic effects in congestive heart failure [82]</td>
</tr>
<tr>
<td><strong>Vasopeptidase inhibitors</strong></td>
<td>Orally active substances inhibiting NEP and angiotensin converting enzyme. Potentiate actions of ANP and block pressor effects of Angiotensin I</td>
</tr>
<tr>
<td>Mixanpril</td>
<td>Hypotensive in the SHR [94]</td>
</tr>
<tr>
<td>Sampatrilat</td>
<td>Hypotensive in humans with essential hypertension [106,107]</td>
</tr>
<tr>
<td>MDL 100,240</td>
<td>Hypotensive and hypotensive effects [105]</td>
</tr>
<tr>
<td>Omapatril</td>
<td>Most clinically developed vasopeptidase inhibitor. Displays substantial hypotensive effects in humans with essential hypertension [108–110]. Has beneficial cardiac and renal haemodynamic effects in patients with congestive heart failure [113]. Concerns about development of angio-oedema [115]</td>
</tr>
</tbody>
</table>
namic effects have been observed and one study compared omapatrilat with ACE inhibition alone with lisinopril on functional capacity and clinical outcome [113]. The authors carried out a prospective, randomised, double-blind, parallel trial of 573 patients. Patients were randomly assigned omapatrilat at a daily target dose of 40 mg (n = 289) or lisinopril at a daily target dose of 20 mg (n = 284) for 24 weeks. The primary endpoint was improvement in maximum exercise treadmill test at week 12. Although there was no difference between the two groups for the primary endpoint, exercise tolerance, omapatrilat displayed a significant benefit in the composite endpoint of death, admission, or discontinuation of study treatment for worsening heart failure. Moreover, the rate of renal dysfunction was significantly less (1.8 vs. 6.1%) in those on omapatrilat. This is of potential beneficial value since renal function frequently deteriorates during the progression of chronic heart failure, and renal impairment is one of the most powerful predictors of prognosis in patients with congestive heart failure [114].

Despite these favorable results, however, there is serious concern about the side-effect profile of omapatrilat and specifically the greater rates of angio-oedema compared with ACE inhibitors alone. Angio-oedema is a well-documented, but rare, serious adverse effect in patients taking ACE inhibitors occurring in 0.1–0.5% of individuals on ACE inhibitors [115]. The prevalence of angio-oedema in those on omapatrilat was dose-dependent and at 20 mg day was more than 3 times as common as that observed with ACE inhibitors [115]. The exact reasons for the development of angio-oedema are not known but may be related to enhancement of bradykinin. Plasma bradykinin concentrations can rise more than 10-fold during acute attacks of angio-oedema associated with an ACE inhibitor [116]. Bradykinin is also degraded by NEP, and hence a dual ACE and NEP inhibitor may well be associated with a higher level of bradykinin and a higher risk of angio-oedema in (genetically?) susceptible individuals. Gastrointestinal side-effects consistent with angio-oedema were also more common in those taking omapatrilat [113].

Clearly, a more detailed analysis of the risk/benefit profile of omapatrilat for the various conditions being considered is required. Nonetheless, given the potential beneficial cardiac and renal haemodynamic effects of vasopeptidase inhibitors (Table 3) it is likely that this class of inhibitors may well become an important approach to the treatment of hypertension and of conditions associated with overt volume overload. A listing of selected substances discussed in this review is given in Table 4.

References


