Inhibition of peptidases in the control of blood pressure

Eiji Kubota, Rachel G. Dean, Leanne C. Balding and Louise M. Burrell

Department of Medicine, University of Melbourne, Austin and Repatriation Medical Centre, Studley Road, Heidelberg 3084, Victoria, Australia

Abstract

The natriuretic peptide and renin–angiotensin systems are physiological counterparts with opposite roles in the regulation of electrolyte balance and blood pressure. In both systems, membrane-bound, zinc-dependent peptidases play an important role in the inactivation or activation of the system. Angiotensin-converting enzyme (ACE) converts angiotensin I into angiotensin II, and neutral endopeptidase (NEP) degrades the natriuretic peptides. Simultaneous inhibition NEP and ACE by a single molecule (a vasopeptidase inhibitor) is a new therapeutic approach in hypertension. Wider applications for vasopeptidase inhibitors being studied include their role as cardioprotective agents in heart failure, as renoprotective agents in chronic renal failure and diabetic nephropathy, and as vasculoprotective agents in endothelial dysfunction and atherosclerosis.

Introduction

The natriuretic peptide and renin–angiotensin systems are physiological counterparts with opposite roles in the regulation of electrolyte balance and

1To whom correspondence should be addressed (e-mail: burrell@austin.unimelb.edu.au)

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blood pressure (Table 1) [1]. Natriuretic peptides are a family of peptides with potent physiological actions, which are released from the heart, brain and endothelial cells. The physiological effects include salt and water excretion (natriuresis and diuresis), relaxation of blood vessels, and antiproliferative and antihypertrophic activities [2]. In many ways, the natriuretic peptides can be regarded as endogenous inhibitors of the renin–angiotensin system as they oppose the actions of angiotensin II (Ang II) on vascular resistance, blood pressure and renal sodium homoeostasis, as well as blunting the secretion of aldosterone by Ang II. One action, however, that the natriuretic peptides and Ang II do share is that they are potent inhibitors of renin release from the kidney.

In both systems, membrane-bound, zinc-dependent peptidases play an important role in the inactivation or activation of the system; the ectoenzyme angiotensin-converting enzyme (ACE; EC 3.4.15.1) is responsible for the conversion of Ang I to Ang II [3], while neutral endopeptidase (NEP; EC 3.4.24.11) is a component of one of the pathways involved in the degradation of the natriuretic peptides [4].

Hypertension or high blood pressure represents a continuing challenge for a number of reasons:
- it is very prevalent and produces a significant global health burden;
- it affects 1 in every 6 members of the population and this incidence rises to 1 in every 2 in those aged 70 years and older;
- it is a major risk factor for heart disease, stroke and renal failure;
- control of blood pressure and continuation of therapy is less than optimal;
- new antihypertensive agents that provide better blood-pressure control reverse structural and functional abnormalities and that have improved tolerability may change this situation.

The use of ACE inhibitors has been a significant advance in the treatment of hypertension [5], while specific NEP inhibitors were developed several years ago [6]. Although these inhibitors were shown to elevate plasma levels of the natriuretic peptides and to cause the expected responses such as diuresis, natriuresis and peripheral vasodilatation under certain experimental conditions, clinical trials in hypertension and cardiac failure had disappointing results. This

<table>
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<th>Table 1. Biological actions of Ang II and natriuretic peptides</th>
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<td><strong>Action</strong></td>
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<td>Vasoconstriction</td>
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<td>Sympathetic activity</td>
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<td>Sodium excretion</td>
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<td>Renin release</td>
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was partly owing to the fact that a decrease in blood pressure activates the renin–angiotensin system, and any increase in natriuretic peptide levels, be it from infusion of natriuretic peptides or inhibition of their breakdown, is unable to overcome an activated renin–angiotensin system. However, in the presence of an inhibited renin–angiotensin system, the biological actions of natriuretic peptide are restored, and this led to the development of compounds that simultaneously inhibit NEP and ACE. Such compounds are known as vasopeptidase inhibitors, and may offer advantages in treating hypertension [7]. The addition of NEP to ACE inhibition potentiates the vasodilator, natriuretic and diuretic actions of natriuretic peptides.

**Renin–angiotensin system**

One of the major advances in the field of the renin–angiotensin system has been the appreciation that it functions as a dual hormonal system, serving both as a circulating and a local-tissue hormone system. All components of the renin–angiotensin system are present in important cardiovascular structures, including the heart, vessels, brain, kidney and adrenal gland. The primary active hormone of the renin–angiotensin system, Ang II, is produced as a result of an enzymic cascade (Figure 1). The glycoprotein angiotensinogen is synthesized by the liver and it is cleaved by the enzyme renin to form Ang I within the circulation. Renin is secreted from the juxtaglomerular cells of the kidneys in response to decreases in blood volume, blood pressure or sodium concentration, and it is the rate-limiting enzyme in the final production of Ang II. In the last step, Ang I is cleaved by the metallopeptidase ACE to form the octapeptide Ang II in both the circulation and tissues. All the known effects of the renin–angiotensin system can be accounted for by the multiple actions of Ang II, which interacts with at least two known membrane receptors, the

![Figure 1. The renin–angiotensin system cascade, and its interaction with kinins and the natriuretic peptide system](image-url)
angiotensin type 1 receptor and the angiotensin type 2 receptor. The well-known physiological effects of Ang II such as vasoconstriction, aldosterone stimulation, and salt and water homoeostasis, appear to be mediated via stimulation of the G-protein-coupled angiotensin type 1 receptor.

ACE

ACE is an ectoenzyme that anchors itself to the plasma membrane by its C-terminal end and has a large extracellular domain. ACE has two active catalytic sites, the N- and C-domains, which are both zinc-dependent but differ in their chloride requirements and catalytic constants. ACE is an integral membrane component of all endothelial cells, occurs in many epithelial cells (gastrointestinal tract, kidney, reproductive tract, placenta, brain), and is expressed on fibroblasts and macrophages [8]. ACE also has a wide substrate-specificity; ACE acts on bradykinin, substance P, opioid peptides, neurotensin, cholecystokinin, bombesin, enkephalins and luteinizing-hormone-releasing hormone *in vitro*, but the effect of ACE on such substrates *in vivo* is not fully understood.

Whether any of the reported benefits of ACE inhibitors involve bradykinin or other ACE substrates is still a highly controversial issue. Only comparative trials with the recently developed new class of Ang II receptor antagonists are likely to settle the issue. However, the data obtained thus far indicate that Ang II receptor antagonists, which do not interfere with bradykinin metabolism, have the same effect as the ACE inhibitors on morbidity and mortality in patients with hypertension and renal disease.

ACE inhibitors and hypertension

ACE inhibitor is the generic name applied to a group of drugs that were first described in 1977 by Ondetti and Cushman [3] and that act by binding competitively to the active catalytic sites of ACE, thereby preventing access to the endogenous substrate. ACE inhibition depends on the co-ordination of the active site with ligands that are present on the inhibitor. All ACE inhibitors (Figure 2) act in an identical manner, but differ in the degree and the length of time of ACE inhibition in different tissues. To improve oral absorption, many second generation ACE inhibitors are ester prodrugs that are metabolized in the liver and gut-wall to release more active diacid derivatives; for example, enalapril is activated to produce the active component enalaprilat.

The indications for ACE inhibitors have widened considerably in scope since they were first introduced for the treatment of hypertension. A series of landmark studies (reviewed in [5]), which started in 1987 with the CONSENSUS study, showed that ACE inhibitors should be used in all grades of heart failure that are associated with left ventricular dysfunction. There is now evidence that ACE inhibitors reduce the rate of progression of renal failure in both diabetic and non-diabetic renal disease, effects that appear to go beyond...
their effects on blood pressure. Ongoing studies will determine whether the mortality benefits seen in heart failure transfer to hypertension, and will clarify the potential effects of ACE inhibition on recurrent myocardial infarction and atherosclerosis.

Clinical trials have shown that antihypertensive treatment improves cardiovascular morbidity and mortality, but it is not known if the benefits are due solely to blood pressure reduction or are specific for different classes of drug. ACE inhibitors lower mean systolic and diastolic blood pressure in hypertensive patients irrespective of age, but they are less effective in hyper-
tensive African–American patients compared with Caucasian patients. This may be related to low renin levels in African–Americans, and if ACE inhibitors are combined with a diuretic, which increases renin levels, they are as effective as in other ethnic groups.

The precise mechanism by which ACE inhibition causes a decrease in blood pressure remains unclear; the acute hypotensive effects of ACE inhibition relate with pre-treatment levels of renin and plasma Ang, but their long-term antihypertensive effect results from more complex mechanisms [9]. ACE has multiple substrates and interacts with other systems that are involved in cardiovascular regulation. Possible mechanisms of action include inhibition of ACE in, for example, the heart, kidneys, adrenal glands and blood vessels, a decrease in plasma aldosterone, suppression of the sympathetic nervous system and accumulation of bradykinin.

**Natriuretic peptides**

The natriuretic peptides are a family of at least three structurally similar peptides including atrial natriuretic peptide (ANP), brain natriuretic peptide and C-type natriuretic peptide [2] (Figure 3). The human ANP gene, which was first sequenced in 1984, is located on chromosome 1 and consists of three exons separated by two introns. Human ANP is derived from a 151-amino-acid precursor, preproANP. Within cardiac myocytes, preproANP is processed rapidly into proANP, which has a high degree of identity between species. The human version of proANP (126 amino acids) is the major constituent of atrial secretory granules, which fuse with the cell surface to release their contents when exposed to hormone-release stimuli. During this process, proANP is thought to be cleaved, by an unidentified enzyme, to yield two products (Figure 3); the N-terminal end becomes ANP_{1-98}, and the 28 amino acids of the C-terminus become the biologically active ANP_{99-126} (or ANP).

The physiological actions of ANP are mediated through guanylate-cyclase-linked receptors upon ligand binding. The natriuretic peptides act as endogenous antagonists of the renin–angiotensin system (Table 1) to cause natriuresis and diuresis, vasodilation and suppression of the sympathetic nervous system; they also inhibit cell growth and decrease the secretion of aldosterone and renin. Natriuretic peptides play an important role in the regulation of cardiovascular, renal and endocrine function, but the therapeutic potential for ANP in hypertension and heart failure is limited as ANP is a peptide and therefore, if administered orally, it will be degraded rapidly in the stomach before it can exert any actions. An alternative approach is to increase endogenous ANP levels by inhibition of its enzymic degradation by NEP.

**NEP**

NEP, which was originally referred to as enkephalinase because of its ability to degrade opioid peptides within the brain, was subsequently shown to be
identical with an already well characterized zinc metallopeptidase that is present in the kidneys [10]. NEP is an integral membrane dimeric glycoprotein with a short intracellular domain, a transmembrane domain that anchors NEP in the plasma membrane and a large ectodomain that includes the zinc-containing active site. As a zinc-dependent metallopeptidase, NEP shares mechanistic similarities with other metallopeptidases including ACE, endothelin-converting enzyme, aminopeptidases and carboxypeptidases [6].

NEP hydrolyses peptide bonds on the amino side of hydrophobic amino-acid residues. In the case of ANP, NEP cleaves the Cys$^{105}$–Phe$^{106}$ bond to disrupt the ring structure and inactivate the peptide [11]. While the role of NEP in the inactivation of ANP has been most extensively studied, it has also been demonstrated to play a role in the metabolism of brain natriuretic peptide and C-type natriuretic peptide. NEP has broad substrate selectivity in vitro and it cleaves enkephalins, endothelin, substance P, kinins, neurotensin, the insulin B-chain, Ang II, calcitonin gene-related peptide and adrenomedullin, as well as the natriuretic peptides. One of the main functions of NEP in vivo is to metabolize the natriuretic peptides.

NEP is located principally within the kidney where it rapidly degrades filtered ANP, thereby preventing the peptide from reaching more distal luminal
sites. NEP is also found in the lung, gut, liver, adrenal glands, brain, heart and vasculature, and is present on endothelial cells and on the surface of neutrophils and leukaemic cells.

**NEP inhibitors**

NEP inhibitor (Figure 2) is the generic term applied to a group of drugs that act by binding competitively to the active site of NEP, thereby preventing access to the endogenous substrate. NEP inhibitors were developed as analgesics (pain killers) because the enzyme metabolizes enkephalins and increases opioid levels, but the current interest focuses on the role of NEP in the metabolism of the natriuretic peptides [6]. As with ACE inhibitors, inhibition of NEP depends on the co-ordination of the active site with ligands that are components of the inhibitor. Many clinically useful NEP inhibitors are prodrugs that are activated by hepatic esterases.

**NEP inhibition in hypertension**

The natriuretic peptides are the major mediators of the cardiovascular and renal effects of NEP inhibition. NEP inhibitors have little effect on blood pressure, natriuresis or diuresis when ANP levels are normal, but when plasma ANP levels are elevated, NEP inhibition produces the expected physiological changes. Unfortunately, long-term NEP inhibition has minimal antihypertensive effects in patients with hypertension [12]; however, experimental studies in both hypertension and heart failure have shown that the addition of an ACE inhibitor restores the haemodynamic and renal benefits of NEP inhibition.

Compounds that simultaneously inhibit ACE and NEP have now been designed, taking into account the similar structural characteristics of the catalytic site of both enzymes (Figure 2). This novel class of drugs, which inhibit the renin–angiotensin system and potentiate the effects of the natriuretic peptides, are known as NEP/ACE inhibitors or vasopeptidase inhibitors [7,13]. Several of these inhibitors are available, including omapatrilat, candoxatril and MDL 100,173.

All of these compounds inhibit ACE and NEP, although the degree of potency against the individual enzymes varies between compounds (Figure 4). We have used radioligand binding assays with the specific NEP inhibitor radioligand \(^{[125}I]\)RB104 and the specific ACE inhibitor radioligand \(^{[125}I]\)MK351A to show that S21402 is a stronger inhibitor of renal NEP than it is of lung ACE in vitro. After oral dosing in rats, it was also found that S21402 was a more potent inhibitor of renal NEP than of renal ACE [14]. By contrast, the inhibition of NEP in rat renal tissue by the vasopeptidase inhibitor omapatrilat is similar to its inhibition of ACE in the same tissue [15]. Because the long-term benefits of ACE inhibitors in preventing or reversing target-organ damage depends on their ability to inhibit ACE in target organs such as the kidney and heart, it will be important to assess whether differences in the degree or the site of NEP inhibition are of importance in determining clinical efficacy.
Vasopeptidase inhibitors and hypertension

Hypertension represents a continuing challenge because of the reasons listed in the introduction, and although ACE inhibitors are firmly established in the treatment of hypertension, 50% of patients require additional therapy. In experimental hypertension, the major advantage of the vasopeptidase inhibitors is their ability to decrease blood pressure independently of body volume or renin status [16], whereas ACE inhibitors are most effective in renin-dependent hypertension, and NEP inhibitors are most effective in low-renin, volume-dependent hypertension. Thus, it is expected that vasopeptidase inhibitors will be effective as single-drug therapies in a wider range of patients compared with selective ACE inhibition [7]. Indeed, early case reports that describe the use of omapatrilat have shown powerful, dose-dependent decreases in systolic and diastolic blood pressure, regardless of age, race or gender. Before such drugs do enter the clinical arena, large-scale randomized controlled trials in humans are necessary.

The precise mechanism by which vasopeptidase inhibition lowers blood pressure is not known. As for the selective ACE inhibitors, the long-term anti-hypertensive effect is likely to result from complex mechanisms, particularly given the multiple substrates for ACE and NEP. Possible mechanisms of action include not only decreasing plasma Ang II levels, but also the accumulation of natriuretic peptides and the inhibition of ACE and NEP at the tissue level. Autoradiographic studies allow the precise localization of NEP and ACE in tissues such as the heart, kidney and blood vessels, and can be used to assess inhibition of NEP and ACE after treatment with vasopeptidase inhibitors. Such studies are important in helping to determine the mechanisms of action of the vasopeptidase inhibitors, which are not clear at present.

Figure 4. Effects of a vasopeptidase inhibitor, S21402, compared with those of the selective ACE inhibitor captopril and the selective NEP inhibitor SCH42354 on the binding of the ligands [125I]MK351A to lung ACE and [125I]RB104 to renal NEP respectively

B, observed binding; B₀, initial binding. Points indicate mean values; n = 3 per curve. The x-axes show the log₁₀ of the concentration (mol/l) of the inhibitor.
Hypertension is also an independent risk factor for renal failure, which is a major public health problem in the Western world. Recent guidelines from the World Health Organization/International Society of Hypertension emphasize that blood pressure levels need to be lower in patients with renal disease and diabetes than in other hypertensive groups [17]. Results from our laboratory suggest that the vasopeptidase inhibitors may help to achieve these more aggressive blood pressure targets, at least in animal models of renal disease [18]. Table 2 summarizes the current state of knowledge with regard to the use of the vasopeptidase inhibitors in experimental cardiovascular disease.

**Summary**

- The natriuretic peptide and renin–angiotensin systems are physiological counterparts.
- In both systems, membrane-bound, zinc-dependent peptidases play important roles in the inactivation or activation of the system.
- ACE converts Ang I into Ang II, and NEP degrades the natriuretic peptides.
- Simultaneous inhibition of the peptidases NEP and ACE by a single molecule (vasopeptidase inhibitor) is a new therapeutic approach in hypertension.
- Wider applications for vasopeptidase inhibitors include their role as cardioprotective agents in heart failure, as renoprotective agents in chronic renal failure and diabetic nephropathy, as well as vasculoprotective agents in endothelial dysfunction and atherosclerosis.

**Table 2. Cardiovascular benefits of vasopeptidase inhibitors**

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<thead>
<tr>
<th>Benefit</th>
<th>Experimental models</th>
<th>Man</th>
</tr>
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<tbody>
<tr>
<td>Decrease blood pressure</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Decrease cardiac hypertrophy/fibrosis</td>
<td>+</td>
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<td>Improve myocardial ischaemia</td>
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<td>Regress atherosclerosis</td>
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<td>Improve endothelial dysfunction</td>
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<tr>
<td>Improve mortality in heart failure</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Slow progression of renal disease</td>
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References