The role of C-type natriuretic peptide in cardiovascular medicine

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Introduction

Since the discovery of atrial natriuretic peptide (ANP) in 1981, the search for further peptides with related activity has continued with vigour. The natriuretic peptide family consists of structurally related peptides with varying degrees of physiological similarity. ANP is primarily secreted from the atria and possesses natriuretic, vasoactive and renin and aldosterone inhibiting properties. The second member, brain natriuretic peptide (BNP), was first isolated from porcine brain in 1988. However, it soon became apparent that BNP was present at higher concentrations in other locations, particularly the ventricular myocardium. BNP appears to share many of the physiological properties possessed by ANP. It was therefore proposed that they might together contribute to the regulation of circulatory homeostasis, essentially functioning as cardiac hormones. Since then attention has focused upon their role in pathological states, particularly heart failure. In chronic heart failure the plasma levels of ANP and BNP are significantly elevated and their measurement may contribute to diagnostic clarity. Further studies have suggested that their levels might also serve as prognostic indicators, particularly post myocardial infarction. More recently it has been proposed that the measurement of plasma BNP may facilitate the optimization of treatment for patients with chronic heart failure. In one study plasma BNP levels were able to predict those patients most likely to gain benefit from beta blockade. In addition, Troughton et al. demonstrated that intensification of conventional drug therapy, in order to reduce plasma aminoterminal BNP levels, resulted in reduced total cardiovascular events when compared to therapy directed by clinical acumen.

Much less is known about C-type natriuretic peptide (CNP) which was first isolated from porcine brain in 1990. It is now known that CNP has a much wider distribution and of particular note both it and its receptor are located in peripheral blood vessels. In vitro and in vivo studies have shown CNP to be a powerful vasorelaxant. This together with its location would theoretically enable CNP to play an important role in local vascular regulation.

In this review we examine the current literature relating to CNP, in particular its cardiovascular role. Wherever possible we will compare and contrast CNP with both ANP and BNP.

Structure

CNP is the most highly conserved of the natriuretic peptides between species. Two mature forms of the peptide exist, being derived from a 126-amino acid preprohormone. Both contain the 17 amino-acid ring common to all members of the natriuretic peptide family. The higher molecular weight CNP-53 predominates in tissues, whereas CNP-22 is found mainly in plasma and cerebrospinal fluid. This has led to speculation that CNP-53 may serve to function, at least in part, as a storage form of the peptide, while CNP-22 circulates in the plasma. Most of the data on the biological effects of the peptide relate to the 22 amino-acid form.

Receptors and signalling (Fig. 1)

The natriuretic peptides exert their effects by interaction with natriuretic peptide receptors (NPR). These are transmembrane proteins containing an extracellular binding domain, together with an intracellular...
particulate guanylate cyclase domain. An important region of the NPR called the kinase homology domain normally inhibits guanylate cyclase. When an appropriate natriuretic peptide binds to the external domain, inhibition of guanylate cyclase ceases, enabling the formation of cyclic guanosine monophosphate (cGMP) from guanosine triphosphate (GTP) from guanosine triphosphate [21]. The subsequent elevation in intracellular cGMP is thought to mediate the various biological actions of the natriuretic peptides. Three types of NPR exist and they differ in their relative affinities for binding the natriuretic peptides. NPR-A has greater affinity for ANP and BNP, whereas NPR-B is more specific for CNP [22]. The third, NPR-C, is thought to act as a clearance receptor and lacks the guanylate cyclase domain [23].

However, this proposed interaction between the natriuretic peptides and the NPR might be an oversimplification. In vitro studies have demonstrated signal-transducing functions for the NPR-C [24]. In addition Takida et al. have shown that natriuretic peptides suppress adrenergic neurotransmission by a pertussis toxin sensitive mechanism, suggesting that GTP-binding proteins are involved in the response [25]. More recently synthetic human CNP-22 has itself been shown to form large cation channels in lipid bilayers [26]. This has led the investigators to speculate that CNP-22 may function as an ion transport pathway for signal transduction. CNP has also been shown to enhance potassium channel activity in vitro, resulting in hyperpolarization of rat mesangial.

Figure 1  Binding of the natriuretic peptide to the appropriate NPR (A or B) results in the release of inhibition of particulate guanylate cyclase by the kinase homology domain. This enables the conversion of guanosine triphosphatase (GTP) to cGMP, which acts as the second messenger, mediating the biological effects of the natriuretic peptides.
cells and porcine coronary artery smooth muscle respectively\(^{27,28}\).

**Metabolism**

There are two mechanisms by which natriuretic peptides are catabolized. Following binding of a natriuretic peptide to the NPR-C, the resulting receptor-ligand complex undergoes endocytosis and subsequent lysosomal hydrolysis. The other involves cleavage by neutral endopeptidase\(^\text{26}\). This enzyme has a wide tissue distribution and in particular is located in the vascular endothelium and at high levels in the kidney\(^\text{29,30}\). In vitro CNP appears to be more rapidly hydrolyzed by neutral endopeptidase than the other natriuretic peptides\(^\text{31}\). Endopeptidase inhibition may be a potential therapeutic intervention by enabling beneficial manipulation of natriuretic peptide levels.

**Location**

Since its discovery in porcine brain, CNP and its receptor NPR-B have been identified in a diverse array of human tissues such as the central nervous system, renal tubular cells and vascular endothelial cells\(^\text{19,32,33}\). The finding of CNP immunoreactivity in cultured human endothelial cells is particularly interesting as the NPR-B receptor is located in high concentrations in adjacent vascular smooth muscle cells\(^\text{15}\). This has led to speculation that CNP may exert its effects locally within the vascular wall.

In contrast to ANP and BNP, initial studies failed to detect CNP mRNA in the human myocardium\(^\text{34}\). Further studies with myocardial tissue have, however, demonstrated mRNA transcripts for the natriuretic peptide receptors, including NPR-B\(^\text{35}\). This has prompted speculation that CNP may have a role in the control of cardiac function. Wei and co-workers have subsequently confirmed the presence of CNP within both atrial and ventricular myocardium by immunohistochemistry and radioimmunooassay\(^\text{36}\). In this study myocardial levels of CNP were significantly elevated in patients with congestive cardiac failure in comparison to controls.

**CNP release**

In vitro studies, using cultured bovine endothelial cells, have shown marked augmentation of CNP mRNA production and subsequent CNP secretion by transforming growth factor\(^\text{37}\). Several other cytokines, including tumour necrosis factor, interleukin-1 and basic fibroblast growth factor also enhance CNP secretion from cultured cells\(^\text{37,38}\). Both tumour necrosis factor-α and interleukin-1 enhance inducible nitric oxide synthase expression and subsequent nitric oxide levels in vascular endothelial and smooth muscle cells. This effect appears to be significantly augmented by ANP, BNP and CNP, and is associated with an increase in intracellular cGMP\(^\text{39}\). Lipopolysaccharide, a bacterial endotoxin, also stimulates in vitro endothelial CNP production\(^\text{38}\). CNP secretion from cultured aortic bovine endothelial cells is also strongly stimulated by both ANP and BNP, the action of BNP being 20 times greater than that of ANP\(^\text{40}\).

Therefore, potential exists for an important in vivo interaction between CNP and these vasoactive substances in the local regulation of vascular tone and growth. Supporting evidence comes from further studies with cultured endothelial cells where CNP was found to inhibit angiotensin II stimulated release of endothelin\(^\text{41}\). Conversely endothelin reduced CNP induced cGMP formation. Thus CNP and endothelin may function as opposing mechanisms in the control of vascular tone.

Whilst little is known about haemodynamic variations which may influence the production of myocardial CNP, data are available for both ANP and BNP. ANP is primarily secreted from storage granules within the atrium and its levels tend to rise in conditions that result in increased atrial stretch, such as volume overload\(^\text{42}\). BNP, although present in the atrium, is found in much larger concentrations in the ventricular myocardium\(^\text{9}\). BNP levels increase with chronic dietary sodium loading in normal man, supporting a volume-related pattern of release\(^\text{43}\). This is further supported by the fall in plasma BNP levels seen during fluid removal by haemodialysis in patients with chronic renal failure\(^\text{44}\). Therefore there may be a direct relationship between ventricular wall stress and BNP secretion, and indeed this may be the reason for its ability to act as a powerful prognostic indicator in various myocardial disease states\(^\text{11,45}\).

**Plasma and urinary CNP levels**

**Normal man**

Basal CNP release into the circulation seems to occur in man. CNP-22 immunoreactivity was first detected at low concentration in normal human plasma by Stingo et al\(^\text{19}\). It has subsequently been studied in various pathological states. However, difficulty arises from the fact that CNP-22 has a short half-life of approximately 2-6 min\(^\text{46}\). In comparison the half-life of BNP is around 22 min, making it a much more amenable peptide to assay\(^\text{47}\). When assessing plasma CNP levels it is therefore essential to process the sample immediately, having collected it in EDTA tubes containing aprotinin (trasyrol), before measuring it with an appropriately validated assay.

**Pathological states**

Plasma CNP is significantly elevated in chronic renal failure\(^\text{48}\) and cor pulmonale\(^\text{49}\). The mechanism of this
increase in these pathological states is not entirely clear. Hypoxaemia is a stimulus for ANP release[56] but whether this can account for elevated levels of plasma CNP in cor pulmonale is not currently known. Alternatively it may represent leakage of CNP from the damaged endothelium. The latter might also explain elevated levels in patients with septic shock, although more specific stimuli might be involved[53]. Septic shock is a condition characterized by peripheral vasodilatation, hypotension and inflammatory immune activation. During the septic early phase, elevated levels of tumour necrosis factor-α and lipopolysaccharide occur, both of which elevate CNP levels in vitro. Several vasouctive mediators have been proposed to contribute to the vasodilatory state and include prostacyclin, bradykinin, nitric oxide and more recently adrenomedullin[52]. Tumour necrosis factor-α, interleukin-1 and lipopolysaccharide are thought to directly induce production of nitric oxide, which activates soluble guanylate cyclase in vascular smooth muscle cells resulting in enhanced cGMP levels[53]. Further evaluation is required to determine whether CNP is a novel mediator involved in the pathophysiology of septic shock.

**Hypertension**

Plasma CNP levels have been studied in hypertensive subjects, but no significant increases were found[54]. This is in contrast to BNP where plasma levels were significantly higher in the hypertensive group. In this study, however, plasma CNP levels did correlate with heart rate and plasma noradrenaline concentrations, raising speculation of sympathetic involvement in CNP release. Similarly in both gestational hypertension and pre-eclampsia plasma CNP levels are not significantly elevated, whereas plasma BNP levels correlate with the blood pressure[55].

Supporting evidence for an interaction between CNP and the sympathetic system comes from in vitro studies using isolated rat tail arteries[56]. In this study exogenous CNP was found to exert an inhibitory neuromodulatory effect on the genetically induced release of the sympathetic cotransmitters noradrenaline and adenosine 5’-triphosphate.

**Chronic heart failure**

Several studies have investigated plasma CNP levels in stable chronic heart failure[56,48,49]. These have failed to demonstrate any significant elevations. This may reflect a true absence of increase in heart failure or that perhaps plasma levels are unreliable indicators of CNP production. In addition only small numbers of patients were involved and classified primarily on the basis of their New York Heart Association classification. It has, however, been suggested that determination of aerobic capacity is necessary to enable proper selection of patients for heart failure studies[57]. It is of interest that urinary excretion of CNP is significantly increased in patients with congestive cardiac failure[53]. Both CNP-22 and CNP-53 were identified in human urine. Since neutral endopeptidase is abundant in the proximal tubular brush border this is more likely to represent enhanced renal production as opposed to increased filtration. This study also demonstrated the presence of CNP, by immunohistochemical staining, in the epithelial cells of all the tubular segments of the human kidney. Supporting evidence comes from studies of acute intravascular overload in dogs. This manoeuvre, like acute heart failure, results in rapid increases in cardiac filling pressures and subsequent elevation of plasma ANP[58]. Borgeson et al. found that whilst acute volume overload resulted in the expected increase in plasma ANP there was no significant increase in plasma BNP or CNP[59]. However, there was a marked increase in urinary CNP but not urinary ANP or BNP. It thus appears that during acute intravascular volume overload there is a differential circulatory and urinary natriuretic peptide response.

A discrepancy therefore exists between studies looking at levels of CNP in patients with chronic heart failure. Whilst Wei et al.[56] demonstrated increased myocardial CNP levels and Mattingly et al. showed that urinary levels of CNP are significantly elevated[53], basal plasma levels appear to remain static in chronic heart failure. This area requires further studies to clarify the exact role of CNP in this condition. Indeed it may be that local tissue levels of CNP are more important in these pathological states, particularly since the NPR-B is located in the smooth muscle cells adjacent to the endothelial site of CNP production. Interestingly the myocardial secretion of ANP in heart failure appears to be different from that of the normal heart. In rats with congestive heart failure, the whole heart content of ANP is diminished whilst plasma levels are significantly elevated when compared to controls[60]. In this study the animals with heart failure demonstrated a decrease in the release of ANP from atrial tissue in response to mechanical and adrenergic stimulation, leading to the suggestion that heart failure may actually deplete cardiac stores of ANP.

In humans, the increments of right atrial ANP concentrations in response to elevations in right atrial pressure induced by exercise, were markedly reduced in patients with chronic heart failure when compared to controls[61].

As mentioned previously, the presence of CNP immuno-reactivity in human kidney has been demonstrated and appears to be primarily located within the tubular epithelial cell. Totsume et al., by immunocytochemical methods, showed that whilst ANP and BNP were localized in the distal tubular segments, CNP was present predominantly in the proximal tubules in human kidney[62]. mRNA for the NPR-B has also been identified within human kidney by means of the polymerase chain reaction[63]. Since urinary CNP is elevated during acute intravascular overload and in patients with chronic heart failure, speculation exists about its potential involvement in a renal natriuretic peptide
system that participates in the regulation of sodium and water balance and the renal circulation.

**Biological actions (Fig. 2)**

*In vitro studies*

**Vascular regulation**

Evidence to support a role for CNP in the regulation of vascular tone has come from in vitro studies using canine arteries and veins[16]. This demonstrated that CNP is a potent vasodilator, with a greater effect on isolated veins than arteries. This is in comparison to ANP whose prime effect was on renal arterial relaxation. Interestingly the vasorelaxant actions of CNP were somewhat attenuated by the presence of an intact endothelium. Several mechanisms could account for this; including diminished clearance or breakdown of the exogenous CNP without the intact endothelial surface or that the endothelium itself might act as a barrier to diffusion of the CNP through to the smooth muscle cells, where the NPR-B receptor is present in significant quantities. In vitro studies in humans have confirmed both the venous and arterial relaxation effects of CNP[64].

Migration and proliferation of aortic smooth muscle cells is thought to be important in the response to vascular injury[65], contributing to vascular restenosis, remodelling and angiogenesis. In vitro, natriuretic peptides have been shown to inhibit vascular smooth muscle and endothelial cell proliferation[66–68]. When compared with ANP, CNP appears to be a more potent inhibitor of smooth muscle cell proliferation in response to various growth factors[69]. Transforming growth factor-β is one such factor and is thought to play a significant role in the control of vascular smooth muscle cell proliferation, particularly after vascular...
injury\textsuperscript{70}. As stated previously transforming growth factor-\(\beta\) markedly augments CNP production in vitro. This therefore raises speculation that the actions of transforming growth factor-\(\beta\) could, at least in part, be mediated by its effect on enhanced CNP production. Ikeda \textit{et al.} demonstrated that CNP significantly inhibited the migration of subcultured rat aortic smooth muscle cells in response to platelet-derived growth factor and fetal calf serum\textsuperscript{71}. This inhibition was paralled by an increase in the cellular level of cyclic GMP. However, in a study using primary cultures of rat aortic smooth muscle cells CNP, together with several nitric oxide donors, stimulated cell migration\textsuperscript{72}

Increased levels of plasminogen activator inhibitor-1 have been reported in both atherosclerotic and balloon-injured vessels\textsuperscript{73}. This may result in the local inhibition of plasminogen, thereby impairing fibrinolysis and promoting thrombosis. Plasminogen activator inhibitor-1 also regulates vascular smooth muscle cell migration\textsuperscript{74}. In cultured rat and human aortic smooth muscle cells CNP and ANP inhibit plasminogen activator inhibitor-1 mRNA expression in response to angiotensin II or platelet derived growth factor (both of which induce its expression under normal circumstances)\textsuperscript{75}. This effect appears to be cGMP dependent. Further work has shown that both BNP and CNP suppress the expression of tissue factor and plasminogen activator inhibitor-1 mRNA induced by angiotensin II in cultured rat aortic endothelial cells\textsuperscript{76}. Tissue factor is an initiator of the blood coagulation cascade. This led the authors to speculate that CNP may have a role in the regulation of coagulation and fibrinolysis by modulating endothelial cells in local lesions.

Regulation of endothelial cell death and survival contributes to vascular pathology. ANP, BNP and CNP induce endothelial cell apoptosis in cultured rat endothelial cells\textsuperscript{77,78}. In contrast endothelin-1 inhibited apoptosis, leading to the suggestion that the balance between these vasodilators and vasoconstrictors not only regulates vascular tone but may also contribute to endothelial cell integrity.

\section*{In vivo studies}

\textit{Animals}

\textit{Cardiovascular and renal actions}

Studies into the physiological effects of CNP have primarily involved the exogenous administration of synthetic CNP. Stingo and co-workers compared the effects of synthetic CNP on cardiovascular and renal function, when given to anaesthetized dogs by continuous and bolus infusions\textsuperscript{79}. They confirmed that CNP is a potent vasoactive peptide, with particular effect on decreasing arterial pressure in association with a decrease in cardiac output. Both right atrial pressure and pulmonary capillary wedge pressures were significantly depressed, although for the latter this only occurred at the highest infusion concentration. Despite its powerful vasodilatory effect there were no significant alterations in heart rate in either protocol, although there was a trend towards higher rates in the bolus treated group. These physiological effects were associated with an increase in plasma cGMP levels. In contrast to both ANP and BNP, CNP administration did not result in enhanced sodium excretion or glomerular filtration rate. The marked decrease in arterial pressure may have contributed to the failure to stimulate natriuresis, and may in part be explained by elevated plasma levels of aldosterone that occurred during CNP infusion. However, there was no concomitant elevation of plasma renin levels despite significant haemodynamic changes, suggesting that CNP may actually inhibit renin release. This is in keeping with the renal effects of exogenous ANP\textsuperscript{82}.

Nevertheless, despite its potent effects on haemodynamics, ANP administration also results in significantly enhanced natriuresis and diuresis. The resulting decrease in venous return results in lowered right atrial pressure and cardiac output. Although CNP infusion results in a similar reduction of venous return, this is more likely to be secondary to a direct vasoactive effect and it is this mechanism which best explains the consistent reduction in cardiac output found during the study.

However, when injected into anaesthetized rats, CNP resulted in a significant decrease in mean arterial blood pressure together with enhanced diuresis and natriuresis\textsuperscript{89}. It should be noted that in the majority of these experiments the animals were anaesthetized. As such, extrapolation of the data from these studies to conscious animals or humans needs to be made with caution.

In vivo studies in dogs have demonstrated a weak positive chronotropic response to exogenous CNP infused directly into the sinoatrial node artery\textsuperscript{80}. This was associated with an increase in the slope of spontaneous diastolic depolarization within the sinoatrial node region. In this study NPR-B mRNA was also detected at high levels of expression in atrial and nodal tissue, when compared to reference lung tissue. The spontaneous depolarization of sinoatrial node cells appears to result from the interaction of several currents\textsuperscript{81}. These include an initial decrease in the delayed rectifier potassium current, followed by enhanced calcium currents. This, together with an enhanced inotropic effect produced by injected CNP on isolated right atrial preparations, have led the authors to speculate that both the positive chronotropic and inotropic effects of CNP are the results of enhanced cardiac calcium channel activity\textsuperscript{80}. They postulate that these actions are mediated via the NPR-B receptors that are present in the right atrial and nodal tissues at significant concentrations.

CNP causes receptor-mediated positive dromotropic effects when directly injected into the atrioventricular artery in autonomically decentralized hearts in anaesthetized dogs\textsuperscript{82}. This resulted in a dose-dependent reduction in atrioventricular conduction time, an effect inhibited by a specific natriuretic peptide receptor.
antagonist (HS-142-1) but not propranolol. Additional studies investigating the inotropic actions of CNP have produced conflicting results, with a negative inotropic effect demonstrated on isolated rat papillary muscles [53]. Studies with ANP have confirmed a more reproducible negative inotropic effect [84,85]. Further evaluation of the inotropic role of CNP in humans is required.

The mechanism by which CNP results in powerful vasodilatation with limited net effect on heart rate remains uncertain. ANP reduces sympathetic tone in the peripheral vasculature by direct suppression of sympathetic outflow from the central nervous system and suppression of release of catecholamines from nerve endings [86,87]. This lowers the activation threshold for vagal afferents, therefore suppressing the tachycardia and vasoconstriction that accompanies a reduction in preload. Although further work is needed to clarify the interaction of CNP with the baroreflex system, a recent study has confirmed that ANP, BNP and CNP augment the baroreflex slowing of heart rate in response to rapid increases in blood pressure in conscious rats [88]. Data are available with regard to the interaction of ANP with the baroreflex control of circulation in humans. ANP primarily appears to reset the baroreflex control of heart rate in a way that favours bradycardia and opposes cardioacceleration [89]. Volpe et al. demonstrated that this modulation seems to be related, at least in part, to the interaction of ANP with angiotensin II [90]. Whilst ANP potentiated the bradycardic reflex response to phenylephrine-induced hypertension and attenuated the tachycardic response to nitroglycerin-induced hypotension, these effects were abolished during angiotensin converting enzyme inhibition.

Local vascular effects
Studies in rabbits have demonstrated that a continuous infusion of exogenous CNP can inhibit intimal thickening after either balloon catheter or air-drying injury [91,92]. In these studies the CNP infusion was continued for between 5 to 14 days post-injury. CNP potently stimulated cGMP production in the injured arteries but not in the intact ones, suggesting that there was an enhanced expression of NPR-B at the sites of vascular injury. Ueno et al. constructed an adenoviral vector expressing CNP and used it to transfect balloon injured rat carotid artery cells [93]. They demonstrated a 90% reduction in neointimal formation when compared to the control group. This occurred without a concomitant rise in plasma CNP. These results suggest that CNP may play a protective role against inflammatory and proliferative changes occurring in injured arteries. This may have important clinical implications particularly with respect to restenosis following balloon angioplasty.

Humans
Cardiovascular and renal actions
Several studies have investigated the effects of exogenous CNP administration in normal man. Hunt et al. infused synthetic CNP at a rate of 5 pmol . kg$^{-1}$ . min$^{-1}$ to achieve plasma levels greater than those found in either physiological or pathological states [94]. In contrast to both ANP [94] and BNP [5] they were unable to demonstrate a significant vasodepressor or natriuretic response. However, there was a small increase in plasma ANP and cGMP, together with a decrease in plasma aldosterone. Although the effect on aldosterone is inconsistent with previous reports in dogs [78], a weak inhibitory effect of CNP on cultured bovine adrenal glomerulosa cells has been demonstrated [95]. In this study the decrease in plasma aldosterone might result from competition between endogenous CNP and endogenous ANP for the NPR-C (clearance receptor), causing elevated plasma ANP levels with subsequent physiological effects. The elevated plasma levels of CNP did not appear to affect the pressor action of a concomitant angiotensin II infusion. This finding has been confirmed with slightly higher infusion rates of CNP in normal man [96]. CNP infused at 10 pmol . kg$^{-1}$ . min$^{-1}$, a level previously demonstrated to have haemodynamic effects in dogs [78], failed to produce any alteration in systemic or pulmonary haemodynamics, nor was there an effect on the aldosterone or pressor responses to infused angiotensin II. In contrast both ANP and BNP are known to attenuate these responses to angiotensin II [97].

More detailed analysis of cardiovascular dynamics during CNP infusion at 2 and 4 pmol . kg$^{-1}$ . min$^{-1}$ has confirmed that there were no alterations in cardiac output, heart rate or arterial pressure [98]. In addition cardiac volumes and dynamics of left and right heart filling were unchanged. In contrast to the study by Hunt et al. [46], there was no effect on the renin-aldosterone axis.

Conflictic results have arisen from studies using much higher doses of CNP. Barr et al. observed a reduction in arterial pressure and cardiac output, but no change in heart rate or plasma aldosterone levels [99]. This was at a CNP infusion rate of 50 ng . kg$^{-1}$ . min$^{-1}$ (approximately 22.75 pmol . kg$^{-1}$ . min$^{-1}$). However, following administration of a bolus of CNP (430 pmol . kg$^{-1}$) there were significant elevations in plasma and urinary cGMP, creatinine clearance, diuresis and natriuresis [100]. In addition there were significant reductions in systolic and diastolic blood pressure, together with an increase in heart rate. However, this was associated with an approximately threefold increase in the plasma levels of ANP and BNP, which again may have resulted from competition for clearance pathways.

Pham et al. compared ANP and CNP intravenous infusions at doses varying between 0·005 and 0·05 μg . kg$^{-1}$ . min$^{-1}$, in normal human subjects [101]. They found a dose dependent increase in natriuresis (but no effect on diuresis or glomerular filtration rate) with CNP, although the effect was less than for ANP. With CNP infusion there was no alteration in blood pressure or forearm blood flow, nor did it produce a significant increase in plasma cGMP. Although both ANP and CNP infusions resulted in elevations of urinary and nephrogenous cGMP, the effect was much greater for
ANP. In contrast, intra-arterially infused CNP results in enhanced forearm blood flow\(^\text{[102]}\). This response is not attenuated in patients with chronic heart failure. Indeed CNP-induced cGMP spillover from the forearm in patients with chronic heart failure was higher than in healthy controls. In comparison ANP was found to be a more potent peripheral vasodilator, but its effect was significantly attenuated in the heart failure group. The latter result is consistent with previous studies’ observations suggesting that ANP receptors on peripheral vascular smooth muscle may be downregulated in patients with chronic heart failure\(^\text{[103]}\). Schiffrin also confirmed a reduction in ANP-binding sites on platelets in patients with severe chronic heart failure and with significantly elevated plasma ANP concentrations\(^\text{[104]}\). A further study has confirmed that the vasodilatory effect on forearm vessels is attenuated in patients with heart failure\(^\text{[105]}\). However, the mechanisms responsible for these alterations are likely to be more complex than pure down-regulation of the ANP receptors since the increases in venous plasma cyclic GMP caused by intra-arterial ANP were comparable in both patients with heart failure and normal subjects. Additional evidence towards an impaired response to ANP in heart failure has come from studies investigating renal responses. Intravenous ANP was found to have little effect on natriuresis or diuresis in patients with heart failure in comparison to controls\(^\text{[106]}\).

How these studies involving CNP infusions that achieve high plasma CNP levels relate to in vivo tissue levels is not known. It is feasible that the tissue level of CNP, in close proximity to its receptor, is greatly different from the readily measurable plasma level.

The natriuretic peptides appear to act as functional antagonists of the renin-angiotensin-aldosterone system. ANP inhibits release of both renin and aldosterone and in vitro inhibits the activity of angiotensin converting enzyme\(^\text{[107–109]}\). To evaluate the role of CNP with respect to the activity of endothelial angiotensin converting enzyme, Davidson et al. measured the differential biological response, in the form of forearm blood flow, to infusions of angiotensin I and II\(^\text{[110]}\). CNP inhibited the vasoconstrictive effect of angiotensin I but not that of angiotensin II, suggesting that CNP may act as a local endogenous regulator of vascular angiotensin converting enzyme.

### Potential therapeutic implications

Advances in the understanding of neurohumoral mechanisms in cardiovascular disease have led onto the potential for new therapeutic strategies. Particular interest has focused on vasopeptidase inhibition with simultaneous inhibition of neutral endopeptidase and angiotensin converting enzyme. This combines the established benefits of suppressing angiotensin II mediated vasoconstriction together with potentiating the vasodilatory natriuretic peptides\(^\text{[111]}\). This approach may be particularly applicable to patients with hypertension and heart failure.

Studies with a recently developed vasopeptidase inhibitor, omapatrilat, have confirmed its efficacy in animal models of hypertension and cardiomyopathy\(^\text{[112,113]}\). In the latter study its effects were compared with captopril in cardiomyopathic hamsters. Omapatrilat improved left ventricular preload and left ventricular remodelling. In addition animals treated with omapatrilat had a 31% increased median survival time compared to the group treated with captopril. Further studies on its safety and efficacy in humans are awaited.

### Conclusions

Since its discovery in porcine brain much more is now known about the location of CNP and its receptor, NPR-B, in human tissue. Despite this, the exact physiological role of CNP in humans and animals is not yet entirely clear. How plasma levels of CNP correlate with local tissue levels is unknown and difficult to fully elucidate without a specific CNP blocking agent. The use of both genetic knockout animal models and models over-expressing appropriate genes have considerably enhanced our understanding of the role of ANP and BNP\(^\text{[114,115]}\). Similar progress for CNP may advance our current understanding of its potential importance. CNP does however appear to have significant interactions with many other important vasoactive substances. This, together with its vascular location and vasorelaxing properties, place it in a prime position to influence local vascular tone and growth.

It is apparent that more work needs to be done to clarify the exact role of CNP in humans. We feel that CNP may prove to be an important peptide, particularly in cardiovascular diseased states such as chronic heart failure and restenosis following angioplasty. With the prospect of commercially available vasopeptidase inhibitors, manipulation of natriuretic peptide levels may prove to be an exciting therapeutic manipulation in the not too distant future.

### References

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