Natriuretic Peptides
Update on Peptide Release, Bioactivity, and Clinical Use

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In the 25 years since de Bold et al demonstrated the existence of an atrial natriuretic factor, investigation of the cardiac natriuretic peptides has produced a maturing knowledge base identifying the cardiac peptides and addressing stimuli for secretion of atrial (ANP) and B-type (BNP) natriuretic peptides, the processing and release of the mature bioactive carboxy terminal peptides, together with their propeptides and amino terminal segments, the nature of natriuretic peptide receptors, and the bioactivities of natriuretic peptides (including natriuresis, vasodilatation, suppression of renin–angiotensin–aldosterone and sympathetic nervous activity, and trophic effects inhibiting vascular and cardiac hypertrophy and fibrosis). Less is known of the more recently discovered C-type natriuretic peptide (CNP) and its cosecreted amino-terminal peptide, NTproCNP.\(^3,4\) Increasingly, measurements of the B-type peptides have found diagnostic and prognostic application in cardiovascular disease.\(^5-7\)

The genes for ANP and BNP are in tandem on human chromosome 1.\(^8\) Upstream regulatory regions identified for the BNP gene include an AP1 binding site, serum response elements, M-CAT and GATA sites.\(^9,10\) Translation results in pre-pro BNP from which a 26 amino acid signal peptide is cleaved to produce the 108 amino acid precursor proBNP. This in turn is processed between amino acid residues 76 and 77 resulting in a 32 amino acid biologically active peptide (BNP) from its carboxy terminal plus the remaining amino terminal peptide sequence (NTproBNP 1 to 76). ANP is synthesized as a 126 amino acid precursor and is stored in granules within atrial tissue.\(^11\) During or shortly after secretion, the precursor is processed to an active 28 amino acid carboxy terminal peptide (ANP) and amino terminal ANP (proANP 1 to 98). The amino terminal propeptides NTproANP, NTproBNP, and NTproCNP have no known biological actions. The mature bioactive human forms of ANP, BNP, and CNP all contain a 17 member amino acid ring bound by cysteine-to-cysteine disulphide bonds with 11 of the 16 residues being conserved across ANP, BNP, and CNP. The 3 peptides have varying length amino terminal and carboxy terminal amino acid residue “tails” with CNP essentially lacking any carboxy terminal extension beyond the ring structure. The actions of ANP and BNP are mediated predominantly via the GC-A receptor and those of CNP via the GC-B receptor. All 3 peptides are subject to varying degrees of degradation by neutral endopeptidase (EC 3.4.24.11) and via uptake by the C (“clearance”) receptor.

ANP and BNP and their receptors are widely distributed in brain, spinal cord, pituitary, kidney, adrenal gland, and vasculature in addition to the heart. Concentrations of immunoactive ANP and BNP within the heart are up to 3 orders of magnitude higher than elsewhere, and within cardiac atria the concentrations of both peptides are 1 to 2 orders of magnitude higher than in ventricle.\(^12\) CNP is also widely distributed mainly in the vasculature with far lower cardiac levels than ANP or BNP.

Diastolic stretch activates BNP promotion in human ventricular tissue.\(^13\) How myocytes sense strain is uncertain but may involve extra-cellular matrix-integrin cell surface attachments\(^14,15\) increasing gene transcription through the binding of transcription factors to upstream GATA elements in the BNP gene. Natriuretic peptide secretion is modulated by endothelin-1 and angiotensin II acting in paracrine-autocrine fashion.\(^16,17\) Estrogen levels and cytokine activity (including interleukin [IL]-1\(^\beta\)) can also alter natriuretic peptide secretory responses.\(^10\) Levels are also influenced, via uncertain pathways, by gender, age, glucocorticoid and thyroid hormone status, and hypoxemia.

Much remains to be discovered concerning factors influencing expression, synthesis, and release of these peptides; and the determinants of receptor-mediated bioactivity. The clinical application of peptide immunoassays requires refinement to account for potentially confounding conditions including impaired renal function and obesity. The remainder of this brief review summarizes relevant reports that have been published in *Hypertension* over the 2004 to 2006 period.

Synthesis and Processing

Secretory Stimuli for Natriuretic Peptides

Lyosphosphatidyl choline (LPC) has joined the ranks of molecular modifiers of natriuretic peptide secretion.\(^18\) This endogenous phospholipid is released from cell membranes during ischemia. In isolated perfused atria LPC attenuated...
release of ANP in response to atrial stretch. Notably, the effect was reduced in hypertrophied versus control atria suggesting cardiac hypertrophy preserves release of ANP in response to some stimuli, including ischemia.

Yuan et al\(^1\) have recently established a relationship between adenosine and ANP release. Adenosine protects the ischemic myocardium by means of coronary vasodilatation and suppression of cardiac contractility. These effects may be mediated in part through release of natriuretic peptides. In isolated perfused beating rat atria, adenosine induced cAMP-dependent ANP release (simultaneously with the onset of negative inotropic effect) in a dose-related fashion. This was attenuated by an adenosine A1 receptor antagonist but not by A2A or A3 antagonists.

### Natriuretic Peptide Receptor Activity

Several advances have occurred in our understanding of factors which influence natriuretic peptide guanylate cyclase-linked receptor activity.

#### GC-A Receptor

ANP inhibits transcription of the GC-A receptor through cGMP-dependent pathways. Hum et al\(^2\) subcloned a 1520-bp fragment of rat GC-A promoter in an expression vector containing the luciferase reporter gene and from serial sequence deletion experiments, concluded that the promoter region for the GC-A gene contains a cGMP response element situated between base pairs −1396 to −1307. Cross competition experiments with mutated oligonucleotides allowed definition of a consensus sequence for the novel cGMP response element which is conserved in human and mouse promoters. Hence, ANP (and presumably BNP which also acts via the GC-A receptor) can downregulate and inhibit the activity of its own predominant receptor through the action of its second messenger, cGMP, on the promoter region of the GC-A receptor gene.

Increases in extracellular osmolality are associated with increased expression and promoter activity of the GC-A receptor gene within rat intermedullary collecting duct kidney cells (IMCD). These events occur via a P38 MAP kinase pathway. Serum and glucocorticoid inducible kinase (Sgk) is thought to be involved in sodium handling in distal tubular segments. Chen et al\(^3\) report exposure of IMCD cells to increasing concentrations of sodium produces an increase in Sgk levels. IMCD transfected with a Sgk vector together with a GC-A luciferase reporter exhibited a 3-fold increment in reporter gene activity. This was blocked by an Sgk mutation and by 8-bromo cGMP. The authors concluded that Sgk mediates the osmotic induction of GC-A gene promoter activity.

Cyclophilin A is a receptor for cyclosporin. It accelerates cis-trans isomerization of prolyl-peptide bonds (PPIase activity). Through these actions, cyclophilin binds and regulates the activity of a number of proteins. Chen et al\(^4\) found cyclophilin A associates with the GC-A receptor and that ANP binding to GC-A releases cyclophilin A from the receptor. Transfection of cyclophilin A leads to inhibition of ANP-induced GC-A activity in cellular preparations suggesting that cyclophilin A is an endogenous inhibitor of GC-A activity. Inhibition of PPIase activity by the drug cyclosporin blocks the inhibitory effect of cyclophilin A on GC-A activity. Hence these authors have demonstrated the presence of an endogenous inhibitor of GC-A activity, cyclophilin A. The fact that cyclosporin blocks this effect carries the possibility that cyclosporin and other agents which bind cyclophilin A, may enhance endogenous natriuretic peptide bioactivity. This is an area that requires further exploration.

#### GC-B Receptor

Rahmutula and colleagues have reported isolation and characterization of the human GC-B gene promoter.\(^2\) Having identified the 5' terminus of the human GC-B gene transcript, the authors generated a series of 5' deletion mutants linked to luciferase reporting and introduced these constructs into rat aortic smooth muscle cells or neonatal rat cardiac fibroblasts. Maximum expression occurred with a 441-bp 5' flanking sequence. Site-directed mutagenesis of the proximal promoter revealed a series of GC rich sequences, 5 of which contributed modestly (approximately 25%) to basal human GC-B promoter activity. A 6th mutation within a GC rich sequence produced a 90% reduction in promoter activity, and this latter sequence associates with SP1 and SP3 in vitro. Mutation at this site leads to loss of binding of SP1 in vitro. Overexpression of SP1 or SP3 in drosophila cells induced enhanced GC-B promoter activity which in turn was nullified by mutation of the SP1 binding site. The authors conclude that the GC-B promoter activity is dominated by a single cluster of SP1 binding elements in the proximal 5' flanking sequence of the gene.

Langenickel et al\(^24\) aimed to determine which residues of the extracellular ligand binding domain of natriuretic peptide receptors were important for dimerization and therefore cGMP induction. Both GC-A and GC-B receptors have an extracellular ligand binding domain, a transmembrane domain, and an intracellular domain which in turn consists of a kinase homology domain, a hinge region, and a guanylyl cyclase domain. In the absence of a ligand, both receptors exist as homodimers or homotetramers. Ligand binds to the ligand binding pocket of the dimer and leads to a conformational change of the receptor. Three intramolecular disulphide bridges between cysteines at positions 53 and 79, 205 and 314, and 417 and 426 determine the ligand binding pocket structure. Consecutive signaling through the transmembrane domain results in ATP binding within the phosphorylated kinase homology domain. ATP binding and phosphorylation are essential for receptor activation and generation of second messenger cGMP. Langenickel et al used dilution mutagenesis to replace cysteines with serines at sites 53, 417, and 426. After this, receptor expression, dimerization, whole cell cGMP response, and guanylyl cyclase activity of cell membranes was determined in stable transfected cos7 cells. The "Y" shaped ligand-binding domain of C417S and C426S mutants were able to bind ligand, but the CNP response is blunted compared with that of wild-type GC-B. In the C53S mutant the contact of the 2 monomers is further from the cell membrane and the resulting "A" shape of the receptor dimer both excludes close tightening of both monomers within the intracellular region and impairs extracellular ligand binding.
These data raise the possibility that yet to be discovered mutations within the coding region of the extracellular domain of GC-A or GC-B could impair receptor signaling and might promote hypertension.

Abbey-Hosch et al demonstrated that sphingosine-1-phosphate (S1P) inhibits GC-B activity. Administration of CNP in whole animal or isolated vascular models induces a reduction in vascular tone and longer term effects include inhibition of vascular smooth muscle cell (VSMC) proliferation and migration. S1P exerts exactly opposite effects. CNP functions as a local regulator in vascular walls and fibroblasts where endothelial cell production of CNP is transcriptionally regulated by paracrine factors including tumor necrosis factor (TNF)-α, IL-1α/β, and transforming growth factor (TGF) β2 as well as by shear stress. CNP influences the tone and proliferation of adjacent smooth muscle cells and adventitial fibroblasts. CNP infusions reduce intimal thickening and restenosis after experimental balloon angioplasty injury to vessels. This report indicates S1P, a novel phospholipid signaling molecule released in micromolar concentrations from activated platelets, inhibits GC-B in both fibroblasts and VSMCs. This appears to be the most potent GC-B desensitizing factor identified to date. S1P stimulates intracellular calcium, vasoconstriction, and VSMC proliferation and migration and therefore may facilitate evolution of atherosclerosis. Cross-talk between CNP and S1P pathways is of potential relevance to wound healing, restenosis, atherosclerosis, and hypertension. Activated platelets release S1P and the exposure of VSMCs to S1P at the time of vessel injury may stimulate VSMC migration and proliferation leading to restenosis. CNP-dependent cGMP elevations antagonise these processes and inhibit restenosis. The interplay between CNP and S1P systems clearly warrants further research.

Left Ventricular Remodeling, Heart Failure, and Clinical Applications

Genetically Modified Animal Models
Mice homozygous for deletion of the proANP gene (Nppa−/−) or the natriuretic peptide GC-A gene, exhibit cardiac hypertrophy and an exaggerated hypertrophic response to volume or pressure overload. The effects of a modest ANP deficiency on development of cardiac hypertrophy, remodeling, and heart failure were studied in mice heterozygous (Nppa+/−) for deletion of the proANP gene. Nppa−/−, Nppa+/−, and Nppa−/− animals underwent transverse aortic constriction or sham surgery. Heart weight varied inversely with Nppa gene number. Collagen deposition was noted with interstitial and perivascular fibrosis seen in Nppa−/− and Nppa+/− but not in Nppa−/+ animals 1 week after aortic constriction. Hence, even moderate chronic reduction in ANP bioactivity promotes cardiac fibrosis in response to hemodynamic stress.

In mice with deletion of the GC-A gene undergoing coronary ligation, higher mortality, a higher incidence of acute heart failure, and impaired water and sodium clearance together with increased cardiac expression of ANP, BNP, TGF-β, and type 1 collagen occurred compared with wild-type controls. Notably, in a double knockout animal in which the angiotensin type 1 receptor had also been deleted, fibrosis was absent but survival was not improved and hypertrophy still occurred. This suggests ANP and BNP attenuate fibrosis during ventricular remodeling by inhibiting the activity of the renin–angiotensin system (RAS). Hence, activation of GC-A by endogenous natriuretic peptides after cardiac injury is cardioprotective with effects mediated through inhibition of RAS activity as well as through RAS-independent pathways. The results suggest the potential use of exogenous ANP or BNP, or pharmacological inhibition of endogenous peptide clearance, to improve short- and long-term outcomes in myocardial infarction.

Natriuretic Peptides as Clinical Markers
The independent prognostic power of the B-type peptides in a wide range of cardiovascular disease and in asymptomatic risk-bearing populations has been established.

CNP
Wright et al examined the potential of plasma NTproCNP as a marker of cardiac function in 305 patients with recent onset dyspnea or peripheral edema presenting to primary care physicians. Plasma NTproCNP peptide concentrations were elevated in heart failure and related to age and renal function. NTproCNP was significantly related to concurrent plasma CNP levels, ANP, NTproANP, B-type peptides, endothelin 1, and adrenomedullin, but not to echocardiographic indicators of ventricular systolic function. Tertile of plasma NTproCNP interacted with tertile of plasma NTproBNP to enhance prediction of heart failure independent of age, gender, renal function, or echocardiographic left ventricular assessment. The findings suggest a possible compensatory response from the peripheral vasculature to heart failure by an endothelium-based vasodilator peptide and mandate further exploration of the role of CNP in heart failure. NTproCNP and CNP appear unlikely to add significant diagnostic power beyond that provided by plasma B-type peptides. However, their elevation adds to our knowledge of the pathophysiology of heart failure and the response of the peripheral circulation in this condition. Notably, data from Langenickel et al support an important role for CNP in maintenance of normal cardiac structure and function. Rats with a partial deficiency of GC-B receptors display progressive cardiac hypertrophy (independent of blood pressure) and elevated heart rate. The hypertrophic phenotype is aggravated by volume overloading, providing evidence linking GC-B signaling to the cardiac response to injury. Looking to the future, these findings suggest that therapeutic agents operating through the GC-B receptor may offer an additional therapeutic target in cardiac injury or overload.

B Type Natriuretic Peptides
Cerebrovascular Disease
In a nested case–control substudy of more than 6000 patients participating in the PROGRESS trial of antihypertensive agents in those with a history of cerebrovascular disease, new congestive heart failure occurred in 258 subjects over a follow-up period of 3.9 years. Both plasma NTproBNP and C-reactive protein predicted onset of heart failure with
the risk ratios between lowest versus highest quartile being 4.5 (2.7 to 7.5) for NT proBNP and 2.9 (1.9 to 4.7) for C-reactive protein. Each marker independently predicted heart failure in multivariate analyses which included an appropriate panel of established predictors. Combined, these markers provided better predictive power than either alone. This paper extends the already substantial evidence base reflecting the consistent association of plasma NTproBNP with prognosis in a broad spectrum of cardiovascular disease.²

Renal Impairment

Two conditions which can partially confound the diagnostic or prognostic utility of measurements of plasma concentrations of B-type peptides include renal dysfunction and the presence of the metabolic syndrome or obesity. Luchner et al³⁸ studied the effect of compensated renal dysfunction on the test performance of both BNP and NTproBNP. In 469 patients, stable after myocardial infarction (MI), renal function and left ventricular ejection fraction were measured. Both peptides were elevated in MI patients with left ventricular ejection fraction of <35% compared with patients in which ejection factor (EF) had been preserved at >45%. Among all myocardial infarction patients the prevalence of renal dysfunction (defined as a glomerular filtration rate ≤85 mL/min) was 24%. Both peptides were significantly elevated in patients with this degree of renal dysfunction compared with those without. Both markers correlated with GFR in univariate and multivariate analyses. When binary cut-off values were stratified according to the absence or presence of renal dysfunction (BNP 75pg/mL and 125pg/mL respectively; NTproBNP 100pg/mL and 350pg/mL respectively) the predictive power of both markers for the detection of left ventricular dysfunction increased substantially. The authors conclude that both peptides are similarly influenced by mild to moderate renal impairment and that renal disease is a cause of elevated marker concentrations in the absence of left ventricular dysfunction. These findings confirm other reports³⁸ and imply that in stable patients, measurements of B-type peptides aimed at detection of left ventricular dysfunction must be interpreted with adjustments according to renal function. The interaction of NTproBNP and renal function on both assessment of left ventricular function and on prognosis has been explored in acute heart failure presenting to the emergency department and in chronic ischemic heart disease.⁷,³⁹,⁴⁰ The test performance of B-type peptides in the diagnosis of heart failure in patients with new onset symptomatic left ventricular dysfunction is sustained despite concurrent renal impairment because of a high “signal to noise” ratio. However, in stable chronic well-treated heart failure or stable ischemic heart disease or other forms of stable cardiovascular disease, the elevation of B-type peptides is more muted³⁸ and selection of screening values of BNP and NTproBNP for rule-in or rule-out of important left ventricular dysfunction will require adjustment according to concurrent renal function.

Obesity

In the normal population, patients with heart failure and in ischemic heart disease cohorts, plasma B-type peptides are inversely related to body mass index.⁷,⁴¹ This has raised concerns that the diagnostic and prognostic utility of the B-type peptides may be impaired in the presence of significant obesity. These concerns have been largely allayed in the context of new onset symptomatic heart failure but are relevant to use of B-type peptide measurements in more chronic and stable settings. In this context, Olsen et al² report NTproBNP levels in patients with metabolic cardiovascular risk factors and the metabolic syndrome. In 2656 people aged 41, 51, 61, or 71 years, randomly selected from the general population, a subset totalling 2070 with no history of cerebrovascular or other arterial events and free of cardiovascular, diabetic, or lipid-related drugs, underwent assessment. Plasma NTproBNP levels were significantly and independently related to gender, age, pulse pressure, and ejection fraction. However, they were independently but inversely related to body mass index, plasma insulin, plasma glucose, and triglycerides. Hence, the metabolic syndrome was associated with lower levels of NTproBNP (35pg/mL versus 48pg/mL; P<0.001) for those with and without the metabolic syndrome.

The inverse association of the B-type peptides with body mass, and now with metabolic indices, remains unexplained. The natriuretic peptides have significant lipolytic activity⁴³,⁴⁴ leading to speculation that the metabolic syndrome is in part a natriuretic peptide “deficiency” state. These underlying questions concerning biological mechanisms remain to be elucidated and are a rich field for further research. The clinical significance of the findings by Olsen et al lies within the fact that in asymptomatic patients with metabolic syndrome, not receiving treatment for cardiovascular risk factors, NTproBNP will be relatively low and unrelated to later cardiovascular risk. This is despite the fact that in other population samples, such as from the Framingham Offspring Study, BNP has been strongly related to later adverse cardiac vascular prognosis.⁴² The latter study did include participants with treated cardiovascular risk factors and ischemic heart disease (albeit with no history of symptomatic heart failure) and this difference in subject selection presumably explains the discrepancy between the conclusions of these 2 reports.

Conclusions

New knowledge of the regulation of synthesis, release, and bioactivity of the cardiac natriuretic peptides (NPs) suggests areas ripe for further research include the interactions of lysophosphatidyl choline, adenosine, cyclophilin A, and shingosine-1-phosphate with peptide release or bioactivity in the settings of hypertension, vascular and cardiac hypertrophy, ischemia, and heart failure. Further findings in these areas may well lead to novel therapies (via actions mediated through both GC-A and GC-B receptors) for a spectrum of cardiovascular disease.

With respect to clinical use of NP immunoassays, much remains unknown and research must continue to explain the mechanisms underlying the inverse relationship between plasma NPs and body mass. Nevertheless, recent work has extended our understanding of the diagnostic and clinical application of plasma NPs and will guide refinement of their use in detection of cardiac dysfunction and in cardiovascular disease.
prognosis in chronic stable disease in addition to their established role in acute heart failure.

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