

# Brain natriuretic peptide in pulmonary arterial hypertension: biomarker and potential therapeutic agent

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**Abstract:** B-type natriuretic peptide (BNP) is a member of the natriuretic peptide family, a group of widely distributed, but evolutionarily conserved, polypeptide mediators that exert myriad cardiovascular effects. BNP is a potent vasodilator with mitogenic, hypertrophic and pro-inflammatory properties that is upregulated in pulmonary hypertensive diseases. Circulating levels of BNP correlate with mean pulmonary arterial pressure (mPAP) and pulmonary vascular resistance (PVR) in patients with pulmonary arterial hypertension (PAH). Elevated plasma BNP levels are associated with increased mortality in patients with PAH and a fall in BNP levels after therapy is associated with improved survival. These findings have important clinical implications in that a noninvasive blood test may be used to identify PAH patients at high-risk of decompensation and to guide pulmonary vasodilator therapy. BNP also has several biologic effects that could be beneficial to patients with PAH. However, lack of a convenient method for achieving sustained increases in circulating BNP levels has impeded the development of BNP as a therapy for treating pulmonary hypertension. New technologies that allow transdermal or oral administration of the natriuretic peptides have the potential to greatly accelerate research into therapeutic use of BNP for cor pulmonale and pulmonary vascular diseases. This review will examine the basic science and clinical research that has led to our understanding of the role of BNP in cardiovascular physiology, its use as a biomarker of right ventricular function and its therapeutic potential for managing patients with pulmonary vascular disease.

**Keywords:** brain natriuretic peptide, pulmonary artery hypertension, biomarker, therapeutic agent

## Introduction

Pulmonary arterial hypertension (PAH) is a progressive vasculopathy characterized by extensive remodeling of the pulmonary circulation that results in narrowing of the arterial lumen and impaired flow-mediated vasodilation. The marked increase in pulmonary vascular resistance limits the rate at which the right ventricle can pump blood through the lungs, causing shortness of breath and reduced functional capacity. The disease can occur at any age, but frequently affects young adults in the third or fourth decade of life who are otherwise healthy. Until recently, there were no specific therapies available to treat this disease and most patients progressed to right ventricular failure and death within 2 to 5 years of diagnosis.<sup>1</sup> In 1995, intravenous epoprostenol became the first drug approved for the treatment of PAH in the United States. Since that time 6 more medications have been approved and several more await consideration by the FDA. These medications have improved survival and exercise capacity, but none have been able to achieve a cure and the majority of patients continue to progress to

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right ventricular failure, albeit at a slower pace. The lack of a cure and the severity with which PAH strikes has generated considerable interest in furthering the understanding of the pathogenesis of this disease and the development of new diagnostic and therapeutic modalities to manage it.

Brain natriuretic peptide (BNP) is one of several members of the natriuretic peptide family that are widely distributed, but evolutionarily conserved throughout the animal kingdom. The natriuretic peptides play important roles in cardiovascular homeostasis including regulation of vascular tone, blood volume, endothelial permeability, and cardiac hypertrophy. Cardiac BNP expression is markedly upregulated in response to ventricular hypertrophy or overload and circulating BNP levels can increase by 1 to 2 orders of magnitude. There is increasing evidence that BNP may be a useful marker for right ventricular dysfunction and predict outcome in patients with PAH. As such, plasma BNP may help guide clinical decision making in patients with newly diagnosed PAH and help gauge response to treatment. BNP is also a pulmonary vasodilator and anti-hypertrophic agent and thus, may have therapeutic potential to inhibit pulmonary vascular remodeling and maladaptive increases in right ventricular mass.

This paper examines the role of BNP in assessing the severity of pulmonary vascular disease and its potential as a therapeutic agent for the treatment of PAH. The synthesis, secretion and biologic properties of BNP, relevant to its role in mediating pulmonary vascular tone, is discussed first followed by a review of its inhibitory effects on pulmonary vascular remodeling, cardiac hypertrophy and endothelial cell apoptosis. Finally, potential therapeutic applications for the treatment of PAH and right heart failure are presented.

## Pulmonary arterial hypertension

Pulmonary hypertension (PH) refers to a variety of conditions in which pulmonary arterial pressure (PAP) is elevated above normal. Mean pulmonary arterial pressure (mPAP) in a healthy adult at rest averages about 14 to 17 mmHg. Pulmonary hypertension is defined as a resting mPAP > 25 mmHg or a mPAP > 30 mmHg during exercise and a pulmonary capillary wedge pressure (PCWP) < 15 mmHg. Providing that cardiac output is not elevated, this results in a pulmonary vascular resistance (PVR) > 3 Wood units. Most patients with PAH have a mPAP that is considerably higher, however. In fact, the mean mPAP of over 600 PAH patients in the French pulmonary hypertension registry was  $55 \pm 15$  mmHg, more than double the cut off level of 25 mmHg.<sup>2</sup> The most common causes of elevated PAP are chronic lung and left sided heart disease. Elevated PAP in these conditions

occurs as the result of hypoxia, loss of the pulmonary vascular bed or elevated pulmonary venous pressure due to impaired left sided heart function. Rarely, PH occurs as the result of abnormal constriction and remodeling of the pulmonary arteries in the absence of any other underlying cardiopulmonary disease. This type of PH represents a true pulmonary artery vasculopathy, described by Dresdale et al in 1951 as primary pulmonary hypertension.<sup>2</sup> Since then, the term secondary PH has been used to describe PH associated with heart and lung disease or other identifiable causes of increased pulmonary vascular resistance. Major advances in our understanding of pulmonary vascular disease led to new terminology proposed during the 4th World Symposium on PH held in 2008 at Dana Point, California that organizes pulmonary hypertension into 5 groups to reflect the major underlying etiologies and sites of injury (Table 1).<sup>3</sup>

PAH is a disease that is characterized by hypertrophy and proliferation of pulmonary vascular smooth muscle and subsequent thickening of the medial wall of mid sized pulmonary arteries and the muscularization of more peripheral, normally non-muscularized vessels.<sup>4</sup> Marked proliferation of endothelial cells in distal pulmonary arteries is also evident and in some areas causes near obliteration of the vascular lumen with evidence of recanalized channels (plexiform lesions). These changes in both the smooth muscle and endothelial layers substantially reduce the cross sectional area of the pulmonary vascular tree.<sup>5</sup> At the same time, pulmonary vascular tone may be increased and the ability of the pulmonary circulation to dilate or recruit underutilized vessels in response to increased flow is reduced.<sup>6</sup> As remodeling of the pulmonary vascular bed progresses, PVR rises, increasing right ventricular afterload and leading to decreased functional capacity and eventually right ventricular failure.<sup>7</sup>

The diagnosis and treatment of PAH is hampered by difficulty in obtaining accurate measurements of PAP. Unlike the systemic circulation where intravascular pressure is easily measured by sphygmometry, there is no accurate, readily available noninvasive test for measuring PAP. Right heart catheterization (RHC) provides the direct measurement of PAP and is vital in the appropriate diagnosis and evaluation of unexplained PH, but it is invasive and expensive and not amenable to repeated measurements that are necessary to monitor disease progression. As a result, the clinician must rely on a combination of indirect assessments of PAP and cardiac output such as transthoracic echocardiography, 6-minute walking distance, and WHO functional class. A simple noninvasive, but objective measure of right

**Table I** Updated clinical classification of pulmonary hypertension (Dana Point 2008)<sup>3</sup>

I. Pulmonary arterial hypertension (PAH)
I.1. Idiopathic PAH
I.2. Heritable
I.2.1. BMPR2
I.2.2. ALK1, endoglin (with or without hereditary hemorrhagic telangiectasia)
I.2.3. Unknown
I.3. Drug- and toxin-induced
I.4. Associated with
I.4.1. Connective tissue diseases
I.4.2. HIV infection
I.4.3. Portal hypertension
I.4.4. Congenital heart diseases
I.4.5. Schistosomiasis
I.4.6. Chronic hemolytic anemia
I.5. Persistent pulmonary hypertension of the newborn
II. Pulmonary Veno-Occlusive Disease (PVOD) and/or Pulmonary Capillary Hemangiomas (PCH)
III. Pulmonary hypertension owing to left heart disease
2.1. Systolic dysfunction
2.2. Diastolic dysfunction
2.3. Valvular disease
IV. Pulmonary hypertension owing to lung diseases and/or hypoxia
3.1. Chronic obstructive pulmonary disease
3.2. Interstitial lung disease
3.3. Other pulmonary diseases with mixed restrictive and obstructive pattern
3.4. Sleep-disordered breathing
3.5. Alveolar hypoventilation disorders
3.6. Chronic exposure to high altitude
3.7. Developmental abnormalities
V. Chronic thromboembolic pulmonary hypertension (CTEPH)
VI. Pulmonary hypertension with unclear multifactorial mechanisms
5.1. Hematologic disorders: myeloproliferative disorders, splenectomy
5.2. Systemic disorders: sarcoidosis, pulmonary Langerhans cell histiocytosis; lymphangioleiomyomatosis, neurofibromatosis, vasculitis
5.3. Metabolic disorders: glycogen storage disease, Gaucher disease, thyroid disorders
5.4. Others: tumoral obstruction, fibrosing mediastinitis, chronic renal failure on dialysis

**Abbreviations:** ALK1, activin receptor-like kinase type I; BMPR2, bone morphogenetic protein receptor type.

ventricular afterload would contribute significantly to the management of PAH patients. Biomarkers that correlate with PAP and/or right sided filling pressures would allow the practitioner insight into how a patient's disease is progressing and allow the physician to evaluate the effect of pulmonary vasodilator therapy. Plasma BNP levels rise in response to

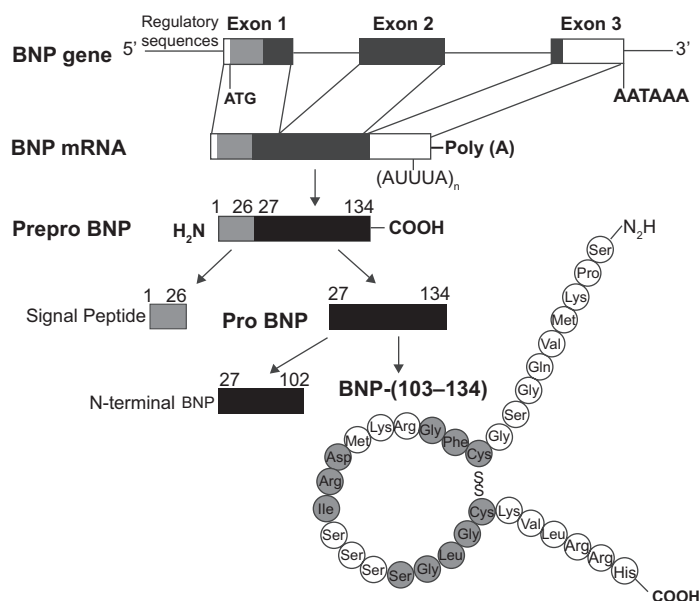
right as well as left atrial or ventricular overload and may be an important tool for assessing right ventricular performance in PAH.

The treatment of PAH has grown considerably in the last decade. Presently, 7 different medications belonging to 3 distinct pharmaceutical classes are available for the management of PAH. However, in the great majority of patients, none of these treatments are capable of restoring normal pulmonary hemodynamics and exercise capacity and have limited ability to improve right heart function. Furthermore, all 3 classes focus primarily on vasodilation or remodeling of the pulmonary circulation. Additional therapies are needed that aid the right ventricle in maintaining adequate systolic function and inhibit maladaptive hypertrophic changes in response to persistent elevation in afterload.

## Brain natriuretic peptide BNP release

Human BNP is a 32-amino acid polypeptide secreted predominantly by the cardiac atria and ventricles. BNP is synthesized as a prohormone of 134 residues that is cleaved to yield a 108-amino-acid prohormone (proBNP1 – 108). The prohormone is subsequently cleaved by an unknown protease to an inactive 76-residue amino-terminal (NT) fragment known as NT-BNP and the 32 amino-acid, carboxyl-terminal 32 fragment that is the biologically active peptide.<sup>8–12</sup> The size of the final, fully processed BNP fragment varies between species. Human, pig, and dog BNP contain 32 amino acids,<sup>13,14</sup> whereas rat and mouse BNP is a 45 amino acid peptide.<sup>8,10,11,15–17</sup> Compared to the sequence homology of atrial natriuretic peptide (ANP) and C-type natriuretic peptide (CNP) that are tightly conserved, the sequence homology of BNP differs across species both in size and amino acid sequence<sup>17,18</sup> (Figure 2). This sequence variability may explain, in part, the variations of BNP biological activity in different species.

Although originally discovered in porcine brain, human BNP is expressed primarily in the cardiac atria and ventricles. The tissue concentration of BNP in the ventricle constitutes only about 1% of the atrium. However, the total BNP mRNA content is almost 3-fold higher in the ventricle.<sup>19</sup> The majority of circulating BNP (>60%) is released from ventricular tissues.<sup>19–22</sup> This is in distinction from ANP, the great majority of which is synthesized and released by the cardiac atria.<sup>19</sup> These findings have prompted some investigators to suggest that ANP and BNP make up a dual cardiac peptide system with ANP the primary atrial peptide and BNP the primary ventricular peptide. In fact, some investigators have suggested



**Figure 1** Structure of the gene and the biosynthetic pathway of human brain natriuretic peptide (BNP). The major storage form of BNP in the heart is the cleaved mature peptide, although in atrial tissue also prohormones may be stored. Reproduced with permission from Barr CS, Rhodes P, Struthers AD. C-type natriuretic peptide. *Peptides*. 1996;17(7):1243–1251.<sup>197</sup> Copyright © 1996 Elsevier.

that BNP be renamed to ventricular natriuretic peptide to denote its greater importance in the cardiovascular system and to distinguish it from ANP.

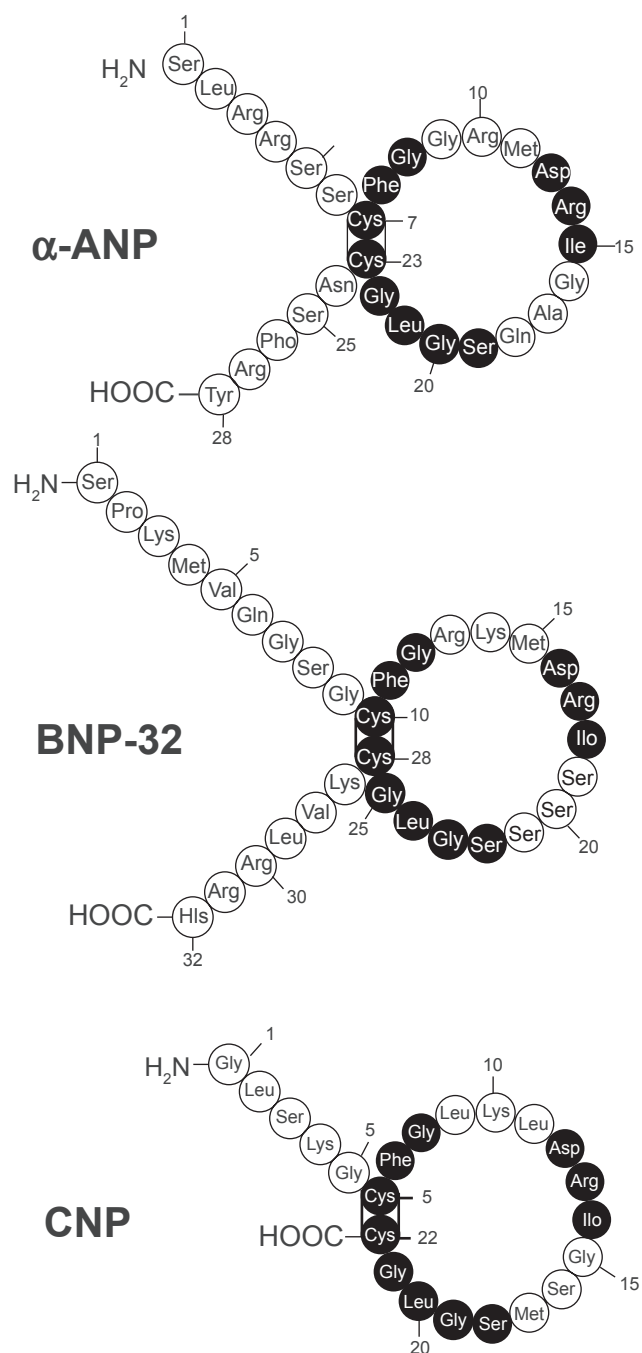
Both atrial and ventricular BNP expression is induced within 1 hr of myocardial stretch from increases in venous volume or arterial pressure.<sup>23,24</sup> Other stimuli that induce cardiac BNP secretion include hypoxia, ischemia, and states of nephrosis and cirrhosis that are associated with an increase in central blood volume.<sup>25–27</sup> Several neuroendocrine mechanisms also mediate cardiac BNP release.<sup>28,29</sup> For example,  $\alpha$ -adrenergic stimulation with phenylephrine results in increases in both BNP gene expression and BNP secretion, whereas myocardial stretch and endothelin-1 (ET-1) induce a marked increase in BNP gene expression alone.<sup>29,30</sup>

Atrial myocytes store pre-synthesized ANP and BNP in secretory granules and release them via a regulated pathway.<sup>31</sup> Unlike ANP, however, most studies in normal subjects show no significant change in BNP levels in response to exercise.<sup>32–35</sup> In patients with cardiac disease such as ischemia, hypertension or heart transplant recipients, exercise does induce a significant increase in BNP secretion.<sup>32–37</sup> These findings suggest that only under pathologic conditions is atrial BNP secreted during exercise-induced heart stress via a regulatory pathway in quantities sufficient to increase circulating levels. Ventricular myocytes also synthesize and secrete both peptides, but through a constitutive pathway.<sup>20</sup> Hence, it is possible that BNP is secreted by ventricular tissue during exercise. Most of the immediate release of BNP likely

derives from atrial stores followed later by gene transcription and new protein synthesis from atrial and ventricular sources. BNP mRNA has a faster turnover rate than ANP due to the presence of mRNA-destabilizing AUUUA repeats in the 3'-untranslated region. This allows the synthesis and secretion of BNP in the ventricles to be closely controlled by mRNA levels according to changing amounts of load on the myocardium. The 5'-untranslated region of the gene possesses shear stress response elements and activation of MAPK and nuclear factor  $\kappa$ B (NF- $\kappa$ B) is thought to be important in upregulating expression.<sup>38</sup> The nuclear transcription factor, GATA 4, plays a dominant role in regulating this process.<sup>39–41</sup> The ability of BNP to be secreted from the heart in response to neurohormonal as well as hemodynamic stimuli and the rapid induction of BNP gene expression in response to ventricular strain make it a robust biomarker of cardiac stress and facilitates its use as a clinical tool.

## BNP receptors

The diverse biological effects of BNP are mediated primarily by a membrane-bound particulate guanylyl cyclase receptor called natriuretic peptide receptor-A (NPR-A). NPR-A is widely expressed in the cardiovascular system including the cardiac atria and the ventricles as well as the aorta and peripheral vasculature, lungs, kidney, skin, platelets, and presynaptic sympathetic fibers.<sup>42–47</sup> NPR-A has much higher affinity for ANP and BNP, than for CNP, but BNP is approximately 10-fold less potent than ANP in



**Figure 2** Amino acid structure of the 3 natriuretic peptides, atrial, brain and c-type natriuretic peptide (ANP, BNP, CNP). All 3 peptides share a similar 17 amino acid loop. Shaded circles represent amino acid sequence that is identical in each peptide. Reproduced with permission from Barr CS, Rhodes P, Struthers AD. C-type natriuretic peptide. *Peptides*. 1996;17(7):1243–1251.<sup>197</sup> Copyright © 1996 Elsevier.

generating particulate cGMP.<sup>48–50</sup> Another membrane bound guanylyl-cyclase linked receptor, NPR-B, demonstrates high affinity for CNP and much lower affinity for ANP and BNP.<sup>49,51</sup> Both NPR-A and NPR-B function as particulate guanylyl cyclases<sup>43,52</sup> distinct from the cytoplasmic soluble guanylyl cyclase stimulated by nitric oxide (NO).<sup>53</sup>

Ligand binding to NPR-A or NPR-B activates the intracellular guanylyl cyclase domain of the receptor and catalyzes the synthesis of cGMP. There are three known proteins that bind cGMP with high affinity and act as downstream targets: cGMP-dependent protein kinase (PKG), cGMP binding phosphodiesterases (PDEs), and cyclic nucleotide-gated ion channels. PKG appears to be the principal intracellular mediator of cGMP signals.<sup>54–58</sup> Natriuretic peptide-induced elevation of intracellular cGMP causes a binding-dependent activation of PKG leading to the catalytic transfer of phosphate from ATP to a serine or threonine residue of target proteins. The phosphorylated proteins then determine the translation of the extracellular stimulus into a specific biological function.<sup>59</sup>

Cyclic nucleotides such as cGMP and cAMP are metabolized by a family of phosphodiesterases (PDE).<sup>60–63</sup> PDE-1, -2, -3, -10, and -11 degrade both cGMP and cAMP; PDE-4, -7, and -8 specifically hydrolyze cAMP; whereas PDE-5, -6, and -9 are highly selective for cGMP.<sup>64</sup> In vascular tissue, PDE-3 and -4 are responsible for most of the degradation of cAMP and PDE-5 is the major metabolic enzyme for cGMP. Despite the relative selectivity of PDE isoforms, both cAMP and cGMP can act as substrate for the other's primary PDE and thereby impede its metabolism. There is clear evidence that this interaction between cyclic nucleotides and PDEs allows for crosstalk and integration between different pathways.<sup>60</sup> PDE-5 is expressed in most vascular smooth muscle, is found in high concentration in the lung, and is increased in the lungs in experimental models of pulmonary hypertension.<sup>65,66</sup> PDE-I in heart, lungs, brain, and smooth muscle is activated by binding of Ca<sup>2+</sup>-calmodulin to cause a decrease in cyclic nucleotide (cGMP or cAMP) concentration. Some PDEs are regulated allosterically by cGMP. For instance, in addition to its catalytic domain, PDE-5 has additional binding sites for both cGMP and PKG that serve to stabilize the enzyme and enhance cGMP metabolism resulting in a feed-forward mechanism

## BNP metabolism and clearance

Circulating natriuretic peptides are removed or metabolized via two major routes. One involves binding of the natriuretic peptide to natriuretic peptide receptor-C (NPR-C). NPR-C has an extracellular domain similar to that of NPR-A and NPR-B, but no intracellular guanylyl cyclase domain. Binding to the receptor is followed by endocytosis and lysosomal degradation,<sup>67</sup> NPR-C has high affinity for all natriuretic peptides with a rank order of ANP ≥ CNP > BNP in both humans and rats<sup>51,68</sup> and has been proposed to function

primarily as a clearance receptor,<sup>46,69</sup> although other biologic effects have been described.<sup>42</sup>

The second mode of metabolism is peptide cleavage and inactivation by neutral endopeptidase 24.11 (NEP).<sup>70</sup> This membrane bound zinc metalloproteinase is found on the luminal surface of endothelial cells and is particularly concentrated at the brush border membranes in the proximal tubule of the kidney. NEP inactivates and degrades both ANP and BNP,<sup>70,71</sup> by opening their ring structures.<sup>72</sup> The relative importance of the two clearance mechanisms is controversial, but it has been shown that in chronic heart failure, the clearance receptor NPR-C may play a lesser role than NEP in natriuretic peptide metabolism.<sup>73</sup> Renal excretion of the peptides has also been described,<sup>74</sup> but probably plays a minor role in regulating circulating concentrations of natriuretic peptides.

The circulating BNP level measured at any given time point is the result of the balance between BNP secretion and degradation. BNP has a plasma half-life of about 20 minutes, which is substantially longer than the 4 minute half-life reported for ANP.<sup>75</sup> The longer half-life may be the result of more resistance to degradation by NEPs and less avid binding to NPR-C than ANP.<sup>76</sup> Healthy individuals have plasma BNP concentrations around 1 fmol/ml (3.5 pg/ml) or approximately one-tenth that of ANP.<sup>77</sup> However, plasma BNP levels in congestive heart failure patients can be 200- to 300-fold higher. The enormous range of plasma BNP concentrations between healthy subjects and patients with heart failure and the rapid induction of BNP expression in response to acute overload or ischemia make it well suited to serve as an indicator of elevated myocardial loading and cardiac stress.<sup>78,79</sup> The amino terminal fragment of proBNP (NT-proBNP) is less susceptible to degradation by NEP and is not cleared by NPR-C. As such, it may represent a more reliable indicator of cardiac BNP expression and secretion than circulating BNP levels. In fact, some studies suggest that plasma NT-proBNP levels may be a more sensitive and specific marker of cardiovascular disease than BNP.<sup>80,81</sup> In patients, with left ventricular (LV) dysfunction NT-proBNP levels are up to 10 times higher than BNP.<sup>82</sup> Unfortunately, NT-proBNP assays are not readily available in most clinical laboratories. A number of factors have been associated with higher BNP levels in addition to ventricular wall stress. Both BNP and NT-proBNP increase with age independent of the increased incidence of diastolic dysfunction.<sup>83</sup> BNP and NT-proBNP levels are also higher in women than men at any age.<sup>84</sup> The association between BNP and renal function is complex. Patients with chronic renal disease

tend to have higher atrial pressure, systemic pressure, and ventricular mass, all of which would be expected to increase BNP synthesis and secretion. As mentioned previously, renal excretion of BNP has been described, but alterations in circulating levels in patients with renal disease are more likely related to decreased clearance by NPR-C and endopeptidases. NT-proBNP, in contrast, is predominantly excreted by the kidney, although plasma NT-proBNP, appears to maintain its clinical utility even in the presence of renal dysfunction.<sup>85,86</sup>

## Use of BNP as a biomarker in PH

### Pulmonary arterial pressure and RV strain

As in systemic hypertension, PAH is a hemodynamic diagnosis. The clinical manifestations of PAH and RV failure are non-specific, and are usually not evident on exam or general diagnostic tests such as chest roentgenogram and electrocardiogram until the disease is fairly advanced. Circulating levels of ANP and BNP correlate with mPAP,<sup>87</sup> but elevations in BNP levels are usually not seen until PAP is high enough to cause right ventricular strain. Therefore, BNP has limited diagnostic value as a screening tool for excluding PAH in populations at low risk for the disease. In select patient groups however, where the risk of PAH is substantially increased, such as those with connective tissue disease, portal hypertension, congenital systemic to pulmonary shunts, or a family history of IPAH, elevated BNP may help identify patients in whom further testing is warranted. For example, BNP may prove useful as a screening test for PAH in systemic sclerosis, where the prevalence of PH has been reported to be 12% to 35% and where PAH treatment has been shown to be beneficial.<sup>88-92</sup> In 2003, Allanore et al obtained plasma NT-proBNP levels and echocardiographic estimates of peak PAP in 40 consecutive patients hospitalized for follow-up care of scleroderma (limited or diffuse disease).<sup>93</sup> All patients were without symptoms of heart failure, had normal LV ejection fraction, and a creatinine clearance >58 mL/min. At baseline, 10 patients (25%) were found to have PAH as defined by a peak right ventricular systolic pressure (RVSP) > 40 mmHg. Thirteen patients had high NT-proBNP values (adjusted for age), including all 10 patients classified as PAH by TTE. There was a moderate correlation between peak RVSP and NT-proBNP level ( $r = 0.44$ ,  $P = 0.006$ ). A high NT-proBNP concentration (cutoff values supplied by the manufacturer and defined as above the 97th percentile) identified PH with a sensitivity of 90%, specificity of 90%, positive predictive value of 69%, and negative predictive value of 96%. Although the sensitivity and specificity of transthoracic echo

for detecting PH is only about 90% and 75% respectively, subsequent studies using right heart catheterization to define PAH have obtained similar results.<sup>94</sup> Mukerjee et al identified found PAH by RHC in 23 of 49 patients with SSc.<sup>95</sup> The mean value of NT-proBNP in patients with PAH was  $3365 \pm 1095$  pg/mL compared to  $347 \pm 174$  pg/mL for patients without PAH. There was a statistically significant correlation ( $P < 0.05$ ) between NT-proBNP values and (i) mPAP ( $r = 0.53$ ), (ii) right ventricular end diastolic pressure ( $r = 0.59$ ) and (iii) PVR ( $r = 0.49$ ). Receiver operator characteristic curve analysis showed that a cut-off value of 395.34 pg/mL had a sensitivity of 0.69 and specificity of 1.0. The same group performed a larger study to assess prospectively the specificity of 395 pg/mL in a larger population and to evaluate the prognostic value of NT-proBNP in a homogeneous group of patients with scleroderma associated PAH (SSc-PAH).<sup>94</sup> The study population included 68 individuals with PAH mPAP  $> 25$  mmHg and PCWP  $< 15$  mmHg and 41 individuals without PAH. The patients without PAH had a lower mean NT-proBNP level than those with SSc-PAH baseline NT-proBNP levels were correlated positively with mPAP ( $r = 0.62$ ;  $P < 0.0001$ ), PVR ( $r = 0.81$ ;  $P < 0.0001$ ), and inversely with 6 minute walking distance (6MWD) ( $r = -0.46$ ;  $P < 0.0001$ ). At an NT-proBNP level of 395 pg/mL, the sensitivity and specificity for predicting the presence of SSc-PAH were 56% and 95% respectively. Thus NT-proBNP estimation in systemic sclerosis-related PAH is a potentially useful diagnostic tool. Similarly, subjects with lung fibrosis and elevated BNP levels ( $n = 20$ ) had significantly higher PAP than those with normal BNP levels (mean pulmonary arterial pressure  $(40.85 \pm 3.2$  mmHg vs  $23.42 \pm 1.44$  mmHg, ( $P < 0.001$ )).<sup>96</sup>

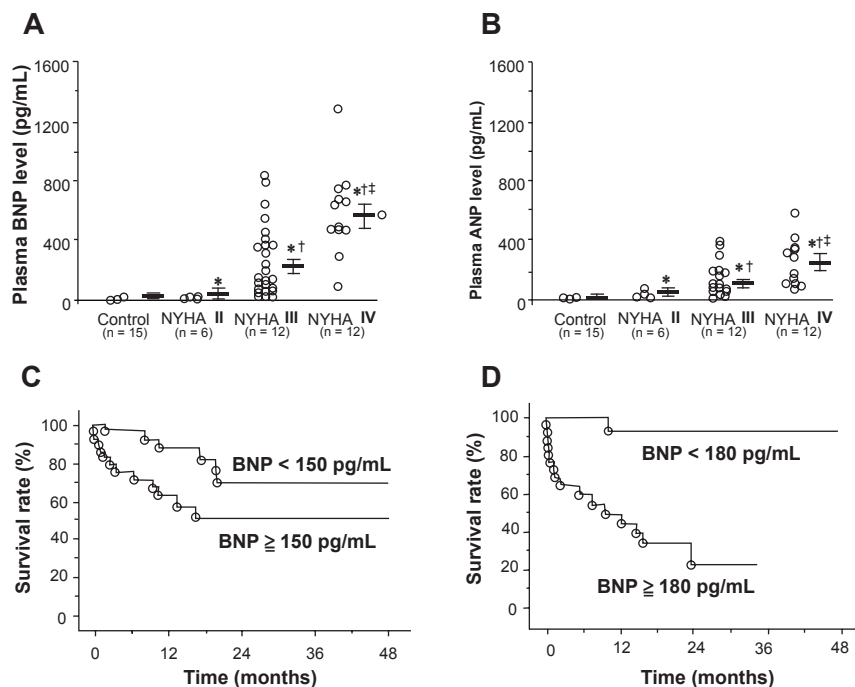
Although TTE is the most commonly used test to diagnose or exclude PH, its specificity is low in early disease.<sup>95</sup> For example, a RVSP  $> 40$  mmHg commonly used cut off level to separate normal from elevated PAP has been found in 6% of individuals older than 50 years of age and in 5% of patients with a body mass index  $> 30$  kg/m<sup>2</sup> in the absence of pulmonary hypertension.<sup>97</sup> In patients such as these, an elevated plasma BNP level may improve the diagnostic accuracy of TTE in screening for PAH. Two recent studies have demonstrated the potential utility of exercise echocardiography in identifying individuals at risk for future development of PH in at risk populations.<sup>98,99</sup> It is interesting to speculate whether increased levels of BNP during exercise would improve the predictive accuracy of this emerging technology.

In addition to PAP, plasma BNP levels are a reliable marker of right heart strain, which is a significant predictor of

morbidity and mortality in PAH and other forms of pulmonary disease causing pulmonary hypertension.<sup>100,101</sup> Elevated BNP is not specific for right or left sided heart disease, but in the absence of left sided heart disease, may prove to be useful adjunct to echocardiography in evaluating right heart stress. In patients with right ventricular pressure overload associated with idiopathic PAH or CTEPH, BNP levels were significantly higher than in controls ( $294 \pm 72$  pg/mL versus  $48 \pm 14$  pg/mL ( $P < 0.05$ )).<sup>100</sup> In this study, BNP levels correlated with, right ventricular end diastolic pressure ( $r = 0.76$ ) as well as mPAP ( $r = 0.73$ ) and total pulmonary resistance (TPR) ( $r = 0.79$ ). Long-term treatment with either prostacyclin or prostaglandin E reduced TPR (from 23 to 15 Wood units) and cut plasma BNP by more than half ( $315 \pm 120$  to  $144 \pm 54$  pg/mL). BNP levels have also been shown to be higher in acute pulmonary embolism and to fall following thromboendarterectomy in patients with CTEPH.<sup>100,102</sup> Conversely, right ventricular dilation is exceedingly uncommon in acute PE when plasma troponin and BNP levels are normal.<sup>103</sup> Souza et al demonstrated a close correlation between PVR and NT-proBNP ( $r = 0.80$ ,  $P < 0.001$ ) in 42 patients with IPAH.<sup>104</sup> BNP levels also correlate with functional capacity in PAH. In one study, BNP levels were inversely correlated with 6MWD ( $r = -0.70$ ;  $P < 0.001$ ) and peak VO<sub>2</sub> ( $r = -0.61$ ;  $P < 0.01$ ), and positive correlation with WHO functional class ( $r = 0.79$ ;  $P < 0.001$ ) in 42 patients with IPAH.<sup>96</sup>

## Survival and response to therapy

Plasma BNP levels have been shown to have significant prognostic capabilities in patients with PAH.<sup>105</sup> One study measured plasma BNP levels in 60 patients with IPAH at initial right heart catheterization and compared the prognostic significance of baseline and follow-up BNP levels with clinical, echocardiographic, and hemodynamic variables. Patients with renal insufficiency were excluded. Measurements were repeated in 53 patients at 3 months. Plasma BNP was increased at baseline and correlated positively with New York Heart Association (NYHA) functional class (Figure 3), mPAP, mean RAP, and TPR, and correlated negatively with cardiac output. Mean follow-up averaged 24 months, during which time 18 patients died of cardiopulmonary causes. Among the noninvasive baseline parameters studied, only plasma BNP was an independent predictor of mortality by multivariate analysis. Kaplan-Meier analysis demonstrated that follow-up BNP provided a more distinct separation of survival curves than did baseline BNP measurement (Figure 3). Survival was markedly improved in those patients with a follow-up BNP level below 180 pg/mL.



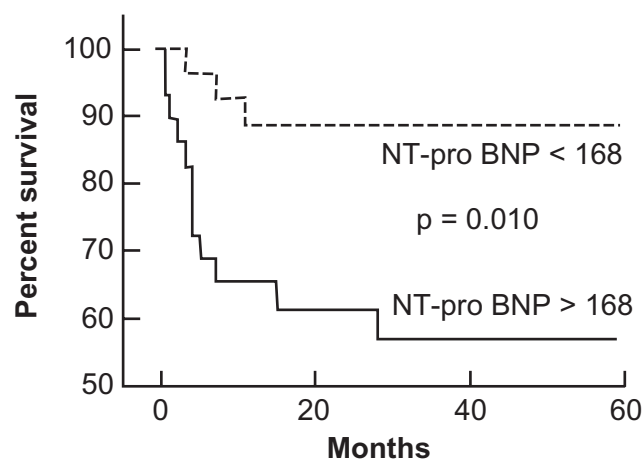
**Figure 3** **A**) and **B**) Correlation between plasma brain natriuretic peptide (BNP) and atrial natriuretic peptide (ANP) and New York Heart Association (NYHA) functional class in patients with pulmonary arterial hypertension. **C**) Effect of plasma BNP levels at time of diagnosis on survival in patients with pulmonary arterial hypertension. **D**) Effect of plasma BNP levels after treatment on survival in the same patients. Reproduced with permission from Nagaya N, et al. Plasma brain natriuretic peptide as a prognostic indicator in patients with primary pulmonary hypertension. *Circulation*. 2000;102(8): 865–870.<sup>105</sup> Copyright © 2002 Elsevier.

In fact, receiver operating characteristic analysis suggested that baseline BNP was at least equal to mPAP and superior to CO in predicting mortality. These findings were substantiated by Fijalkowska et al who demonstrated in their 36 IPAH patients that a serum NT-proBNP level of  $\geq 1400$  pg/mL identified patient with a poor long term prognosis estimated by Kaplan-Meier cumulative survival curves.<sup>106</sup>

Plasma NT-proBNP levels have also been shown to predict survival in patients with PH (Figure 4). The use of NT-proBNP to predict survival was assessed in 68 patients with SSc-PAH<sup>94</sup> over a mean duration of 10 months (range 1 to 18 months). The mean NT-proBNP level in patients without PAH was significantly lower than in those with SSc-PAH ( $P = 0.0002$ ). For every order of magnitude increase in NT-proBNP level in patients with PAH, the risk of death increased 4-fold ( $P = 0.002$  for baseline level and  $P = 0.006$  for follow-up level). Baseline NT-proBNP levels correlated directly with mPAP ( $r = 0.62$ ;  $P < 0.0001$ ), PVR ( $r = 0.81$ ;  $P < 0.0001$ ), and inversely with 6MWD ( $r = -0.46$ ;  $P < 0.0001$ ). Plasma NT-proBNP levels also identified differences in functional class among patients with SSc-PAH. The 13 patients (19%) in WHO functional class II had mean N-TproBNP levels of  $325 \pm 388$  pg/mL, whereas the 53 patients (78%) in WHO

classes III and IV had mean NT-proBNP levels that were 5-fold higher ( $1677 \pm 2835$  pg/mL,  $P = 0.02$ ).

The correlation of circulating BNP levels with pulmonary hemodynamics, right heart strain and survival has generated interest in using it as a noninvasive assessment of response



**Figure 4** Effect of plasma N-terminal pro-brain natriuretic peptide (NT-proBNP) levels on survival in patients with pulmonary arterial hypertension. Reproduced with permission from Andreassen AK, Wergeland R, Simonsen S, Geiran O, Guevara C, Ueland T. N-terminal pro-B-type natriuretic peptide as an indicator of disease severity in a heterogeneous group of patients with chronic precapillary pulmonary hypertension. *Am J Cardiol*. 2006;98:528–529.<sup>198</sup> Copyright © 2002 Elsevier.



to therapy. In an early study of nine patients with IPAH who underwent prostaglandin therapy,<sup>87</sup> plasma BNP levels fell in conjunction with TPR after a mean follow-up of 35 days (TPR  $23 \pm 4$  to  $15 \pm 3$  Wood units,  $P < 0.05$ ; plasma BNP  $315 \pm 120$  to  $144 \pm 54$  pg/mL  $P < 0.05$ ). In a larger follow-up study by Nagaya and colleagues, BNP measurements were repeated after 3 months of vasodilator therapy in 49 patients with idiopathic PAH.<sup>105</sup> Changes in plasma BNP were associated with changes in RV end-diastolic pressure and TPR but not mPAP or right ventricular dimension. During prostacyclin therapy, plasma BNP significantly decreased in survivors but increased in non-survivors. Park and colleagues studied 20 patients with PAH on epoprostenol and similarly found that BNP may help predict those who are refractory to treatment.<sup>107</sup> Leuchte et al found that BNP levels parallel changes in pulmonary hemodynamics and functional parameters, including 6MWD, in 30 patients with PAH.<sup>108</sup> Progression of the disease was accompanied by an increase in BNP levels, whereas improvement of pulmonary hemodynamics and functional status was accompanied by a significant decline in plasma BNP concentrations. Findings from studies such as these suggest that serial plasma BNP could be useful in monitoring the efficacy of therapy in PAH patients and this approach is reflected in recent guidelines from the American College of Cardiology that suggest the use of plasma BNP levels in assessing the risk of deterioration in patients with PAH.<sup>109</sup> Although one-time measures of circulating BNP may be difficult to interpret, serial BNP testing may provide a non-invasive measure to help identify patients who are failing their current therapeutic regime and predict RV decompensation before it becomes clinically apparent. This is supported by the finding that higher BNP levels at presentation with pulmonary embolism is associated with increased mortality in two recent studies with large cohorts of patients.<sup>110,111</sup> These findings suggest that in pulmonary embolism at least, BNP can help identify patients with increased right ventricular strain at higher risk for acute decompensation.

### **Effect of BNP on right ventricle and pulmonary circulation**

In addition to being a biomarker of elevated PAP and right heart failure, BNP has many biologic properties that could be beneficial in combating progressive pulmonary vascular disease. These properties have piqued the interest of investigators and clinicians in BNP as a potential therapeutic target in the management of PAH. Before discussing therapeutic approaches, a review of the current understanding of

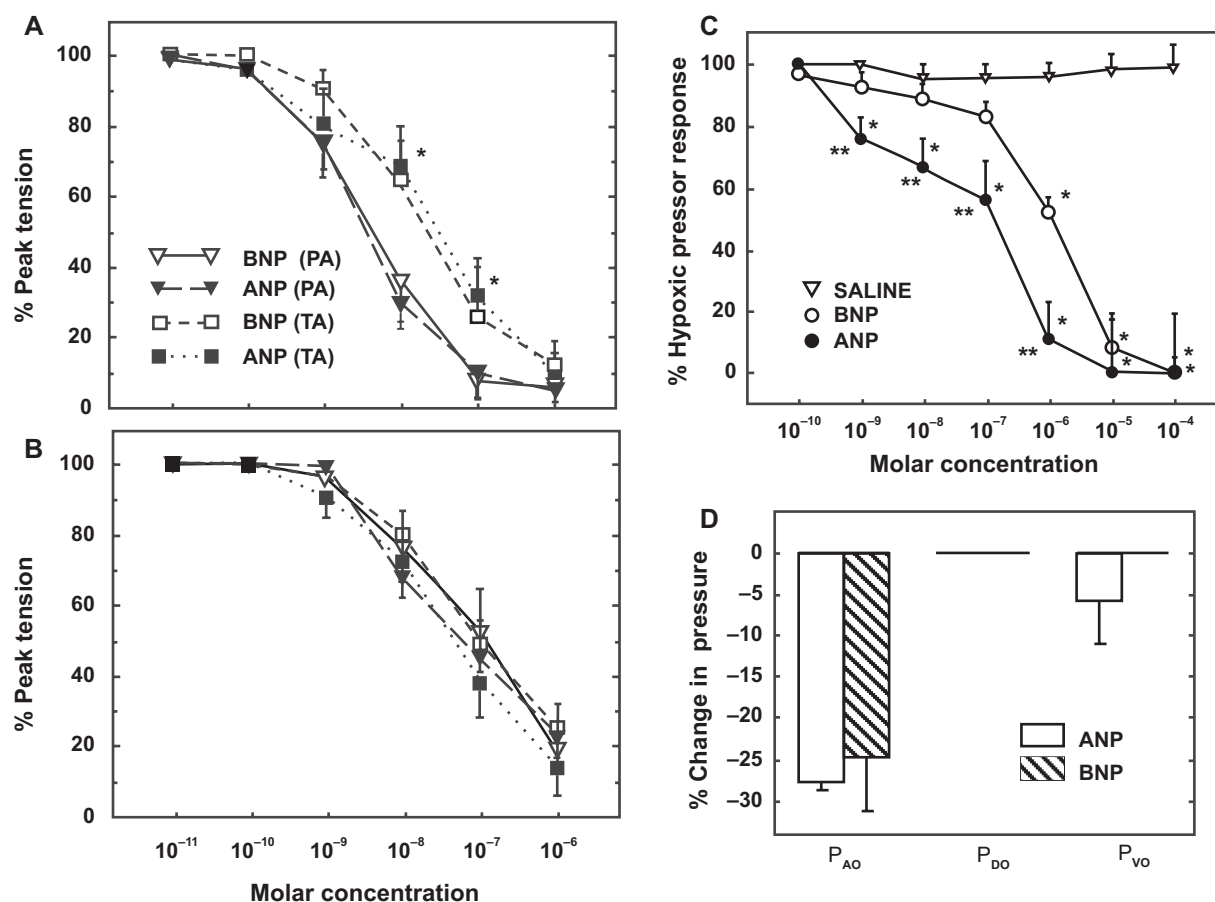
BNP effects on the heart and pulmonary circulation is presented.

### **Effect on PA pressure**

BNP relaxes precontracted isolated pulmonary arteries and blunts hypoxic pressor responses in isolated perfused rat lungs with a potency similar to that of ANP (Figure 5).<sup>112</sup> Although circulating BNP levels are an order of magnitude lower than ANP, the percent increases in plasma BNP levels and right heart BNP expression during exposure to chronic hypoxia are similar for both peptides.<sup>112</sup> These data suggest that BNP, in concert with ANP, may play a role in modulating pulmonary hypertensive responses and protecting the right heart from the development of hypoxic pulmonary hypertension. In one study, BNP was more effective than ANP at blunting pulmonary hypertension in rats exposed to 3 weeks of hypoxia.<sup>113</sup> Furthermore, mice deficient in NPR-A, the receptor that mediates the pulmonary vasodilator effects of both ANP and BNP develop greater pulmonary hypertension and right ventricular hypertrophy than wild type mice after exposure to chronic hypoxia.<sup>114,115</sup> These studies suggest that ANP and BNP signaling through NPR-A play important roles in regulating pulmonary vascular tone and in the pathogenesis of hypoxia-induced pulmonary hypertension. Interestingly, chronic hypoxia causes a significant downregulation of NPR-C expression in pulmonary vascular smooth muscle in vitro and a marked decrease in pulmonary clearance of ANP in intact rats.<sup>116,117</sup> This may represent a compensatory response of the lungs to increase the circulating levels of natriuretic peptides in the pulmonary vasculature and attenuate the effect of hypoxic pulmonary hypertension.

### **Effect on pulmonary vascular smooth muscle proliferation**

In addition to its vasorelaxant effects, BNP has antiproliferative effects on vascular smooth muscle cells in culture and thus may play a role in inhibiting pulmonary vascular remodeling.<sup>118</sup> ANP has been shown to inhibit the effect of several growth factors on the proliferation of smooth muscle cells isolated from the systemic vascular bed, however few studies have examined the effect of the natriuretic peptides on inhibition of pulmonary vascular smooth muscle cell proliferation. Arjona et al found that BNP inhibited proliferation of serum stimulated pulmonary vascular smooth muscle cells and increased generation of intracellular cGMP.<sup>119</sup> In preliminary studies we have shown that ANP inhibits PDGF-induced proliferation of pulmonary vascular smooth muscle cells as well and that this effect is absent in cells isolated from NPR-A knock



**Figure 5** Vasodilator effect of atrial and brain natriuretic peptide (ANP, BNP) on pulmonary arterial rings isolated from normoxic **A**) and hypoxia-adapted rats **B**) and preconstricted with phenylephrine. **C**) Vasodilator effect of ANP and BNP on acute hypoxic pulmonary vasoconstriction in isolated rat lungs exposed to acute hypoxia. **D**) Distribution of change in pulmonary vascular resistance following administration of ANP and BNP in isolated rat lungs exposed to acute hypoxia. Pressure in the pulmonary arteries, veins, and capillaries were assessed by occluding the pulmonary artery catheter ( $P_{AO}$ ), pulmonary venous catheter ( $P_{VO}$ ) and both catheters (double occlusion,  $P_{DO}$ ), respectively.<sup>112</sup>  
**Abbreviations:** PA, pulmonary artery; TA, thoracic aorta.

out mice.<sup>120</sup> Together, these studies suggest that ANP and BNP are capable of modulating proliferation of pulmonary vascular smooth muscle via signaling through NPR-A and elevation of cGMP. These findings are consistent with reports showing that pulmonary vascular remodeling is reduced in rats given ANP or BNP infusion during exposure to chronic hypoxia<sup>113, 121</sup> and that mice with gene-targeted disruption of ANP or NPR-A have greater pulmonary vascular remodeling under normoxic conditions and after hypoxic exposure than wild-type littermates.<sup>114, 115, 122</sup>

### Effect on right ventricular hypertrophy

The natriuretic peptide system also plays an important role in inhibiting maladaptive hypertrophic responses in cardiac myocytes. Numerous studies have demonstrated that cardiac hypertrophy in response to a sustained increased in ventricular afterload is associated with a marked increase in ventricular ANP and BNP mRNA expression. This response

is seen in both the right and left ventricle in response to a variety of hypertrophic stimuli such as chronic hypoxia, monocrotaline-induced pulmonary hypertension or aortic banding.<sup>122–124</sup> Initially, this finding was attributed to the activation of a program of fetal genes during hypertrophy of cardiac myocytes.<sup>125</sup> As a result, increased ventricular expression of ANP and BNP was considered a marker of maladaptive cardiac hypertrophy. However, other studies have shown that the degree of hypertrophy is inversely related to ANP expression and genetic disruption of ANP, BNP or NPR-A results in increased biventricular cardiac mass under baseline conditions and exaggerated hypertrophic responses to aortic banding or chronic hypoxia.<sup>114, 122, 124, 125</sup> In addition, hearts of mice with disrupted ANP/BNP-NPR-A signaling have increased deposition of collagen and decreased contractility suggesting that the natriuretic peptides play important roles in mediating maladaptive cardiac hypertrophic effects.<sup>126</sup>

Although BNP and ANP appear to signal through the same receptor, mice with targeted deletion of BNP exhibit a different phenotype than ANP-deficient mice. Mice without ANP are hypertensive at sexual maturity and this has not been demonstrated in mice without BNP. Thus, gene deletion experiments suggest that ANP and BNP have distinct physiologic effects and that ANP and BNP may play complementary roles in the regulation of cardiovascular homeostasis through NPR-A.

BNP has been shown to inhibit proliferation of cardiac fibroblasts in culture.<sup>127</sup> This observation was validated *in vivo* when ventricular pressure overload caused by abdominal aortic banding induced an increase of multi-focal fibrotic lesions in the ventricles in mice lacking BNP, but not in wild-type mice, despite a similar increase in ventricular mass between the two genotypes.<sup>128</sup> In this study, ventricular fibrosis was associated with a marked increase in expression of angiotensin converting enzyme, transforming growth factor- $\beta$ 3 (TGF $\beta$ 3), and pro- $\alpha$ 1(I) collagen, factors that are implicated in the generation and progression of ventricular fibrosis.<sup>128</sup> Finally, BNP has been shown to almost completely abolish TGF $\beta$ -induced increases in collagen secretion from cardiac fibroblasts.<sup>129</sup> Taken together, these results indicate that BNP exerts antifibrotic as well as antihypertrophic effects on the heart and plays an important role in mitigating maladaptive cardiac hypertrophic responses.

The mechanism involved in BNP regulation of fibroblasts is unclear. Kapoun et al found that BNP treatment inhibited TGF $\beta$ -induced expression of genes related to fibrosis, (collagen 1, fibronectin, CTGF, PAI-1, and TIMP3, myofibroblast conversion (alpha-smooth muscle actin 2 and nonmuscle myosin heavy chain), proliferation (PDGFA, IGF1, FGF18, and IGFBP10), and inflammation (COX2, IL6, TNF $\alpha$ -induced protein 6, and TNF superfamily).<sup>129</sup> Other studies have found that ANP and BNP modulate the matrix metalloproteinases, enzymes that play important roles in regulating the production of extracellular matrix proteins.<sup>129-131</sup>

It can be difficult to separate the antihypertrophic effect of BNP from its vasorelaxant properties. Studies of BNP and NPR-A knockout mice typically show increases in systemic arterial and pulmonary arterial pressure as well as cardiac hypertrophy, although the hypertrophic effect of disrupted NPR-A signaling has been more pronounced than its effect on arterial pressure. To differentiate the cardiac from the vascular effects of ANP and BNP, Holtwick et al developed mice with conditional, selective deletion of NPR-A in cardiomyocytes mice.<sup>120</sup> The endocrine, blood pressure- and volume-regulating effects of ANP were found to be preserved in these

mice but significant cardiac hypertrophy was still observed. These findings suggest that NPR-A signaling indeed functions as counter-regulatory hypertrophic circuit in the heart under both basal and pressure-overload conditions.<sup>132</sup> Interestingly, in humans, certain ANP- and BNP-receptor polymorphisms have been associated with left ventricular mass in essential hypertension.<sup>133</sup> In addition, a deletion in the 5'-flanking region of the gene encoding NPR-A has been associated with hypertension and hypertrophy.<sup>134</sup>

## Effect on pulmonary endothelial proliferation and apoptosis

Preservation of endothelial function is critical in combating pulmonary vascular disease.

Recent studies demonstrate that the NO/sGC/cGMP pathway complements natriuretic peptide/pGC/cGMP such that suppression of one pathway decreases the sensitivity of the other.<sup>135,136</sup> The reciprocal regulation of natriuretic peptides and nitric oxide suggest that natriuretic peptides could play a key role in treating diseases like PAH that are associated with deficiencies in NO production. Interactions between natriuretic peptides and ET-1 may also be important. BNP can directly inhibit ET-1 synthesis as well as functionally antagonize ET-1-induced vasoconstriction and cardiomyocyte hypertrophy.<sup>137</sup> These molecular interactions may play a significant role in the cardioprotective function of natriuretic peptides in PAH. Furthermore, classic heterologous desensitization of NPR-A, ie, the ability of other cGMP-elevating enzymes to desensitize NPR-A, does not seem to occur.<sup>138</sup> This supports important reciprocal regulation of vascular tone by natriuretic peptides and NO, which activate the particulate and soluble isoforms of guanylate cyclase, respectively.<sup>135,136</sup>

Impaired apoptosis of pulmonary vascular endothelial cells may play an important role in the pathogenesis of pulmonary arterial hypertension. Tudor et al have proposed that impaired apoptotic responses in pulmonary vascular endothelial cells favors the emergence of apoptosis-resistant endothelial cells leading to uncontrolled proliferation in PAH.<sup>4,139,140</sup> ANP, BNP and CNP are all capable of inducing apoptosis in cultured rat endothelial cells,<sup>141</sup> but the extent to which they modify apoptosis under such circumstances has not been fully defined. During ischemia/reperfusion in rodents and humans, 5% to 30% of cardiac myocytes in the area at risk undergo apoptosis within 16 hours.<sup>142</sup> Kato et al reported that ANP significantly inhibits apoptosis in rat cardiac myocytes and that this effect is mediated by the increase in cGMP followed by activation of the

Akt/PI3K pathway.<sup>143</sup> Conversely, ANP has been shown to induce cell cycle entry in terminally differentiated chick cardiac myocytes, and to induce apoptosis in a model of neonatal rat cardiac myocytes.<sup>144</sup> Therefore, it appears that natriuretic peptides can modulate apoptosis in cardiac myocytes as well as endothelial cells, but to what extent this occurs in pulmonary vascular endothelial cells and/or the right ventricle in PAH is uncertain.

## Effect of BNP on inflammation and oxidative stress

The pathological lesions in IPAH include areas of marked intima remodeling and near complete obliteration by fibrous tissue. Intravascular and perivascular inflammatory infiltrates have also been described, suggesting that vascular inflammation may contribute to the pulmonary vascular remodeling seen in some forms of PAH. BNP has the ability to modulate the production of inflammatory mediators in activated macrophages such as leukotriene B<sub>4</sub>, prostaglandin E<sub>2</sub> and the cytokines (TNF $\alpha$ , IL-12 and IL-10).<sup>145</sup> Interestingly, NPR-A knockout mice exhibit decreased neutrophil infiltration to cardiac tissue after ischemic injury compared with wild-type mice by decreasing activation of the transcription factor NF- $\kappa$ B.<sup>146</sup>

Oxidative stress also contributes to the pathophysiology of inflammation in the cardiovascular system.<sup>147</sup> Reactive oxygen species are produced in the lung tissue of patients with severe pulmonary hypertension as a consequence of tissue hypoxia<sup>148,149</sup> or ischemia<sup>150</sup> or through the activation of inflammatory cascades and increased production of inflammatory cytokines.<sup>151,152</sup> BNP reduces the production of free radicals, such as reactive oxygen- and nitrogen species from activated macrophages thereby reducing oxidative stress during inflammation.<sup>145</sup> This may represent another potential mechanism by which BNP could protect against the progression of vascular disease and endothelial dysfunction in PAH.

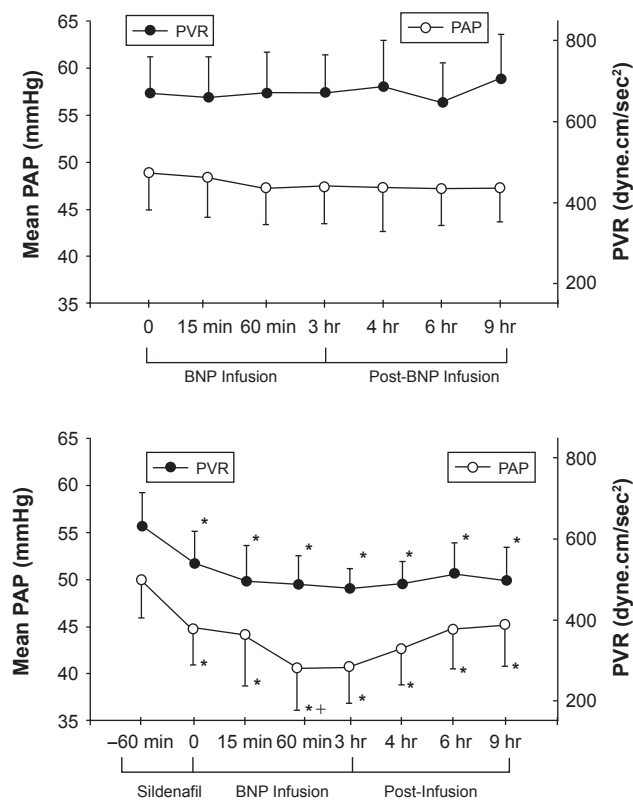
## Potential role for BNP in treatment of PH

As described above, BNP influences a variety of biologic processes in a manner that has the potential to benefit patients with PAH. Unfortunately, the peptide structure of BNP results in rapid degradation in the gastrointestinal tract following ingestion, making oral delivery challenging. Intravenous infusion of BNP is feasible using recombinant human BNP developed for the treatment of acute decompensated heart failure (nesiritide; Natrecor<sup>®</sup>). Inconvenience, expense and

potential safety concerns of continuous intravenous infusion are barriers to conducting even small proof of concept clinical trials. Although it should be acknowledged that long-term intravenous infusion has been used successfully for prostacyclin therapy in PAH. Most of the information available on the use of BNP to treat cardiovascular disease derives from clinical trials in congestive heart failure and acute renal failure and a handful of reports of its acute effects on the pulmonary circulation.<sup>153,154</sup> As a result, there are limited data on the efficacy or safety of BNP for the treatment of PAH.

BNP has biological effects that are similar to those of ANP, but its circulating levels are much lower and in the absence of any physiological derangements, small increases in plasma BNP levels are unlikely to have significant cardiovascular effects. This is supported by most human studies in which physiological doses of BNP given to healthy volunteers have little effect on blood pressure, heart rate, angiotensin II, or aldosterone synthesis.<sup>155–160</sup> However, at supra-physiological doses, BNP has been shown to have hypotensive and diuretic properties. For example, infusion of pharmacological doses of BNP into normal subjects leads to a rapid and sustained reduction in mean arterial blood pressure.<sup>161</sup> The fall in blood pressure is not solely a consequence of vasodilation, but also related to reduced stroke volume and intravascular volume. Pharmacologic doses of BNP have also been shown to have an acute pulmonary vasodilator effect in humans. Intravenous infusion of human BNP (10 pmol kg<sup>-1</sup> min<sup>-1</sup>) blunts the acute pulmonary vasoconstrictor response to hypoxia<sup>162</sup> and reduces PA pressure in patients with cor pulmonale.<sup>163</sup> These preliminary studies demonstrate that BNP is capable of reducing elevated PAP in some forms of pulmonary vascular disease.

Clinical experience with BNP infusion in patients with PAH is extremely limited. Michaels et al reported the hemodynamic effects of intravenous nesiritide infusion in five patients with precapillary PH.<sup>164</sup> At 15 and 30 minutes following infusion, there was a decrease in systemic arterial pressure but no change in right-sided heart pressures or CO. Klingler et al found that a 3-hour BNP infusion alone, although well tolerated in adult patients with PAH, had no effect on mPAP or PVR.<sup>165</sup> However, the combination of sildenafil followed by BNP infusion caused a statistically significant reduction in both parameters that persisted for up to 6 h after stopping the BNP infusion (Figure 6). The reduction in mPAP with sildenafil plus BNP was at least as great as that seen with any of the other pulmonary vasodilators used in this trial and greater than that observed 1 hour



**Figure 6** Upper panel: Mean pulmonary arterial pressure (PAP) and pulmonary vascular resistance (PVR) in patients with pulmonary arterial hypertension during a 3-hour infusion of human BNP (nesiritide) and 6 hours after infusion was completed. Lower panel: Effect of BNP infusion on PAP and PVR when given 1 hour after sildenafil.<sup>165</sup>

after sildenafil alone. Thus, BNP may be able to potentiate the acute vasodilator effects PDE-5 inhibitors.

In addition to its diuretic and acute pulmonary vasodilator effect, the natriuretic peptides generally oppose the actions of angiotensin II, ET-1, and the sympathetic nervous system<sup>137</sup> and thereby have the potential to improve right ventricular function in patients with PAH. Nesiritide administration leads to a rapid vasodilatory effect and decrease in both right and left ventricular filling pressures and pulmonary capillary wedge pressures<sup>166</sup> and has been shown to have therapeutic potential in slowing progression of right ventricular dysfunction.<sup>167</sup>

There is increasing recognition that left heart failure may have a relative natriuretic deficient or resistant state. Elevated plasma levels of ANP and BNP are found in patients with congestive heart failure and the magnitude of increase coincides with the severity of heart failure.<sup>168–170</sup> Studies of early LV dysfunction have demonstrated that natriuretic peptides play an important role in maintaining cardio-renal homeostasis through systemic actions that antagonize volume overload.<sup>171</sup> Gene deletion studies confirm that NPR-A

signaling protects against heart failure induced by volume overload in mice.<sup>172</sup> However, in severe symptomatic HF, there is sodium and water retention associated with increased systemic vascular resistance and high cardiac filling pressures despite extremely high plasma concentration of immunoreactive ANP and BNP. The discrepancy in plasma levels of natriuretic peptides and their apparent lack of effect on cardio-renal physiology is not clearly understood, but may be due to impaired NP synthesis and release, a down regulation of NPRs at the tissue level, or increased metabolism of cGMP. Animal studies have shown an up-regulation of PDE expression in left ventricular failure, causing impaired cGMP activity despite high stimulation of the guanylyl cyclase-linked NPRs.<sup>173</sup> Supaporn et al have reported similar increases in PDE-1 and PDE-5 in severe congestive HF in humans.<sup>174</sup> Abnormal processing of proBNP into less active forms has also been described in congestive heart failure.<sup>175</sup> Resistance to natriuretic peptides may explain the variable response rate and the need for high doses that characterized the early clinical trials of BNP in congestive heart failure.<sup>176–179</sup> Recent analyses of clinical trial data of nesiritide have raised concerns about adverse renal effects.<sup>180,181</sup> The use of nesiritide at the dosages described in the package insert (0.01 to 0.03  $\mu\text{g kg}^{-1} \text{min}^{-1}$ ) was noted to be associated with a dose-dependent increase in serum creatinine. Furthermore, this was exacerbated by systemic hypotension a known adverse effect of nesiritide. This clinical experience needs to be considered in future trials of BNP in pulmonary hypertension.

As discussed previously, BNP has significant anti-hypertrophic and anti-fibrotic effects on myocytes. It is conceivable that prolonged administration of BNP may mitigate maladaptive hypertrophic responses in the right ventricle. In a small randomized trial of PAH, Wilkins et al<sup>182</sup> found that patients treated with sildenafil had a significant reduction in right ventricular mass as measured by MRI that was not found in those who received the endothelin receptor antagonist bosentan, despite a similar degree of improvement in 6MWD. Further augmentation of cGMP with the combination of BNP infusion and PDE inhibition may inhibit RV hypertrophy and fibrosis and help to preserve RV function. This idea is supported by the report Khush et al of 10 patients with precapillary PH that had acute elevations in pulmonary NO and cGMP levels post nesiritide infusion.<sup>183</sup>

To advance the use of BNP as a therapy for PAH, more practical methods will likely be needed to increase plasma BNP levels. Subcutaneous administration of BNP has been used successfully in animal and in clinical studies to increase

plasma BNP and its second messenger 3',5'-cyclic guanosine monophosphate (cGMP) with subsequent natriuresis and reduction in cardiac filling pressures.<sup>184-189</sup> In one canine study,<sup>188</sup> tolerance was not observed following chronic administration of BNP. Development of orally available peptides has long been a challenge, given various barriers to protein absorption and penetration.<sup>190</sup> Recently, Cataliotti et al<sup>191</sup> reported the application of proprietary technology that enabled oral delivery of BNP by covalently attaching short, amphiphilic oligomers to peptides. In normal conscious dogs, this novel oral conjugated human BNP activated cGMP and exerted hypotensive effects.<sup>191</sup> Recently, Cataliotti et al<sup>192</sup> further demonstrated in a canine model of acute hypertension that a more advanced oral conjugated human BNP significantly increased cGMP and lowered MAP. The availability of an orally active NP such as BNP could lead to a much wider application of these peptides. An alternative approach would be to impede degradation of endogenous BNP through NEP inhibition. Neutral endopeptidase is a major enzymatic pathway for the degradation of BNP and also plays a role in kinin and adrenomedulin breakdown.<sup>193</sup> Inhibition of NEP increases plasma levels of BNP<sup>193</sup> and has been shown to inhibit hypoxic pulmonary hypertension in rats,<sup>194</sup> although the mechanism by which this was achieved is not known.

In summary, although few clinical data currently exist, there is evidence to suggest that enhancement of BNP activity, especially in combination with other agents that increase cGMP signaling has the potential to aid in the treatment of PAH.<sup>195,196</sup> Perhaps most intriguing of the pleiotropic effects of BNP is its antiproliferative/antifibrotic properties and the potential for reversing the maladaptive remodeling associated with PAH.

## Summary

BNP is an integral part of the natriuretic peptide system that functions as a hormonal regulator of cardiovascular homeostasis. Like ANP, it is capable of relaxing pulmonary vascular smooth muscle, especially under condition in which vascular tone is increased. It also shares with ANP diuretic, antimitogenic, antifibrotic and antihypertrophic effects that have the potential to limit pulmonary vascular remodeling and maladaptive hypertrophic responses in the right ventricle. Right ventricular expression and circulating levels of BNP rise markedly in patients with pulmonary hypertension and correlate with severity of disease and mortality. Changes in BNP expression are more prominent in the ventricle than ANP expression, particularly during times

of increased ventricular overload. This ventricular response, along with its slower clearance from the circulation appears to be responsible for BNP acting as a better biomarker of ventricular function than ANP. Monitoring of BNP and its precursor NT-ProBNP provides important objective information regarding the ability of the right ventricular to compensate for increased after load as pulmonary vascular disease progresses. The prognostic significance of plasma BNP on survival in pulmonary hypertension is independent of hemodynamic variables and provides the practitioner another tool with which to assess prognosis and response to therapy. Findings from in vitro and intact animal studies suggest that administration of exogenous BNP is capable of blunting pulmonary vasoconstriction and inhibiting the development of pulmonary hypertension, right ventricular hypertrophy and pulmonary vascular remodeling. However, lack of a convenient method for achieving sustained increases in circulating BNP levels has impeded the development of BNP as a therapy for treating pulmonary hypertension. Further study is needed to determine if BNP can potentiate the pulmonary vasodilator effects of phosphodiesterase inhibitors and blunt or reverse maladaptive hypertrophic responses in the right ventricular. New technologies that allow transdermal or oral administration of the natriuretic peptides have the potential to greatly accelerate research into therapeutic use of BNP for cor pulmonale and pulmonary vascular diseases.

## Abbreviations

ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CTEPH, chronic thromboembolic pulmonary hypertension; cGMP, dependent protein kinase; cGMP, cyclic guanosine monophosphate; CNP, C-type natriuretic peptide; CO, cardiac output; ET-1, endothelin-1; FDA, Food and Drug Administration; MPAP, mean pulmonary arterial pressure; NEP, neutral endopeptidase; NO, nitric oxide; NPR-A, natriuretic peptide receptor-A; NPR-B, natriuretic peptide receptor-B; NPR-C, natriuretic peptide receptor-C; NYHA, New York Heart Association; PAH, pulmonary arterial hypertension; PAP, pulmonary arterial pressure; PCWP, pulmonary capillary wedge pressure; PDE, phosphodiesterase; PH, pulmonary hypertension; PKG, PVR, pulmonary vascular resistance; RVSP, right ventricular systolic pressure; RHC, right heart catheterization; SSc-PAH, scleroderma associated PAH; TGF b, transforming growth factor-b; TPR, total pulmonary resistance; TTE, transthoracic echocardiogram; WHO, World Health Organization.

## Disclosures

The authors declare no conflicts of interest.

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