

Recent advances in natriuretic peptide research

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Abstract

The natriuretic peptides are a family of related hormones that play a crucial role in cardiovascular and renal homeostasis. They have recently emerged as potentially important clinical biomarkers in heart failure. Natriuretic peptides, particularly brain natriuretic peptide (BNP) and the inactive N-terminal fragment of BNP, NT-proBNP, that has an even greater half-life than BNP, are elevated in heart failure and therefore considered to be excellent predictors of disease outcome. Nesiritide, a recombinant human BNP, has been shown to provide symptomatic and haemodynamic improvement in acute decompensated heart failure, although recent reports have suggested an increased short-term risk of death with nesiritide use. This review article describes: the current use of BNP and its inactive precursor NT-proBNP in diagnosis, screening, prognosis and monitoring of therapy for congestive heart failure, the renoprotective actions of natriuretic peptides after renal failure and the controversy around the therapeutic use of the recombinant human BNP nesiritide.

Keywords: natriuretic peptides • heart failure • renoprotective effect • nesiritide • biomarkers

Introduction

Natriuretic peptides are a family of hormones, sharing similar chemical structure (a characteristic 17-amino acid ring structure, stabilized by a cysteine bridge, which contains several invariant amino acids and variable C- and N-terminal tails) and biological function, with relevant effects in cardiovascular physiology and pathology. The study of natriuretic peptides began 50 years ago, when electron microscopy revealed the presence of secretory granules in cardiac atrial cells containing atrial natriuretic peptide (ANP) as demonstrated by de Bold and coworkers in the early 80s [1]. From 1988 to 1990, two more

members of the natriuretic peptide family, the brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP) were identified in porcine brain [2, 3]. More recently a new peptide that shares structural and functional characteristics with the previous natriuretic peptides was identified in the venom of the green mamba (*Dendroaspis angusticeps*) and received the denomination of *Dendroaspis* natriuretic peptide (DNP) [4]. In addition, other peptides with similar cardiovascular effects have been identified in mammals, including urodilatin, a peptide derived from alternative cleavage of pro-ANP in the distal

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tubules of the kidney, where it exerts its natriuretic actions [5], or the intestinal epithelium derived peptides, guanylin and uroguanylin, which participate in water absorption [6].

The classic physiological role of natriuretic peptides includes promotion of renal excretion of sodium (natriuresis) and water (diuresis). Natriuretic peptides also exert autocrine and paracrine effects within circulation, such as vasodilatation by relaxing vascular smooth muscle cells, regulation of renin, progesterone, endothelin and vasopressin secretion [7].

ANP and BNP are 28- and 32-amino acid peptides, respectively, mainly synthesized and released by atrial (in the case of ANP) and ventricular cardiomyocytes (for BNP) in response to blood pressure and volume loading [8, 9]. In addition, ANP and BNP secretion from the ventricles increases associated to a number of ventricular dysfunctions, and the extent of ANP and BNP release under these circumstances is in relation to the severity of the pathology, that has led to the use of both natriuretic peptides as diagnostic tests for heart failure [10, 11]. ANP and BNP are synthesized as larger molecules that are subsequently cleaved to yield the active peptide hormone and the biologically inactive N-terminal peptide fragment [12, 13]. Both natriuretic peptides circulate in the blood reducing vascular tone and promoting diuresis/natriuresis to lower blood volume and pressure. ANP and BNP are removed from the circulation by two different mechanisms: receptor-mediated internalization and proteolytic degradation by neutral endopeptidase, that has been shown to take place in the kidneys, vascular endothelium, lungs and heart [11]. Circulating half-life of ANP is approximately 3–5 min, whereas the half-life of BNP is significantly greater, about 23 min., and even greater is that of the inactive terminal fragment of BNP, NT-proBNP, from 60 to 120 min, which is relevant to their value as diagnostic tests [11].

CNP, the third natriuretic peptide identified, is mainly expressed in the nervous system and vascular endothelial cells [14, 15], where CNP exerts autocrine and paracrine actions on vascular tone and muscle cell growth [15, 16]. The cardiovascular effects of CNP are more likely mediated by its vascular local effects or by central actions on vasopressin and adrenocorticotropine release than to its natriuretic and diuretic effects, that are weaker than that of ANP and BNP [16, 17]. CNP gene expression is stimulated by several vasoactive mediators, such as interleukin 1 β ,

vascular endothelial growth factor, transforming growth factor, tumour necrosis factor- α and insulin [15, 18–20].

DNP is a recently isolated 38-amino acid peptide that shows structural and functional properties of the previously identified members of the natriuretic peptide family. Although DNP purification from human blood has not yet been achieved, DNP immunoreactivity has been reported in human plasma [21]. DNP-like immunoreactivity has been found in rat aorta, carotid artery and kidney [22, 23], where it has been found to induce vasorelaxation and inhibition of vascular smooth muscle cell proliferation [23].

Natriuretic peptides regulate cardiovascular homeostasis by the occupation of three membrane receptors; two are guanylyl cyclase-coupled receptors, known as natriuretic peptide receptor (NPR)-A and NPR-B, while NPR-C lacks enzymatic activity and among other functions acts as a clearance receptor [24, 25]. NPR-A is activated by ANP, BNP and DNP [25, 26], NPR-B shows high affinity and is activated by CNP and, finally, the NPR-C binds all natriuretic peptides [25].

NPR-A and NPR-B are single-transmembrane receptors of approximately 120 kD with a similar basic structure that consist of a variable extracellular natriuretic peptide-binding region, a conserved intracellular kinase homology domain and a guanylyl cyclase domain with enzyme activity. Occupation of NPR-A and NPR-B induces cellular responses through the elevation of intracellular cGMP levels, which, in turn, leads to the activation of a cGMP-dependent protein kinase that phosphorylates a target protein in serine or threonine residues and mediates the specific physiological function. Signal transduction activated by these receptors is terminated by cGMP phosphodiesterases that modulate the intracellular concentrations of cGMP and the duration and magnitude of the responses [27].

NPR-C is a transmembrane receptor with an extracellular domain containing the natriuretic peptide-binding region, a transmembrane domain and a cytosolic domain. Two different subtypes of NPR-C, of approximately 67 and 77 kD in size, have been identified [28, 29]. NPR-C has been involved in peptide clearance, removing natriuretic peptides from the circulation [30] and in the mediation of natriuretic peptide-induced inhibition of cAMP synthesis, an effect that requires the involvement of a heterotrimeric G protein [7, 31, 32]. We have provided evidence

that the 77 kD NPR-C-like protein is involved in peptide internalization whereas the 67 kD NPR-C-like protein is likely involved in adenylyl cyclase inhibition [15].

Natriuretic peptides as a 'biomarker' of congestive heart failure and acute renal failure/insufficiency

In the past decade, much attention has been given to the investigation of natriuretic peptides testing in the evaluation of patients with different pathologies, such as congestive heart failure (CHF). Studies in patients with CHF showed that both ANP and BNP secretion from ventricular myocytes increases in relation to the rigor of dysfunction [10]. These findings led to investigate whether the plasma levels of ANP and BNP may assist in the diagnosis of patients with heart failure. Since half-life of BNP is greater than that of ANP comparison of the diagnostic value of ANP and BNP have generally favoured BNP [33]. As reported above, the inactive N-terminal fragment of BNP, NT-proBNP, has an even greater half-life than BNP; thus, most of the clinical investigations concerning natriuretic peptides as biochemical markers for CHF has focused on BNP or NT-proBNP.

Basal plasma levels of BNP have been established between 5 and 50 pg/ml, while the corresponding values for NT-proBNP are from 7 to 160 pg/ml. The approved cut-off levels to consider an abnormal range are 100 pg/ml for BNP and 125 pg/ml for NT-proBNP (450 pg/ml for individuals older than 75 years old), although they vary according to age, sex and estimated glomerular filtration rate [11]. Levels of both BNP and NT-proBNP are elevated under a number of pathological situations including cardiac dysfunctions, such as CHF, diastolic dysfunction, valvular heart disease, atrial fibrillation and non-cardiac pathologies, such as acute pulmonary embolism, pulmonary hypertension, sepsis or hyperthyroidism [11, 34–36].

Four major applications of BNP and NT-proBNP testing in patients with CHF have been presented in the last few years, including diagnosis, screening, prognosis and monitoring of therapy. Different studies have reported the efficacy of BNP and NT-proBNP tests increasing the diagnostic accuracy of heart failure [37, 38]. The sensitivity and specificity of the test is especially high at a cut-off of 76 pg/ml BNP

[11], and NT-proBNP has been proved to be particularly important in decreasing the overdiagnosis of heart failure that occurs in primary care [38]. For NT-proBNP, levels over 450 pg/ml (over 900 pg/ml for patients older than 50 years old) are sensitive and specific for heart failure; however, if the value is below 300 pg/ml, heart failure is highly unlikely with a negative predictive value of 99% [39]. In addition, recent studies have reported that BNP is a significant prognostic indicator in patients diagnosed with heart failure and in asymptomatic patients [40]. NT-proBNP has been found to be a stronger risk biomarker for cardiovascular disease and death than C-reactive protein [41]. NT-proBNP and BNP might be used as blood tests to identify the 'high-risk' subject with cardiovascular risk, which would be a great advance prompting more aggressive primary prevention [42]. However, a number of limitations have been reported about the prognostic value of BNP and NT-proBNP. In patients with advanced CHF, atrial fibrillation does not alter NT-proBNP levels [43]. BNP or NT-proBNP can also be used as a guide for therapy in heart failure. For this use, it should be taken into account that therapies that reduce clinical disorders in heart failure also reduce BNP levels and those that improve heart failure conditions act primarily through mechanisms that are linked with changes in natriuretic peptide levels. In addition, BNP and NT-proBNP levels do not decrease as rapidly in response to therapy as might be expected from their short half-lives [44], suggesting that the natriuretic peptide system need some time to autoregulate. Considering this, BNP and NT-proBNP levels might guide the clinician to adjust the treatment in order to achieve a plasmatic level of these agents below a critical value.

Sepsis is commonly complicated with myocardial dysfunction. Sepsis and septic shock is associated with an elevation of cardiac troponin levels that has been shown to indicate left ventricular dysfunction and a poor prognosis [45]. Whereas cardiac troponins are a good biomarker for myocardial dysfunction in patients with severe sepsis or septic shock the use of natriuretic peptides, including BNP, as an indicator of prognosis remains controversial.

Renal insufficiency has also been shown to affect the plasmatic levels of both BNP and NT-proBNP both in children and adult patients. The levels of BNP are correlated with renal function, so that an increase reaching about 200 pg/ml have been reported in patients with reduced creatinine clearance (below

60 ml • min⁻¹ • 1.73 m⁻²) [46]. Similar observations have been reported with NT-proBNP and a recommended reference value of 1200 pg/ml has been shown for patients with reduced creatinine clearance [47]. It has been shown that peritoneal dialysis does not alter plasma NT-BNP or BNP levels in patients with renal failure, whereas both blood urea nitrogen and creatinine levels declined as expected, which should be taken into account if plasmatic levels of these hormones are used as a guide to the management of patients with renal failure [48].

NT-pro-BNP and BNP have been recently presented as useful biomarkers for coronary artery disease and left ventricular hypertrophy in patients with chronic kidney disease, where detection of coronary artery disease and left ventricular hypertrophy in these patients has remained elusive despite the greater prevalence in patients with chronic kidney disease [49].

Renoprotective effect of natriuretic peptides after acute renal failure

Natriuretic peptides, especially ANP, have long been reported to have the potential to restore renal function after ischaemic injury, and have been shown to counteract renal sympathetic nerve activity in renal function.

ANP overexpression has been shown to exhibit protection against gentamycin-induced nephrotoxicity, as demonstrated by daily subcutaneous administration of gentamycin, for 10 days, in Sprague Dawley rats either treated with an intravenous injection of adenovirus (Ad.RSV-ANP), carrying the human ANP gene, the first day of gentamycin administration, or not treated (control) for comparison. This finding raises the possibility of using ANP gene therapy for the treatment of drug-induced renal failure [50]. In addition, ANP has been reported to exert a protective effect on the outcome of acute renal failure in animals when infused over short periods of time, while it has been shown that prolonged infusion of ANP does not alter the course of acute renal failure [51]. On the other hand, infusion of Wistar rats with ANP exerted little renoprotective effect against endotoxin-induced acute renal failure, since ANP infusion did not improve the hyponatruresis and oliguria induced by endotoxin administration [52].

Recent studies have pointed out that continuous infusion of synthetic human ANP is effective for preventing acute renal failure, which is a major problem occurring immediately after liver transplantation and requiring haemodialysis [53].

BNP has also been shown to induce renoprotective actions under pathological conditions. This is the case of a model of transgenic mice overexpressing BNP that show reduced glomerular injury than control mice during the development of *diabetes mellitus*. Glomerular hyperfiltration is an early haemodynamic alteration and one of the key mechanisms of the pathogenesis of diabetic nephropathy. This observation suggests that renoprotective effects of natriuretic peptides may prevent the progression of diabetic nephropathy, although further studies are necessary to establish therapeutic strategies to palliate renal complications associated to diabetes mellitus [54].

Further evidences for the renoprotective role of natriuretic peptides come from studies where the peptide hydrolysis is inhibited. Neutral endopeptidase is an endothelial cell surface zinc metallopeptidase and the major pathway involved in the degradation of the natriuretic peptides [55]. Inhibition of neutral endopeptidase increases levels of ANP, BNP and CNP, and has been shown to offer a therapeutic advantage in the treatment of hypertension, heart failure and endothelial dysfunction [56]. Endopeptidase inhibitors reduce vasoconstriction and improve sodium/water balance; as a result these inhibitors decrease peripheral vascular resistance and blood pressure and improve local blood flow [57]. Endopeptidase inhibitors also reduce the activity of the angiotensin-converting enzyme (ACE), leading to a reduction of vasoconstrictor and proliferative mediators, such as angiotensin II and increase local levels of bradykinin [57]. Recent studies have reported that ANP reduces angiotensin II-induced renomedullary interstitial cells proliferation and extracellular matrix synthesis in diabetic subjects more efficiently in neutral endopeptidase-deficient mice, which provide evidence for the beneficial effect of inhibition of neutral endopeptidase in attenuating abnormal cell growth associated with diabetic nephropathy [58].

In addition to the renoprotective effect, neutral endopeptidase inhibition, in combination with inhibition of the ACE activity, has been shown to reduce blood pressure in spontaneously hypertensive rats. This is the case of omapatrilat, a potent vasopeptidase inhibitor that exerts anti-hypertensive effects by inhibition

Table 1 Beneficial and deleterious effects of nesiritide in cardiovascular and renal systems

Beneficial effects	Deleterious effects	Refs.
Vascular vasodilatation, reduction of cardiac pre-load and after-load, increase cardiac output		60–62
Enhances glomerular filtration rate and filtration fraction.		63
Increases cardiac stroke volume		64–66
	Possible risk of short-term (30 days) death in patients with acute decompensated heart failure	71, 74, 75
No risk of higher mortality after 30 or 180 days		76
Enhances diuresis and natriuresis		63
	Nesiritide infusion for 24 hrs or more is associated with worsening renal function	71
Decreases pulmonary capillary wedge pressure and right atrial pressure		64–66
No arrhythmogenic, no induce tachyphylaxis or increase myocardial oxygen consumption		67

of the neutral endopeptidase and ACE at the tissue level, which results in beneficial effects on the cardiovascular structure [59]. The dual metalloprotease inhibitors of ACE and neutral endopeptidases, called vaso-peptidase inhibitors, provide an advance over individual ACE or neutral endopeptidase inhibitors, and might represent a new and attractive therapeutic strategy for the treatment of cardiovascular disease.

Nesiritide – current benefits/hazards of drug usage

Nesiritide is a recombinant form of human BNP and its amino acid sequence is identical to that of endogenous human BNP. Administration of nesiritide results in venous, arterial and coronary vasodilatation, reducing cardiac the pre-load and after-load, which increase cardiac output without direct inotropic effects [60–62]. In addition, nesiritide increases glomerular filtration rate and filtration fraction, suppresses the renin-angiotensin-aldosterone axis, and enhances diuresis and natriuresis [63]. Nesiritide is currently used in the treatment of acute decompensated heart failure, where it has been shown to decrease pulmonary capillary wedge pressure, pulmonary artery pressure, right atrial pressure and

systemic vascular resistance, as well as increase cardiac index and stroke volume index [64–66].

In comparison with nitroglycerin, treatment with nesiritide did not induce the appearance of tachyphylaxis and other adverse effects [63]. Unlike inotropes, the beneficial haemodynamic effects produced by nesiritide do not cause an increase in myocardial oxygen consumption, an important consideration for patients with acutely decompensated heart failure. Since nesiritide is not an inotrope, it does not affect myocardial contractility, as does the agonists of β -adrenergic receptors or the inhibitors of phosphodiesterase III. As a result, nesiritide does not exert arrhythmogenic activity [67].

Studies aimed to determine the impact of early initiation of acute decompensated heart failure therapy with nesiritide on subsequent outcomes have confirmed its beneficial effects reducing the severity of the associated complications, reducing the mean total hospital length of stay and the requirement of transfer to the intensive care unit [68]. In comparison with dobutamine and milrinone, nesiritide therapy was associated with a lower in-hospital mortality rate and shorter length of stay. In addition, total health care costs with nesiritide were decreased compared with the other drugs, since although the acquisition cost of nesiritide was higher than that of milrinone and dobutamine, nesiritide has been shown as a

more cost-effective treatment option for patients with acute decompensated heart failure [69, 70].

Despite its beneficial effects on the cardiovascular system, recent studies have raised the question of safety with nesiritide therapy. In three randomized controlled trials Sackner-Bernstein and coworkers expressed concern of possible short-term (30-day) risk of death after nesiritide use for the treatment of acute decompensated heart failure [71]. As mentioned by the authors, there are a number of limitations to this analysis. First, the three randomized controlled trials used the Nesiritide Study Group Efficacy Trial (NSGET), the Vasodilation in the Management of Acute Congestive heart failure (VMAC), and the Prospective Randomized Outcomes Study of Acutely Decompensated CHF Treated Initially in Outpatients with Natrekor (PROACTION) were not designed to determine whether nesiritide is associated with risk of death. In addition, there was not complete information concerning the use of additional medications or procedures through the 30-day period of the study [71]. The same group has reported that nesiritide significantly increases the risk of worsening renal function in patients with acute decompensated heart failure, although whether renal dysfunction reflects haemodynamic effect or renal injury is unclear [72]. Concerning this issue, more recent studies have reported that the effect of nesiritide on renal function strongly depends on the infusion times, so that nesiritide infusion time ≥ 24 hrs is associated with elevated markers of worsening renal function in patients with acutely decompensated heart failure compare with infusion of less than 24 hrs [73]. Several studies have reported that an increase in serum creatinine levels, such as that observed with nesiritide, predicts a higher risk of death even when that increase is transient [74, 75].

Unlike these previous studies, a more recent meta-analysis has reported that nesiritide is not associated with a higher 30- or 180-day mortality [76]. Given the limitations of the meta-analyses, without randomized controlled trials powered to evaluate mortality, Arora and coworkers suggest that it is premature to abandon the use of nesiritide [76]. A summary of the current information concerning the benefits and deleterious effects of the use of nesiritide is provided in Table 1. Future clinical trials are necessary to address the concerns raised and provide a better understanding of the actions of nesiritide in the management of acute decompensated heart failure. In

addition, recent studies have reported that NPR-B, not NPR-A, which is the receptor of nesiritide, is the predominant NPR in the failing heart, suggesting that drugs directed towards both NPRs might provide a greater benefit than nesiritide *per se* [77].

The clinical role of natriuretic peptides either as biomarkers for diagnosis, prognosis or monitoring of therapy, or as therapeutic strategies for cardiovascular and renal disorders has gained acceptance over the last decade. Despite specific basic studies and clinical trials necessary to better understand the possibilities of natriuretic peptides in therapeutic interventions, the use of these peptides to treat cardiovascular dysfunction seems to be most promising.

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