Review

Current biochemistry, molecular biology, and clinical relevance of natriuretic peptides

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Summary The mammalian natriuretic peptide family consists of atrial (ANP), brain [B-type; BNP] and C-type natriuretic peptide (CNP) and three receptors, natriuretic receptors-A (NPR-A), -B (NPR-B) and -C (NPR-C). Both ANP and BNP are abundantly expressed in the heart and are secreted mainly from the atria and ventricles, respectively. By contrast, CNP is mainly expressed in the central nervous system, bone and vasculature. Plasma concentrations of both ANP and BNP are elevated in patients with cardiovascular disease, though the magnitude of the increase in BNP is usually greater than the increase in ANP. This makes BNP a clinically useful diagnostic marker for several pathophysiological conditions, including heart failure, ventricular remodeling and pulmonary hypertension, among others. Recent studies have shown that in addition to BNP-32, proBNP-108 also circulates in human plasma and that levels of both forms are increased in heart failure. Furthermore, proBNP-108 is O-glycosylated and circulates at higher levels in patients with severe heart failure. In this review we discuss recent progress in our understanding of the biochemistry, molecular biology and clinical relevance of the natriuretic peptide system.

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Introduction

The natriuretic peptide system consists of three distinct endogenous peptides: atrial natriuretic peptide (ANP), brain (or B-type) natriuretic peptide (BNP) and C-type natriuretic peptide (CNP), as well as three receptors: natriuretic peptide receptor-A (NPR-A or guanylyl cyclase-A), natriuretic peptide receptor-B (NPR-B or guanylyl cyclase-B) and natriuretic peptide receptor-C (NPR-C or clearance receptor). Recent advances suggest that through its pleiotropic effects, this system plays key roles in the regulation of blood pressure and body fluid volume. In this review, we discuss the current understanding of the biochemistry, molecular biology and clinical relevance of natriuretic peptides.

Structures of the genes and peptides

ANP

Kirsch reported in 1956 that mammalian atrial myocytes contain granules with morphologies similar to the granules found in endocrine cells [1]. However, the significance of these granules was not recognized until 1981, when de Bold et al. reported that intravenous injection of crude myocardial extract induces a natriuretic effect in rats [2], suggesting the heart contains a natriuretic hormone. These findings ultimately led to the identification of the complete amino acid sequence of ANP in mammalian atrial tissue [3]. Subsequent studies showed that ANP is synthesized and secreted from the heart into the circulation as a cardiac hormone in response to atrial stretch. The major molecular form of circulating ANP is a 28-amino acid peptide (α-ANP) with a ring structure formed by an intramolecular disulfide linkage (Fig. 1).

ANP gene contains three exons. Exon 1 encodes the 5'-untranslated region, a 25-amino acid signal peptide and the first 16 amino acids of proANP. Exon 2 encodes most of the proANP sequence, and exon 3 encodes the terminal tyrosine and 3'-untranslated region (Fig. 2). ANP mRNA is translated to 151-amino acid preproANP. Thereafter, the 25-amino acid signal peptide is removed, yielding 126-amino acid proANP (γ-ANP) [4]. Because the tissue molecular form of ANP in the atrium and ventricle is proANP, it is presumed that the proteolytic conversion of proANP to ANP occurs during secretion.

BNP

BNP was first isolated from porcine brain extracts in 1988 by Sudoh et al. [8]. Soon after its discovery, however, the highest concentrations of BNP were shown to be in the heart, where it acts as a cardiac hormone [9]. BNP peptides and cDNA clones from a variety of species have now been isolated and sequenced. The predominant circulating form of BNP is a 26-, 45- and 32-amino acid peptide in pig, rat and human, respectively [10]. Thus, whereas the structure of ANP is relatively well conserved among species, the structure of BNP differs. The mature active molecular form of human BNP is BNP-32 (Fig. 1). Like ANP gene, BNP gene contains three exons (Fig. 3). Exon 1 encodes a 26-amino acid signal peptide and the first 15 amino acids of proBNP. Exon 2 encodes most of the proBNP sequence, and exon 3 encodes the terminal tyrosine and the 3'-untranslated region. BNP mRNA is translated to 134-amino acid preproBNP, after which the 25-amino acid signal peptide is removed, yielding 108-amino acid proBNP-108. In contrast to ANP, atrial and ventricular tissue contain two molecular forms of BNP: proBNP-108 and BNP-32. BNP-32 is dominant (~60%) in atrial tissue, while
proBNP-108 is dominant (∼60%) in ventricular tissue [11]. It is thought that cleavage of proBNP into BNP-32 and N-terminal proBNP-76 occurs in the trans-Golgi network [12] and that BNP-32 and N-terminal proBNP are then released into the circulation via a constitutive pathway. However, recent studies have shown that proBNP-108 is also present in human plasma and that the proBNP-108/BNP-32 ratio is increased in cases of severe heart failure [11]. We discuss the molecular forms of plasma BNP in detail in “Molecular complexity of plasma BNP — increase of proBNP-108 in heart failure” and “A new hypothesis of processing of proBNP” sections.

**CNP**

CNP was first isolated from porcine brain in 1990 [13]. Composed of 22 amino acids, the ring structure of CNP is highly homologous with those of ANP and BNP, but CNP uniquely lacks the carboxy-terminal extension (Fig. 1). Based on molecular cloning of CNP cDNA and the genes from various species, it has been predicted that the structure of CNP is identical among species and is the most highly conserved in the natriuretic peptide family. CNP-53, which has an amino-terminal extension of 31 amino acids, is a second endogenous form of CNP. Also first isolated from the porcine brain, CNP-53 has now been identified in both human and rat brain.

Like the other natriuretic peptide genes, CNP gene contains three exons. Exon 1 encodes a 23-amino acid signal peptide and the first 7 amino acids of proCNP. Exon 2 encodes the rest of the proCNP sequence, and exon 3 encodes the 3' untranslated region (Fig. 4). PreproCNP is comprised of 126 amino acids, the first 23 of which are cleaved as a signal peptide. The resulting 103-amino acid proCNP is further processed to CNP-53 and/or CNP, the potencies of which...
are essentially the same. Furin is thought to cleave proCNP into CNP-53 [14], whereas PC2 and/or PC1/3 are thought to cleave proCNP into CNP. CNP-53 is generally the major endogenous tissue molecular form [15].

Tissue distribution and gene expression

ANP

ANP is abundantly expressed in the heart, such that tissue levels of ANP are 250–1000-fold higher in atrium than in ventricle. The atrium is the major site of ANP synthesis, and ANP mRNA levels are considerably higher in the atria than in the ventricles. High levels of both ANP mRNA and ANP are found in the ventricles of neonatal rats, but these levels rapidly decline in parallel with the change in cardiac contractile protein isoforms that occurs after birth [4]. As a result, healthy adult ventricular tissue actually produces little ANP. However, ventricular ANP expression is re-induced in the hypertrophied and failing heart, making the ventricle a substantial source of circulating ANP in cases of severe heart failure [16].

ANP mRNA has also been detected in a variety of extracardiac tissues, including the hypothalamus, pituitary gland and lung [17], but the extracardiac expression is generally weak. Within the central nervous system, the highest ANP concentrations are in the hypothalamus and septum. The major molecular forms of ANP as a neuropeptide are α-ANP(4–18) and α-ANP(5–28) [18]. Thus, the molecular forms of the ANP neuropeptide differ from those of the ANP cardiac peptide. Finally, urodilatin, an N-terminal 4-amino acid-extended form of α-ANP derived from γ-ANP, was identified in urine. Its site of production is the kidney [19].

BNP

Concentrations of BNP and its mRNA are much lower in the human cardiac ventricle than in the atrium, but the total content of BNP and its mRNA in the ventricle accounts for 30% and 70% of that in the whole heart, respectively [20]. A clinical study also showed that plasma BNP levels are higher in the anterior interventricular vein and the coronary sinus than in the aortic root, suggesting BNP is a cardiac hormone that is predominantly synthesized in and secreted from the ventricle [21]. In addition, no appreciable amount of BNP has been detected in the rat or human brain, suggesting that the tissue distribution of BNP differs among species.

ProBNP-108 is most likely cleaved by a processing enzyme to BNP-32 and NT-proBNP-76 as it is secreted [10]. Levels of myocardial BNP mRNA and circulating BNP and NT-proBNP-76 are obviously increased as compared with those of ANP in patients with congestive heart failure, which suggests BNP functions as an emergency defense against ventricular overload in disease states.

One of the characteristic features of BNP mRNA that sets it apart from ANP mRNA is a conserved sequence consisting of repeated AUUUA units in the 3′ untranslated region (Fig. 3). The presence of this sequence accelerates the degradation of BNP mRNA in a manner similar to that seen with lymphokine genes and oncogenes [22]. Thus, BNP gene expression is regulated differently from ANP gene expression and is thought to dynamically change, depending on the physiological and pathophysiological conditions.

CNP

CNP is distributed throughout the brain in rats and humans, and a substantial amount is present in pituitary gland in rat. Consequently, CNP was initially thought to act as a neuropeptide [22]; however, subsequent studies showed that CNP is also synthesized in kidney, bone, blood cells, blood vessels and heart [23]. For example, cultured vascular endothelial cells (ECs) reportedly show significant CNP gene expression and peptide secretion [24]. CNP secretion from ECs is stimulated by such cytokines as transforming growth factor, tumor necrosis factor, interleukin-1, basic fibroblast growth factor, and lipopolysaccharide. This suggests CNP
may be active in the vascular wall under various pathological conditions [25]. On the other hand, initial studies failed to detect CNP mRNA in human or rat heart, and the low levels of CNP immunoreactivity detected in pig and human hearts were thought to reflect cross-reactivity with ANP or products from coronary arterial ECs [26]. Subsequent immunohistochemical and reverse transcription polymerase chain reaction analyses confirmed the presence of CNP and its mRNA in both the atrium and ventricle, and a recent in vitro study verified that a significant amount of CNP is expressed in and secreted from cultured rat cardiac fibroblasts, but not myocytes, indicating a cardiac expression profile that is distinctly different than those of ANP and BNP [27].

**Molecular mechanism regulating natriuretic peptide gene expression**

**Transcriptional regulation of ANP gene expression**

Studies using transgenic mice carrying a 500-bp segment of the 5′ flanking region (5′-FR) of the human ANP gene fused to a gene encoding SV40 large T antigen, a 2.4-kbp 5′-FR segment of human ANP gene fused to the chloramphenicol acetyltransferase gene, or a 638-bp or 3-kbp 5′-FR segment of the rat ANP gene fused to the luciferase gene have shown that the proximal 5′-FR of the ANP gene is sufficient to recapitulate the spatial and temporal expression of the endogenous ANP gene, and that the region contains sequences important for the regulation of ANP gene expression in the heart [28–30]. Indeed, expression of a reporter gene driven by the proximal 5′-FR of the ANP gene in atrial or ventricular cardiac myocytes at different developmental stages showed that the region confers proper spatial and temporal activity to the ANP promoter [31,32]. The proximal 5′-FR of the ANP gene contains multiple transcription factor-binding sites, including two CArG boxes, two NKEs, three TBEs, two GATA sites, an A/T-rich element and a phrenylephrine-responsive element (PERE), to which the transcriptional factors SRF, NKX2.5, Tbx5, GATA4/6, MEF2C and Zfp260 have been shown to bind [33,34]. These transcriptional factor-binding elements contribute singly and cooperatively to the basal and inducible activation of ANP promoter activity in cardiac cells [33,34]. In addition, neuron-restrictive silenced element (NRSE), hypoxia-response element (HRE) and glucocorticoid responsive element (GRE), which are located outside the proximal promoter, also reportedly mediate inducible ANP gene transcription [33,36]. In that regard, differences in the expression pattern of the proximal 5′-FR of the ANP gene and the intact endogenous ANP gene highlight the importance of regulatory elements outside the proximal 5′-FR [33]. It also should be noted that the ANP gene is located 8 kbp and 12 kbp downstream of the BNP gene on the same chromosome in humans and mice, respectively (human, chromosome 1; mouse, chromosome 4) [37,38].

**Transcriptional regulation of BNP gene expression**

The 5′-FR of the BNP gene has also been studied so as to better understand the regulatory mechanisms governing the gene’s cardiac-specific and inducible expression. A study using transgenic mice carrying a 5′-FR segment of the human BNP gene extending from −1818 to +100, or from −408 to +100, coupled to a luciferase gene (−1818hBNPluc and −400hBNPluc, respectively) showed that the proximal region of the human BNP promoter is sufficient to mediate ventricle-specific expression [39]. The luciferase activity of −1818hBNPluc was also greater in ventricular myocytes than in atrial myocytes [40]. In addition, deletion analysis showed that the region extending from −127 to −40 of the human BNP 5′-FR confers cardiac-specific expression [40]. This proximal region of the human BNP promoter contains potential GATA, M-CAT and AP-1/CRE-like elements, which are conserved among humans, rats and mice [41].

All three of the aforementioned elements are known to regulate cardiac-specific gene expression, and have been shown to mediate both basal and inducible BNP gene expression [41]. Other sites located in relatively distal regions of the human 5′-FR, including NRSE (−552), shear stress-responsive elements [SSRE] (−652, −641 and 161), thyroid hormone-responsive element (TRE) (−1000) and the nuclear factor of activated T-cells (NF-AT) binding site (−927), have also been shown to participate in inducible activation of the human BNP promoter [41,42]. Among these elements, the transcriptional repressor element NRSE, which is located at −552 in the human BNP promoter and is conserved in the rat and mouse BNP promoters, represses basal BNP promoter activity and mediates hypertrophic signaling evoked with extracellular matrix [43]. A transcriptional repressor, neuron restrictive-silencer factor (NRSF, also named as REST), binds to NRSE, thereby repressing promoter activity. Interestingly, NRSE is also located in the 3′ untranslated region of the ANP gene and is involved in basal and endothelin-1-inducible activation of human ANP transcription [35]. Moreover, cardiac-restricted inactivation of NRSF through overexpression of a dominant-negative NRSF driven by the cardiac-specific a-MHC promoter leads to up-regulation of ventricular ANP and BNP gene expression, cardiomyopathy and sudden death, which confirms the importance of NRSF in the regulation of cardiac gene expression and cardiac function [36]. Although BNP gene expression is reportedly under the control of SRF, as is the case with ANP gene expression, a functional CArG box had not been identified in the proximal 5′-FR of the BNP gene [44]. However, we recently identified a conserved and functional CArG box in the BNP 5′-FR, and showed that Rho- and actin dynamics-dependent signaling activates BNP gene expression through this element by inducing translocation of a novel SRF co-factor, myocardin-related transcription factor (MRTF)-A (also named as MAL or MKL1) [45,46].

**Transcriptional regulation of CNP gene expression**

Transcriptional regulation of CNP has received much less attention than that of ANP and BNP. It is known, however, that two GC-rich elements in the CNP promoter play an important role in regulating CNP gene expression [47]. TSC22D1, a leucine zipper protein, and STK16 (TSF-1), a protein possessing both DNA-binding ability and serine-threonine kinase activity, have been identified as transcription factors that bind to the GC-rich element [48,49].
ANP and BNP as diagnostic markers of cardiovascular disease

Plasma levels of ANP and BNP are increased under such pathological conditions as heart failure, myocardial infarction, hypertension, left ventricular hypertrophy and pulmonary hypertension.

Heart failure

Initial studies demonstrated that plasma ANP levels increase in proportion to the severity of symptomatic congestive heart failure [50]. Similarly, levels of both ANP and BNP increase and their secretion profiles vary according to the underlying cardiac condition in patients with severe heart failure [51]. Several studies have also shown that plasma ANP levels are significantly elevated in asymptomatic patients with left ventricular dysfunction. Conversely, the severity of heart failure has been evaluated based on plasma BNP levels, which closely correlate with NYHA functional class and hemodynamics [21]. In addition, plasma ANP levels increase in response to increases in right and/or left atrial pressure, while increases in BNP reflect the degree of ventricular overload. Plasma BNP levels are also useful for identifying patients with asymptomatic left ventricular dysfunction, and several investigators have found that they can serve as diagnostic markers of left ventricular systolic dysfunction both in the general population and in patients with cardiovascular disease [52]. Furthermore, low BNP levels can be used to rule out congestive heart failure in symptomatic individuals with higher negative predictive values. In the urgent care setting, rapid measurement of BNP values is useful for establishing or excluding a diagnosis of congestive heart failure in patients with acute dyspnea [53]. Thus, measuring BNP levels is useful not only for evaluating the severity of heart failure, but also for excluding and screening for left ventricular dysfunction and heart failure. For these reasons, authoritative guidelines on the clinical diagnosis and management of heart failure recommend measuring BNP [54–56].

Acute myocardial infarction

Plasma ANP levels are already elevated by the time patients with acute myocardial infarction are admitted to hospital and decrease thereafter. By contrast, although plasma BNP levels are significantly elevated at admission, they do not peak until 12–24 h after the onset of infarction; they then decline and peak once again 5–7 days later [57]. Furthermore, despite a gradual decline, BNP levels remain significantly elevated during the chronic phase, reflecting any permanent impairment of left ventricular function and/or remodeling that occurs. BNP levels return to normal or near normal in patients without left ventricular remodeling after successful early coronary reperfusion [58]. The height of the second peak during the acute phase serves as a valuable index of left ventricular remodeling in patients with acute myocardial infarction [59], as myocardial hypoxia, intracellular acidosis and myocardial stretch all contribute to the increase in natriuretic peptide levels during ventricular remodeling [60].

Hypertension and left ventricular hypertrophy

Several studies have shown that plasma ANP and BNP levels are higher in patients with hypertension than in normotensive individuals [61], and are higher in hypertensive patients with left ventricular hypertrophy than in those without left ventricular hypertrophy [62]. In addition, BNP levels are significantly higher in patients with left ventricular concentric hypertrophy than in those with eccentric hypertrophy or concentric remodeling, or in patients with essential hypertension but a normal left ventricular structure [63]. Antihypertensive therapy that reduces blood pressure leads to a decline in BNP levels and a reduction in left ventricular mass [64]. Measurement of BNP is also useful for detecting the patients with left ventricular hypertrophy in the general population [65].

Pulmonary hypertension

Plasma ANP and BNP levels are elevated in patients with right ventricular overload, such as that caused by pulmonary hypertension, or right ventricular volume overload, such as that caused by an atrial septal defect. Interestingly, ANP is dominant in atrial septal defects [66], whereas BNP is dominant in pulmonary hypertension [67]; but when pulmonary hypertension accompanies an atrial septal defect, BNP is dominant. ANP and BNP levels correlate with mean pulmonary arterial pressure, right atrial pressure, right ventricular end-diastolic pressure and total pulmonary resistance in patients with pulmonary hypertension [67]. ANP and BNP levels decline together with a reduction in total pulmonary resistance after long-term therapy with prostaglandin derivatives [68]. Plasma BNP levels are also elevated in patients with acute pulmonary embolism. In these patients, elevated BNP is associated with right ventricular overload as well as increased mortality. Thus, pressure overload in the right atrium and ventricle stimulates ANP and BNP secretion independently of etiology, and plasma levels of these peptides are good indices of the severity and effects of treatment in patients with right ventricular overload. These results suggest that the measurement of BNP is a useful means of assessing disease severity, the effect of drug treatment, and the prognosis of patients with cardiovascular disease.

Molecular complexity of BNP in plasma — increased proBNP-108 in heart failure

ProBNP-108 is thought to be cleaved to BNP-32 and N-terminal proBNP-76 in an equimolar fashion by a processing enzyme as it is secreted from ventricular myocytes. However, recent studies have shown that, in addition to NP-32 and NT-proBNP-76, proBNP-108 also circulates in human plasma and that the level of this peptide is increased in heart failure [69,70]. What is more, other studies have shown that the assay kit currently being used for BNP-32 also recognizes proBNP-108 and that there is a high degree
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Figure 5  Schematic representation of the processing and glycosylation of proBNP in cardiomyocytes. Some of the biosynthesized proBNP-108 is O-glycosylated within the Golgi apparatus. If O-glycosylation does not occur at Thr-71, proBNP-108 is cleaved to BNP-32 and NT-proBNP-76 by furin within the trans-Golgi network. BNP-32 and NT-proBNP-76 are then secreted in an equimolar fashion. If O-glycosylation occurs at Thr-71 of proBNP, glycosylated-proBNP-108 is not cleaved by the processing enzyme, and uncleaved glycosylated proBNP-108 is secreted into the circulation.

of cross-reactivity [71], which suggests the BNP level measured using this assay system is the sum of the BNP-32 and proBNP-108 levels. We recently showed that both proBNP-108 and BNP-32 circulate in the plasma of patients with heart failure and that the proBNP-108/BNP-32 ratios vary greatly, depending on the patient’s heart failure status [11]. This ratio is markedly higher in patients with heart failure caused by ventricular overload, as well as in those with decompensated heart failure, but the ratio is not increased in patients with atrial overload. In addition, proBNP-108 is a major molecular form in human ventricular tissue. These findings are consistent with the hypothesis that proBNP-108 is the major molecular form of BNP in the ventricle and that proBNP-108 levels increase in response to ventricular overload [11].

On the other hand, proBNP-108 does not induce cGMP production as effectively as BNP-32. In mild to moderate heart failure, the plasma cGMP level increases in proportion to the severity of the heart failure, and its level correlates with plasma BNP. In severe heart failure, however, the increases in cGMP level are attenuated relative to the disease state, so that the plasma cGMP levels no longer correlate with the BNP levels [72]. Apparently, the increase in the hormonally less active proBNP-108 that occurs in severe heart failure results in a relative deficiency in natriuretic peptide.

A new hypothesis of processing of proBNP

Recent studies have shown that proBNP-108 is O-glycosylated and that the degree of glycosylation depends on heart failure severity [73,74]. The clinical relevance of proBNP-108 glycosylation remains unclear, however. Various stimuli, including pressure overload, volume overload and ischemia, stimulate BNP gene transcription, after which the transcript is translated in the endoplasmic reticulum to produce preproBNP. As the protein is processed in the Golgi network, removal of its signal peptide generates proBNP-108, which can then be posttranslationally glycosylated to varying degrees at several sites in its N-terminal region (Ser36, Thr37, Thr44, Thr48, Thr53, Ser58 and Thr71). The O-glycosylated proBNP-108 is then transported to the trans-Golgi network, where it is cleaved to BNP-32 and NT-proBNP-76, probably by furin [75]. Both BNP-32 and NT-proBNP-76 are thought to be secreted via a constitutive pathway without storage in secretory granules.

How glyco-proBNP-108 is secreted without processing under conditions of severe heart failure is not fully understood. One recent study has shown that O-glycosylation at Thr-71, which is situated close to the cleavage site, impairs proBNP-108 processing by furin in HEK293 cells, a cell line derived from human embryonic kidney cells [76] (Fig. 5). Because the effect of O-glycosylation was only evaluated with furin in that vitro study, the actions of other potential processing enzymes are still unknown. Also unknown is whether similar glycosylation occurs in cardiac myocytes in both the atrium and ventricle. Further studies using cardiac myocytes will be required to elucidate the precise mechanism by which proBNP-108 is processed.

Conflict of interest

None of the authors have a conflict of interest.

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