

Biology of the B-Type Natriuretic Peptide: Structure, Synthesis and Processing

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Abstract

In 1988, a new member of the natriuretic peptide family was isolated. BNP is a circulating hormone playing key role, together with the sympathetic nervous system and other hormones like an endogenous system in the regulation of the body fluid homeostasis and blood pressure. The function and physiological role of B type natriuretic peptide is pleiotropic. BNP is synthesized from polypeptide precursors. BNP and N-terminal proBNP are the products of pro BNP processing. In many cardiovascular diseases natriuretic peptides serve not only as marker for diagnosis and prognosis but they have therapeutic importance. In the last years the potential use of the elevated BNP levels for diagnosis of pre-eclampsia was examined. In our review, we discuss the current understanding of molecular biology, biochemistry and clinical relevance of natriuretic peptides.

Keywords: B-type natriuretic peptide; Biologic function; Genetic; Cardiovascular diseases

Introduction

Brain natriuretic peptide (BNP) is a member of natriuretic peptide family [1-3]. The molecules of this family have a common ring structure, which consists of 17 amino acids. This structure is thought to be essential for mediating receptor binding and the biological activity of natriuretic peptides [4,5]. In 1988, Sudoh et al. [6] purified BNP first time from porcine brain extracts. The highest concentrations of BNP were measured however in the heart, where it acts as a cardiac hormone [7]. It is regulated by increased wall stress, causing significant decrease in blood pressure. The half-life of the BNP is about 20 minutes. It has been isolated and a variety of species of BNP peptides and cDNA clones are sequenced. Although the structure of ANP is relatively well conserved among species, the structure of BNP differs. The predominant circulating form of BNP is a 26-, 45- and 32-amino acid peptide in pig, rat and human, respectively. BNP exerts its physiological actions by binding to natriuretic peptide receptor A (NPR-A) on the surface of the target cells [8]. This receptor contains an intracellular guanylyl-cyclase domain. Activation of NPR-A leads to elevation of cyclic GMP (cGMP) levels, which mediates biological effects: vasodilatation, natriuresis, diuresis, inhibition of the aldosterone synthesis and lipolysis. The BNP and its mRNA concentrations are much higher in the human cardiac atrium than in the ventricles. However, given the great ventricular mass which is 70% of the total content, all cardiac BNP and its mRNA derives from the ventricles [5]. Plasma BNP levels in the anterior interventricular vein and in the coronary sinus are higher than in the aortic root. The tissue distribution of BNP differs among species. In humans, extracardiac sources of BNP have been found in the lungs, kidneys, brain, aorta, adrenal glands and in fetal membranes (both in amnion and chorion) in much smaller concentrations than in the atrias. Levels of myocardial BNP mRNA and circulating BNP as well as NT-proBNP-76 are 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.

Structure of the NPPB gene and Molecular Forms of BNP

The BNP gene (Natriuretic Peptide Precursor B gene, NPPB gene)

is positioned on the short arm of the human chromosome 1 [9,10]. The BNP gene contains three exons. 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 histidine and the 3'-untranslated region. BNP mRNA is translated to 134-amino acid preproBNP, after which the 26-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]. The cleavage of proBNP into BNP-32 and N-terminal proBNP-76 occurs in the trans-Golgi network by furin, which is a subtilisin-like prohormone convertase [12]. The biological function of NT-proBNP is still unknown. BNP-32 and N-terminal pro-BNP are then released into the circulation via a constitutive pathway. Recent studies have shown that proBNP-108 is also present in human plasma and the proBNP-108/BNP-32 ratio is increased in cases of severe heart failure [13]. Among the important fragments of BNP that circulate is BNP 3-32, which results from the cleavage of BNP1-32 by dipeptidyl peptidase IV. It does not change the resistance of human BNP to further degradation by human neutral endopeptidase. Recently, it was reported that BNP 1-32 is cleaved by the metalloprotease meprin A to BNP 8-32, which has a lower bioactivity in the kidneys [14]. There is an alternative spliced transcript for proBNP, which has an intronic retention. This transcript contains the first and second exons and the second intron. It encodes and alters BNP. It has an additional 34 amino acid from the second intron, but missing 5 amino acid from exon3 [15]. This 61 amino acid BNP cannot stimulate cGMP production and has no vasorelaxing effect.

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Received November 17, 2012; Accepted November 19, 2012; Published November 21, 2012

Citation: Szabó G (2012) Biology of the B-Type Natriuretic Peptide: Structure, Synthesis and Processing. Biochem Anal Biochem 1:e129. doi:10.4172/2161-1009.1000e129

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Transcriptional Regulation of the NPPB Gene Expression and Post-translational Modification of proBNP

The 5'-flanking region of the human NPPB gene was subcloned in 1996 to evaluate gene expression. It has been studied to understand the mechanisms regulating the gene's cardiac-specific and inducible expression. Several single nucleotide polymorphisms and complex mutations were identified, some of which were shown to be associated with cardiovascular physiopathology. By the single nucleotide polymorphism rs198389 (T-381C) in the promoter region of the NPPB gene, the homozygotes for C allele show a higher prevalence for atherosclerotic renovascular hypertension [16]. Kosuge et al. [17] found association with the (TTTC)_n microsatellite polymorphism in the 5'-flanking region of the NPPB gene and essential hypertension. This polymorphism is connected with elevated BNP levels in pre-eclampsia [18]. Another complex mutation in this region was reported to have impact on the antihypertensive effects of sodium nitroprusside in patients with hypertension [19].

Unlike ANP, proBNP gene expression must be upregulated before it can be released into the blood. Transcription of the NPPB gene is activated in response to increased stretching of the myocardium caused by pressure and volume overload. Cytokines, neurohormones, endothelin-1, angiotensin II were shown to activate transcription. 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 [20]. The luciferase activity of -1818hBNPluc was also greater in ventricular myocytes than in atrial myocytes [21]. Deletion analysis showed that the region extending from -127 to -40 of the human BNP 5'-flanking region confers cardiac-specific expression. 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 [22]. All of these elements are known to regulate cardiac-specific gene expression, and have been shown to mediate both basal and inducible BNP gene expression. Other sites located in relatively distal regions of the human BNP 5'-flanking region, 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 [23]. Among these elements, the transcriptional repressor element NRSE, which is located at -552 in the human BNP promoter represses basal BNP promoter activity and mediates hypertrophic signaling evoked with extracellular matrix [24]. A transcriptional repressor, neuron restrictive-silencer factor (NRSF), binds to NRSE repressing promoter activity. NRSE is located in the 3' untranslated region of the ANP gene and is involved in basal and endothelin-1-inducible activation of human ANP transcription [25]. Recently, it was identified as a conserved and functional CArG box in the BNP 5'-flanking region, 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) [26].

Regulation of BNP gene expression also occurs at post-transcriptional level. There is a characteristic sequence in the BNP mRNA that makes a difference from ANP mRNA. It is a conserved sequence consisting of repeated AUUUA units in the 3' untranslated

region). The presence of this sequence accelerates the degradation of BNP mRNA similar to that seen with lymphokine genes and oncogenes [27]. BNP gene expression is regulated dynamically, depending on the physiological and pathophysiological conditions, differently from ANP gene expression.

Post-translational modifications of proBNP have connections with the O-glycosylation. There are seven identified sites of O-glycosylation in recombinant proBNP. It appears that the level of endogenous Nt-proBNP and proBNP glycosylation in humans depend on high interindividual variability.

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