The Journal Of The International Federation Of Clinical Chemistry And Laboratory Medicine

eJIFCC

10. NATRIURETIC PEPTIDES IN ASSESSMENT OF VENTRICULAR DYSFUNCTION

Maksimiljan Gorenjak, MSc

Department for Laboratory Diagnostics, Teaching Hospital, Maribor, Slovenia

1.1 Introduction

The natriuretic peptide (NP) family is comprised of four peptide hormones, each with a common 17 amino acid ring structure. The precursor prohormone for each is encoded by a separate gene. The tissue-specific distribution and regulation of each peptide is different.

Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are similar in their ability to promote natriuresis and diuresis and act as vasodilators. These actions are counterbalanced by the vasoconstrictive and sodium-retaining actions of the renin-angiotensin-aldosteron system. Both systems help to maintain sodium and fluid volume homeostasis in a healthy cardio-renal environment. C-type natriuretic peptide and urodilatin act predominately as vasodilators. Each NP has antimitogenic activity in both the cardiovascular system and other organ systems. This implies that the NPs may modulate growth within the vascular wall in disorders such as atherosclerosis, hypetension, and postangioplasty restenosis.

Atrial natriuretic peptide is produced mainly in the atria. Increased atrial wall tension, reflecting increased intravascular volume, is the dominant stimulus for its release. Cleavage of human pro-ANP releases a 98-amino acid amino-terminal fragment (NT-proANP), as well as a 28-amino acid carboxy-terminal fragment that is mature ANP which is the biologicaly active form of ANP. It has been suggested that a transmembrane serine protease highly expressed in the heart converts proANP into ANP and NT-proANP. Both fragments circulate in the plasma. Little NP is produced by ventricular tissue in normal adults, but it is present in ventricular tissue of fetuses and neonates and in hypertrophied ventricles.

The ANP gene is also expressed in the kidney, where alternative processing of the precursor generates a 32-amino acid substance- urodilatin, one of the factors that regulate sodium and water handling in the kidney.

Brain natriuretic peptide was isolated from porcine brain in 1988. This 32-amino acid peptide also contained a 17-amino acid ring (Figure 1). The name brain natriuretic peptide quickly became something of a misnomer when it was discovered that the highest level of BNP was in the ventricular myocardium. Human proBNP contain 108 amino acids; processing releases a mature 32-amino acid molecule of BNP and an amino terminal fragment NT-proBNP (1-76 amino acids sequence).

A third peptide, C-type natriuretic peptide (CNP), a paracrine hormone with high concentrations in the vascular endothelium, belongs to this family as well. There are two forms of CNP consisting of either 53 or 22 amino acids; both lack an amino acid tail at the carboxy terminal.

Another member of the natriuretic peptide family is urodilatin which is localized in the kidneys and secreted into urine. It is a paracrine factor involved in the local regulation of the body fluid volume and water-electrolyte excretion by regulating water and sodium reabsorption. Urodilatin is a differentially processed form of precursor proANP. ANP and CNP are highly conserved across species, whereas BNP shows species specificity.

The natriuretic peptides are ligands for three different natriuretic peptide receptors (NPR), that are named A, B and C, with designation not corresponding to their relative affinities for ANP, BNP and CNP. All three receptors are widely distributed in target tissues and have been localized in the kidney, heart, vascular endothelium, adrenals, and throughout the central nervous system. The NPR are transmembrane proteins, members of the receptor guanyl cyclase family. The clearance of NPs from the circulation occurs via two mechanisms; first, via NPR-C receptor-mediated endocytosis, followed by lysosomal degradation; second, via degradation by the zinc-containing enzyme, neutral endopeptidase, a non-specific membrane-bound enzyme present in the kidney and vascular beds that cleaves NPs and opens the ring structure, thus inactivating the peptides.

1.2 Heart failure

Heart failure (HF) is a multisystem disorder which is characterized by abnormalities of cardiac, skeletal muscle, and renal function; stimulation of the sympathetic nervous system; and a complex pattern of neurohormonal changes.

HF is the major cause of cardiovascular morbidity and mortality and is one of the main causes of hospitalization in industrial countries. Currently, the prevalence of symptomatic HF in the general European population ranges from 0.4 to 2.0 percent. To provide cost-effective treatment for these patients, rapid and accurate differentiation of congestive heart failure from other causes of

eJIFCC2003Vol14No2pp089-091

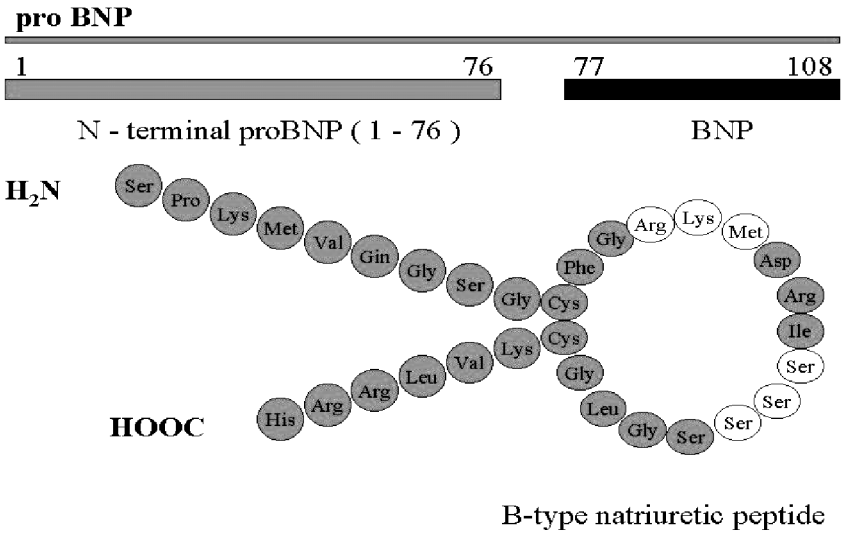

Figure 1. Brain natriuretic peptide (BNP) is synthesized as a high molecular weight precursor; Biologicaly active form BNP has amino acid sequence 77-108 and the N-terminal proBNP the sequence 1-76. Lower diagram gives the amino acid sequence and 17 amino acid ring structure that is common to all natriuretic peptides

dyspnoea must be accomplished. However, HF is often difficult to diagnose in the emergency department or urgent care setting. The symptoms may be nonspecific, and physical findings are not sensitive enough to use as a basis for an accurate diagnosis. Although echocardiography is considered the gold standard for the detection of left ventricular dysfunction, it is expensive. Is not always easily accessible, and may not always reflect an acute condition. Misdiagnosis can be life-threatening, because treatments for congestive heart failure are hazardous to patients with other conditions, such as chronic obstructive pulmonary disease, that have the same primary symptoms at presentation.

Early diagnosis and treatment of HF are important factors in reducing morbidity and mortality associated with the disease.

1.3 Diagnostic and prognostic use of ANP and BNP

NP determination is a useful addition to the standard clinical investigation of patient with ventricular dysfunction. During several years much work has demonstrated that NPs are the biochemical markers of choice for diagnosing and risk stratification of patients with HF. In side-by-side comparison, NPs were superior to other neurohormones, such as catecholamines, renin, angiotensin and aldosterone. The NP assays discriminated very well between controls and patients with end-stage HF. BNP measurement appears to be accurate for diagnosing HF in emergency department patients who present with acute dyspnoea. Increased NP plasma concentrations are, however, also frequently found in asymptomatic patients with left ventricular dysfunction. Therefore, these peptides have also been suggested as potentially useful early HF markers.

BNP is elevated in post myocardial infarct patients with impaired left ventricular function and BNP appears to be superior to ANP for identifying left ventricular systolic dysfunction. The very high negative predictive value of BNP in the detection of these diseases gives the best insight into how this test might be used clinically.

NP may be even more useful as prognostic indicators than as diagnostic markers. Elevated plasma levels of BNP have been suggested as an independent predictor of the severity of congestive HF and of mortality in patients with chronic HF.

The value of NPs has already been recognized by their inclusion in the recent European guidelines for the diagnosis of chronic HF.

1.4 Measurement of natriuretic peptides

The developing of a sensitive, precise, and accurate method for NPs measurement has been difficult because of the structural, metabolic, and physiological characteristics of these proteins. Therefore, several important points should be taken into account when discussing the pathophysiological relevance of particular NP assay:

- The goal should be to assess the activity of a specific hormone system; therefore, only the biologically active substances of this system should be assayed.

- Although ANP and BNP bind to same specific receptor, they have different types of metabolism and biological activity, and their production and secretion may be regulated differently in humans.
- ANP and BNP are stored in granules in form of proNP, which are split into an active form of ANP and BNP and their N-terminal fragment. Several studies have demonstrated that N-terminal proANP and N-terminal BNP have also some biological activity. However, these molecules exhibit (at least in part) different biological properties as well as different mechanism of action with respect to the ANP/BNP system.
- NPs are degraded both in vivo and in vitro by several proteases. EDTA and protease inhibitors (aprotinin) are generally added to whole blood samples to inhibit this degradation. Some recent studies have suggested that the use of protease inhibitors may be not necessary, at least for BNP and proANP.

The determination of the biologically active form of NPs (ANP and BNP) is generally performed by means of competitive immunoassays, such as RIA or EIA; recently some noncompetitive immunoassays have been developed. The methods of this second generation are two-site (sandwich) immunometric assays, using two specific monoclonal antibodies or antisera prepared against two sterically remote epitopes of the ANP and BNP peptide chain.

Noncompetitive assays are generally more precise and sensitive than their respective competitive assays and are not significantly affected by nonspecific interference. These facts suggest that non-competitive immunoassays for ANP and BNP may be more suitable for clinical routine than competitive assays.

Theoretically, developing an immunoassay for N-terminal peptide fragments of proANP and proBNP should be easier, because these peptides have higher plasma concentrations. However, these immunoassays may also be affected by several analytical problems, mainly concerning the assay specificity. Because there is no a general consensus on the best method for NPs assay, at the present time each laboratory must choose the methods and the peptides to measure that meet its own clinical requirements as well as to other issues, such as stability of both analytes and materials, ease of measurement, and costs.

1.5 Preanalytical factors

ANP has very short half-life time (3 minutes) and is poorly stable *in vitro* and thus appears suitable only for point-of-care measurement under routine conditions. By contrast, BNP (20 min.), NT-proANP (60 min.), and NT-proBNP (60-120 min.) are sufficiently stable in EDTA-containing plastic tubes to be sent to the laboratory without special care. When handling NP, glassware should be avoided or must be carefully siliconized to avoid adsorption of NP to the wall. The larger N-terminal prohormone fragments are more stable and have a longer biological half-life and requirements for blood sampling are less critical. They are also less sensitive to the rapid fluctuations caused by short-term stimuli of secretion, such as change in body posture, exercise, or acute volume load.

So far, preanalytic influences on NP concentrations are not completely clear. Therefore, blood sampling should be performed under standardized conditions. It has been reported by some investigators that BNP values are higher in healthy women than in healthy men, and that there is a positive relationship between BNP values and age.

1.6 Conclusions

Studies of the NP family have increased our understanding of blood-volume regulation and blood-pressure control. The natriuretic peptides defend against excess salt and water retention, inhibit the production and action of vasoconstrictor peptides, promote vascular relaxation, and inhibit sympathetic outflow. These actions lead to a reduction in blood pressure that is most apparent in states of volume excess. NPs may be most useful clinically as a rule-out test due to consistent and very high negative predictive values.

The value of NP assays has already been recognized by their inclusion in the recent European guidelines for the diagnosis of chronic HF. At present time there is no consensus on the best method for NP assay, although non-competitive immunoassays may be more suitable for clinical routine than competitive assays. Therefore, each laboratory must choose the methods and the peptides to assay that meet its own clinical requirements.

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