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Atrial natriuretic peptides in plasma

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ABSTRACT

Measurement of cardiac natriuretic peptides in plasma has gained a diagnostic role in the assessment of heart failure. Plasma measurement is though hampered by the marked instability of the hormones, which has led to the development of analyses that target N-terminal fragments from the prohormone. These fragments are stable in plasma and represent surrogate markers of the actual natriuretic hormone. Post-translational processing of the precursors, however, is revealing itself to be a complex event with new information still being reported on proteolysis, covalent modifications, and amino acid derivatizations. In this mini-review, we summarize measurement of the principal cardiac hormone, e.g. atrial natriuretic peptide (ANP) and its precursor fragments. We also highlight some of the analytical pitfalls and problems and the concurrent clinical "proof of concept". We conclude that biochemical research into proANP-derived peptides is still worthy of attention and that new biological insight may change our chemical perception of the markers.

1. Introduction

"When the right thing can only be measured poorly, it tends to cause the wrong thing to be measured well. And, it is often much worse to have a good measurement of the wrong thing, especially when it is so often the case that the wrong thing will, in fact, be used as an indicator of the right thing, than to have a poor measure of the right thing". With such precision, the statistician John Tukey expressed his experience with scientific data. Notably, this statement is also valid in the clinical setting, where for instance blood borne markers are often used as surrogate measures of a complex and often poorly understood biology but still reduced to mere numerical numbering. One classic simple example is blood glucose as a measure of diabetes pathophysiology. Natriuretic peptides as markers of the heart failure syndrome should also be considered only as surrogate measures albeit their usefulness in both the diagnosis and monitoring of disease has been documented.

From a molecular point of view, John Tukey's statement should also awaken scrutiny. As reviewed below, we now use plasma measurement of peptide fragments from the natriuretic peptide prohormones as markers of the bioactive compounds; to a large extent simply because specific immunoassays are more easily developed for these fragments, which are more stable analytes in plasma. But clinical documentation as to what end of the prohormones is truly the best measure in the heart failure syndrome remains to this day almost unaddressed.

In this mini-review, we first summarize the measurement of the principal cardiac hormone atrial natriuretic peptide (ANP) and its precursor fragments. Then, we highlight on selected analytical pitfalls. For measurement of the related B-type natriuretic peptide in plasma, we refer to earlier reviews on the subject [1–3]. We conclude that further research into plasma measurement of proANP-derived peptides is still worthy of scientific attention.

2. Historical ANP

Atrial natriuretic factor, or ANF, was logically named after its primary site of production, the cardiac atria [4]. When infused into animals, atrial tissue extracts elicit prompt natriuresis. The natriuretic factor was identified as a small peptide containing a disulfide bridge (and by many hence renamed ANP), which is essential for receptor binding and physiological effects [5]. In the circulation, ANP circulates without binding to plasma proteins, which is often the case for labile hormones. The ANP gene is also expressed in other tissues [6–8], but this extracardiac production seems to have little impact on plasma ANP concentrations when compared to the cardiac release.

The first immunoassay for ANP in plasma was reported in 1985, where the assay was based on antiserum from the Peninsula Laboratories [9]. Although the assay epitope was not defined, antibody detection of $ANP_{1–28}$ seemed to require the amino-terminal decasequence of the peptide. Plasma concentrations in healthy individuals were (and still are) in the low picomolar range [10]. ANP in plasma stored at −20 °C is, however, unstable which suggests problems in samples handled at room temperature. Accurate ANP measurement in plasma requires delicate handling of the blood sample itself, rapid separation of chelated

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plasma, and storage at −80 °C if analysis is to be performed at a later time. From a practical point of view, even more troublesome is the need for plasma extraction prior to immunoassay measurement, as plasma contains unspecific protein interference [11]. While this is achievable in research projects, it is almost impossible in the everyday measurement of ANP in patient plasma.

3. ProANP measurement

Given the laborious work with preanalytical sample handling and extraction in ANP measurement, one alternative strategy would be to measure another peptide fragment from the prohormone, which is analogous to C-peptide measurement in assessing insulin production [12]. For proANP, this strategy was first reported in 1989, where antibodies raised against $proANP_{1-30}$ and $proANP_{79-98}$ were used [13,14]. Notably, these sequences were deduced from cDNA sequences and not based on identified protein/peptide fragments. Generally, the plasma concentration of the N-terminal fragment(s) is(are) higher than for the bioactive ANP peptide, typically around 4-fold when using the early immunoassays. The preanalytical stability, however, was much better than for ANP, which rapidly led to the concept of a useful marker in heart failure that could be handled by routine laboratories [15]. This resulted in a battery of research assays being introduced by diagnostic companies, most often in the form of ELISAs. The assay validation was often incomplete with little information on specificity and unspecific interference. One consequence was that the molar concentrations between assays varied to an extent that biology itself could not explain. Moreover, ELISA and RIA are not suitable for everyday diagnostics in acute settings, which again only allowed for testing in already collected samples, e.g. clinical studies. Prospective studies were not reported for the proANP peptidology.

One automated analysis was reported in 2004, which generated new interest in proANP-derived peptides [16]. This method is based on antibodies raised against the mid-region of proANP, where the dogma was (and still is) that little proteolytic degradation occurs. Hence, this region represents a simple and stable analyte. From this methodology, a series of clinical reports have been published; essentially the measurement seems to match that of the related proBNP [17,18] with one major difference: Mid-regional proANP concentrations are much higher than that of proBNP-related peptides in non-cardiac patients and thus allow for information on decreased concentrations compared to reference individuals [19]. Notably, this information may be important for instance in obesity and diabetes, where the paradoxical decrease has gained considerable interest in connection to metabolic hormones and their regulation [20,21].

4. Total measurement of ANP expression

One analytical approach in quantitating the total sum of proANP-derived peptides in plasma is to measure a fragment that is specifically generated by exogenous proteolysis. This approach is well-established in proteomic identification by mass spectrometry. In brief, plasma is subjected to proteolysis by trypsin for instance, and a particular fragment derived from this process can then be a target for conventional immunoanalyses [22]. One beneficial side effect from this procedure is that unspecific assay interference is eliminated, which allows for a more accurate measurement of the peptide in question [23]. For proANP, we have developed such a methodology, which serves as a specialized analysis in research [24]. The technique is, as for ANP measurement, cumbersome and not applicable for everyday clinical measurement.

5. Post-translational proANP processing

Our general understanding of cardiomyocyte peptide processing is a relatively new area of interest. The early data suggested fairly simple processing of the prohormones, typically by endoproteolytic cleavage of the precursor in to an N-terminal fragment and the C-terminal natriuretic hormone (Fig. 1). New technology, however, has documented a more complex processing and harbored new thoughts in to clinical measurement. One early step after translation from mRNA to polypeptide is the cleavage of the signal peptide from the preprohormone. The

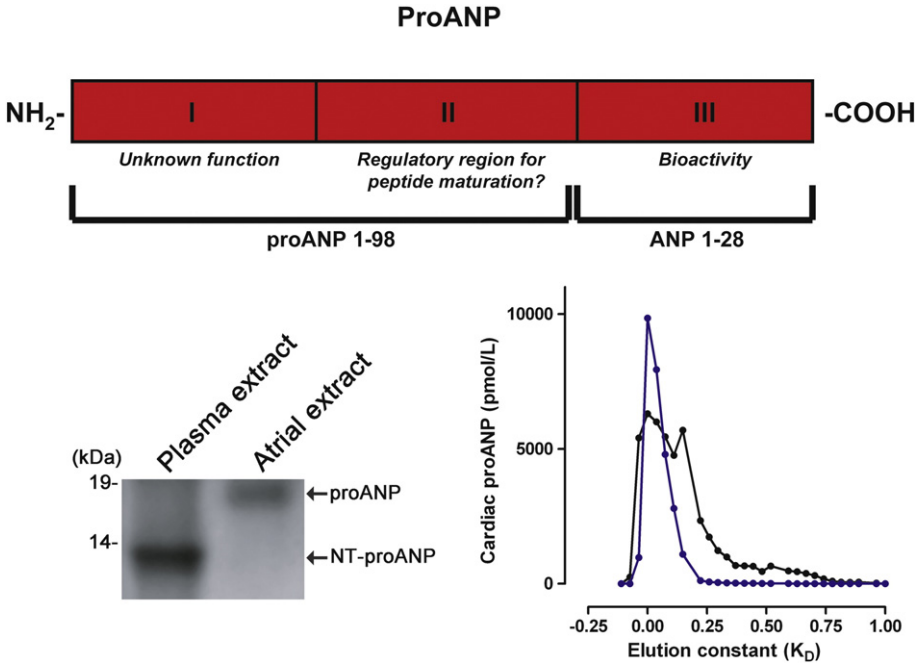

Fig. 1. This figure illustrates the human proANP structure, where we postulate that the midregion may be important for granular export and endoproteolytic processing. The Western blot (lower left) shows N-terminal proANP in porcine atrial extract and plasma. Note that different forms of proANP can be differentiated by this method. The gel chromatography on the lower right shows the elution profile of proANP in porcine atrial extract. The blue line depicts immunoreactivity for midregional proANP and the black line for N-terminal proANP (modified from Ref. [24] with permission).