1. Introduction

In the 20 years which have passed since the first description by de Bold of a natriuretic and diuretic substance produced in the heart [1], a large body of research has firmly established the position of the heart as an endocrine organ. Two peptides, both containing a 17-amino acid ring structure, have been a focus of much interest: atrial natriuretic peptide (ANP), and brain (or B-type) natriuretic peptide (BNP). A third member of this family of peptides, C-type natriuretic peptide, has been described; it is mainly produced by the endothelium and not by the heart, has no diuretic or natriuretic activity, and will not be discussed here.

ANP is produced mainly in the cardiac atria, while BNP, originally isolated from porcine brain, was soon reported to be mainly produced in the cardiac ventricles. In recent years, however, it has become clear that in fact both ANP and BNP are produced both in atria and in ventricles, and that ANP is normally predominant; under pathological conditions production of BNP rises strongly in both atria and ventricles and plasma concentrations may overtake those of (also risen) ANP [2,3]. Both are formed as pre-pro-polypeptides. Pro-atrial natriuretic peptide is a 126-amino acid peptide stored in atrial granulae; upon secretion it is cleaved by a serine protease into equimolar amounts of the active ANP (amino acids 99–126) and the inactive N-terminal fragment N-ANP (amino acids 1–98) [4]. Further degradation of N-ANP may lead to several smaller fragments some of which may also have biological activity [5]. Less is known about the procession of BNP; it is believed to be more constitutively expressed. Pro-brain natriuretic peptide is a 108-amino acid peptide also cleaved into equimolar amounts of the 32-amino acid active BNP and an inactive N-terminal fragment, N-BNP. ANP, BNP, N-ANP and N-BNP all circulate in plasma. In animals, the structure of the natriuretic peptides is often very different, especially for BNP, which is much less conserved than ANP.

The triggering factor for release/production of ANP and BNP is an increase in stretch and/or pressure, but neurohumoral factors such as angiotensin II and endothelin may also play a role. Most effects of ANP and BNP are mediated through binding to the A-type natriuretic peptide receptor, which activates guanyl cyclase, leading to the formation of cyclic guanosine monophosphate (cGMP). Clearance of ANP and BNP from the blood is effected in two ways: through a special clearance receptor, the C-type natriuretic peptide receptor, and through enzymatic degradation by neutral endopeptidase. The inactive N-terminal fragments have no specific clearance receptor. As a result they have a longer half-life, especially N-ANP, which therefore circulates in plasma in much higher concentrations than ANP. Also, the concentration is thereby more stable, being less influenced by short bursts of secretion.

In the following, we will briefly discuss the physiology of ANP and BNP, the methodology for measurements, the (potential) use of such measurements in clinical practice, and pharmacological manipulation, based on what is known at present and on our own experiences over the past 10 years in collaboration with many colleagues.

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2. Physiological effects of ANP and BNP

The principal function of ANP and BNP is to protect the cardiovascular system from volume overload. ANP and BNP exert their effects by interaction with the subtype A-natriuretic peptide receptor resulting in an increase in intracellular cGMP concentration. The subtype A-receptor is expressed in a variety of tissues, including kidney, blood vessels, adrenal glands, heart, lungs, adipose tissue, eye, and the pregnant uterus and placenta [6,7]. The other two subtypes are the B-receptor, for which C-type natriuretic peptide has by far the highest affinity, and the C-receptor, which is the clearance receptor. Natriuretic peptides have both natriuretic and diuretic effects. In addition, these peptides cause intravascular volume contraction by inducing a shift of fluid from the capillary bed to the interstitium, resulting in a decrease in preload and blood pressure [8–10].

The natriuretic action of ANP is effected by several mechanisms. In isolated kidneys as well as in normal human subjects ANP increases glomerular filtration rate (GFR), indicating that the renal haemodynamic effect of ANP in part accounts for its natriuretic action. Contrary to ANP, and notwithstanding a comparable natriuretic effect, BNP does not increase GFR in humans [11]. Apart from increasing GFR, ANP directly inhibits sodium transport in the proximal tubule and in the inner medullary collecting duct [12–14]. Furthermore, ANP as well as BNP inhibit aldosterone release from adrenal cells [15]. Moreover, there is evidence that both natriuretic peptides inhibit renal renin release. Because of these various actions natriuretic peptides, from a functional point of view, may be regarded as the natural antagonists of the sodium and volume conserving and blood pressure elevating renin–angiotensin system [16,17].

In human subjects information about the physiological role of ANP has indirectly been obtained by studying the effects of prolonged low-dose infusions of ANP or BNP on sodium balance and systemic haemodynamics [18]. From this study it appears that an approximately two-fold increase in plasma ANP concentration is sufficient to induce a negative sodium balance, a fall in systolic and diastolic blood pressure as well as an increase in heart rate. The effects of equimolar low-dose infusions of ANP and BNP, resulting in plasma levels of the respective peptides as observed in mild-to-moderate heart failure (5–6-fold increase), have been compared in a study performed in hypertensive subjects. This study revealed that the natriuretic and blood pressure lowering effects of BNP were 2–3-fold those of ANP [19]. Interestingly, despite the greater natriuretic effect of BNP, urinary cGMP excretion was lower with this peptide than with ANP.

Important information about the physiological role of the natriuretic peptides has further been obtained in experimental studies with HS-142-1, a selective antagonist of the natriuretic peptide A and B receptor. Studies with HS-142-1 performed in dogs have confirmed a role for the endogenous natriuretic peptides in the control of renal sodium excretion and in the natriuretic response to volume expansion [20,21].

3. Methodology

3.1. Blood sampling conditions

With many neurohormonal parameters, measurements are influenced by body position during blood sampling or by venepuncture itself. With the natriuretic peptides, this is not a great source of variation: blood sampling after 30 min of sitting gave the same values for ANP, BNP and N-ANP as after 30 min of bed rest, although sampling blood directly upon arrival or while standing resulted in somewhat higher values (least for N-ANP) [22]. This is in line with results of measurements after exercise, where N-ANP increased by only 5%, versus 59% for ANP, 38% for BNP and 24% for N-BNP [23].

3.2. Measurement

Ever since the discovery of the natriuretic peptides much effort has been put into the development of reliable methods for measuring them. All methods are based on immunoassays, mostly radioactive ones. Various research groups developed their own in-house radioimmunoassays (RIAs), but several immunoassay kits have now become commercially available [24–30]. For many reported methods a prior extraction step from plasma using SepPak C-18 minicolumns is necessary. Since this extraction step leads to losses in recovery (often 20–30%), is laborious, and is time-consuming, methods have been developed which obviate the need for prior extraction and which can be performed directly with plasma, giving results in 1–2 days.

Since the characteristics of antibodies can differ greatly, outcomes of measurements can also vary greatly. Apart from the natriuretic peptide of interest, varying amounts of fragments, with partial or full cross-reactivity, may also circulate in plasma, and thus can influence the outcome of measurements. With (extraction) RIA kits for ANP (Nichols, Wijchen, The Netherlands) and BNP (Peninsula, Belmont, CA, USA) e.g. we determined normal ranges of 15–35 and 7–16 pmol/l, respectively, while using the non-extraction sandwich immunoradiometric assay (IRMA) kits from Shionoria (Osaka, Japan) normal ranges of 1–15 and 1–10 pmol/l, respectively, were determined for ANP and BNP. Correlations are however good: in a series of 48 samples, measured with both methods for ANP and BNP, correlation coefficients of 0.83 and 0.91 were found. Usually similar results are obtained regarding elevations under pathophysiological conditions. An example is shown in Table 1, where (mean±S.E.) values of ANP and BNP are given in 20 controls and in 20 patients with congestive heart failure, measured with the above-
Table 1
Comparison of ANP and BNP as measured with radioimmunoassays and with immunoradiometric assays

<table>
<thead>
<tr>
<th></th>
<th>Control RIA</th>
<th>Control IRMA</th>
<th>Congestive heart failure RIA</th>
<th>Congestive heart failure IRMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANP (pmol/l)</td>
<td>39.1±3.4</td>
<td>4.6±0.8</td>
<td>256.1±48.9</td>
<td>59.4±12.0</td>
</tr>
<tr>
<td>BNP (pmol/l)</td>
<td>12.1±0.6</td>
<td>4.4±0.5</td>
<td>102.9±16.4</td>
<td>147.0±25.3</td>
</tr>
</tbody>
</table>

* Data are means±S.E. in 20 control subjects and in 20 patients with congestive heart failure.

mentioned RIA as well as IRMA methods. The elevations in CHF are obvious with both methods, although the percentage increases compared to control vary. It is thus imperative that for each method normal ranges are established; data from different methods cannot be compared directly.

In general, the methods used for ANP measurements give the largest differences, although most report upper levels of normal <15 pmol/l. BNP normal ranges differ less, and normal values are commonly reported to be lower than 12 pmol/l. The concentration of N-ANP is higher due to the longer half-life; with a non-extraction RIA from Biotop (Oulu, Finland) we have determined a normal range of 150–500 pmol/l, which seems to agree reasonably well with most other methods available. Some commercial kits for N-BNP have recently become available, but few if any experimental data are known at present. In-house methods report upper levels of normal <15 pmol/l, barely higher than BNP concentrations. An upper limit of 200 pmol/l however has been reported with an immunoluminometric method [31].

When looking at normal ranges, it should be borne in mind that the concentrations of natriuretic peptides increase with age, and there have also been reports that females generally have higher ANP and BNP concentrations than males [27,32].

Transformation of different units of measurement can be done using the following: 1 pmol/l of ANP=3.1 pg/ml; 1 pmol/l of BNP=3.5 pg/ml; 1 pmol/l of N-ANP=10.5 pg/ml, and 1 pmol/l of N-BNP=8.6 pg/ml.

3.3. Stability

The best way for reliable ANP and BNP measurements is to collect blood in chilled EDTA-tubes (1.9 mg/ml) also containing the protease inhibitor aprotinin (Trasylo; 100 kIU/ml), to prepare plasma as soon as possible (within 1 h, in the meantime kept on ice), and to store the plasma preferably at −70°C, or for up to 2 months at −20°C. Under these conditions, both ANP and BNP are stable, in contrast to a single report on ANP, refuted by many others [33–41]. Stability is less in the absence of aprotinin. The N-terminal peptides are more stable than the C-terminal ones. In whole blood (containing aprotinin), BNP has been reported to be stable for up to 3 days at room temperature, and N-ANP for up to 4 days, which allows for uncooled mail transport [42,43].

4. Use of natriuretic peptide measurements

4.1. Congestive heart failure (CHF)

In view of the localization and secretion mechanism of ANP and BNP, it is not surprising that elevated plasma levels of these hormones are found in conditions of increased cardiac wall stress. In CHF, circulating concentrations of both peptides (as well as of the N-terminal ones) are clearly elevated [44–49]. The elevation reflects the severity of the condition. An example is given in Fig. 1, where ANP and N-ANP median values of 74 patients, classified as NYHA class 0 (n=7), I (n=13), II (n=43) and III (n=11) are depicted; the trend is highly significant for both peptides [50]. In CHF, ANP is higher in patients with atrial fibrillation, than in patients with a sinus rhythm [51]. During long-standing atrial fibrillation ANP levels decrease again and may even fall to very low levels due to deficient ANP production by the degenerated, standstill atria [52].

It has become increasingly clear that measurement of one or more of the natriuretic peptides can be very helpful in the often still difficult diagnosis of incipient or mild CHF [53,54]. For example, in a study for diagnosis of CHF in GP practices in a suburb of Rotterdam, it was shown that combining history taking and a medical examination with measurement of ANP, BNP or N-ANP resulted in a receiver operating characteristic (ROC) of 0.87–0.92 for correct diagnosis of heart failure, similar to ROC values for more elaborate examinations including chest X-ray and echocardiography [55].

Of interest is whether natriuretic peptides are useful...
markers for the very early detection of cardiac damage or heart failure when patients are still asymptomatic. There is limited evidence that this may be the case. For instance, in a population-based study performed in 80 asymptomatic patients with impaired left ventricular function (ejection fraction 0.5±0.1), 26 patients with a persistently low ejection fraction (0.4±0.1) 1.3 years after the first examination had elevated ANP and BNP concentrations (60 and 20 pmol/l, respectively). In the 54 patients in which the ejection fraction had regressed to a normal value (0.7±0.1), the ANP and BNP concentrations (34 and 13 pmol/l, respectively) were not different from normal control values [56]. Repeated determination of atrial natriuretic peptides may also be of value for the early detection of cardiac damage induced by chemotherapy, radiation or other conditions. In a follow-up study in 56 breast cancer patients, treated with anthracycline-containing adjuvant chemotherapy, it was shown that 17 patients with exertional dyspnea, as an early sign of heart damage, had significantly higher N-ANP plasma levels than the patients without dyspnea [57]. Similarly, in 20 patients with carcinoid syndrome (where heart failure is a cause of high mortality) N-ANP was elevated in 70% of the patients [58].

Although a normal natriuretic peptide concentration virtually excludes the presence of heart failure (negative predictive value >90%), the reverse is not true. Elevated levels have a positive predictive value of only 30–40%, as other conditions like renal failure or pulmonary embolism may elevate the concentration of natriuretic peptides as well. For the diagnosis of CHF it is still open to discussion which of the natriuretic peptides can most profitably be used. For reasons of in-vitro and in-vivo stability in blood, the N-terminal peptides may have advantages, but at this time more experience has been obtained with BNP [59,60].

Several studies have clearly shown that natriuretic peptides are excellent prognostic indicators for survival in heart failure [61–64]. Our own results in 372 patients with congestive heart failure, followed-up for 5 years, indicate that ANP, BNP and N-ANP are superior over the more classical neurohormones like noradrenaline and renin [65]. It is interesting that several studies seem to indicate that natriuretic peptides have a higher predictive value for survival of CHF than ejection fraction.

Follow-up of treatment in CHF is another useful reason for measuring natriuretic peptides: a continued increase in plasma concentrations would be indicative of unsuccessful, and a decrease of successful, treatment [66]. A first encouraging study in which pharmacotherapy for heart failure was guided by plasma concentrations of N-BNP has recently been published [67].

With the advent of rapid simple methods for on-the-spot measurements of natriuretic peptides [such as the recently launched Triage system for BNP (Biosite Diagnostics, La Jolla, CA, USA), which provides results in 15 min [68]], it is foreseen that plasma levels of atrial natriuretic peptides will be used with increasing frequency both for diagnosis and follow-up of patients with CHF.

4.2. Acute coronary syndromes

In myocardial infarction ANP and BNP concentrations are also elevated, and are of prognostic value for indicating patients most at risk [69–76]. N-ANP and N-BNP, measured a few days after myocardial infarction, appear to have a better predictive value in this respect than ANP.

In patients with unstable angina pectoris BNP, but not ANP, concentrations were found to be four times higher as compared to values obtained in patients with stable angina pectoris or healthy control subjects [77].

4.3. Right ventricular overload

As mentioned previously, elevations in plasma ANP and/or BNP concentrations might also give information about the condition of the right ventricle. Indeed, in asymptomatic patients with right ventricular pressure overload due to congenital heart disease both BNP and ANP plasma levels were significantly increased, and correlated inversely with right ventricular ejection fraction as determined by magnetic resonance imaging [78].

Elevated plasma BNP concentrations have also been found in patients with an acute pulmonary embolism [79,80].

4.4. Renal failure

In chronic renal failure natriuretic peptides are often markedly elevated [81–83]. Overfilling, a diminished clearance and myocardial dysfunction may all contribute to this elevation. Plasma concentrations in patients with end-stage renal failure can vary widely. In seven patients on chronic haemodialysis, we found predialysis plasma ANP concentrations ranging from 90 to 2617 pmol/l and plasma BNP concentrations ranging from 6 to 2335 pmol/l. One hour after dialysis concentrations had declined by (median values of) 32% for ANP and 7% for BNP, most likely indicating that overfilling contributed to the elevated levels. This is supported by other studies showing a decrease in plasma ANP and BNP concentrations after hemodialysis [84,85]. Of interest is that an impaired renal function in CHF is a strong independent predictor of mortality, and that it is inversely related to increased levels of N-ANP [86]. In patients with chronic renal failure concentrations of BNP have been found to be independent prognostic parameters for survival, just as in CHF [85].

4.5. Hypertension

In hypertensive patient reports on whether natriuretic peptides are elevated are not uniform, but most studies report an elevation [87–89]. To some extent this may be
related to the absence or presence of left ventricular hypertrophy (LVH) [90–95]. If hypertension is complicated by left ventricular hypertrophy atrial natriuretic peptides are usually elevated, and as expected, plasma BNP concentration correlates better with left ventricular mass than with plasma ANP concentration.

In elderly, never-previously treated hypertensive subjects without overt heart failure, both ANP and BNP concentrations have been shown to be elevated in those subjects with concentric LVH, but not in those without LVH or in those with eccentric remodelling of the left ventricle [96–98]. Measurement of BNP as a marker for left ventricular dysfunction has been advocated [99–102]. In a recently published population-based study in 610 middle-aged subjects plasma BNP concentration as compared to control subjects were significantly and markedly increased in subjects with an increased LV mass as well as in subjects with systolic dysfunction [103]. After performing a multivariate analysis the positive and negative predictive value of BNP as a non-invasive marker to detect LVH was respectively 32 and 86%, respectively. This means that a substantial number of positively tested subjects will not suffer from this condition if BNP is used as a screening test for LVH. Thus, although BNP determination may be useful as a screening test to detect LVH in the hypertensive population, its value as a screening test in a population-based study for the detection of LVH remains doubtful.

5. Pharmacological manipulation

Administration of ANP has been successfully used for increasing natriuresis and diuresis in patients with heart failure [19,104–106]. As natriuretic peptides have to be given by a continuous intravenous infusion, an important alternative to ANP administration would be drugs that can increase the concentrations of ANP and/or BNP. Two alternative therapeutic options are now available.

The first is the use of drugs that inhibit neutral endopeptidase, the enzyme responsible for break-down of ANP. One such drug, candesartan, indeed increases ANP concentrations [107]. Much more promising is the development of combined neutral endopeptidase/angiotensin-converting enzyme inhibitors of which omapatrilat presently is investigated in phase II and III clinical trials in patients with CHF and hypertension. In a recently published study performed in patients with CHF 12-weeks of administration of omapatrilat appeared to have advantages over lisinopril [108]. Whether in the long run the effect of agents that increase endogenous concentrations of natriuretic peptides will be counteracted by an increase in the expression of the clearance receptor, the alternative way of degrading ANP, remains uncertain.

A second method may be the use of beta-blockers. Beta-blockers are now used with increasing frequency in patients with CHF. The mechanism by which beta-blockers improve myocardial function and survival in CHF is still not completely understood. In our own hands, administration of bisoprolol, a beta1-selective beta-blocker to 24 patients with hypertension increased ANP concentrations from 50.1 to 83.0 pmol/l, and BNP from 6.6 to 14.7 pmol/l, while administration of losartan, an angiotensin II receptor antagonist, did not effect ANP or BNP concentrations (Fig. 2) [109]. If such increases in atrial natriuretic peptides are therapeutically beneficial, they might lead to improvement of CHF, and thereby to a decrease in ANP and BNP secretion. This might well explain that, despite clinical improvement, chronic beta-blocker treatment in patients with advanced CHF is not associated with changes in plasma ANP or N-ANP concentrations [110].

In conclusion, measurements of plasma N-ANP and/or N-BNP are of increasing importance for assessing the condition of the heart, as is serum creatinine concentration for renal function. Natriuretic peptide measurements will become invaluable tools for diagnosis of diseases as CHF and pulmonary embolism, for early and timely detection of the beginning of ventricular or atrial problems in conditions associated with an increased risk of heart failure, for identifying heart failure patients most at risk, and for the follow-up of the effects of treatment in CHF. Pharmacological manipulation aimed to increase the concentrations of ANP and BNP and thereby enhancing their natriuretic, diuretic and vasodilatory effects in patients with heart failure as well as hypertension is underway and will become of increasing interest.

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