RAPID COMMUNICATION

Pulsatile secretion of atrial natriuretic peptide and brain natriuretic peptide in healthy humans

Erling B. PEDERSEN*, Henrik B. PEDERSEN† and Kaare T. JENSEN*

*Department of Medicine, Holstebro Hospital, DK-7500 Holstebro, Denmark, and †Institute of Physics and Astronomy, Aarhus University, Aarhus, Denmark

ABSTRACT

Both atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are involved in sodium and water homeostasis in healthy humans. The plasma concentrations of the natriuretic peptides can be used to differentiate between dyspnoea of cardiac and pulmonary origin, and the degree of elevation of the peptide levels in the plasma in heart failure is a measure of the severity of the disease. However, the patterns of secretion of ANP and BNP are not clear either in healthy humans or in patients. The purpose of the present study was to test the hypotheses that both ANP and BNP are secreted in pulses in healthy humans, and that this phenomenon can be revealed by determination of ANP and BNP in peripheral venous blood samples. In 12 healthy subjects, blood samples were drawn every 2 min through an intravenously inserted plastic needle over a period of 2 h. Plasma concentrations of ANP and BNP were determined by RIAs, and the results were analysed for pulsatile behaviour by Fourier transformation. Pulsatile secretion of ANP was seen in 10 out of 12 subjects [v = 0.028 min⁻¹ (median; range 0.013–0.047 min⁻¹)], i.e. a pulse of ANP with an interval of 36 min (range 21–77 min). Pulsatile secretion of BNP was seen in nine out of 12 patients [v = 0.021 min⁻¹ (range 0.013–0.042 min⁻¹)], i.e. a pulse of BNP with an interval of 48 min (range 24–77 min). The main conclusion is that the secretion patterns of both ANP and BNP are pulsatile in most healthy humans. Consequently, it is important to study whether pulsatile secretion also occurs in heart failure in order to obtain the most informative predictive values both in the differential diagnosis of dyspnoea and in the evaluation of the severity of the disease.

INTRODUCTION

Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) belong to the family of natriuretic peptides, which have natriuretic, diuretic and vasodilatory properties. ANP and BNP are synthesized in the atria and ventricles respectively of the heart. Both ANP and BNP are involved in sodium and water homeostasis in healthy humans, in arterial hypertension and in diseases involving sodium and water retention, as seen in heart failure, liver cirrhosis and nephrotic syndrome [1,2]. Measurement of ANP and BNP concentrations has recently been documented to be clinically relevant, helping in the differential diagnosis of dyspnoea caused by either heart disease or pulmonary disease [3]. The plasma concentrations of ANP and BNP were elevated in heart failure and normal in chronic lung disease, with a rather high discriminatory power. BNP appeared to be a more accurate indicator than ANP. However, the patterns of secretion of ANP and BNP have not yet been clarified either in healthy humans or in patients. It has been suggested that the secretion of ANP is pulsatile [4,5], while nothing is known about the secretion pattern

Key words: atrial natriuretic peptide, brain natriuretic peptide, Fourier transformation, pulsatile secretion, radioimmunoassay.
Abbreviations: ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CV, coefficient of variation.
Correspondence: Professor E. B. Pedersen.

© 1999 The Biochemical Society and the Medical Research Society
of BNP. More information about the secretion of these peptides is necessary, especially if plasma concentrations of the hormones are to be used in future clinical practice in the differential diagnosis of dyspnoea.

We hypothesize that ANP and BNP are secreted in a pulsatile manner in healthy humans, and that these pulses can be determined in the peripheral venous blood. The aims of the present study were: (1) to measure ANP and BNP in peripheral blood by sampling every 2 min over 2 h, and (2) to analyse whether the secretion pattern is pulsatile by using Fourier transformation of the ANP and BNP measurements.

METHODS

Subjects
Healthy control subjects were included according to the following criteria: (1) male, and (2) age 20–40 years. Exclusion criteria were: (1) a history or clinical signs of diseases of the heart, lungs, liver, kidneys, brain or endocrine organs, (2) arterial hypertension, (3) neoplastic disease, (4) medical treatment, (5) unwillingness to participate, and (6) abnormal laboratory screening, including blood haemoglobin, blood white cell count, plasma creatinine, plasma alanine aminotransferase, plasma glucose, urinary albumin and urinary glucose. The study was approved by the local Medical Ethics Committee, and all participants gave informed consent.

Experimental procedures
All subjects arrived at the laboratory at 09.00 hours, and rested in the supine position until 12.00 hours. In the period 10.00–12.00 hours, blood samples were drawn at 2 min intervals from an intravenously inserted catheter. Each blood sample was collected within < 30 s. Blood samples were drawn into polypropylene tubes containing 50 µl of 30 % (w/v) EDTA and 75 µl of Trasylol (10 000 units/ml). On each occasion, 7 ml of blood was drawn and used for the determination of both ANP and BNP concentrations, i.e. a total amount of 420 ml of blood was drawn. Blood pressure and pulse rate were measured every 15 min throughout the entire study. The amount of blood drawn was replaced by an equal amount of isotonic NaCl infused into another peripheral vein.

RIAs
The concentration of ANP in the plasma was determined by RIA, as previously described [6]. ANP was extracted from the plasma by means of Sep-Pak C18 cartridges. For RIA, rabbit anti-ANP antibody was obtained from the Department of Clinical Chemistry, Bispebjerg Hospital, Copenhagen, Denmark. The minimum detection level was 0.5 pmol/l of plasma. The coefficient of variation (CV) was 12 % (interassay).

The concentration of BNP in the plasma was measured by an RIA developed in our laboratory [7]. Immunoreactive BNP was extracted from the plasma by use of Sep-Pak C18 cartridges eluted with 80 % (v/v) ethanol in 4 % (v/v) acetic acid. RIA was performed using a rabbit anti-BNP antibody developed in our laboratory. There was no cross-reactivity with ANP. The minimum detection level was 0.55 pmol/l. The CV was 11 % (interassay).

The intra-assay variations were determined using double determinations of three different plasma pools in all assays for both ANP and BNP. The S.D. was calculated according to the formula:

$$S.D. = \frac{1}{2m} \sum_{i=1}^{m} d_i^2$$

where $m$ is the number of double determinations and $d$ is the difference between the two determinations.

Data analyses
To quantify the hypothesis that ANP and BNP are secreted in a pulsatile manner, we carried out the following parameterization of the measurement:

$$X(t) = at + b + \sum_{i} c_i \cos(2\pi \nu_i t) + d_i \sin(2\pi \nu_i t)$$

$$= at + b + \sum_{i} C_i \sin(2\pi \nu_i t + \phi_i)$$

where $X(t)$ represents either ANP or BNP and $t$ is the time of drawing the blood sample. The parameters $a$ and $b$ account for long-term variations and normal levels of ANP or BNP of the subject. The summation extends over possible pulsation frequencies ($\nu_i$), and $c_i$ and $d_i$ are the cosine and sine amplitudes respectively. In the second line of this equation, the parameterization has been rewritten to include only sine functions with amplitudes $C_i = (c_i^2 + d_i^2)^{1/2}$ and phases $\phi_i = \arctan(d_i/c_i)$ (note that $\nu_i \in [0;2\pi]$). It was the aim of the analysis to clarify whether pulsatile secretion was significant and, if so, to determine the dominating frequency and the corresponding amplitude.

For each subject, the collected data have the form $(t_k, ANP_k, BNP_k)$, where $t_k$ is the time of sample collection, $ANP_k$ and $BNP_k$ are the corresponding peptide concentrations found from RIA, and $k = 0, 1, \ldots, n-1$, where $n = 60$ is the number of samples. The analysis proceeded by first considering the variations in ANP and BNP as a function of time by visual inspection, since a dominant pulsatile secretion should be clearly visible. Secondly, the coefficients $a$ and $b$ were determined by linear regression.
to the data \((t_k, X_k)\), where \(X\) is either ANP or BNP. Thirdly, the pulsation frequencies and amplitudes were obtained using the method of Fourier transformation [8]. Before performing the Fourier transformation, the linear term \((at + b)\) is subtracted, thus leaving the data to be analysed in the form \((t_k, X_k - (at_k + b))\). This subtraction does not affect the determination of \(v, C\) and \(\mathcal{I}\), since Fourier transformation is a linear operation. To reduce the wiggles on the peaks in the Fourier-transform spectrum, we use the Welch apodization function [8]:

\[
 w_k = 1 - \left[ \frac{k - \frac{1}{2}(N-1)}{\frac{1}{2}(N-1)} \right] ^2 
\]

In summary, the Fourier-transform spectrum \([v, C]\) was computed as:

\[
 v_i = \frac{i}{N\Delta t} \\
 C_i = \frac{1}{N} \sum_{k=0}^{N-1} w_k [X_k - (at_k + b)] e^{-j \Delta T k} ; \quad i = 0 \ldots N-1
\]

where \(j = (-1)^{\frac{1}{2}}\). The phases \((\cdot)\) are of no importance in this study, and are not calculated explicitly.

The sampling time of 2 min allows determination of frequencies smaller than the Nyquist frequency \((v_{Ny} = 0.25 \text{ min}^{-1})\) from the Fourier-transform analysis [8]. However, variations in the measured peptide concentrations caused by the uncertainty of the RIA will result in oscillations that will appear at the higher frequencies in the Fourier-transform spectrum. In consequence, only frequencies \(v_i < v_{Ny}/4\) were considered significant, i.e. we required each pulsation period to be documented by at least eight data points. Further, for a frequency \((v_i)\) to be considered significant, it was required that the corresponding amplitude \(C_i > 1.5 < C >\), where \(< C >\) is the average Fourier amplitude.

**RESULTS**

**Demographics**

Twelve men with a mean age of 25 years (range 21–36 years) were studied. Blood haemoglobin was 9.09 ± 0.70 mmol/l (mean ± S.D.), plasma creatinine was 84 ± 7 \(\mu\)mol/l, plasma sodium was 141 ± 1.1 mmol/l, and the 24 h urinary excretion of sodium was 102 ± 56 mmol/l. Average body weight was 80.2 ± 12.5 kg both before and after the study period.

**Intra-assay variation and sensitivity of the ANP and BNP measurements**

Three different pools of control plasma corresponding to different levels of ANP and BNP were analysed as double determinations in all assays of ANP and BNP.

For ANP the CV was 6.7\%, with 1 S.D. = 0.309 pmol/l, using 65 double determinations. For BNP the CV was 6.3\%, with 1 S.D. = 0.0963 pmol/l, using 58 double determinations. The sensitivity was calculated, as the smallest detectable difference at the 95\% confidence limit, to be 0.44 pmol/l for ANP and 0.14 pmol/l for BNP.

**Analysis of pulsatile secretion of ANP and BNP**

In Figure 1(a), the ANP concentration in subject no. 6 is shown as a function of time. From visual inspection, it is evident that oscillations are present in the ANP concentration. The broken line shows a linear fit to the ANP...
values, and the full curve shows the ANP data after frequency components \( v > v_{Ny} / 4 \) have been removed by digital filtering [8]. The corresponding Fourier-transform spectrum is displayed in Figure 1(b). A significant peak is seen at \( v = (3.8 \pm 1.5) \times 10^{-2} \text{ min}^{-1} \), where the error bar corresponds to the full width at half-maximum of the peak. Analysis of pulsation in the ANP level for all subjects is summarized in Table 1. We report only the main peak in the Fourier-transform spectra.

In Figure 2(a) the BNP concentration in subject no. 5 is shown as a function of time. From visual inspection it is evident that oscillations are present in the BNP level. The broken line shows a linear fit to the BNP values, and the full curve shows the BNP data after frequency components \( v > v_{Ny} / 4 \) have been removed by digital filtering [8]. The corresponding Fourier-transform spectrum is displayed in Figure 2(b). A significant peak is seen at \( v = (3.4 \pm 1.4) \times 10^{-2} \text{ min}^{-1} \), where the error bar corresponds to the full width at half-maximum of the peak. The analysis of pulsation in the BNP level for all subjects is summarized in Table 2. We report only the main peak in the Fourier-transform spectra.

Pulsatile secretion of ANP was seen in 10 out of 12 subjects \( [v = 0.028 \text{ min}^{-1} \text{ (median), range } 0.013-0.047 \text{ min}^{-1} \text{), i.e. a pulse of ANP with an interval of 36 min (range 21-77 min)}] \). Pulsatile secretion of BNP was observed in nine out of 12 patients \( [v = 0.021 \text{ min}^{-1} \text{ (range } 0.013-0.042 \text{ min}^{-1} \text{), i.e. a pulse of BNP with an interval of 47 min (range 24-77 min)}] \). All subjects had pulsatile secretion of either ANP or BNP. No significant difference was found between the oscillation intervals for ANP (36 min) and BNP (47 min).

**Blood pressure and pulse rate**

Blood pressure and pulse rate did not change significantly during the study period in any of the subjects: at 0 min,
DISCUSSION

All healthy control subjects in this study had rhythmic oscillations in the concentration of at least one of the natriuretic peptides produced in the heart. The interval between the oscillations in the plasma concentrations of ANP and BNP were 40–50 min on average, and was not significantly different between the two hormones.

The ideal test situation in which to evaluate pulsatile secretion would be continuous measurement of the test substance very close to the place of secretion. For ANP and BNP, this would imply insertion of a catheter close to or in the sinus coronarius for a considerable period of time. Such a study would not be justified in healthy people for ethical reasons. In the present study we have chosen an experimental procedure with very frequent blood sampling from a peripheral vein during a period of 2 h, sensitive RIAs with high precision, and Fourier transformation to reveal oscillation as a function of time. Each of the two RIAs has a coefficient of intra-assay variation that is sufficiently low, i.e. the magnitude of the oscillations exceeded the methodological variation. The conditions for using Fourier transformation are fulfilled. Thus the experimental set-up and methodology are adequate and robust. Our results showed that the procedure was also sufficiently sensitive to demonstrate pulsatile secretion, and thus to support our hypotheses.

Diurnal variations in the plasma concentration of ANP have been reported previously, but considerable disagreement exists with regard to the time at which the peak concentration occurs during a 24 h period. Peaks have been reported to be at 20.00 hours [9], at 11.00 hours, 18.00 hours and 05.00 hours [10], and at 11.00 hours or 13.00 hours [11]. We have measured BNP in the plasma every 3 h during a 24 h period in healthy control subjects, but we did not disclose any diurnal pattern [7], and we are not aware of other studies on a possible circadian rhythm for BNP. Most likely, the disagreement with regard to the time at which peak levels of ANP occur in plasma might be attributed to the existence of faster oscillations in ANP levels in the plasma, which cannot be demonstrated by blood sampling with intervals of 0.5 h or more, but require blood sampling with shorter intervals. According to the present results, rhythmic oscillations in the plasma concentrations of natriuretic peptides are normal phenomena for both ANP and BNP. This has been suggested previously for ANP [4,5], but the present results are the first to show pulsatile secretion of BNP.

Oscillations in the plasma concentrations of ANP and BNP might be attributed to oscillation in either secretion or degradation. Degradation is dependent on enzymic activity either of the clearance receptors or of neutral endopeptidases. However, there is no evidence that the activity of these enzymes is regulated in a rhythmical fashion that could be responsible for the oscillations seen in the plasma concentrations of ANP and BNP. Thus the oscillations most probably reflect a pattern of rhythmic secretion. This would be in accordance with the fact that ANP and BNP are stored intracellularly in granules. When the contents of the granules are extruded from the cells, this might be measured as a pulse of the hormone. A similar pulsatile secretion pattern has been demonstrated...
for other hormones, i.e. insulin and glucagon [12] and thyrotropin [13].

The mechanism of regulation of the pulsatile secretion of ANP and BNP is not known. We have demonstrated previously that prostacyclin infusion (epoprostenol) stimulates the release of ANP [14], but, in addition to the well known stimulus by distension of the atrial wall, also endothelin, arginine-vasopressin, catecholamines and presumably other hormones directly stimulate the secretion of ANP. However, at present it is far from clear how BNP secretion is regulated.

It is important to take into consideration the fact that the pattern of secretion of ANP and BNP is pulsatile in most healthy people, and probably also in patients, when these hormones are used to differentiate between dyspnoea of cardiac and pulmonary origin [3] and in the evaluation of the severity of heart failure. Based on the present results, it is necessary (1) to study patients with heart failure to examine whether they demonstrate pulsatile secretion of ANP and BNP, and (2) if pulsatile secretion is present, to analyse how often and for how long blood sampling should be carried out in order to obtain reproducible results for levels of ANP and BNP to allow correct evaluation of the severity of the disease.

ACKNOWLEDGMENTS

This study was supported by The Foundation for Medical Research in the Counties of Ringkøbing, Ribe and Southern Jutland, and The Novo Nordic Foundation. Chief laboratory technician Lisbeth Mikkelsen and laboratory technician Elsebeth Larsen are thanked for skilful assistance.

REFERENCES


Received 17 March 1998/19 April 1999; accepted 7 May 1999