Is the secretion of atrial natriuretic peptide in man under neural control?

G. McDowell, M. Cave, A. Bainbridge, M. Danton, C. Shaw, K. D. Buchanan, J. Wallwork, S. Large and D. P. Nicholls

Department of Medicine, Royal Victoria Hospital, Belfast; \textsuperscript{1}Cardiac Surgery Unit, Papworth Hospital, Cambridge, U.K.

Aims
Previous work has described short-term variation in the circulating plasma level of atrial natriuretic peptide (ANP), but the mechanism remains unknown. Our aim was to investigate the role of cardiac innervation in this variability.

Methods and Results
Blood samples were obtained from the right atrium via a pulmonary artery flotation catheter every 2 min over a 90 min period. Seven patients who underwent cardiac transplantation by the standard biatrial technique (partial innervation) and ten patients who underwent transplantation by the bicaval technique (total denervation) were studied. ANP levels were measured by radioimmunoassay. The median ANP levels were somewhat higher in the biatrial group compared to the bicaval group [470 (150–1095) vs 216 (100–605) pg mL\(^{-1}\); median (range); \(P=\text{ns}\)], and both were much higher than normal levels in the pulmonary artery (40 (24, 56) pg mL\(^{-1}\); median and interquartile range). In both transplant groups circulating plasma ANP levels showed considerable variability. The median number of ‘peaks’ and ‘troughs’, as counted by visual inspection, were not significantly different between the two groups. Computer analysis identified 12–16 and 6–15 ‘pulses’ in the biatrial and bicaval group, respectively. Further analysis revealed that pulse amplitude, height and area were significantly higher in the biatrial compared to the bicaval group.

Conclusion
It would appear that variability of circulating plasma levels of ANP is preserved despite complete or partial cardiac denervation, and so a neural mechanism does not appear to account for such variation.

Introduction
The existence of a natriuretic hormone regulating fluid balance and opposing the action of the renin-angiotensin–aldosterone system was suspected for many years\cite{1,2} before it was established that rat atrial extracts produce a diuresis\cite{3} and the existence of the circulating hormone atrial natriuretic peptide (ANP) was demonstrated\cite{4}. ANP acts as a diuretic\cite{5} and vasodilator\cite{6}. Circulating plasma levels are increased in heart failure\cite{7,8}, probably as a compensatory mechanism for the vasoconstriction characteristic of that condition. After cardiac transplantation it might be expected that ANP levels would fall along with symptomatic improvement and increased exercise capacity\cite{9}, but several studies have shown that levels remain elevated\cite{10,11,12}.

The main source of ANP in both animals\cite{13} and man\cite{13,14} is the right atrium, where the peptide precursor is stored in granules and membrane-associated caveolae\cite{15}. These granules are still present after denervation\cite{16,17}. The main stimulus to ANP secretion is thought to be right atrial stretch and an increase in transmural pressure\cite{18} but the response to volume loading after denervation is variable between species. The response is abolished in the denervated (pithed) rat\cite{19}, whereas in the dog the increase in circulating ANP in response to atrial stretch is preserved, although diuresis and natriuresis are reduced\cite{19,20}. In man, after denervation by transplantation, the relationship between circulating plasma levels of ANP and right atrial pressure is lost\cite{21}, again suggesting that pressure by itself is not the only control mechanism for ANP release.

Previous research has described short term variations in the peripheral circulating plasma concentrations of ANP which do not appear to coincide with changes in heart rate or right atrial pressure\cite{22}. It is possible that...
and all were on immunosuppression with prednisolone, cyclosporine and azathioprine. All patients were in sinus rhythm at the time of study, biatrial (n=7) or bicaval (n=10) heart transplantation. A ventricular pacemaker in situ.

The aim of this study was to compare the effects of partial and complete cardiac denervation[23–25]. The aim of this study was to compare the effects of partial and complete cardiac denervation[23–25]. The aim of this study was to compare the effects of partial and complete cardiac denervation[23–25].

### Methods

#### Subjects

Seven patients who underwent cardiac transplantation by the standard biatrial technique and 10 patients who underwent transplantation by the bicaval technique were studied when they re-attended for their routine post-transplant biopsy (see Table 1). The choice of operation was selected for the individual patient by prospective randomization. The mean time since transplantation was 36 months. All patients were in sinus rhythm at the time of study, and were on standard post-transplant medications, including immunosuppression comprising cyclosporine, azathioprine and prednisolone.

#### Methods

Each subject attended the laboratory after an overnight fast. A sheath was placed under local anaesthetic into the right internal jugular vein and a pulmonary artery flotation catheter positioned with its tip in the right atrium. Blood sampling (2 ml) was performed from the right atrium following 30 min supine rest, and then every 2 min over 90 min. Samples were collected into bottles containing EDTA, and plunged immediately on ice. Following centrifugation at 3000 rpm for 10 min at 4 °C, plasma was decanted and stored at −20 °C pending analysis.

### Assay

An in-house radioimmunoassay was used to measure circulating plasma levels of ANP99,126 following Sep-pak plasma extraction as previously described[22]. Briefly, dried extract was reconstituted with 250 µl of assay buffer (40 mM sodium phosphate buffer, pH 7.4). A 300 µl assay was used. Sample (100 µl) was incubated with 100 µl of antibody, at an initial titre of 1:12000, for 24 h at 4 °C. Following this pre-incubation, radio-labelled ANP (100 µl) was added as tracer. Following a further 48 h incubation at 4 °C, separation was effected by the addition of 1 ml of Dextran coated charcoal. The mixture was then centrifuged at 3000 rpm for 25 min at 4 °C, the supernatant decanted and the radioactivity in the pellet counted on an automatic LKB gamma counter. The intra-assay co-efficients of variation were 7·8%, (mean ANP concentration 32 pg . ml⁻¹), 8·2% (mean ANP concentration 50 pg . ml⁻¹) and 7·9% (mean ANP concentration 637 pg . ml⁻¹). The inter-assay co-efficients of variation were 9·15% (mean ANP concentration 32 pg . ml⁻¹), 9·5% (mean ANP concentration 50 pg . ml⁻¹) and 9·3% (mean ANP concentration 637 pg . ml⁻¹) All samples from each patient were assayed as a single batch.

### Ethics

Ethics approval was granted by the Huntingdon Local Research Ethics Committee. Written informed consent was given by all subjects.

### Statistics

Non-parametric tests were used because of the small numbers involved and the consequent difficulty in proving a normal distribution of data. Differences between groups were analysed by Mann–Whitney U-test. Variability of ANP levels was assessed by counting ‘peak’ and ‘trough’ levels as defined as lying outside 2SD from the mean, calculated from the intra-assay coefficient of variation (CV) of 8·2%. In addition, the frequency and characteristics of pulses were determined by the PULSAR computer program which identified peaks by height and duration from a smoothed baseline using the assay CV across the standard curve as a scale factor, as
previously described\textsuperscript{[22,26]}. Variables were correlated with peptide concentration and other measurements using Spearman’s correlation coefficient. A \( P \) value of less than or equal to 0.05 was accepted as statistically significant.

Results

Sampling was completed in 11 of the 17 subjects. In six the catheter blocked during the procedure, but the results available have been included. Table 2 shows the results of ANP analysis. The median ANP level was somewhat higher in the biatrial group compared to the bicaval group, but this did not reach statistical significance (\( P = 0.06 \)). In both the biatrial and bicaval groups plasma ANP levels from the right atrium were greatly raised when compared to our previously reported normal value of 40 (24.56) pg·ml\(^{-1}\) (median, interquartile range) from the pulmonary artery\textsuperscript{[23]}, but nevertheless showed considerable variability (Figs 1 and 2 respectively). The median number of peaks and troughs, as counted by visual inspection, were not significantly different between the two groups. Within the same group the number of peaks and troughs were not significantly different. In addition to visual inspection, computer analysis using the PULSAR program identified 12–16 pulses and 6–15 pulses in the biatrial and bicaval group respectively, but the difference between the two groups did not reach statistical significance (Table 2). The number of pulses were significantly higher than the number of peaks identified by visual inspection in both the biatrial (\( P = 0.01 \)) and the bicaval (\( P = 0.007 \)) groups. Further analysis revealed that peak amplitude (\( P = 0.04 \)), height (\( P = 0.01 \)) and area (\( P = 0.03 \)) were significantly higher in the biatrial group compared to the bicaval group. The peak length was similar in both groups while frequency tended to be higher in the biatrial group, but this did not reach statistical significance (Table 3).

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Right atrial concentration of hANP (pg·ml(^{-1})) in post transplant patients over a 90 min sampling period, with the median (range) number of peaks, troughs and pulses. Between group comparisons by Mann–Whitney U-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biatrial group</td>
</tr>
<tr>
<td>ANP</td>
<td>470 (150–1095)</td>
</tr>
<tr>
<td>Peaks</td>
<td>9 (8–12)</td>
</tr>
<tr>
<td>Troughs</td>
<td>9 (8–12)</td>
</tr>
<tr>
<td>Pulses</td>
<td>14 (12–16)</td>
</tr>
</tbody>
</table>

Discussion

Increased circulating levels of ANP following cardiac transplantation using the standard biatrial technique have been reported by other investigators\textsuperscript{[10–12,21]} and the results of this investigation support these previous

\begin{figure}
\centering
\includegraphics[width=\textwidth]{anp_profile.png}
\caption{Profile of circulating plasma ANP levels (pg·ml\(^{-1}\)) obtained over a 90 min period from the right atrium of a patient post biatrial cardiac transplantation. The upper and lower lines represent ± 2SD from the mean. Values above the line are termed ‘peaks’ and below ‘troughs’.
}
\end{figure}
In the present study the slightly higher ANP levels observed in the biatrial as opposed to the bicaval group may relate to the increased atrial mass present; similarly, peak amplitude, height and area were greater in the biatrial group. In addition, the short-term variation in plasma levels of ANP we have previously reported in normal control subjects and patients with heart failure[22] was also observed in both transplant groups. The number of peaks and troughs recorded was similar, ranging from 8–12 and 3–13 respectively, in both the biatrial and bicaval transplant groups. In both groups the variability was confirmed by computer analysis, which revealed about 13 ‘pulses’ over the 90 min period (approximately one pulse every 7 min). A possible limitation of the study is the use of the right atrium as the sampling site. During the course of the 90 min study, the position of the catheter tip could vary, and the level of ANP in the effluent from the coronary sinus would be higher than elsewhere. However, such variation is likely to be random, whereas the computer analysis detected definite patterns of a similar type and duration to those previously shown in other vascular sites.

Previous studies have shown that the bicaval transplantation technique restores physiological atrial function, retains atrial dimensions[27,28] and reduces the incidence of tricuspid valve regurgitation[29], despite producing complete cardiac denervation. It remains unclear therefore why circulating plasma levels of ANP remain elevated after such transplantation, when the haemodynamic abnormalities have improved. Cyclosporin-mediated sodium and water retention may also account for the higher than normal circulating plasma levels of ANP[30], but the drug is used after both types of operation to prevent rejection. Other mechanisms have been proposed, including diastolic

---

**Figure 2** Profile of circulating plasma ANP levels (pg. ml$^{-1}$) obtained over a 90 min period from the right atrium of a patient post bicaval cardiac transplantation. The upper and lower lines represent ± 2SD from the mean. Values above the line are termed ‘peaks’ and below ‘troughs’.

**Table 3** Table showing pulse characteristics as assessed by PULSAR analysis in patients post transplant. Results are expressed as median (range). Between group comparison by Mann–Whitney U-test

<table>
<thead>
<tr>
<th>Pulse</th>
<th>Biatrial groups</th>
<th>Bicaval groups</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitude (pg . ml$^{-1}$)</td>
<td>202 (131–378)</td>
<td>109 (63–286)</td>
<td>0.04</td>
</tr>
<tr>
<td>Height (pg . ml$^{-1}$)</td>
<td>604 (314–1208)</td>
<td>310 (154–765)</td>
<td>0.01</td>
</tr>
<tr>
<td>Length</td>
<td>0.07 (0.06–0.07)</td>
<td>0.07 (0.06–0.09)</td>
<td>ns</td>
</tr>
<tr>
<td>Area</td>
<td>209 (184–507)</td>
<td>136 (61–441)</td>
<td>0.03</td>
</tr>
<tr>
<td>Frequency</td>
<td>9.55 (8.18–10.91)</td>
<td>8.86 (8.11–11.25)</td>
<td>ns</td>
</tr>
</tbody>
</table>
increase in renal dysfunction [37]. Post-transplant hypertension [34,35], mild rejection [36] and plasma renin activity, aldosterone and ANP circadian rhythm findings that may be responsible [38]. Altered processing or degradation of ANP in ventricles or atria could be a possible cause [38]. Previous research has shown disappearance of circadian or short term variation in other hormone-secretory systems. The major molecular form of ANP observed in ventricular extracts from transplant recipients remains the high molecular weight storage form ANP 

Recent evidence suggests that increased secretion of ANP may occur in the transplanted heart. The major molecular form of ANP observed in ventricular extracts from transplant recipients remains the high molecular weight storage form ANP [39] ANP [126] but whereas ventricular production of ANP in the normal heart is rare, increased ventricular ANP synthesis has been reported in the failing heart and in the transplanted heart [40,41] so that the ventricles may contribute to the total circulating plasma ANP pool after transplantation.

It has been established that cardiac innervation is not required for ANP release [41,42] but the role of cardiac innervation in the variable pattern of ANP secretion has not been investigated previously. The results from the present study would suggest that cardiac innervation is not responsible for the the variable pattern of ANP secretion, as the pattern is still observed after the bicaval technique, which results in cardiac denervation due to total extirpation of the recipient’s heart. Indeed, it is possible that sympathetic denervation may serve to increase circulating plasma levels of ANP [42]. The mechanism of the short term variability in plasma ANP levels therefore remains unclear, but it is not related to changes in heart rate or right atrial pressure [22]. The role of circadian or short term variation in other hormone-secretory systems on the secretion of ANP may also be responsible. Previous research has shown disappearance of plasma renin activity, aldosterone and ANP circadian rhythms following orthotopic heart transplantation [63] which could relate to constant activation of the renin-angiotensin-aldosterone system due to cyclosporin and glucocorticoid immunosuppression therapy.

A recent study has confirmed variability in circulating plasma levels of BNP as well as ANP [44], and has established the mathematical basis for time analysis. The mechanism of such variation is therefore of increasing interest, and our study would indicate that cardiac innervation is not involved.

The authors gratefully acknowledge the financial assistance of the Northern Ireland Chest, Heart and Stroke Association.

References


