Atrial Natriuretic Peptide in Humans
Production and Clearance by Various Tissues
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TIM G. YANDLE, AND SIEGFRIED JANS

SUMMARY Although it is assumed that the human heart secretes atrial peptides, direct proof is not available. We therefore measured immunoreactive atrial natriuretic peptide levels in coronary sinus blood and simultaneously in femoral arterial and venous blood from patients before and during stepwise incremental atrial pacing of up to 200 beats per minute. Since the fate of circulating atrial peptides is unknown, we also measured immunoreactive atrial natriuretic peptide concentrations in arterial and venous blood across the liver, kidney, lower limb, and lung in patients undergoing cardiological investigation. Peptide levels in coronary sinus blood were higher than in samples from the femoral artery or vein. As the heart rate was accelerated by atrial pacing, peptide concentrations increased in coronary sinus blood and to a lesser extent in peripheral samples. Whereas the levels in venous blood draining the liver, kidney, and lower limb were approximately 50% of those in arterial blood, concentrations were similar in samples drawn simultaneously from the pulmonary artery and the aorta. These results show that the human heart produces immunoreactive atrial natriuretic peptide and that secretion increases with atrial tachycardia. The liver, kidney, and lower limb remove the peptide from arterial blood, but there is little change in its concentration during circulation of blood through the lungs. (Hypertension 8 [Suppl II]: II-11-II-15, 1986)

KEY WORDS • tachycardia • heart • coronary sinus

THE finding of specific granules in mammalian atrial tissue1 and the identification of at least three peptides in atrial extracts with natriuretic activity2 suggest that the heart may have an endocrine function. It remains to be established, however, that α-atrial natriuretic peptide (αANP), the most potent of the three peptides, is produced by the human heart. If indeed the heart is the secreting organ, the factors that stimulate release of the peptides are open to speculation. One possible stimulus is tachycardia, since it has long been known that polyuria accompanies paroxysmal tachycardia in some patients.3 Equally uncertain is the fate of circulating αANP. To address these questions, we developed a radioimmunoassay technique to measure plasma immunoreactive αANP (IR-ANP) in blood samples obtained from different vascular beds, including the coronary sinus, during routine diagnostic cardiac catheterization. We obtained simultaneous samples of arterial and venous blood across the heart, lung, liver, limb, and kidney to determine the sites of production and clearance of IR-ANP. In addition, we measured the effect of atrial tachycardia on IR-ANP levels in coronary sinus, arterial, and limb vein blood.

Methods
The protocols were approved by the Hospital Ethical Committee, and patients gave informed consent.

Protocol 1: Atrial Pacing
We studied five patients (three men and two women) aged 50 to 68 years who were undergoing electrophysiological investigation of syncope (two patients) or supraventricular tachycardia (three patients). Medications were stopped 48 hours before the study, which was performed after overnight fasting and 1 hour after 20 mg of diazepam had been administered orally. A pacing catheter was positioned in the right atrium under fluoroscopic control. A cannula was placed in the right femoral artery for blood sampling and continuous recording of blood pressure. Venous samples were obtained through the sheath of a femoral vein catheter. Another catheter was positioned in the proximal portion of the coronary sinus under fluoroscopic control.
for blood sampling and for measurements of coronary sinus flow by the thermal dilution method. After catheter placement the patients rested for 15 minutes; baseline recordings were then made of coronary sinus flow and arterial pressure, and blood for measurements of IR-ANP was drawn simultaneously from the femoral artery, femoral vein, and coronary sinus. Right atrial pacing was then started at 100 beats/min and increased by 20 beats/min at intervals of 3 minutes to a maximum of 200 beats/min. Blood sampling and other recordings (see above) were repeated at the end of each 3-minute period of pacing and 5 and 15 minutes after termination of pacing. Formal diagnostic electrophysiological testing was then carried out. One patient (Case 4) spontaneously developed atrial flutter immediately after termination of atrial pacing. Since there was no clinical or hemodynamic disturbance, measurements were repeated over a period of 20 minutes in this patient before the arrhythmia was terminated by injection of the antiarrhythmic drug flecainide acetate.

**Protocol 2: Peptide Levels in Various Vascular Beds**

One group of subjects consisted of eight patients who were admitted to the hospital for cardiological investigation and underwent cardiac catheterization for assessment of valvular heart disease (four patients), cardiomyopathy (two), or coronary artery disease (two). One patient also had chronic renal failure. Medications were stopped at least 24 hours before the procedure, which was performed after an overnight fast and 1 hour after oral administration of diazepam, 20 mg. One catheter was placed in the central aorta through a femoral artery, and another was passed from a femoral vein into the inferior vena cava. The latter catheter was positioned sequentially in a renal vein, a hepatic vein, and the pulmonary artery under fluoroscopic control. At each catheter position, 5 ml of venous blood was drawn at the same time as arterial blood (from the aorta) for measurements of IR-ANP. Two samples were taken from the renal vein and the pulmonary artery (along with matching arterial blood samples) in all patients, but single samples were obtained from the hepatic vein. Routine cardiac catheterization was then carried out. In four of the eight patients, a sample of femoral venous blood (and a matching arterial sample) was also obtained. All plasma samples from these eight patients were extracted (see below) before radioimmunooassay of IR-ANP.

Another group consisted of six patients undergoing electrophysiological investigation for syncope (two patients) or paroxysmal tachycardia (four). Cannulae were placed in a femoral artery and the proximal portion of the coronary sinus. Blood was drawn simultaneously from the coronary sinus and the femoral artery on two occasions 15 minutes apart. Plasma was assayed for IR-ANP without prior extraction (see below).

**Assay for Immunoreactive Atrial Natriuretic Peptide**

Blood samples (5 ml) were added to chilled tubes containing an inhibitor solution (20 kallikrein inhibitor units of Trasylol per 10 µl of 15% Na₂ ethylenediaminetetraacetic acid per milliliter of blood) and were centrifuged immediately at +4 °C. Plasma was stored at -80 °C until it was extracted or assayed. For radioimmunoassay, an antibody directed to the C-terminal region of αANP (Peninsula Laboratories, Belmont, CA, USA) was used, and αANP from Bachem (Torrance, CA, USA) was used as standards and for iodination by the chloramine-T method. Labeled αANP was purified on a Sephadex G25 column, and the peak containing iodinated hormone was further purified by reverse phase high-pressure liquid chromatography. Plasma (100 µl, either extracted or unextracted) or standard αANP (5-160 pg per tube) was incubated with 100 µl of antiserum solution for 21 hours at +4 °C in assay buffer. Labeled αANP (14,000 cpm in 100 µl of assay buffer) was added and incubated for a further 24 hours at +4 °C. Bound αANP and free αANP were separated by addition of 100 µl of normal rabbit serum solution and 100 µl of goat antirabbit immunoglobulin G antiserum, and the bound fraction was counted in a gamma counter.

**Plasma Extraction**

Some plasma samples were extracted before radioimmunoassay. For this purpose, 0.5 to 5 ml of plasma was run slowly through a Sep-Pak cartridge (Millipore-Waters, Milford, MA, USA) prewashed with 10 ml of methanol and 10 ml of 4% acetic acid, and then washed with 10 ml of 4% acetic acid. ANP was eluted with 3 ml of ethanol/water/acetic acid (80:20:4). The eluate was dried under a stream of air at 37 °C, dissolved in assay buffer, and stored at -80 °C until it was assayed.

**Results**

**Assay of Immunoreactive Atrial Natriuretic Peptide**

When peripheral venous plasma was assayed without extraction, dilution curves were not parallel to the standard curve, whereas after Sep-Pak extraction, the curves were parallel. Duplicate plasma samples assayed with and without extraction were highly correlated, as indicated by the linear regression formula $y = 1.01x - 84.5 (r = 0.99, n = 76)$. These findings are consistent with nonspecific interference from some component of plasma when unextracted samples were assayed. Recovery of unlabeled αANP, added to fresh plasma to achieve levels between 0 and 1600 pmol/l, was linear and averaged 90% after Sep-Pak extraction, and recovery of labeled ANP was similar (88%). The slope of the standard curve was $-1.16 ± 0.03$ (mean ± SEM), and the amount of standard αANP required for 50% displacement was $14.7 ± 0.5$ fmol (mean ± SEM). These values, determined from 20 consecutive assays, were obtained from a four-parameter logistic function. The minimum amount of αANP that could be distinguished from zero (2 SD from zero binding) was 0.6 fmol per tube. In practice, the detection limit was also affected by variation in “blank” values from Sep-Pak cartridges. Blank values were determined in samples of distilled water (1 ml), which were extracted and assayed in an identical manner to
plasma samples. The mean blank value was 1.5 ± 0.28 (SD) fmol per tube (n = 10), which gave a practical detection limit of 2.1 fmol per tube or 5 pmol/l when plasma volumes of 2 ml were used. Results have not been corrected for blank values or adjusted for recovery. Less than 3% of iodinated α-ANP was bound by unextracted plasma in the absence of antiserum, indicating negligible nonspecific binding. The intraassay coefficient of variation from six analyses of one plasma sample (mean, 19.2 pmol/l) was 10.1%, and the interassay coefficient of variation for 11 sequential extractions and assays of a single sample (mean, 21 pmol/l) was 13.8%. The venous IR-ANP level in extracted plasma from 19 healthy volunteers on an unrestricted sodium diet was 14 ± 1 pmol/l (mean ± SEM).

Protocol 1: Atrial Pacing

The studies were completed without incident. The mean arterial pressure rose with atrial pacing in three patients and fell in two, and coronary blood flow increased from a baseline value of 148 ± 32 ml/min to a maximum of 385 ± 49 (mean ± SEM). Levels of IR-ANP in coronary sinus plasma were in general much higher than in samples drawn simultaneously from the femoral artery or vein (Figures 1 and 2). The exception was Patient 5 in whom the tip of the coronary sinus catheter was noted on fluoroscopy to be in the great cardiac vein. In this patient, IR-ANP levels were similar in plasma obtained from the three sites before and during the pacing procedure. During incremental cardiac pacing in the other patients, the largest increases in coronary sinus IR-ANP levels occurred at heart rates of 140 beats/min and higher, with later and smaller rises in peripheral levels (see Figures 1 and 2). This pattern was similar whether unextracted plasma (see Figure 1) or extracted plasma (see Figure 2) was used. Patient 4 developed atrial flutter (atrial rate, 280 beats/min; ventricular rate, 120–140 beats/min) after cardiac pacing, and in this instance coronary sinus IR-ANP levels remained high while peripheral levels continued to rise (see Figure 2). With few exceptions, IR-ANP levels were higher in plasma from femoral artery blood than in plasma from the femoral vein, and this arteriovenous ratio increased further during cardiac pacing (see Figures 1 and 2). Levels of IR-ANP were higher in unextracted plasma than in extracted plasma (see Figure 2), but the pattern of change with cardiac pacing and the coronary sinus/arterial/venous ratios were similar with and without extraction.

Protocol 2: Peptide Levels in Various Vascular Beds

Plasma IR-ANP levels in venous and arterial samples are shown for each patient, along with clinical details, in Table 1. Although there was a wide range of individual IR-ANP levels, the ratios of venous to arterial concentrations were similar for the same vascular beds in most patients. As shown in Figure 3, the ratio of venous to arterial IR-ANP was approximately 50% across the liver, kidney, and lower limb. In contrast, concentrations were almost identical across the lung (i.e., in samples obtained from the pulmonary artery and aorta), giving a "venous"/arterial ratio of close to 1 (see Figure 3). That the heart produced IR-ANP was shown by the high level in blood draining the heart (coronary sinus), as compared with the levels in simultaneously obtained arterial samples (mean ratio, 3.91; see Figure 3).
TABLE 1. Immunoreactive Atrial Natriuretic Peptide (IR-ANP) Levels in Arterial and Venous Plasma

<table>
<thead>
<tr>
<th>Patient no., age (yr), sex</th>
<th>Diagnosis</th>
<th>Medications</th>
<th>Aorta*</th>
<th>Pulmonary artery</th>
<th>Hepatic vein</th>
<th>Renal vein</th>
<th>Femoral vein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 48, F</td>
<td>CAD</td>
<td>Vitamins</td>
<td>113 ± 10</td>
<td>125</td>
<td>68</td>
<td>37</td>
<td>—</td>
</tr>
<tr>
<td>2, 48, F</td>
<td>Renal failure</td>
<td>—</td>
<td>143 ± 6</td>
<td>130</td>
<td>117</td>
<td>97</td>
<td>—</td>
</tr>
<tr>
<td>3, 68, M</td>
<td>Valve disease</td>
<td>Furosemide</td>
<td>154 ± 3</td>
<td>183</td>
<td>79</td>
<td>62</td>
<td>—</td>
</tr>
<tr>
<td>4, 45, M</td>
<td>CAD</td>
<td>Atenolol, Nifedipine, Digoxin, Aspirin</td>
<td>11 ± 2</td>
<td>14</td>
<td>5</td>
<td>9</td>
<td>—</td>
</tr>
<tr>
<td>5, 54, M</td>
<td>Cardiomyopathy</td>
<td>Furosemide</td>
<td>170 ± 9</td>
<td>172</td>
<td>59</td>
<td>55</td>
<td>77</td>
</tr>
<tr>
<td>6, 31, M</td>
<td>Valve disease</td>
<td>—</td>
<td>52 ± 3</td>
<td>51</td>
<td>32</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>7, 25, M</td>
<td>Valve disease</td>
<td>—</td>
<td>11 ± 1</td>
<td>9</td>
<td>4</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>8, 55, M</td>
<td>Cardiomyopathy</td>
<td>—</td>
<td>15 ± 1</td>
<td>15</td>
<td>5</td>
<td>5</td>
<td>8</td>
</tr>
</tbody>
</table>

Levels of IR-ANP were measured after Sep-Pak extraction (see Methods). CAD = coronary artery disease.

Discussion

We have developed a radioimmunoassay for the measurement of αANP in human plasma. Since we do not have access to βANP or γANP, we do not know the cross-reactivity of the antiserum with these larger peptides. Preliminary results with a Sephadex G50 column suggest that almost all IR-ANP in coronary sinus plasma elutes as a single peak at the same position as that of pure synthetic αANP. Furthermore, analysis of a separate extract of coronary sinus plasma by high-performance liquid chromatography showed a dominant peak eluting at a position identical to that of standard αANP (T. G. Yandle et al., unpublished data). These results suggest that the main immunoreactive peptide in coronary sinus plasma is the 28-amino acid αANP, although confirmatory information is needed.

Unextracted peripheral plasma appeared to contain nonspecific factors that interfered in the assay, so that values reported without extraction are overestimated by approximately 80 pmol/l. Nevertheless, some useful information was gained, since we observed a close correlation between IR-ANP levels in extracted and unextracted samples over a wide range of concentrations. Furthermore, fluctuations in IR-ANP levels in coronary sinus blood were strikingly similar whether or not plasma was extracted before the assay (see Figure 2). Finally, we have found comparable ratios of IR-ANP levels in blood from different vascular beds whether or not extraction was used. It is clearly preferable, however, that plasma be extracted before immunoassay in future studies.

We observed that IR-ANP concentrations were higher in coronary sinus samples than in femoral artery or vein blood. Moreover, coronary sinus IR-ANP levels rose during incremental atrial pacing, and a sharp increase was noted at around 140 to 180 beats/min. Cessation of atrial pacing was associated with a rapid decline in coronary sinus IR-ANP levels. The exception was Patient 4 in whom atrial flutter developed after pacing, and IR-ANP concentrations remained elevated (see Figure 2). The only patient who did not have a gradient between peptide levels in coronary sinus and peripheral samples was Patient 5, in whom
In summary, we have shown that the human heart produces IR-ANP and that its production is increased by atrial pacing. There is extensive clearance of IR-ANP during the passage of blood through the kidneys, liver, and lower limb, but levels of IR-ANP are altered little in the pulmonary circulation.

Note added in proof: The unpublished observations of T. G. Yandle et al. mentioned in the text will be published in *Life Sciences* in 1986.

Acknowledgments

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References

Atrial natriuretic peptide in humans. Production and clearance by various tissues.
I G Crozier, M G Nicholls, H Ikram, E A Espiner, T G Yandle and S Jans

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