Effects of atrial natriuretic peptide on systemic haemodynamics and cardiac function in normal man

MICHAEL J ALLEN, SUSAN M GILMOUR, MERVYN SINGER, E DAVID BENNETT
From the Department of Medicine One, St. George’s Hospital Medical School, London, SW17

ABSTRACT Atrial natriuretic peptides (ANP) reduce blood pressure. Animal experiments suggest that this depressor action results from a reduction in cardiac output rather than peripheral vascular resistance but it is unresolved whether this is wholly due to their effect of reducing left ventricular filling or whether they have a negatively inotropic effect. We have therefore investigated the effects of ANP in normal man using Doppler measurements of ascending aortic blood flow. Six normal volunteers underwent infusions of placebo and incremental doses of ANP in the range 0.25 to 12 μg min−1. Each infusion was given for 15 min and measurements made both in the supine and erect positions (passive tilt).

In both positions ANP had dose dependent effects of increasing heart rate (HR) and maximal acceleration whilst lowering an index of systemic vascular resistance (ISVR). In the erect position ANP also lowered systolic blood pressure. In the 30 min after completion of the infusions there were significant decreases in peak velocity and cardiac output with increases in ISVR in both positions, but HR fell and diastolic pressure increased only when supine. During the course of the experiment mean haematocrit (SEM) increased from 43.9 (1.2) to 46.7 (1.0), indicating a mean reduction in plasma volume of 10.5%. This occurred despite a negative fluid balance of only 31 (7) ml over the 2 h.

These data suggest that ANP is not negatively inotropic and that, at pharmacological doses, it is an arteriolar dilator of rapid offset and reduces cardiac filling pressures by a mechanism of slower offset.

Atrial natriuretic peptides (ANP) have been shown to reduce blood pressure in both experimental animals and man.1-4 This effect has only been demonstrated at pharmacological doses. Specific ANP receptors exist on vascular smooth muscle which result in the release of cyclic GMP via the activation of particulate, membrane bound guanylate cyclase.5-6 The effect of ANP in isolated vessel strips is to cause smooth muscle relaxation7 and it has been demonstrated that at plasma levels similar to those found in heart failure or above, alpha human ANP, infused directly into brachial arteries of normal volunteers, will increase forearm blood flow in a dose dependent manner.8 In contrast to these findings whole animal experiments have demonstrated that the reduction in blood pressure from ANP is not due to a reduction in vascular resistance but to a reduction in cardiac output.9-12

It is now established that ANP will reduce atrial pressures and plasma volume independent of its diuretic effects.13 The mechanism of this response is likely to be net efflux of fluid from the vascular compartment either by increased vascular permeability or by altered Starling forces across the capillary beds. It is unresolved whether the reduction in cardiac output seen with ANP is wholly due to this fall in cardiac filling or whether it has a direct cardiodepressant effect. This study was therefore designed to examine the effects of ANP on both systemic haemodynamics and cardiac function using Doppler measurements of ascending aortic blood flow.
Haemodynamic effects of atrial peptide

Methods

Six normal male volunteers (age range 20-24, weight range 58-78 kg) were recruited and written informed consent was obtained. Approval for the study was given by the District Medical Ethics Committee. Volunteers were maintained on their usual sodium intake but alcohol and caffeine were prohibited on the study days.

After the insertion of a peripheral venous cannula each subject spent the next 30-45 min in the supine position until a stable heart rate and blood pressure were obtained. During this preliminary period saline was infused at a rate of 60 ml·h\(^{-1}\). The subjects then received a placebo followed by an incremental infusion of alpha human atrial natriuretic peptide (Peninsula Laboratories, St Helens, UK) at doses of 0.25, 1.0, 4.0 and 12.0 \(\mu\)g·min\(^{-1}\), each for 15 min. On completion of the ANP infusions subjects continued to receive saline at a rate of 60 ml·h\(^{-1}\) for a further 30 min. Heart rate, blood pressure and Doppler measurement of aortic blood flow were performed after 10 min at each dose in the supine position and after a further 5 min of each dose in a position of passive 80° head-up tilt. Similar measurements were continued for two 15 min periods after completion of ANP. Both the subject and the Doppler operator were told that the infusions, including placebo, were given in random order to which they were blind. In fact the infusions were given in ascending order of dose in each case.

ANP and placebo infusions were made up in a solution of saline (9 g litre\(^{-1}\)) and Haemaccel (Hoechst), which had previously been passed through a 0.2 µm filter. Each solution was diluted such that the volume infused in each 15 min period was 15 ml (13.5 ml saline + 1.5 ml Haemaccel). All fluids were given using an infusion pump (Vickers Medical).

Blood pressure was measured by ultrasonic recorder (Arteriosonde 1225, Roche). Ascending aortic blood flow was assessed by suprasternal Doppler ultrasound (Exerdop, Quinton, Seattle, USA) which gives values for peak velocity (PV), maximal acceleration (MA), and stroke distance (SD). SD is the area under the velocity waveform. Minute distance (MD) is the product of SD and heart rate. If multiplied by the aortic cross sectional area these two values (SD and MD) are equal to stroke volume and cardiac output respectively. As aortic cross sectional area would not be expected to change significantly in this study, SD and MD provide linear indices of stroke volume and cardiac output. Mean arterial pressure (diastolic + \(1/3\) pulse pressure) \(\times 100\) divided by MD was taken as an index of systemic vascular resistance (SVR).

Blood was sampled for haematocrit estimation in five subjects during infusion of saline and on completion of the highest dose of ANP and was measured by Coulter counter. Plasma for ANP assay was sampled on completion of each dose from the sixth subject. Blood was collected into chilled polypropylene tubes containing aprotonin (400 kallikrein inactivator units per ml blood) and the potassium salt of ethylenediamine acetate (K-EDTA) as an anticoagulant, immediately centrifuged and the plasma stored at \(-20^\circ\text{C}\) until assayed. Stored plasma was extracted using a Sep-Pak C18 cartridge and assayed within one month using the technique previously described.\(^{14}\)

To assess the extent to which changes in maximal acceleration can be affected by afterload reduction, six further subjects (age range 26-49 years, weight range 60-80 kg) each received three intravenous bolus doses of phentolamine in the range 2 to 8 mg. Subjects were rested in a warm room lying in a position of 15° head-up tilt for 30-45 min and the experiment was started when stable heart rate, blood pressure and Doppler signal were obtained. Heart rate, blood pressure and aortic blood flow were measured as previously described 1 min post injection. Phentolamine was given in incremental doses with at least 10 min between injections.

Statistical Analysis

Results are expressed as mean (SEM). Dose response and offset curves were constructed for each measured variable and examined by analysis of variance (anova). Post hoc Student’s paired \(t\) tests were applied to identify where changes had occurred when anova was significant. Changes were considered significant when \(p<0.05\).

Results

Dose Response

Compared with placebo, ANP produced a dose dependent increase in heart rate (fig 1) from 64.3(2.2) to 76.3(4.2) \(\text{min}^{-1}\) in the supine position (\(p<0.001\)) and from 86.5(6.5) to 96.8(7.5) \(\text{min}^{-1}\) in the erect position (\(p<0.01\)). Post hoc \(t\) tests show that this increase only became significant at the highest dose of ANP in the supine position and at the two highest doses when erect. Heart rate was significantly higher when erect than supine at all stages (\(p<0.001\)).

Diastolic blood pressure tended to decrease in both postures but neither trends were significant (fig 1). DBP was significantly higher in the erect than the supine position (\(p<0.001\)). In contrast to this, systolic blood pressure was not significantly affected by posture alone, at 107(5) mm Hg supine and 112(5) mm Hg erect or by ANP in the supine position, but it was significantly lowered by ANP when erect, from 112(5) to 92(7) mm Hg on placebo to 92(7) mm Hg on the highest dose of ANP.
Heart rate
(heart rate)

Systolic
blood
pressure

Diastolic
blood
pressure

Placebo
Log dose (μg min⁻¹)
Offset

FIG 1 Changes in (a) heart rate, (b) systolic blood pressure and (c) diastolic blood pressure in 6 normal volunteers during infusion of placebo, incremental doses ANP, and 15 and 30 min after ANP in the supine () and erect () positions. Results are means. Bars indicate SEM.

Both PkV and MD were significantly higher in the supine position that the erect position (p<0.001 for each) but no significant changes were seen following ANP in either position (figs 2 and 3). PkV was 0.94(0.09) m·s⁻¹ when supine at rest and 0.68(0.06) m·s⁻¹ when erect. MD was 902(157) cm supine and 638(72) cm erect.

Maximal acceleration was greater when supine than erect (p<0.001). With ANP it showed a non-significant increase from 22.2(2.4) to 24.0(1.5) m·s⁻² in the supine position and a significant increase from 15.7(1.6) to 19.0(1.2) m·s⁻² when erect (p<0.001). This increase was only significant at the highest dose (fig 2).

Our index of systemic vascular resistance was significantly lower in the supine compared with the erect position (p<0.001). In the erect position it showed a non-significant reduction with ANP from 14.8(2.2) to 11.2(1.1), whilst in the supine position it fell from 10.2(2.0) to 8.1(0.7), p<0.05 (fig 3). Two of our subjects felt faint when the highest dose of ANP was combined with passive upright tilt. This was associated with hypotension and a relative bradycardia which reverted quickly on assumption of the supine position.

During the course of the ANP infusions blood haematocrit increased from 43.9(1.2) to 46.7(1.0)% (p<0.02, Student's paired t test). This is equivalent to a reduction in plasma volume of 10.5%. This occurred despite a negative fluid balance of only 31(7) ml.

Plasma ANP levels in pg·ml⁻¹ during infusion in one subject were:

<table>
<thead>
<tr>
<th>Placebo</th>
<th>6.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25 μg/min</td>
<td>49.5</td>
</tr>
</tbody>
</table>
Haemodynamic effects of atrial peptide

**FIG 3** Changes in (a) minute distance (MD) and (b) index of systemic vascular resistance (ISVR) in 6 normal volunteers during infusion of placebo, incremental doses ANP and 15 and 30 min after ANP in the supine (□) and erect (■) positions. Results are means. Bars indicate SEM.

OFFSET
After completion of the ANP infusions and whilst saline was infused at the same rate the offset of any ANP effects was measured. Heart rate fell from 76(4.2) to 60(2.9) min⁻¹ (p<0.005) in the supine position and from 96(0.7) to 94.3(7.1) when erect (NS) (fig 1). Diastolic blood pressure rose from 59(1) to 69(3) mm Hg in the supine position (p<0.05) and from 67(5) to 73(5) mm Hg when erect (NS) (fig 1). Systolic blood pressure was not significantly altered (fig 1).

Peak velocity fell from 0.95(0.06) to 0.83(0.04) m s⁻¹ in the supine position (p<0.05) and from 0.70(0.03) to 0.56(0.04) m s⁻¹ when erect (p<0.005), fig 2. Maximal acceleration fell from 24.0(1.5) to 19.8(1.0) m s⁻² in the supine position (p<0.05) and from 19.0(1.2) to 13.8(0.9) m s⁻¹ when erect (p<0.001), fig 2.

Minute distance fell from 958(87) to 693(93) cm in the supine position (p<0.05) and from 692(51) to 547(39) cm when erect (p<0.05), fig 3. The index of systemic vascular resistance increased from 8.1(0.7) to 13.0(1.8) in the supine position (p<0.05) and from 11.2(1.1) to 15.1(1.4) when erect (p<0.05), fig 3.

**PHENTOLAMINE RESPONSE**
Phentolamine produced a dose dependent fall in ISVR and increase in MA. Figure 4 shows the relationship between these measurements for the three doses of 2, 4 and 8 mg. The regression line of these data (R=0.97) is close to the line of identity.

**Discussion**
This study demonstrates dose and posture dependent effects of ANP. Most of the haemodynamic effects that we have seen have occurred only at the highest two doses, which are clearly in the pharmacological range. The lower two doses are equivalent to a physiological level and a level similar to those found in cardiac failure or other fluid overload states.

We have seen differing responses in the supine and erect positions. In the supine position ANP had minimal effects on blood pressure but did produce a relative tachycardia, if anything an increase in cardiac output, and a definite reduction in systemic vascular
resistance; all findings consistent with an arteriolar dilator. Similar changes are seen in the erect posture except there was also a significant fall in systolic blood pressure and an increase in maximal acceleration. The reduction in systemic vascular resistance during ANP infusion was associated with a reflex tachycardia in our subjects. This is in contrast to recent suggestions that ANP may be sympatholytic, although this study does not rule out the possibility that it may be sympatholytic at physiological plasma levels or even with levels found in fluid overload.

MA is dependent on both the isotropic state of the heart and on afterload. ISVR fell and so with the ANP data alone it is not possible to exclude a negatively inotropic effect of ANP. Phentolamine is an alpha blocker devoid of any direct cardiac effect in normals. We have demonstrated a reciprocal relationship between ISVR and MA during vasodilatation with phentolamine. From this relationship it is possible to predict the change of MA that will occur with a given dilator effect. With ANP we saw a mean reduction of ISVR of 15% in the supine position and 16% in the erect position. From the phentolamine relationship we would expect 15% increase in MA in response to this fall in ISVR. We actually saw a 23% increase in the supine position and a 24% increase in the erect position. These data refute the suggestion that ANP is negatively inotropic.

During the 30 min period after completion of ANP we saw a recovery of heart rate in the supine but not the erect position and a recovery of blood pressure in both positions. We also saw marked reductions in PKV and MD. These variables were not only lower than on the highest dose of ANP but were lower than at that at the start of the experiment. The converse changes occurred in systemic vascular resistance. We would suggest that these changes are consistent with a withdrawal of the arteriolar dilator effect of ANP but persistence of reduced cardiac filling. During infusion of ANP the cardiac output may have been maintained due to a balanced pre-load and after-load reduction. This reduction in cardiac filling could be due either to a contraction of plasma volume or to an increase in venous capacitance. Most of the available evidence suggests that ANP is arterioselective, and changes in plasma volume therefore provide a more likely explanation. ANP has been shown to have no venodilator effects in whole animals, isolated human saphenous veins or human dorsal hand veins in vivo. Our subjects experienced a mean plasma volume contraction of about 10.5% without an appreciable diuresis. The contraction of plasma volume with ANP is known to be independent of its diuretic effects and may relate to an increase in efflux of fluid from the intravascular space due to either an increase in capillary permeability or a change in the Starling forces across the capillary bed. The latter explanation is perhaps the more likely, and is what one would expect from a dilator which is arterioselective, increasing flow into capillary beds but not dilating the capillary venous outflow. There is, however, some evidence to support the hypothesis that ANP increases capillary permeability at least in glomerular capillaries.

We conclude that ANP is not negatively inotropic. At plasma levels within the physiological range it has no effect on systemic haemodynamics and cardiac function. At pharmacological doses it reduces blood pressure by a direct arteriolar dilator effect of rapid onset and offset and by reducing cardiac filling, probably by a contraction of plasma volume, which is a mechanism of slower offset.

References
Haemodynamic effects of atrial peptide


