Atrial Natriuretic Peptide Regulates Regional Vascular Volume and Venous Tone in Humans

Matthias Schmitt, Andrew J.M. Broadley, Angus K. Nightingale, Nicola Payne, Prasad Gunaruwan, Justin Taylor, Leong Lee, John Cockcroft, Allan D. Struthers, Michael P. Frenneaux

Objective—To date, the contribution of basal atrial natriuretic peptide (ANP) levels to resting vascular function in humans is unknown. In the present study we sought to investigate the role of ANP in regulating regional vascular volume and venous tone in healthy subjects.

Methods and Results—We used radionuclide plethysmography to examine the effects of ANP and the ANP-receptor antagonist A71915 on forearm vascular volume. Creating pressure/volume relations, we determined changes in vascular volume, compliance, and tone. Performing dose-ranging studies, we additionally assessed the potency and specificity of A71915 in the forearm resistance vasculature. Equilibrium blood pool scintigraphy was then used to assess the effects of systemic administration of A71915 on regional intestinal vascular volume. Infusion of ANP increased forearm vascular volume in a dose-dependent manner (maximum 20%; \( P<0.001 \)), exerting a maximum venodilating effect at plasma levels similar to that seen in heart failure. A71915 increased venous tone, thereby decreasing vascular volume by \( 9.6\pm1.1\% \), \( P<0.001 \) (forearm), and \( 2.6\pm0.5\% \), \( P=0.01 \) (intestinal beds). At an infusion ratio of 50:1, A71915 almost completely abolished the effects of ANP on forearm blood flow.

Conclusions—ANP locally regulates regional vascular volume and tone without affecting compliance. (Arterioscler Thromb Vasc Biol. 2003;23:1833-1838.)

Key Words: veins ■ vascular volume ■ A71915 ■ receptor antagonism

Atrial natriuretic peptide (ANP) plays an important role in cardiovascular control, being a major determinant of sodium homeostasis, plasma volume, and blood pressure (BP). Although intraarterial infusion of ANP produces vasorelaxation of resistance vessels,\(^1,2\) this may not be the underlying mechanism of the fall in BP that accompanies systemic ANP infusion. Animal studies\(^3,4\) have shown that the fall in BP and cardiac output are almost invariably associated with a fall in central venous pressure (CVP) and, in fact, an increase in total peripheral resistance. Indeed, Groban et al\(^5\) demonstrated in humans that low-dose systemic infusion of ANP reduced CVP without affecting BP. It is now generally accepted that the fall in cardiac output and BP after systemic infusion of ANP is a consequence of reduced preload.\(^6\) Possible mechanisms include volume contraction as a result of diuresis, increased capillary filtration,\(^7\) and direct venodilation, even though no venorelaxant effects of ANP were observed in dorsal hand veins\(^2\) and saphenous veins.\(^8\) Given that atrial stretch is the principal stimulus for ANP release,\(^9\) the apparent lack of a direct effect on venous tone in humans seems counterintuitive. In addition, the contribution of basal levels of ANP to human resting vascular function is unknown, but acceptance of ANP as a physiologically important vasoactive hormone in health clearly depends on its contribution being significant.

We hypothesized that, despite its lack of action on conduit veins, ANP may act as a venodilator on the small veins and venules that constitute most of the capacitance vasculature. To study the effect of ANP on vascular function in general and venous tone in particular, we performed 4 experiments. In experiment 1, we evaluated the effect of incremental doses of intraarterial ANP on forearm vascular volume (FVV) and venous tone. In experiment 2, we assessed the contribution of basal ANP plasma levels to FVV and venous tone by intraarterial infusion of the NPR\(_A\)-selective ANP-receptor antagonist A71915. In experiment 3, we additionally explored the specificity and potency of A71915 by measuring forearm blood flow (FBF) during intraarterial coinfusion of incremental doses of this receptor antagonist and either ANP or sodium nitroprusside (SNP). In experiment 4, we determined whether the potential effects of basal ANP on vascular volume were specific to the forearm by assessing the effects of intravenous infusion of A71915 on regional vascular volume in the physiologically important intestinal bed.

Methods

Subjects

Four groups of 7 healthy volunteers with no history of or evidence of cardiovascular disease or other cardiovascular risk factors were...
Assessment of Venous Function

Changes in forearm and intestinal vascular volume (IVV) were assessed by equilibrium blood-pool scintigraphy. Combining equilibrium blood-pool scintigraphy with a standard occlusion technique (radioisotope plethysmography), as described previously,\textsuperscript{10,11} we also determined forearm venous compliance from which venous tone was derived.

Briefly, a dynamic image of the forearm was continuously acquired and a region of interest (ROI) was defined (Figure 1A). The count in the ROI, obtained with no occluding pressure, was taken as baseline or unstressed vascular volume (UVV). Forearm pressure volume relationships (PVRs) were then constructed by inflating upper arm cuffs for 1 minute to pressures of 10, 20, and 30 mm Hg. Scintigraphic vascular volumes were plotted against cuff pressure to form venous PVR. Linear regression was performed for each PVR, and a linear model was accepted if \( R^2 \geq 0.9 \). The slope of each PVR is a measure of venous compliance. A parallel upward shift in PVR indicates a decrease in venous tone, whereas a parallel downward shift indicates an increase. To allow presentation of grouped data, results are presented as percentages.

Continuous data are presented as mean±SEM.

Baseline Characteristics of Subject Groups

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
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<td>Age, y (range)</td>
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<td>50 (27 to 66)</td>
<td>32 (27 to 36)</td>
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<td>7/0</td>
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<td>Forearm volume, mL</td>
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<td>1580±105</td>
<td>1671±82</td>
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<td>LV ejection fraction, %</td>
<td>57±3</td>
<td>51±4</td>
<td>59±3</td>
<td>50±4</td>
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<td>Serum Na(^+), mmol/L</td>
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<td>140.2±0.8</td>
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<td>140.1±0.7</td>
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<tr>
<td>Urinary Na(^+), mmol/L</td>
<td>105±13</td>
<td>124±25</td>
<td>112±16</td>
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<td>Serum creatinine, μmol/L</td>
<td>96±10</td>
<td>83±4</td>
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<td>104±9</td>
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<td>Serum urea, mmol/L</td>
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<td>Diastolic</td>
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<td>71±7</td>
<td>69±7</td>
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</tbody>
</table>

Experiment 1: Effect of Intrabrachial ANP on UVV and Venous Tone in the Forearm

An antecubital vein in each arm was cannulated with an 18-gauge cannula. After red cell labeling with \(^{99m}\)Technetium, a 27-gauge steel needle, sealed with dental wax to a 16-gauge epidural catheter, was inserted into the brachial artery of the left arm of 7 volunteers (group 1) under sterile conditions and kept patent by continuous infusion of saline at a rate of 1.0 mL/min. The left arm was positioned on the face of a gamma camera (Elscint Apex 215 mol/L). The right arm was also placed on a gamma camera (ADAC-Transcam) and thus served as a control for the left arm. Thirty minutes after needle insertion, 2 baseline venous PVRs were recorded. Thereafter, incremental ANP (Clinalfa) infusions at concentrations of 0.05, 0.1, 0.5, 1, 2.75, and 5 μg/mL commenced at 1 mL/min. At each dose, 1 PVR was performed after a 5-minute run-in period. Venous sampling from the infused arm was attempted at baseline and before the end of each infusion step for measurement of plasma ANP concentration. Ten milliliters of blood was collected in chilled polypropylene tubes containing 400 μL of propylalcohol-conserved aprotinin. Plasma was immediately separated and frozen at −70°C. Of a possible 49 samples, 37 could be obtained without a visible degree of hemolysis. Natriuretic peptide analysis was performed in 2 assay runs and measured by radioimmunoassay as described elsewhere.\textsuperscript{12} Results from an additional 4 samples had to be excluded because duplication of results was poor, even on repeat testing.

Experiment 2: Effect of Intrabrachial A71915 on UVV and Venous Tone in the Forearm

The set up for this experiment was identical to that of experiment 1. Thirty minutes after needle insertion, 2 baseline venous PVRs were recorded in an additional 7 volunteers (group 2). Thereafter, incremental A71915 (Clinalfa) infusions at concentrations of 0.5 and 1 μg/mL commenced at 1 mL/min. At each dose, 1 PVR was recorded after a 20-minute run-in period. Because the binding affinity of A71915 to the guanylate cyclase-coupled ANP-receptor (NPR-A) is 22 times lower than that of ANP,\textsuperscript{13} the infused concentrations of A71915 were chosen to produce approximately 50:1 and 100:1 ratios of A71915 and basal ANP in the venous effluent. The lengths of run-in periods in experiments 1 and 2 were chosen to allow a steady state to be reached, based on pilot data.

Experiment 3: Effect of Intrabrachial A71915 on FBF

In this experiment, FBF was recorded simultaneously in both arms by strain-gauge venous occlusion plethysmography as described previously.\textsuperscript{14} The left brachial artery of 7 volunteers (group 3) was...
cannulated as before and kept patent with a 15-minute infusion of saline. Thereafter, ANP was infused at 0.1 μg/min followed by coinfusion with A71915 at 0.5, 1.2, and 5 μg/min to achieve approximate ANP:A71915 ratios of 1:5, 1:10, 1:20, and 1:50 in the brachial artery. To assess whether the inhibitory effect of A71915 on ANP-induced vasodilation was specific or nonspecific, more than 1 week later, 5 of the 7 volunteers underwent a second study to assess the effects of A71915 (5 μg/min) on changes induced by SNP (1 μg/min) (David Bull Laboratories) in FBF. In all FBF studies, measurements were made during the last 90 seconds of each 6-minute infusion period, and the ratio in FBF between arms was expressed as percent change (ΔFBF%) from baseline (FBF during saline infusion).

Experiment 4: Effect of Intravenous A71915 on IVV
Measurement of regional IVV was performed in all 7 volunteers (group 2) from experiment 2 more than 90 minutes after completion of their forearm study, plus an additional 7 volunteers (group 4) who acted as time controls but did not receive A71915. Volunteers were positioned supine on a bed. The gamma camera (ADAC-Transcam) was positioned approximately 2 cm above each volunteer’s anterior abdominal wall. The bladder, the lower edge of the liver, and the bifurcation were used as landmarks. Lead strips attached to the skin facilitated the monitoring of a constant ROI (Figure 1B). Volunteers were instructed to breathe regularly and not to move. A constant patient-camera position was maintained throughout. At baseline, 5 mL of saline was injected intravenously followed by an infusion at 1 mL/min, during which a 200-second scintigram was recorded. The 7 subjects of group 2 were then injected with 5 μg A71915 in 5 mL saline followed by an infusion of A71915 at 1 μg in 1 mL/min, during which another 200-second scintigram was recorded. The 7 time control volunteers underwent the same protocol but received only saline. Because the count from a region is directly proportional to the quantity of blood in that region, changes in count rate (corrected for physical decay) are proportional to changes in regional vascular volume.

Statistics
Data are expressed as mean±SEM. In experiments 1 and 2, the effects of ANP and A71915 on FVV in the infused arm were assessed by 2-way ANOVA with post-hoc comparison to baseline. A value of P<0.05 was considered significant. Original data (counts per second) were analyzed when assessing drug effects. Conversion to percentage was then undertaken to allow presentation of grouped data. When additionally presenting corrected data in experiment 2, changes in the infused arm (venoconstriction) were first corrected for systemically occurring venoconstriction and converted into percentage before assessed by 2-way ANOVA as above. In experiment 3, changes in FBF were assessed either by 2-way ANOVA (ANP dose-ranging studies) or the nonparametric Wilcoxon test (SNP) as appropriate. In experiment 4, regional IVV was analyzed by unpaired t test.

Results
Subject Characteristics
Grouped baseline characteristics are shown in the Table. There were no significant changes in BP, heart rate, hematocrit, or plasma proteins in any of the groups in any experiment.

Experiment 1: Effect of Intrabrachial ANP on UVV and Venous Tone in the Forearm
Intraarterial infusion of incremental ANP concentrations caused a dose-dependent parallel upward shift in the PVR in all 7 subjects, indicating venodilation in the infused arm (Figure 2). ANP infusion of 0.05 μg/min caused a modest but nonsignificant 6% (P=0.1) increase in UVV. ANP infusions of 0.1, 0.5, 1, 2.75, and 5 μg/min caused significant increases in UVV of 8% (P<0.05), 13% (P<0.001), 14% (P<0.001), 18% (P<0.001), and 20% (P<0.001), respectively, compared with baseline in the same arm (Figure 3). UVV decreased gradually in the noninfused arm during the study by 12±6% (P=NS versus baseline) during the initial infusion rate and by 43±8% (P=0.01 versus baseline) at the final/peak infusion step.

Baseline plasma ANP level was 14.6±8.4 pg/mL. To additionally investigate the relation between venous ANP and venodilation, we grouped the effect on UVV according to ANP levels in the venous effluent from the infused arm (Figure 4). High physiological/low pathophysiological ANP levels (25 to 100 pg/mL) were associated with a nonsignificant 11±4% (P=0.1) increase in UVV. Infusions achieving pathophysiological (101 to 400 pg/mL) and high pathophysiological (401 to 600 pg/mL) concentrations caused a significant 18±6% (P<0.05) and 19±5% (P<0.05) increase in UVV, respectively. Pharmacological levels (>600 pg/mL) caused a significant 16±3% increase in UVV (P<0.01).

Experiment 2: Effect of Intrabrachial A71915 on UVV and Venous Tone in the Forearm
Infusion of A71915 caused a dose-dependent parallel downward shift of PVR (Figure 5) in all but 1 volunteer, reflecting...
increased venous tone. Infusion of 0.5 and 1 μg/min caused 7.7±2.3% (P<0.01) and 16.1±3% (P<0.01) reductions in UVV in the infused arm, respectively. UVV decreased gradually in the noninfused arm during the study by 3.3±2.4% (P<NS) at 0.5 μg/min and by 7.0±3.2% (P=0.08) at 1 μg/min. When correcting for the reduction in vascular volume observed in the contralateral arm, the net effect of A71915 infusion was 4.4±1.5% (P<0.01) and 9.6±1.1% (P<0.001), respectively.

Experiment 3: Effect of Intrabrachial A71915 on FBF
The results of the dose-ranging studies are shown in Figure 6. At an infusion ratio of 50:1, A71915 almost completely abolished the effects of ANP on FBF. SNP increased FBF by 111±29% (P=0.05). A71915 did not attenuate the SNP-induced increase in FBF.

Discussion
In this study we provide direct evidence that ANP is a local regulator of FVV and IVV in healthy human subjects. This is in keeping with a previous study demonstrating that systemic ANP infusion increases intestinal blood content15 but contrasts with other reports that ANP has minimal or no activity on conduit veins.2,8 Venodilator activity was seen across a wide range of physiological and pathophysiological plasma levels with a near maximum venodilating effect at plasma levels similar to those seen in severe heart failure (between 100 and 400 pg/mL). Importantly, we show for the first time that basal ANP levels influence vascular volume and resting venous tone in both forearm and intestinal beds. The forearm studies exclude ANP-mediated central sympathetic withdrawal as the mechanism.

Previous Studies Assessing the Effects of ANP on Venous Function
Several studies showed that ANP persistently reduced CVP,3,4 even at very low doses, without measurable changes in BP.5 This was in keeping with a primary venodilating ANP effect. However, other studies failed to detect a direct venodilating action. Holtz et al16 observed that systemic infusion of ANP had little or no effect on venous tone in anesthetized dogs despite potent effects on arterioles. Studies in human dorsal hand veins2 and saphenous veins8 also failed to demonstrate any significant effect. Doorenbos et al.17 using a strain-gauge technique, investigated the effect of brachial artery infusion of ANP on venous compliance. ANP infusion alone had no effect, but it antagonised the effects of angio-
tensin II on venous compliance during coinfusion. Ando et al. used a water displacement plethysmography technique to compare the effects of brachial artery infusion of SNP and a single dose of ANP on the forearm vascular bed. Both agents shifted the forearm PVR upwards, but ANP had less marked effects than SNP and simultaneously increased capillary filtration. Because this technique measures total limb volume (ie, tissue plus blood volume), it is difficult to be certain about the relative contributions of venodilation and increased tissue fluid.

How Can These Discrepancies Be Explained?
It has become increasingly clear that conduit veins in general and dorsal hand veins in particular behave differently from the physiologically important small veins and venules. Furthermore, much of the in vitro work assessing the vascular effects of ANP has been carried out on mammary arteries and saphenous vein grafts harvested from patients undergoing bypass operations.8 The vascular response in these specimens may not reflect the in vivo response in healthy people. In addition, the absolute number and proportional representation of natriuretic peptide receptor subtypes (NPRA, NPRB, and NPRC) vary from tissue to tissue,18 and such differences may well account for the different response between the small veins and venules and large conduit veins. Moreover, some human studies used doses of ANP that resulted in plasma concentrations far above those seen in health or disease.19 Because of the consequent fall in BP, baroreflex-mediated constrictor activity may not reflect the in vivo response. Our observation of an increase in plasma NE levels attributable to systemic drug effects or prolonged supine posture would tend to blunt the magnitude of the measured change in tissue volume. It is clear that the findings of this study cannot simply be extrapolated to the syndrome of chronic heart failure, because there is evidence of tolerance to ANP in situations associated with chronic elevation of ANP levels.23 Furthermore, it is important to note that the observations cannot be explained simply on the basis of increased arterial inflow. We and others have previously shown that limb PVR is unaffected by large increases in flow.10,11 Finally, in experiment 1, the documented increase in count rate does not represent increased extravasate as a consequence of increased capillary permeability, because 95% to 98.5% of the technetium was bound to erythrocytes and vascular volume returned to below baseline within 30 minutes after the cessation of ANP infusion. The measurement therefore reflects changes in vascular volume. Indeed, increased capillary permeability would tend to blunt the magnitude of the measured change in venous tone using this technique by increasing tissue attenuation.

In conclusion, we provide direct evidence that ANP is a regulator of regional vascular volume and venous tone in

Study Limitations
Observations in the contralateral arm merit comment. During infusion of A71915, vascular volume decreased in both arms, although significantly more in the infused arm. Because of this unidirectional behavior, changes in vascular volume and venous tone in the infused arm were corrected for changes in the contralateral arm. In contrast, infusion of ANP increased vascular volume in the infused arm whereas there were opposing effects in the contralateral arm. Expressing volume changes in the infused arm in relation to the changes in the contralateral arm, as is convention in assessing FBF using venous occlusion plethysmography, would therefore have been potentially misleading and was consequently not performed in the ANP dose-response study. The contralateral arm venoconstriction, initially surprising, is a consistent phenomenon during our studies. Potential mechanisms include increased sympathetic outflow caused by discomfort arising during the lengthy study and reflex-mediated vasoconstriction via baroreceptors or cardiopulmonary receptors in response to subtle changes in BP or central blood volume attributable to systemic drug effects or prolonged semirecumbence. Our observation of an increase in plasma NE levels (data not shown) is consistent with any of these mechanisms. Furthermore, it has previously been shown that prolonged venous congestion per se significantly reduces venous vascular volume in the forearm of healthy volunteers.24 Consequently, our measurements of the venodilating ANP action in the infused arm are likely to underestimate the true magnitude of its effect, because ANP actions were opposed by the effects of increased sympathetic stimulation.

Effects of ANP-Receptor Antagonism
Acceptance of vasoactive compounds as physiologically significant depends on the demonstration of changes in vascular tone in response to blockade of basal levels. This has traditionally been achieved using receptor antagonism. A71915, an antagonistic ANP analogue, has emerged as the most potent inhibitor of ANP-stimulated, NPRA-mediated, cyclic guanylate monophosphate production.11 Brunner and Woelkert used A71915 to show that basal ANP contributes to regulation of coronary and total peripheral resistance in rodents. To our knowledge, the present work is the first to assess the effects of ANP-receptor antagonism in a human in vivo study. We provide direct evidence that blocking the effects of basal ANP levels increases venous tone, thereby decreasing vascular volume. Furthermore, we provide evidence that A71915 inhibits ANP-induced increases in FBF in a dose-dependent, NPRA-receptor–mediated fashion.

Alternative Explanations for Increased Vascular Volume?
ANP has well-documented effects on sympathetic nerve activity.20 Vatta et al demonstrated that ANP plays a role in the modulation of norepinephrine (NE) metabolism in the rat hypothalamus and adrenal medulla, affecting storage, release, and uptake of NE. They concluded that ANP acts as an inhibitor of noradrenergic neurotransmission. Also, because angiotensin II is known to potentiate peripheral sympathetic activity,22 the mechanism in veins is believed to be presynaptic, the well-documented effects of natriuretic peptides on the modulation of norepinephrine (NE) metabolism in the rat hypothalamus and adrenal medulla, affecting storage, release, and uptake of NE. They concluded that ANP acts as an inhibitor of noradrenergic neurotransmission. Also, because angiotensin II is known to potentiate peripheral sympathetic activity22 (the mechanism in veins is believed to be presynaptic), the well-documented effects of natriuretic peptides on reducing angiotensin II levels could indirectly reduce NE release. However, withdrawal of sympathetic activity was not the mechanism of venodilation in this study, either at a central or a peripheral level, because we observed venoconstriction in the contralateral arm, and venodilation in the infused arm occurred despite an increase in plasma NE in the venous effluent from this arm (data not shown).
healthy subjects. Importantly, we show that basal ANP levels contribute significantly to resting venous tone and that plasma levels similar to those seen in severe heart failure exert a powerful venodilator effect.

Acknowledgments
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References
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