Attenuation of Reflex Forearm Vasoconstriction by α-Human Atrial Natriuretic Peptide in Men

Akira Takeshita, Tsutomu Imaizumi, Naonori Nakamura, Hideki Higashi, Takeshi Sasaki, Motoomi Nakamura, Kenji Kangawa, and Hisayuki Matsuo

This study examined whether atrial natriuretic peptide (ANP) modulates reflex forearm vasoconstriction in humans. Synthetic α-human ANP (α-hANP) was infused at a rate of 0.03 μg/kg/min in 8 healthy men (mean age 23 ± 0.7 years, mean ± SEM). The α-hANP decreased systolic blood pressure and central venous pressure (CVP) but did not significantly alter resting heart rate and forearm vascular resistance (FVR). The magnitudes of reflex increases in FVR during lower body negative pressure (LBNP) at −110, −20, and −40 mm Hg were less during infusion of α-ANP than those magnitudes during infusion of saline solution. The slope of the regression line relating changes in CVP and those in FVR was less during infusion of α-hANP than the slope during infusion of saline solution. Forearm vascular responses to intra-arterial infusion of norepinephrine at doses of 100, 200, and 500 ng/min did not significantly differ during infusion of α-hANP and saline solution. These results suggest that α-hANP attenuates cardiopulmonary baroreflex control of FVR in normal men. (Circulation Research 1987;61:555-559)

Subjects and Methods

Subjects

Studies were performed on 8 young, healthy volunteers (mean age, 23 ± 0.7 years, mean ± SEM). All subjects were male. The study protocol was explained, and informed consent was obtained from each subject.

Measurements of Forearm Blood Flow, Arterial and Central Venous Pressure, and Heart Rate

Forearm blood flow was measured using a mercury-in-silastic strain gauge plethysmograph with venous occlusion technique. The strain gauge was placed approximately 5 cm below the antecubital crease. The pressure in the venous occlusion or congesting cuff was 40 mm Hg. Circulation to the hand was arrested by inflating a cuff around the wrist until pressure was suprasystolic during determination of forearm blood flow. Forearm blood flow was taken as the average of 4–8 flow measurements made at 15-second intervals. From the data, calculation of forearm blood flow was performed independently by two of the authors, and the average value was used for statistical analysis. The blood pressure was measured in the other arm with a sphygmomanometer. All blood pressure measurements were performed by one individual to minimize observer variation. Forearm vascular resistance was calculated by dividing mean arterial pressure (diastolic pressure plus one third of the pulse pressure in mm Hg) by forearm blood flow (ml/min/100 ml of forearm volume); these values are expressed as units throughout this report. Heart rate was calculated from the electrocardiogram. Central venous pressure was obtained from a catheter introduced into an antecubital vein and advanced into an intrathoracic vein. The pressure was measured with a pressure transducer (MPU 0.5, Toyo

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Supported in part by grants from the Japanese Ministry of Education, Science and Culture.

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Received October 20, 1986; accepted May 8, 1987.
Baroreflex Control of Forearm Vascular Resistance

Reflex vasoconstriction in the forearm was examined during LBNP. The subject's body below the iliac crest was enclosed in a chamber that was sealed and connected to an adjustable vacuum. LBNP was applied at -10, -20, and -40 mm Hg, which produced graded decreases in central venous pressure and reflex increases in forearm vascular resistance. The slope of the regression line relating changes in central venous pressure and changes in forearm vascular resistance was calculated by the least-squares method. Correlation coefficients were greater than 0.879.

Forearm Vascular Responses to Norepinephrine

Forearm blood flow was measured during intra-arterial infusion of norepinephrine at rates of 100, 200, and 500 ng/min. Each dose of norepinephrine was infused for 2 minutes. Forearm vascular resistance was calculated from forearm blood flow and mean arterial pressure. The arterial pressure was measured in the other arm with a sphygmomanometer.

Preparation of α-hANP

The α-hANP was synthesized as previously described. The homogeneity of α-hANP used in this study was confirmed by reverse-phase, high-performance liquid chromatography and amino acid analysis. The α-hANP was dissolved in saline solution with 10% lactose, which was sterilized by passage through a 0.22-µM Millipore filter (Millipore Corp., Bedford, Mass.). The α-hANP synthesized and sterilized in this manner has been safely given to humans.

Protocol

The study was performed with the subjects supine in the postabsorptive state in a warm and quiet room. After the placement of catheters and a strain-gauge plethysmograph, at least 15 minutes were allowed for the subjects to become accustomed to the study conditions before beginning the protocol.

Blood pressure, central venous pressure, and forearm blood flow were measured at rest, with LBNP at -10, -20, and -40 mm Hg, and with intra-arterial infusion of norepinephrine at doses of 100, 200, and 500 ng/min. The measurements were performed, first, during intravenous infusion of saline solution and were repeated during infusion of α-hANP at a dose of 0.03 µg/kg/min. The measurements at rest were performed 15 minutes after the beginning of saline solution or α-hANP infusion. The study was performed with only one dose of α-hANP because the amount of α-hANP that could be used in humans was limited.

Calculation and Statistical Analysis

A paired t test was used for comparisons of the results during infusion of saline solution and α-hANP. Forearm vascular responses to norepinephrine during infusion of saline solution and α-hANP were compared by two-way analysis of variance. \( p \leq 0.05 \) was considered significant. All data are expressed as mean ± SEM.

Results

Table 1 summarizes resting values and changes with LBNP during intravenous infusion of saline solution and infusion of α-hANP.

<table>
<thead>
<tr>
<th>Changes during LBNP</th>
<th>0 mm Hg</th>
<th>-10 mm Hg</th>
<th>-20 mm Hg</th>
<th>-30 mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>126±5</td>
<td>-0.5±1.0</td>
<td>-2±1</td>
<td>-7±2*</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>69±3</td>
<td>1±1</td>
<td>2±1</td>
<td>4±1‡</td>
</tr>
<tr>
<td>mBP (mm Hg)</td>
<td>88±3</td>
<td>0.6±0.2</td>
<td>1.0±0.3</td>
<td>-0.3±1.0</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>57±1</td>
<td>0.0±0.1</td>
<td>1.2±1.0</td>
<td>11±2$</td>
</tr>
<tr>
<td>CVP (mm Hg)</td>
<td>3.7±0.5</td>
<td>-2.4±0.2$</td>
<td>-4.2±0.3$</td>
<td>-6.3±0.5$</td>
</tr>
<tr>
<td>FoBF (ml/min/100 ml)</td>
<td>4.1±0.4</td>
<td>-1.4±0.3$</td>
<td>-1.7±0.3$</td>
<td>-2.2±0.3$</td>
</tr>
<tr>
<td>FoVR (units)</td>
<td>24.4±3.5</td>
<td>13.5±2.8$</td>
<td>20.0±4.3$</td>
<td>34.9±4.6$</td>
</tr>
</tbody>
</table>

α-hANP

<table>
<thead>
<tr>
<th>Changes during LBNP</th>
<th>0 mm Hg</th>
<th>-10 mm Hg</th>
<th>-20 mm Hg</th>
<th>-30 mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>120±5*</td>
<td>-0.1±0.4</td>
<td>-3±1</td>
<td>-12±2*‡</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>67±3</td>
<td>0.0±0.3</td>
<td>-0.3±1.0</td>
<td>0.3±2.0</td>
</tr>
<tr>
<td>mBP (mm Hg)</td>
<td>84±3*</td>
<td>0.1±0.3</td>
<td>-1±1</td>
<td>-3.5±2.0</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>60±1</td>
<td>0.0±0.1</td>
<td>1.7±1.0</td>
<td>7±1*$</td>
</tr>
<tr>
<td>CVP (mm Hg)</td>
<td>1.3±0.3$</td>
<td>-1.4±0.3$</td>
<td>-2.9±0.3$</td>
<td>-4.6±0.5$</td>
</tr>
<tr>
<td>FoBF (ml/min/100 ml)</td>
<td>3.6±0.3</td>
<td>-0.7±0.1$</td>
<td>-0.9±0.1$</td>
<td>-1.3±0.1*$</td>
</tr>
<tr>
<td>FoVR (units)</td>
<td>25.0±2.0</td>
<td>6.0±0.8$</td>
<td>0.5±1.5$</td>
<td>14.3±2.3$</td>
</tr>
</tbody>
</table>

SBP, systolic blood pressure; DBP, diastolic blood pressure; mBP, mean blood pressure; HR, heart rate; CVP, central venous pressure; FoBF, forearm blood flow; FoVR, forearm vascular resistance.

*p = 0.05 saline vs. α-hANP, †p = 0.01 saline vs. α-hANP, $p = 0.05 rest vs. LBNP, and $p = 0.01 rest vs. LBNP.
and \(\alpha\)-hANP. The \(\alpha\)-hANP decreased systolic and mean blood pressure and central venous pressure but did not alter diastolic blood pressure, heart rate, forearm blood flow, and forearm vascular resistance at rest.

LBNP at \(-10\) and \(-20\) mm Hg decreased central venous pressure and forearm blood flow but increased forearm vascular resistance before and during intravenous infusion of saline solution and \(\alpha\)-hANP. Systolic, diastolic, and mean blood pressure and heart rate did not change significantly with LBNP at \(-10\) and \(-20\) mm Hg during infusion of either saline solution or \(\alpha\)-hANP. The decreases in central venous pressure and forearm blood flow and the increases in forearm vascular resistance with LBNP at \(-10\) and \(-20\) mm Hg were less during infusion of \(\alpha\)-hANP than during infusion of saline solution.

LBNP at \(-40\) mm Hg decreased systolic blood pressure, central venous pressure, and forearm blood flow but increased heart rate and forearm vascular resistance during infusion of saline solution and \(\alpha\)-hANP. Diastolic blood pressure increased with LBNP at \(-40\) mm Hg during infusion of saline solution but not during infusion of \(\alpha\)-hANP. The decrease in systolic blood pressure with LBNP at \(-40\) mm Hg was greater during infusion of \(\alpha\)-hANP than during infusion of saline solution. The decreases in central venous pressure and forearm blood flow and the increases in heart rate and forearm vascular resistance with LBNP at \(-40\) mm Hg were less during infusion of \(\alpha\)-hANP than during infusion of saline solution. The slope of the regression line relating changes in central venous pressure and those in forearm vascular resistance with LBNP at \(-10\), \(-20\), and \(-40\) mm Hg was less \((p\leq0.05)\) during infusion of \(\alpha\)-hANP \((-3.5\pm0.8)\) than during infusion of saline solution \((-6.0\pm1.0)\) (Figure 1).

Forearm vascular responses to intra-arterial norepinephrine did not significantly differ during infusion of saline solution and \(\alpha\)-hANP (Figure 2). The increases in forearm vascular resistance with norepinephrine at doses of 100, 200, and 500 ng/min were 5.7 ± 1.6, 19.5 ± 2.7, and 37.5 ± 5.4 during infusion of saline solution and 2.3 ± 2.1, 14.2 ± 3.7, and 26.6 ± 5.0 during infusion of \(\alpha\)-hANP. The increase in forearm vascular resistance with each dose of norepinephrine did not differ during infusion of saline solution and \(\alpha\)-hANP.

**Discussion**

The results of this study indicate that \(\alpha\)-hANP infused at a rate of 0.03 \(\mu\)g/kg/min lowered central venous pressure as well as arterial pressure and attenuated the slope of the regression line relating changes in central venous pressure and those in forearm vascular resistance with LBNP. These results suggest that \(\alpha\)-hANP dilates veins as well as arteries and attenuates reflex sympathetic activation mediated by the cardiopulmonary baroreceptors in humans.

**Effects on Resting Hemodynamics**

An intravenous infusion of \(\alpha\)-hANP decreased systolic and mean blood pressure but did not alter resting forearm vascular resistance. Considering the vasodilator effect of ANP, one may expect reduced forearm vascular resistance during infusion of \(\alpha\)-hANP. A previous study has demonstrated that direct intra-arterial infusion of \(\alpha\)-hANP causes vasodilatation of the forearm resistance vessels. However, in this study, intravenous infusion of \(\alpha\)-hANP significantly lowered central venous as well as systolic arterial pressure. The reduction in the stimulus level to the cardiopulmonary as well as arterial baroreceptors must have caused reflex sympathetic activation. We believe that, during infusion of \(\alpha\)-hANP, reflex neurogenic vasoconstriction mediated by the baroreceptors may have competed with the direct vasodilator effect of \(\alpha\)-hANP so that resting forearm vascular resistance did not change. In fact, during infusion of saline solution, LBNP at \(-10\) mm Hg lowered central venous pressure to the level comparable to that at rest during infusion of \(\alpha\)-hANP. Forearm vascular resistance during LBNP at \(-10\) mm Hg with saline infusion was higher \((p<0.05)\) than that at rest with infusion of \(\alpha\)-hANP.
The α-hANP lowered central venous pressure by 2.4 ± 0.2 mm Hg in our subjects. Diuresis caused by α-hANP must have contributed in part to the decrease in central venous pressure. However, changes in central venous pressure were measured 15 minutes after the beginning of α-hANP infusion. Urine output was not measured, but it appears that the magnitude of the decrease in central venous pressure is disproportionate to what might be expected from diuresis occurring during a period of 15 minutes. The decrease in central venous pressure caused by α-hANP has been reported in experimental animals. We have recently observed that α-hANP significantly decreased mean circulatory filling pressure in rats with bilateral nephrectomy, suggesting that α-hANP may dilate veins as well as arteries in rats (H. Higashi et al, unpublished observation). It is conceivable that α-hANP may cause venodilatation in humans as well as in rats.

Studies in experimental animals have shown that heart rate may not increase with hypotension during infusion of α-hANP. In our subjects, heart rate did not significantly increase during infusion of α-hANP. This finding differs from the results in previous human studies that have shown a small but significant increase in heart rate after a bolus injection or during infusion of synthetic ANP. However, the dose of ANP used in this study was much smaller than those used in previous studies. Thus, the difference is likely to have resulted from the difference in the speed and magnitude of the reduction in blood pressure. Failure to significantly increase heart rate under conditions of hypotension may suggest altered baroreflex control of autonomic nervous system caused by α-hANP.

Effects on Forearm Vascular Responses to LBNP

The major finding of this study is that reflex forearm vasoconstriction in response to LBNP was markedly attenuated during infusion of α-hANP as compared with that during infusion of saline solution. LBNP at −10 and −20 mm Hg did not significantly alter blood pressure and heart rate but decreased central venous pressure. Thus, forearm vasoconstriction with these levels of LBNP was likely to be mediated by the cardiopulmonary baroreceptors. LBNP at −40 mm Hg decreased systolic blood pressure as well as central venous pressure and increased heart rate. Thus, the arterial as well as cardiopulmonary baroreceptors may have contributed to forearm vasoconstriction with LBNP at −40 mm Hg. However, it has been suggested that the arterial baroreceptors have a minor role in forearm vasoconstriction with LBNP as opposed to splanchnic vasoconstriction, which is largely determined by the arterial baroreceptors. Thus, we consider that forearm vasoconstriction with LBNP at −10, −20, and −40 mm Hg was largely determined by the cardiopulmonary baroreceptors and was triggered by the decreases in intra-arterial and ventricular diastolic pressure with LBNP.

To assess the stimulus level to the cardiopulmonary baroreceptors, changes in central venous pressure during LBNP were measured. Decreases in central venous pressure at LBNP at −10, −20, and −40 mm Hg were less during infusion of α-hANP than those during infusion of saline solution. Thus, attenuated forearm vasoconstriction with LBNP during infusion of α-hANP must have resulted in part from smaller decreases in central venous pressure with LBNP. However, the slope of the regression line relating changes in central venous pressure and those in forearm vascular resistance with LVBP was less during infusion of α-hANP than that during infusion of saline solution. The latter finding cannot be accounted for by the difference in the decreases in central venous pressure with LBNP. This finding suggests that reflex sympathetic activation in response to deactivation of the cardiopulmonary receptors is attenuated by α-hANP.

We need to consider the possibility that a less steep slope of the regression line during infusion of α-hANP might have been caused by nonspecific mechanisms such as the difference in baseline central venous pressure, baseline forearm vascular resistance, or forearm vascular responses to norepinephrine. However, a previous study has shown that changes in baseline central venous pressure caused by nitroglycerin or trapidil did not alter the slope or reflex forearm vasoconstriction with LBNP. Baseline vascular resistance may also influence vascular responses to sympathetic stimulation, but baseline forearm vascular resistance did not differ between infusion of α-hANP and saline solution. We examined forearm vascular responses to intra-arterial norepinephrine. The dose-response curve with intra-arterial infusion of norepinephrine during infusion of α-hANP did not significantly differ from that during infusion of saline solution. The increase in forearm vascular resistance caused by norepinephrine at a dose of 500 ng/min was slightly smaller during infusion of α-hANP than that during infusion of saline solution, but this difference was not significant.

On the basis of these considerations, the results suggest that α-hANP modulates cardiopulmonary baroreflex control of forearm vascular resistance in humans. The α-hANP attenuates reflex sympathetic activation to the forearm in response to the decrease in central venous pressure. During infusion of α-hANP, LBNP at −40 mm Hg produced a greater decrease in systolic blood pressure and a smaller increase in heart rate as compared with the results during infusion of saline solution. These results are in agreement with the view that α-hANP attenuates baroreflex control of autonomic nervous activity.

The blood level of immunoreactive ANP (1R-ANP) during infusion of α-hANP was not determined in this study. However, Hirata et al have reported that intravenous infusion of α-hANP at a rate of 0.025 μg/kg/min for 20 minutes in normal volunteers causes an eightfold increase in blood 1R-ANP to an average of 500 pg/ml. In contrast, we have found in middle-aged subjects that the decreases in endogenous 1R-ANP during LBNP were much smaller; 1R-ANP decreased on average from 55 pg/ml at control to 45, 34, and 33 pg/ml with LBNP at −10, −20, and −40
mm Hg, respectively (unpublished observation). The decreases in 1R-ANP with tilting were also smaller. Thus, it is unlikely that changes in endogenous ANP during LBNP had significantly influenced the alteration of reflex control of forearm vascular resistance during infusion of synthetic α-hANP.

In summary, the results of this study indicate that α-hANP decreases central venous pressure as well as arterial pressure in humans. The results also suggest that α-hANP may attenuate reflex sympathetic activation mediated by the cardiopulmonary baroreceptors. These results are of interest in considering possible roles of ANP in control of circulation in humans. First, it has been suggested that the release of ANP from atrial myocytes is stimulated by the elevation of intra-arterial pressure. Released ANP lowers central venous pressure. Thus, there seems to be a negative feedback system in this respect in humans. Second, in humans and intact animals, the direct vasodilator and natriuretic effects of ANP compete with reflex sympathetic activation triggered by the decreases in central venous pressure and arterial pressure. The results of this study suggest that such compensatory neural mechanisms that counteract with the direct effects of ANP on blood vessels and kidney may be attenuated by ANP.

Acknowledgments

We appreciate the technical assistance of Ms. Tomoko Hirokawa and the secretarial assistance of Ms. Mieko Itoyama.

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Key Words • cardiopulmonary baroreflex • strain-gauge plethysmograph • central venous pressure • forearm vascular resistance
Attenuation of reflex forearm vasoconstriction by alpha-human atrial natriuretic peptide in men.
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Circ Res. 1987;61:555-559
doi: 10.1161/01.RES.61.4.555

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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