Atrial natriuretic factor potentiates forearm reflex vasoconstriction induced by cardiopulmonary receptor deactivation in man

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ABSTRACT Previous evidence suggests that atrial natriuretic factor (ANF) interferes with the autonomic control of circulation. In the present study we investigated whether ANF modulates forearm vasoconstriction reflexly induced by cardiopulmonary receptor unloading in man. For this purpose, the hemodynamic response to -20 mm Hg lower body negative pressure (LBNP) was assessed under control conditions and during the constant infusion of α-human ANF (0.5 µg/kg bolus followed by 0.05 µg/kg/min) in seven normal subjects. ANF infusion resulted in a slight reduction in blood pressure and right atrial pressure, did not modify heart rate or forearm vascular resistance, but significantly potentiated the reflex increase in forearm vascular resistance during LBNP (+25 ± 9% under control conditions vs +40 ± 12% during ANF, p < .05). In an attempt to clarify the mechanisms underlying the enhanced reflex vasoconstriction during infusion of ANF, in five additional subjects we demonstrated that there was a comparable vascular reflex response to LBNP under control conditions and during nitroglycerin infusion at a dose that induced a reduction in atrial pressure comparable to that observed during ANF. Finally, in seven additional subjects we found that ANF infusion did not alter the reflex hemodynamic responses elicited by carotid baroreceptor unloading induced by a +60 mm Hg increase in external neck pressure. We conclude that during the infusion of a pharmacologic dose of ANF the reflex forearm vasoconstriction in response to selective cardiopulmonary receptor unloading is potentiated. This effect does not seem to be related to the hemodynamic actions of the peptide or to interference with the sympathetic control of peripheral circulation.


BESIDES its characteristic diuretic and natriuretic effects,1–3 the exogenous administration of atrial natriuretic factor (ANF) has remarkable interactions with the renin-angiotensin-aldosterone system,4 vaso- pressin,5–7 and catecholamines,8,9 and it also has important cardiovascular effects.10–13 In this latter regard, it has been hypothesized that ANF exerts its cardiovascular effects by interacting with autonomic control mechanisms.14,15 Such a hypothesis is supported by several experimental observations. In particular, a number of studies have shown that the blood pressure–lowering effect of an infusion of ANF is not consistently associated with the expected reflex tachycardia.10,12,13,15–17 In addition, it has been reported that the hypotension caused by the peptide can be markedly attenuated by preventive bilateral vagotomy,10,15,18 whereas the concomitant denervation of arterial baroreceptors potentiates the depressor response.19,20 Finally, we have reported that during ANF infusion the bradycardiac reflex response to phenylephrine-induced hypertension is enhanced.15

These and other observations indicating that ANF might interfere with the autonomic control of circulation prompted us to investigate whether ANF modulates the vasoconstrictive reflex response evoked by venous pooling in man.

The present study describes the influence of the exogenous infusion of ANF on the vascular reflex responses produced in the forearm by simulated orthostatic stress induced by applying negative pressure to the lower portion of the body at a level known to deactivate predominantly cardiopulmonary receptors.
Methods

Subjects and general procedures. The study was performed in healthy volunteers of both sexes. Informed consent was obtained from each subject before the study. The study protocol was approved by the research committee of our institution. In the week preceding the study the subjects were maintained on a standard daily diet containing 1500 ml of fluids, 130 meq of sodium, and 60 meq of potassium. Two days before the study the subjects were made familiar with the experimental procedure and in the day preceding the study they abstained from caffeine, alcohol, smoking, and strenuous exercise.

The studies were always performed in the morning, after an overnight fast, with the subject in the supine position, in a quiet room with the temperature kept constant between 22° and 24° C.

Experimental protocols

ANF and lower body negative pressure (LBNP). This protocol was carried out in seven subjects (mean age 39 ± 4 years; six men, one woman). A heparinized polyethylene catheter was introduced through an antecubital vein of each and positioned in the right atrium for direct continuous recording of right atrial pressure through a pressure transducer connected to a polygraph.

Blood sampling for hormonal measurements was performed through a peripheral venous line, while a separate polyethylene catheter was inserted into a contralateral antecubital vein for drug and fluid administration. Heart rate was continuously monitored by an electrocardiographic lead and blood pressure was measured at 60 sec intervals throughout the study with a Vitastat automatic blood pressure recorder. The internal diameter of the brachial artery was determined by means of transcutaneous pulsed Doppler velocimetry (Echovar Doppler Pulsed, Alvar Electronic, Montreuil, France). The Doppler velocimeter operated at a frequency of 8 MHz and pulsed at 15 kHz, and was equipped with two original features as previously described.21

The probe position was adjusted over the brachial artery and fixed throughout the study by means of a stereotaxic device placed around the arm. Flow through the brachial artery was measured as previously described.21 The random variability between repeated measurements with this method was 7 ± 2%.

In man, arterial flow of superficial arteries deduced from concomitant pulsed Doppler measurements of diameter and blood velocity has been found to correlate strongly with the brachial artery-to-femoral arterial flow measured by strain-gauge plethysmography. Forearm vascular resistance (in mm Hg/ml/sec) was calculated by dividing mean arterial pressure (diastolic pressure plus one-third of pulse pressure in mm Hg) by brachial artery blood flow. A constant saline infusion (2 ml/min) was maintained throughout the study to ensure a venous line for drug administration as well as to replace fluid losses.

A LBNP chamber similar to that previously described from our laboratory21 was placed over the lower portion of each subject’s body from the iliac crest down to apply LBNP in an adjustable vacuum.

After baseline measurements (blood pressure, heart rate, right atrial pressure, forearm vascular resistance, plasma levels of ANF), −20 mm Hg LBNP was applied, and this level of LBNP was maintained for 15 min. This level of LBNP has been previously shown to predominantly affect the discharge of cardiopulmonary receptors.22, 23 Measurements for comparison with baseline were obtained during the steady-state phase of the maneuver (i.e., the average of six measurements obtained during the last 3 min of the maneuver). After 30 min of recovery, measurements were repeated to obtain a new baseline. A constant infusion of ANF (α-human-1-28 atrial natriuretic peptide, Bissendorf Peptide Gmbh, West Germany) (0.5 μg/kg bolus, 0.05 μg/kg/min) was then started and maintained for 30 min.

A set of measurements was obtained between 12 and 15 min from the beginning of the infusion to evaluate the direct hemodynamic effects of the peptide. Finally, during the infusion of ANF −20 mm Hg LBNP was applied again and maintained for 15 min. Measurements were obtained during the last 3 min of the maneuver.

The reproducibility of the responses evoked by LBNP was assessed in a separate group of subjects by applying −20 mm Hg LBNP at two times separated by a 30 min interval during the infusion of saline.

Nitroglycerin and LBNP. To rule out the possibility that the influence of ANF on the vascular reflex responses to LBNP was a mere consequence of the hemodynamic changes caused by the peptide, in five normal subjects (mean age 38.4 ± 2; three men, two women) the protocol described above was repeated with the difference that a 30 min constant infusion of nitroglycerin (NTG) instead of ANF was used. The dose of NTG (0.5 μg/kg/min) had been titrated during the course of preliminary experiments on the basis of a hemodynamic response equipotent to that caused by ANF (in terms of blood pressure, atrial pressure, and vascular resistance).

Effect of ANF on carotid baroreceptor deactivation. To explore the possibility that the influence of ANF on the reflex response to LBNP was mediated through interference of the peptide with the sympathetic efferent control of vascular resistance, in seven additional normal subjects (mean age 42 ± 3 years; five men, two women) we studied the effects of ANF on the reflex sympathetic activation provoked by selective carotid baroreceptor unloading. This was produced by a reduction in carotid transmural pressure evoked by a 60 mm Hg increase in external neck pressure produced by means of a variable-pressure pneumatic neck chamber similar to that previously described from our laboratory.24 The maneuver lasted for 4 min and measurements were obtained during the steady-state phase. The responses were expressed as percent changes from control. The procedure was performed under control conditions and during a constant infusion of ANF at the same dose used in the first protocol.

ANF assay. Plasma levels of ANF were determined by radioimmunoassay, as previously described,25 with rabbit antisera (RAS 8798, Peninsula Lab. Europe), iodinated human ANF (2000 Ci/mmol, Amersham), and α-hANP (Bissendorf) as a standard. ANF was separated from plasma by use of C-18 cartridges. The recoveries, determined in each plasma sample by the addition of a minimal amount of radioelabeled ANF, ranged from 57% to 85%. The peptide retained on C-18 was eluted by means of 80% aqueous CH3CN. The eluates lyophilized, and were reconstituted in radioimmunoassay (RIA) buffer (phosphate buffer 0.1M, pH 7.4). Bound/free separation was carried out by use of charcoal-dextran. Intra-assay and interassay coefficients were 6.6% and 10.5%, respectively. The RIA sensitivity was 1 fmol/tube.

Statistical analysis. Data were analyzed by one-way analysis of variance and the t test for repeated measurements was adopted for comparisons within the same group. The Bonferroni method was used for simultaneous comparison of the responses. Linear regression analysis was used to plot the changes in vascular resistance versus the changes in right atrial pressure during LBNP. The comparison of the regression lines so obtained was performed by standard methods.26 Data are presented as the mean ± SE.

Results

ANF and LBNP. The hemodynamic effects of −20 mm Hg LBNP observed under control conditions and during ANF infusion are shown in figure 1. In the basal
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FIGURE 1. Hemodynamic effects of −20 mm Hg LBNP under control conditions and during constant infusion of ANF (0.5 μg/kg + 0.05 μg/kg/min). MBP = mean blood pressure; RAP = right atrial pressure; HR = heart rate; FVR = forearm vascular resistance; B₁ and B₂ = baseline conditions. *p < .05 vs C; ▲ p < .05 vs ANF.

state, LBNP did not modify blood pressure or heart rate, significantly reduced right atrial pressure (by 28 ± 5%), and increased forearm vascular resistance (by 25 ± 9%). Plasma levels of ANF fell from 28 ± 6 to 20 ± 6 fmol/ml (p < .05). After 30 min of recovery all variables returned to baseline, and in particular, the plasma levels of ANF (35 ± 8 fmol/ml) did not differ from those observed under control conditions. After 15 min of infusion of ANF, the plasma levels of the peptide rose to 1432 ± 322 fmol/ml (p < .001 vs baseline), blood pressure and right atrial pressure fell slightly, and heart rate and forearm vascular resistance were not significantly modified. When LBNP was applied during ANF infusion, right atrial pressure showed a further reduction (by 35 ± 11% from ANF), which was associated with a marked reflex rise in forearm vascular resistance (40 ± 12%) that was significantly greater (p < .05) than that observed under control conditions. Blood pressure, heart rate, and plasma levels of ANF did not show further changes during this maneuver.

A significant correlation was obtained by plotting the responses (% of changes from control) in atrial pressure vs those observed in vascular resistance evoked by LBNP both under control conditions (r = .754, n = 7, p < .05) and during ANF (r = .946, n = 7, p < .001). Although the difference between the two regression lines so obtained did not achieve statistical significance, the slope (f (x) = 4.03 + 1.10x) of the regression line obtained during ANF tended to be higher than that obtained under control conditions (f (x) = 1.92 ± 0.84x), thus suggesting an enhanced reflex response independent of the initial value for atrial pressure.

To partially clarify the possible mechanisms by which ANF potentiated the reflex forearm vasoconstriction in response to LBNP two further studies were carried out.

NTG and LBNP. The results of these experiments, which had been planned to investigate the possible role of the fall in atrial pressure on the reflex vascular responses, are shown in figure 2. Under control conditions, the hemodynamic responses to LBNP were comparable to those observed under the control conditions of the ANF protocol. In particular, in the presence of a fall in right atrial pressure by 35.8 ± 12%, forearm vascular resistance rose by 24 ± 5%, with no change in blood pressure and heart rate. As observed in the ANF studies, plasma ANF levels fell during LBNP from 29.5 ± 17 to 16.2 ± 10 fmol/ml (p < .05). The infusion of NTG, which was started after the establishment of a new baseline, caused a reduction in blood pressure and right atrial pressure and a slight increase in heart rate and it did not significantly modify forearm vascular resistance. Plasma levels of ANF were reduced during NTG infusion (from 28.2 ± 18 to 16.8 ± 11 fmol/ml, p < .05). At the steady state of LBNP during NTG infusion, blood pressure did not change significantly, heart rate was not modified, right atrial pressure fell further by 31.8 ± 12%, and there was a reflex rise in forearm vascular resistance (31 ± 16%) that was not different from that observed under
control conditions. Finally, plasma ANF levels further fell to 10.4 ± 6.4 (p<0.5 vs baseline).

Effect of ANF on carotid baroreceptor deactivation. The results of this study, which was undertaken to explore a possible interaction of ANF with the sympathetic control of vascular tone, are presented in table 1. The hemodynamic and vascular reflex responses to the reduction in carotid transmural pressure evoked by the increase in external neck pressure did not differ under control conditions and during the steady-state phase of the ANF infusion (ANF plasma levels 12.8 ± 2.4 fmol/ml).

The reproducibility of the responses evoked by LBNP was assessed in a separate group of subjects (n = 7) undergoing two sessions of −20 mm Hg LBNP separated by a 30 min interval during saline infusion. The steady-state hemodynamic responses observed in the two sessions were not statistically different (mean arterial pressure = −1.1 ± 1 vs −1.4 ± 1 mm Hg, right atrial pressure = −2.4 ± 0.2 vs −2.55 ± 0.4 mm Hg, forearm vascular resistance = +24.1 ± 5 vs +28.4 ± 6 mm Hg/ml/sec).

Discussion

The results obtained in the present study demonstrate that the forearm vasoconstriction reflexly induced by the selective deactivation of cardiopulmonary receptors is potentiated during the exogenous infusion of a pharmacologic dosage of synthetic ANF. They also suggest that the enhanced vascular reflex response might be mediated through an augmented vagal tone.

The LBNP technique has been widely used to investigate the physiologic role of low-pressure baroreceptors located in the cardiopulmonary area. In particular, Zoller et al.22 and Johnson et al.23 have demonstrated that at low levels of LBNP the reduction in central venous pressure is associated with forearm vasoconstriction, without changes in mean or pulse arterial pressure or heart rate, thus suggesting that selective inhibition or unloading of cardiopulmonary baroreceptors triggers reflex increases in forearm vascular resistance. On the basis of these previous experiences, we used a low level of LBNP (−20 mm Hg) to look specifically at the eventual interference of ANF with the function of cardiopulmonary receptors, and then to

TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ANF</th>
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<tbody>
<tr>
<td>MBP (%)</td>
<td>9.6±2.1</td>
<td>10.2±4</td>
</tr>
<tr>
<td>HR (%)</td>
<td>7.2±2</td>
<td>9.2±3</td>
</tr>
<tr>
<td>FVR (%)</td>
<td>62±8</td>
<td>73±18</td>
</tr>
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MBP = mean blood pressure; HR = heart rate; FVR = forearm vascular resistance.

No differences between control and ANF values were significant.
investigate the reciprocal interaction of two regulatory systems (hormonal and neural) located in the cardiopulmonary area. We also decided to study the reflex responses in the forearm vascular bed, since it has been shown that cardiopulmonary rather than arterial baroreflexes plays the major role in controlling forearm vascular responses during venous pooling in man.27 The results obtained under control conditions with −20 mm Hg LBNP show that this level of LBNP causes a reduction in atrial pressure with reflex forearm vasoconstriction, without concurrent significant changes in blood pressure and heart rate, thus confirming predominant deactivation of cardiopulmonary receptors.

The major finding of the present study is the enhanced forearm reflex vasoconstriction observed during infusion of ANF (at a dose previously shown to be biologically effective in humans28), which results in circulating levels of the peptide largely exceeding the "physiologic" range. The observation of enhanced vasoconstriction during ANF infusion might appear to contradict earlier reports that the peptide has potent vasorelaxant activity on precontracted preparations in vitro29, 30 and in some vascular beds31 or in vasoconstricted preparations of hypertension.12 However, there is also evidence that ANF does not dilate resistance-sized isolated arteries32 and does not cause vasodilation in vivo,12, 33, 34 and that it may even be associated with vasoconstriction12, 33 that most likely is reflex-like. The dose of ANF used in our study induced a slight reduction in right atrial pressure and in arterial pressure, but did not modify forearm vascular resistance per se. This latter observation, while excluding a direct vasodilator effect of ANF, minimizes the possibility that the enhancement of the reflex vasoconstriction was also due to a direct vasoconstrictive action of the peptide.

The finding that when LBNP was applied during ANF infusion right atrial pressure fell further raises the possibility that a lower initial level of atrial pressure might have amplified the response to cardiopulmonary receptor unloading. This latter mechanism is also minimized by the observation that when the LBNP-induced individual changes in right atrial pressure and in forearm vascular resistance were correlated, the slope of the regression line obtained during infusion of ANF tended to be steeper than that obtained under control conditions. This suggests that, independent of initial atrial pressure, the reflex vasoconstrictive responses observed during infusion of ANF are more marked than those found in the basal state. To further investigate this aspect, however, we also performed a series of experiments devoted to evaluation of the influence of a lower initial right atrial pressure on LBNP-induced forearm vasoconstriction. This hypothesis was tested by reducing venous return to the heart with NTG at a dose, determined during preliminary experiments, equivalent to the dose of ANF in terms of reduction of right atrial pressure. The results of this study showed no potentiation of cardiopulmonary receptor-mediated vasoconstriction during NTG. In this regard, it must be pointed out that systemically administered NTG, at this dose, had no vasodilator effect in the forearm, or at least any effect was counterbalanced by reflex vasoconstriction caused by arterial baroreceptor deactivation. These findings are consistent with the observation that a reduction in the initial central venous pressure induced by different pharmacologic agents35 and changes in the initial value of vascular resistance are unable to modify the magnitude of the vascular response to vasomotor stimuli.36

In another group of subjects we evaluated the possibility that the sympathetic efferent discharge to peripheral vessels was increased during ANF. In fact, other authors8 and we13 have reported increased plasma levels of norepinephrine during the infusion of similar doses of ANF in humans. On the other hand, the present data show that the infusion of ANF has no effect on the arterial baroreceptor–induced reflex activation of the sympathetic nervous system, as demonstrated by the lack of influence of the peptide on systemic and regional hemodynamic reflex responses induced by selective deactivation of carotid baroreceptors. In this regard, it should also be noted that in normal man ANF selectively reduces the pressor activity of epinephrine.9 Therefore, the possibility that ANF might have potentiated the reflex vasoconstriction in response to simulated orthostatic stress via an enhanced sympathetic reflex response is unlikely, although an interference of ANF with the arterial baroreceptor control of circulation cannot be completely ruled out. In fact, the deactivation of carotid baroreceptors by increased external neck pressure could be associated with reflex activation of the aortic arch baroreceptors. Despite the fact that only a slight tendency for a reduction in arterial pressure was noted with LBNP or ANF, the possibility that aortic arch baroreceptors contribute to the reflex responses to LBNP or ANF cannot be excluded.

The possibility that renin or vasopressin might have been involved in the modified reflex vascular response observed during ANF seems unlikely. In fact, it has been shown that ANF antagonizes the secretion of both these vasoconstrictors.5–7, 37

An alternative mechanism through which ANF
could potentiate the reflex response to the deactivation of cardiopulmonary receptors is based on the reported vagomimetic activity of both the crude atrial extract and the synthetic peptide. Indeed, experiments in the rabbit performed in our laboratory have shown that vagotony, but not atropine, markedly attenuates the hypertensive effect of the peptide, suggesting that ANF sensitizes sensory receptors with vagal afferents. In this view, it could be hypothesized that ANF might potentiate the baroreflexes arising from cardiopulmonary receptors with vagal afferents that exert a tonic inhibitor activity. The mechanical unloading of these receptors, therefore, should result in an enhancement of the peripheral vasoconstriction. It is known that the reflex vascular response elicited by venous pooling is mainly due to the deactivation of cardiopulmonary receptors with vagal afferents. In fact, cardiopulmonary receptors with sympathetic afferents mediate mainly excitatory influences. LBNP should reduce the discharge of these receptors and thereby promote an inhibitory response rather than an excitatory reflex response, as observed in our study.

The present results provide the first evidence that exogenous ANF has the ability to interfere with the neural control of peripheral circulation in man. It must be pointed out that we observed such an effect of ANF at a dose that resulted in circulating levels much higher than the physiologic ones, and also higher than the pathologic levels observed under conditions such as congestive heart failure. Therefore, the possible physiologic meaning of our findings is unclear. However, it could be speculated that the excessive vasoconstriction that occurs under clinical conditions characterized by volume expansion might be partially accounted for by an interference of the extremely high circulating levels of ANF with the cardiopulmonary receptor-related control mechanisms of the peripheral circulation.

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