Pressure Dependence of Atrial Natriuretic Peptide During Norepinephrine Infusion in Humans

DOMINIK E. UEHLINGER, TAJ ZAMAN, PETER WEIDMANN, SIDNEY SHAW, AND MARKUS P. GNÄDINGER

SUMMARY The relative contribution of increased blood pressure (BP) or norepinephrine (NE), or both, to the stimulatory effect of an NE pressor infusion on circulating immunoreactive atrial natriuretic peptide (ANP) was evaluated in 10 healthy young men. They were studied during an infusion of NE, which was applied initially alone and then in combination with sodium nitroprusside. NE infusion rate was increased in four 30-minute intervals to a final dose of 200 ng/kg body weight per minute, leading to 12-fold higher plasma NE levels than were seen during control conditions. This increased mean BP (from a mean basal value of 94 ± 3 to 119 ± 4 [SEM] mm Hg; p<0.001) and plasma immunoreactive ANP (from 50 ± 7 to 112 ± 17 pg/ml; p<0.001), whereas heart rate decreased (p<0.001). The NE infusion was continued at the highest dose and an additional infusion of sodium nitroprusside was started to titrate mean BP in 30-minute intervals down to control values; a mean sodium nitroprusside dose of 0.95 μg/kg/min restored mean BP to 93 ± 4 mm Hg (p<0.001), decreased plasma immunoreactive ANP to basal values (51 ± 4 pg/ml; p<0.001), increased heart rate (p<0.001), and left plasma levels of NE largely unchanged. Plasma protein and hematocrit rose about 5 to 6% (p<0.001) during the NE infusion and then decreased about 3 to 4% (p<0.001 and p<0.01) when sodium nitroprusside was added. No consistent changes were observed for plasma levels of epinephrine, dopamine, sodium, and potassium. These results suggest that a pressor infusion of NE increases plasma immunoreactive ANP levels mainly through hemodynamic changes.

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KEY WORDS • atrial natriuretic peptide • norepinephrine • blood pressure • humans

Atrial natriuretic peptides (ANPs), originating mainly from myocytes of the heart auricles,1,2 may produce a broad spectrum of cardiovascular, renal, and endocrine actions.1,4 In normal humans, the biologically active α-human ANP (αhANP; 28 amino acid residues) is released into the circulation.2-6 Atrial distention secondary to variations in central blood volume or pressure, or both, is thought to play the major role in the control of ANP secretion. Thus, in normal subjects, plasma levels of immunoreactive ANP (irANP) rise in response to acute loading with saline or water,6,7,9,10 maneuvers causing a central shift of volume (e.g., water immersion11 or changes from the upright to the supine position10,12), or increases in afterload caused by acutely increased blood pressure (BP).13

Apart from a stretch-related mechanism14 certain hormonal factors may also modulate ANP release. Studies in vitro revealed a distinct stimulatory effect of arginine vasopressin, acetylcholine, and epinephrine.15 In humans, a pressor infusion of norepinephrine (NE) tended to elevate circulating irANP more than did an equipressor dose of angiotensin II.13 Therefore, the present study was designed to further assess whether and to what extent a pressor-independent component may stimulate plasma irANP during an NE excess in normal subjects.

Subjects and Methods

Ten healthy young men (age range, 22–27 years; mean age, 24 ± 2 [SD] years) were studied. All had a BP below 140/90 mm Hg and a normal body weight (72 ± 7 kg) for their height (180 ± 6 cm); none was
taking any medications. After a short clinical examination and after providing informed consent, the subjects were instructed to continue their usual diet but to avoid very salty food or adding salt to their food for the last 5 days preceding the study. The day before the study, all subjects collected a 24-hour urine sample for the determination of sodium, potassium, and creatinine excretion. The following procedure was performed after an overnight fast.

The subjects emptied their bladder at 0730 and from then on remained supine until the end of the study. Plastic cannulas were inserted in an antecubital vein of each arm. During an initial 60-minute equilibration period, a slow intravenous infusion of 5% dextrose (0.1 ml/min) was administered by a calibrated infusion pump (Perfusor V, Braun, Melsungen, West Germany). The dextrose infusion was then replaced by levaterenol bitartrate in 5% dextrose (NE; 200 ng/kg/ml), which was started at a dose of 20 ng/kg/min (0.1 ml/min) and increased to 40 and 100 ng/kg/min, respectively, at 30-minute intervals. Thereafter, the NE infusion solution was replaced by a solution containing a 10-fold higher NE concentration (2000 ng/kg/ml), which was infused slowly (0.1 ml/min) at a constant dose of 200 ng/kg/min until the end of the study. Initially, this later dose of NE was infused alone; after 30 minutes a concomitant infusion of sodium nitroprusside (SNP) in 5% dextrose (Nipride; Roche, Basel, Switzerland; 1.25 μg/kg/ml) was started at a dose of 0.25 μg/kg/min. The SNP dose was increased stepwise at 30-minute intervals until the mean arterial BP decreased to the control values obtained before starting the NE infusion. The following SNP doses were necessary to achieve this effect: 0.75 μg/kg/min in three subjects, 1.0 μg/kg/min in six subjects, and 1.25 μg/kg/min in one subject. At the end of this step, the SNP infusion was stopped and the NE infusion was continued at a stable rate (200 ng/kg/min) for another 30 minutes.

Blood samples for the determination of plasma irANP, NE, epinephrine, and dopamine were drawn at 50 and 60 minutes of the equilibration period as well as at 20 and 30 minutes of each of the 30-minute infusion steps with either NE alone or in combination with SNP. Additional blood samples for the determination of hematocrit, plasma protein, sodium, potassium, and creatinine were drawn at the same times during the equilibration period and, except for plasma creatinine, during 1) the last infusion step with NE alone, 2) the last infusion step with the combination of NE and SNP, and 3) the very last infusion step of the study, when NE was again infused alone. Blood removed was always replaced immediately by a similar volume of 0.9% saline given intravenously. Each blood sampling was preceded by five recordings of BP and heart rate (HR) at 1-minute intervals. The mean of each five measurements was used for statistical analysis.

BP was determined with the automatic device Tonometer (Speidel & Keller, Jungingen, West Germany), which was calibrated daily against a standard sphygmomanometer. Hematocrit was determined in triplicate by the microcrit method. Plasma and urine sodium and potassium levels were determined by flame photometer. Creatinine concentration was determined by Greiner autoanalyzer (Greiner SA, Langenthal, Switzerland), and plasma protein concentration was established using the biuret method. Plasma NE, epinephrine, and dopamine levels were determined by high performance liquid chromatography with electrochemical detection following extraction from plasma using a modification of the method of Smedes et al.16

For determination of plasma irANP, 10 ml of blood was collected in precooled glass tubes containing disodium EDTA (1 mg/ml blood). The blood samples were mixed and immediately centrifuged at 4°C, and the plasma was stored at −20°C until assay. Plasma (2 ml) was extracted by first passing the samples through C18 octadecyl silica cartridges (Sep-Pak C18, Waters Associates, Milford, MA, USA), preactivated with 10 ml of methanol and 10 ml of triethylamine acetate buffer (20 mM, pH 4.0) and then eluting the absorbed hormone with 4 ml of 86% methanol in 4% acetic acid. The efficiency of the extraction procedure was estimated by recovery of synthetic ohANP added to the plasma. When synthetic ohANP, 4 to 500 pg/ml, was added, plasma recovery was 91 ± 4% (n = 8) after the extraction and radioimmunoassay procedures. Radioimmunoassay of irANP was performed using a rabbit anti-ANP antibody (Peninsula Laboratories Europe, Merseyside, UK). This antibody shows complete cross-reactivity with ohANP and rat atriopeptin III but has no cross-reactivity with somatostatin, oxytocin, or vasopressin. The standard buffer was 0.1 M Tris HCl, pH 7.4, containing 0.1% bovine serum albumin (radioimmunoassay grade), 0.1% Triton X-100 and 0.01% Na azide. 125I-ohANP was used as tracer (Amersham Buchler, Braunschweig, West Germany; specific activity, 2000 μCi/mmol), and synthetic ohANP (Bissendorf, Wedemark, West Germany) was used to construct standard curves. Incubation was performed for 48 hours at 4°C. Bound and free 125I-ohANP were separated by added dextran-coated charcoal. With the use of this procedure, the lowest concentration of irANP detected was 4 pg/tube; the 50% intercept was at 30 pg/tube. Interassay variation was 15.5% (n = 6), and intra-assay variation was 7.8% (n = 6). All plasma values were calculated from an extracted standard curve after correction for non-specific binding of tracer. In normal subjects, the ANP-like immunoreactive material in plasma was previously characterized by reverse-phase high performance liquid chromatography and found to correspond largely to ohANP.4

Statistical analysis was performed using the software package SAS (Statistical Analysis System, Version 5.0, Cary, NC, USA). The methods used included analysis of variance and the Ryan-Einot-Gabriel-Welsch multiple F test for comparison between mean data under different conditions. Since natural logarithmic transformations rather than absolute values followed a Gaussian distribution, the natural logarithm of plasma irANP, NE, epinephrine, dopamine, and pro-
tein values were used for statistical analysis. Values are reported as means ± SEM.

Results

Since values of any measured variables did not differ significantly between 50 and 60 minutes of the initial equilibration period or between 20 or 30 minutes of the NE and SNP infusion steps, the mean of the two determinations was used for further analysis. The NE infusion increased plasma levels of NE about 12-fold (from basal values of 26 ± 3 to 318 ± 20 ng/dl) 30 minutes after reaching the highest dose. A further slight, but statistically insignificant increase in plasma NE was observed on continuing the infusion to the end of the study (Figure 1 and Table 1). BP increased during the NE infusion (from basal values of 119/81 ± 4/3 to 153/102 ± 5/4 mm Hg; p<0.001) 30 minutes after starting the final dose of 200 ng/kg/min (see Figures 1, 2, and Table 1); HR decreased from 54 ± 2 to 44 ± 2 beats/min.

When the SNP infusion was added to the NE infusion, a mean dose of 0.95 ± 0.05 /µg/kg/min was required to decrease mean BP to 93 ± 4 mm Hg (not significantly different from basal values of 94 ± 3 mm Hg; see Figure 1). Systolic BP decreased only slightly (from 153 ± 5 to 144 ± 7 mm Hg; p<0.001) and did not reach basal values (p<0.001) 30 minutes after starting the highest dose of SNP, whereas diastolic BP dropped below basal values (from 102 ± 5 to 68 ± 3 mm Hg; p<0.001 vs basal values); HR rose slightly above basal values (see Figure 2 and Table 1). Thirty minutes after cessation of the SNP infusion, BP increased again to values comparable to those measured before the SNP infusion was started, whereas HR tended to decrease again (see Figure 2 and Table 1).

Plasma levels of irANP increased (p<0.001) during the NE infusion from basal values of 50 ± 7 to maximal values of 112 ± 17 pg/ml, and were restored (p<0.001) to 51 ± 4 pg/ml at the end of the SNP infusion. Thirty minutes after cessation of the SNP infusion, plasma levels of irANP tended to rise again, but they were still significantly lower (67 ± 6 pg/ml; p<0.001) as compared with the values measured before the SNP infusion was started (see Figure 1 and Table 1).

Methodological testing with 10 /µg/ml concentrations of NE or SNP revealed no interference with either the SEP-PAK extraction or radioimmunoassay of ANP. Thus, the extraction and radioimmunoassay of synthetic ANP added to 2 ml of plasma over the range of 15 to 250 pg/ml in the presence of 10 /µg/ml of NE or SNP gave values that were 100.85 ± 0.54 and 100.12 ± 0.99%, respectively, of the concentrations measured in the absence of either NE or SNP (n = 4).

Plasma protein and hematocrit increased by about 5 and 6% (p<0.001) during the NE infusion, decreased again by about 3 and 4% when SNP was added (p<0.001 and p<0.01), but did not fully recover 30 minutes after discontinuing the infusion (p<0.05 and 0.01; see Table 1).

No consistent changes were observed for plasma levels of epinephrine, dopamine, sodium, and potassium (see Table 1). There was a tendency for a slight decrease in plasma potassium (p<0.05) and an increase in plasma epinephrine (not significant) during the SNP infusion, whereas plasma sodium tended to decrease during the NE infusion (p<0.01).

During the day preceding the investigation, urinary sodium and potassium excretion averaged 167 ± 2 and 81 ± 10 mmol/24 hours, respectively, whereas creatinine clearance was 107 ± 6 ml/min.

Percent changes of plasma irANP from basal values correlated with changes in mean BP (r = +0.72, p<0.001) and HR (r = −0.67, p<0.002) at the end of the NE infusion alone and in combination with the SNP infusion. There was also a tendency for a correlation with changes of hematocrit (r = +0.48, p<0.05) but not with plasma protein.

Discussion

The results of this study complement our previous observation that a pressor infusion of NE acutely increases the levels of circulating irANP in normal subjects. Furthermore, when the NE-induced rise in mean BP was eliminated by the concomitant infusion of SNP, plasma irANP returned to control values.

With the use of reverse-phase high performance liquid chromatography, the irANP detected by our assay was previously shown to correspond largely to cANP, the presumed active peptide of the ANP system in humans. In the present study, plasma irANP.
TABLE 1.  Clinical, Endocrine, and Metabolic Variables During Intravenous Infusions of Norepinephrine Alone and Combined with Sodium Nitroprusside

<table>
<thead>
<tr>
<th>Variable</th>
<th>Basal values</th>
<th>NE infusion</th>
<th>SNP infusion</th>
<th>After SNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean infusion rates</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE (ng/kg/min)</td>
<td>—</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>SNP (µg/kg/min)</td>
<td>—</td>
<td>0.95</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>BP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>119 ± 4</td>
<td>153 ± 5*</td>
<td>144 ± 7*†</td>
<td>154 ± 6*</td>
</tr>
<tr>
<td>Diastolic</td>
<td>81 ± 3</td>
<td>102 ± 4*</td>
<td>68 ± 31‡</td>
<td>98 ± 4*</td>
</tr>
<tr>
<td>Mean</td>
<td>94 ± 3</td>
<td>119 ± 4*</td>
<td>93 ± 4‡</td>
<td>116 ± 4*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>54 ± 2</td>
<td>44 ± 2*</td>
<td>58 ± 3‡†</td>
<td>49 ± 2‡</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>irANP (pg/ml)</td>
<td>50 ± 7</td>
<td>112 ± 17*</td>
<td>51 ± 4‡</td>
<td>67 ± 6‡§</td>
</tr>
<tr>
<td>Norepinephrine (ng/dl)</td>
<td>26 ± 3</td>
<td>318 ± 20*</td>
<td>351 ± 20*‡</td>
<td>367 ± 31* §</td>
</tr>
<tr>
<td>Epinephrine (ng/dl)</td>
<td>1.9 ± 0.3</td>
<td>2.2 ± 0.3</td>
<td>2.8 ± 0.3</td>
<td>2.8 ± 0.4</td>
</tr>
<tr>
<td>Dopamine (ng/dl)</td>
<td>7.0 ± 1.4</td>
<td>5.7 ± 1.3</td>
<td>4.7 ± 0.9</td>
<td>5.6 ± 1.2</td>
</tr>
<tr>
<td>Protein (g/L)</td>
<td>65.5 ± 0.7</td>
<td>70.2 ± 1.0*</td>
<td>68.1 ± 0.1*†</td>
<td>68.8 ± 0.9*¶</td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>140.6 ± 0.6</td>
<td>139.9 ± 0.5*</td>
<td>140.0 ± 0.4‡</td>
<td>139.9 ± 0.6*</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>4.1 ± 0.1</td>
<td>4.1 ± 0.1</td>
<td>3.9 ± 0.1§</td>
<td>3.8 ± 0.1</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>45 ± 1</td>
<td>47 ± 1*</td>
<td>46 ± 1‡†</td>
<td>46 ± 1*‡</td>
</tr>
</tbody>
</table>

Values are means ± SEM. NE = norepinephrine; SNP = sodium nitroprusside; irANP = immunoreactive ANP. 
* p < 0.001, † p < 0.01, § p < 0.05, compared with basal values. 
‡ p < 0.001, †‡ p < 0.01, †§ p < 0.05, compared with values before SNP.

![Figure 2](http://hyper.ahajournals.org/)

**Figure 2.**  Systolic and diastolic BP and heart rate before and during intravenous infusions of norepinephrine alone and combined with sodium nitroprusside. Values are means ± SEM.

averaged 50 ± 7 pg/ml under control conditions and 112 ± 17 pg/ml during the NE pressor infusion. The 130% increase in circulating irANP cannot be explained by the slight hemoconcentration during the NE infusion, as suggested by the 6 to 7% rises in hematocrit and plasma protein. The presumed basic mechanism for a stimulation of ANP secretion is an increased atrial wall distention when right (and possibly also left) atrial pressure rises. In fact, significant positive correlations between plasma irANP levels and right or left atrial, pulmonary, or left ventricular pressure have been described in humans.17,18 NE increases BP mainly through an increase in total peripheral vascular resistance, with unchanged or even decreased cardiac output. This increase is accompanied by an increase in left atrial pressure. In addition, pulmonary arterial pressure is elevated and the venous capacity is decreased by venous vasoconstriction, both of which lead to an increased right atrial pressure.19 As a significant positive correlation between mean arterial pressure and central venous pressure during infusions of NE has been described in healthy subjects.20 Therefore, the increase in plasma irANP during the NE infusion could be explained by an increase in left and right atrial pressure.

NE and SNP may both modify regional blood flow. Thus, concomitant changes in the clearance of ANP theoretically could contribute to the observed changes in plasma irANP during these infusions. Furthermore, recent findings suggest that additional mechanisms may play a role in the control of ANP secretion. Increases in HR are accompanied by high plasma irANP levels,11,12 and β-adrenergic receptor agonists have been shown to stimulate the release of ANP in vitro from rat atrial tissue13 or an isolated heart preparation.21 In our study, as expected, the baroreceptor reflex mechanism decreased rather than increased HR during the NE pressor infusion; however, the very high plasma NE levels of about 320 ng/dl should have produced a potent β-adrenergic receptor stimulation. When the NE infusion was complemented by the concomitant infusion of the potent vasodilator agent SNP,
the latter readily allowed the restoration of mean BP to control values (93 ± 4 mm Hg vs pre-NE infusion values of 94 ± 4 mm Hg). This change was accompanied by a fall in plasma irANP (from 112 ± 17 to 51 ± 4 pg/ml), although plasma levels of NE remained markedly elevated (351 ± 20 ng/dl). Despite comparable mean BP average, values of HR and systolic BP were slightly higher (+10% and +20%, respectively) and diastolic BP was 15% lower at the end of the last SNP infusion step than they were under basal control conditions. This constellation suggests that, at the end of the SNP infusion, central venous pressure and atrial pressure could have been somewhat lower than they were under basal conditions. Therefore, a slight direct effect of α-adrenergic receptor stimulation on the secretion of ANP cannot be excluded.

Since 12-fold elevated plasma concentrations in this study failed to maintain the increased irANP levels when mean BP was normalized, the regulatory relevance of a direct adrenergic receptor stimulation could, at most, be minimal. A possible direct inhibitory influence of SNP on ANP secretion is unknown but could only be excluded in vitro. We conclude that a pressor infusion of NE increases plasma levels of irANP mainly through hemodynamic changes.

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