Atrial Natriuretic Peptide Decreases Circulatory Capacitance in Areflexic Rats

NICK C. TRIPPODO, FRANCIS E. COLE, EDWARD D. FROHLICH, AND ALLAN A. MACPHEE

The short-term hemodynamic response to atrial natriuretic peptide appears to be partly mediated by decreased venous return, which could result from increased circulatory capacitance or decreased blood volume. To determine if rat atrial natriuretic peptide 99-126 (0.5 μg/kg/min IV for 30–70 minutes) dilated capacitance vessels or decreased blood volume, mean circulatory filling pressure (measured during brief circulatory arrest by inflating an intraatrial balloon) and blood volume (51Cr-erythrocytes) were measured in anesthetized rats. Mean circulatory filling pressure, central venous pressure, and blood volume decreased by 0.4 mm Hg, 0.5 mm Hg, and 3.4 ml/kg, respectively. To determine the total circulatory pressure-volume relationship without influence from autonomic reflexes, mean circulatory filling pressure and blood volume were measured in spinal-cord-transected rats before and immediately after infusing or withdrawing 5 ml blood. Atrial natriuretic peptide decreased mean circulatory filling pressure, central venous pressure, and blood volume by 0.9 mm Hg, 1.7 mm Hg, and 8.0 ml/kg, respectively, and displaced the pressure-volume relationship toward the pressure axis by decreasing extrapolated unstressed volume. Similar results were obtained in spinal-cord-transected rats that had initial vascular tone restored to a greater level by norepinephrine infusion. In anephric rats, atrial natriuretic peptide decreased central venous pressure by 0.3 mm Hg and blood volume by 1.6 ml/kg. The results indicate that short-term infusion of atrial natriuretic peptide reduced circulatory capacitance in rats and suggest that this reduction resulted from 1) diminished blood volume due to urinary fluid loss followed by passive vascular recoil and 2) active venoconstriction. These findings are consistent with the hypothesis that intravascular volume contraction and increased resistance to venous return are factors that contribute to the diminished venous return following atrial natriuretic peptide. (Circulation Research 1986;59:291-296)

Atrial natriuretic peptides (ANP) lower arterial pressure by a complex mode of action. Following acute administration, ANP can cause a transient peripheral vasodilation, particularly in the kidneys, usually lasting less than 5 minutes. In some reports decreased total peripheral resistance occurred after 10 minutes. ANP also lowered arterial pressure by reducing cardiac output within 5 minutes after bolus injection as well as after 10–75 minutes of continuous infusion.

The mechanism by which ANP decreases cardiac output has not been established. Although ANP in large doses depressed myocardial performance through coronary vasoconstriction in isolated hearts, this mechanism is unlikely to be the primary cause of the reduced output in intact animals since increased atrial pressure is not a feature of the hemodynamic response to ANP. To the contrary, decreased left and right atrial pressures associated with decreased stroke volume have been reported. Moreover, bolus injection of atrial peptide at a maximum diuretic dose did not alter cardiac performance in anesthetized rats. These observations suggest that reduced myocardial preload resulting from diminished venous return is the primary mechanism that lowers cardiac output.

Since ANP relaxes vascular smooth muscle in vitro (reviews: Maack et al., Cantin and Genest, Needleman et al.), it is possible that an ANP-induced relaxation of smooth muscle in vivo with subsequent increased vascular capacitance might be responsible for the observed decrease in central venous pressure. However, as pointed out by Rothe, central venous pressure is not necessarily indicative of capacitance vessel tone. A more definitive measurement is required to determine whether ANP increases circulatory capacitance. This study reports the effects of ANP on the relation between mean circulatory filling pressure and blood volume in rats, thus utilizing an established measure of whole body circulatory capacitance (reviews: Gow, Rothe).

Materials and Methods

Intact Rats

Sixty male Sprague-Dawley rats weighing 260–395 g were anesthetized with 100 mg/kg thiothitarbarbital, I.P. (Inactin; BYK Gulden Konstanz, West Germany). Catheters were placed in the trachea, abdominal aorta via the left femoral artery, thoracic inferior vena cava via the left femoral vein, and right jugular vein for infusing vehicle or ANP. In 22 of these rats, a balloon-
tipped catheter was placed in the right atrium via the right jugular vein for measuring mean circulatory filling pressure (MCFP) (described below). In 17 rats a catheter for collecting urine was placed in the urinary bladder via a small midline incision.

The rats were allowed to stabilize for 20–30 minutes after surgery. Arterial and venous pressures were measured with Gould-Statham transducers and a Beckman or Grass recorder. Blood volume (described in detail below) was measured by injecting $^{51}$Cr-tagged erythrocytes IV, allowing a minimum of 15 minutes for mixing and taking duplicate 24-μl blood samples for each measurement. Baseline measurements were taken, and then vehicle (0.9% sodium chloride) or rat ANP 99–126 (0.5 μg/kg per minute) was infused at 0.02 ml/min. Measurements were repeated after 30 minutes of infusion.

**Spinal-Cord-Transected Rats**

To determine the total circulatory pressure–volume relationship with minimal interference from autonomic reflexes, the spinal cord was transected in rats as previously described. Male Sprague-Dawley rats (376–448 g) were anesthetized for the placement of catheters as described above. An additional catheter was placed in the left common carotid artery for rapid infusion or withdrawing blood. The spinal cord was transected by aspiration between the first and second thoracic vertebrae. Completeness of spinal transection was verified at the end of each experiment as described previously. Although respiratory control remained intact, a Harvard Apparatus Rodent Respirator was used to insure adequate ventilation (2 ml tidal volume, 80 strokes/minute). Rectal temperature was maintained at 36–37°C with lamps. Vehicle or test substance was infused for 30 minutes before the first measurements were taken. During this period $^{51}$Cr-tagged erythrocytes were injected and allowed to mix. For measuring blood volume, 5 ml of fresh blood from donor rats. The time required to infuse or withdraw blood and measure MCFP was 15 seconds. Blood volume was measured immediately before each blood volume change, correcting for loss of $^{51}$Cr radioactivity due to previous sampling or blood removal, and 5 ml was added or subtracted to this measured blood volume to obtain the blood volume during each MCFP measurement. Immediately after each blood volume change, 5 ml of blood was removed or returned to the rat. MCFP and blood volume were measured during a second “control” state after the blood volume changes were complete. After each MCFP measurement 10 minutes were allowed for stabilization before the next measurements were made. The MCFP–blood volume relationship for each rat was determined from four data points (two at “control” state and two at ±5 ml of blood volume) by linear regression using the method of least squares. The correlation coefficients ranged from 0.96 to 1.00.

The following variables were derived from the equation of the MCFP–blood volume line for each rat: total circulatory compliance (reciprocal of the slope); extrapolated unstressed circulatory volume (volume axis intercept); and total circulatory capacity (blood volume at MCFP of 8.0 mm Hg). In this report the term capacitance is used interchangeably with capacity (ml/kg).

**Anephric Rats**

To determine if ANP changed blood volume in the absence of diuresis, cardiovascular catheters were placed in male, anesthetized Sprague-Dawley rats (291–377 g) as described above, except for the atrial balloon. Both kidneys were extirpated through a midline incision. $^{51}$Cr-tagged erythrocytes were injected 15 minutes after surgery and control measurements were taken 15 minutes later. Vehicle or ANP 99–126 (0.5 μg/kg per minute) was infused IV for 30 minutes and measurements were repeated.

**MCFP, Blood Volume, Peptide**

MCFP was measured by inflating the intraatrial balloon for approximately 15 seconds, arresting the circulation and allowing venous pressure to increase and reach a plateau within 5 seconds in the intact rats and 12 seconds in the spinal-cord-transected rats. MCFP was determined from the venous and arterial pressures during circulatory arrest. The assumptions and limitations of this method for measuring vascular capacitance have been discussed previously.

For measuring blood volume, erythrocytes were labelled each day by incubating 1 ml of fresh blood from donor rats with 50 μCi of $^{51}$Cr at room temperature for 30 minutes. The erythrocytes were washed and resuspended in isotonic saline and injected IV (approximately 12 μCi in 0.2 ml). At least 15 IVs were allowed for mixing. Arterial blood was collected in duplicate micro-hematocrit tubes precalibrated to 24 μl. Radioactivity of each sample was recorded for 10 minutes in a Packard gamma scintillation spectrometer.

Rat ANP 99–126 (Peninsula Laboratories, Belmont, Calif.) represents the 28-amino-acid C-terminus of proANP 1–126 and is the major circulating form of ANP in the rat. The peptide was stored in lyophilized form at −70°C and dissolved in vehicle immediately before use. Purity and biological activity was examined by HPLC and rat diuretic assay, respectively. HPLC chromatograms showed a single peak and the rat diuretic assays indicated the expected activity (a urine output of 400–800 μl during the 10 minutes following bolus injection of 1 μg ANP).

**Statistics**

Data are expressed as mean ± SEM and were analyzed by Student’s t test for group or paired compari-
effects of Rat ANP 99-126 and Norepinephrine on Total circulatory compliance (ml/kg per mm Hg)

Effects of Rat ANP 99-126 and Norepinephrine on TABLE 2.

Mean circulatory filling pressure (mm Hg)

Central venous pressure (mm Hg)

Mean arterial pressure (mm Hg)

Blood volume (ml/kg)

Hematocrit (%)

Urine volume (ml/kg)

Values are means ± SEM. Number per group = 5.

*p < 0.001, †p < 0.05.

Body weights were 313 ± 9 and 313 ± 8 g in the ANP and vehicle groups, respectively. MAP, mean arterial pressure; CVP, central venous pressure; MCFP, mean circulatory filling pressure; BV, blood volume; Hct, hematocrit; UV, urine volume.

A two-way analysis of variance was used for multiple group comparisons.

Results

ANP infusion decreased mean arterial and central venous pressures, MCFP, and blood volume in intact rats and increased hematocrit and urine output (Table 1). Except for blood volume, which increased slightly, these variables did not change from baseline values in the vehicle-infused group.

In the spinal-cord-transected rats, ANP infusion, either in the presence or absence of norepinephrine infusion, decreased mean arterial and central venous pressures, MCFP, and blood volume and increased hematocrit (Table 2). To obtain the mean values in Table 2 for mean arterial pressure, central venous pressure, MCFP, blood volume, and hematocrit, the values in both "control" states before and after blood volume changes were averaged for each rat. The total circulatory pressure–volume relationship was also altered by ANP infusion; extrapolated unstressed circulatory volume (volume axis intercept) and total circulatory capacity (blood volume at MCFP of 8 mm Hg) decreased, whereas total circulatory compliance (reciprocal of the slope) did not change (Table 2). These results indicate a parallel shift of the pressure–volume curve toward the pressure axis, i.e., a decrease in the volume axis intercept without a change in slope (Figure 1).

A different pattern of changes in these variables was induced by norepinephrine infusion in the spinal-cord-transected rats. Mean arterial pressure, MCFP, and hematocrit increased, blood volume decreased, and central venous pressure did not change (Table 2). Norepinephrine infusion decreased extrapolated unstressed circulatory volume, total circulatory compliance, and total circulatory capacity, indicating a shift in the pressure–volume curve toward the pressure axis.

Table 2. Effects of Rat ANP 99-126 and Norepinephrine on Total Circulatory Pressure–Volume Relationship in Spinal-Cord-Transected Rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>A Vehicle</th>
<th>B ANP</th>
<th>C Norepinephrine</th>
<th>D Norepinephrine + ANP</th>
<th>P† A + B vs C + D</th>
<th>P‡ A + C vs B + D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>391 ± 8</td>
<td>401 ± 5</td>
<td>395 ± 11</td>
<td>400 ± 13</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>66 ± 3</td>
<td>64 ± 3</td>
<td>121 ± 8</td>
<td>87 ± 4</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Central venous pressure (mm Hg)</td>
<td>2.7 ± 0.1</td>
<td>1.0 ± 0.5</td>
<td>2.1 ± 0.3</td>
<td>1.6 ± 0.3</td>
<td>NS</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mean circulatory filling pressure (mm Hg)</td>
<td>6.7 ± 0.4</td>
<td>5.8 ± 0.4</td>
<td>7.7 ± 0.4</td>
<td>7.0 ± 0.2</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Blood volume (ml/kg)</td>
<td>56.4 ± 1.2</td>
<td>48.4 ± 0.7</td>
<td>50.3 ± 1.6</td>
<td>43.8 ± 1.3</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>45.1 ± 0.9</td>
<td>49.0 ± 1.0</td>
<td>49.4 ± 0.8</td>
<td>52.0 ± 0.9</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Extrapolated unstressed circulatory volume (V'o) (ml/kg)</td>
<td>34.7 ± 0.8</td>
<td>28.5 ± 0.1</td>
<td>28.4 ± 1.3</td>
<td>23.0 ± 1.6</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total circulatory compliance (ml/kg per mm Hg)</td>
<td>3.3 ± 0.2</td>
<td>3.5 ± 0.1</td>
<td>3.0 ± 0.2</td>
<td>3.1 ± 0.1</td>
<td>&lt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Total circulatory capacity (ml/kg)</td>
<td>61.1 ± 1.0</td>
<td>56.6 ± 0.7</td>
<td>52.5 ± 2.2</td>
<td>48.0 ± 1.6</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Number per group = 5.

*p = 0.9% NaCl at 0.02 ml/min; ANP = rat atrial natriuretic peptide 99-126 at 0.5 µg/kg per minute; norepinephrine at 0.6 µg/kg per minute.

†Probability that the difference between the norepinephrine-treated groups and the groups not receiving norepinephrine is due to chance.

‡Probability that the difference between the ANP-treated groups and the groups not receiving ANP is due to chance. NS = not significant (p > 0.05).
The relation between mean extrapolated unstressed circulatory volume (on volume axis) and mean total circulatory capacity (at 8 mm Hg) in four groups of spinal-cord-transected rats (Table 2). The symbols for each group indicate the means ± SEM of mean circulatory filling pressure and blood volume at "control" state (middle symbols) and at ±5 ml of blood volume. The SEM bars are too small to be depicted on the graphs. The lines were drawn through the filled circles. The vehicle group (O) received 0.9% sodium chloride at 0.02 ml/min. The ANP group (△) received rat atrial natriuretic peptide 99-126 at 0.5 μg/kg per minute. The norepinephrine group (●) received norepinephrine at 0.6 μg/kg per minute. The norepinephrine + ANP group (▲) received both norepinephrine and ANP at the above doses.

Discussion

In the intact rats ANP decreased MCFP by 0.4 mm Hg (Table 1). However, since blood volume also decreased (3.4 ml/kg), three possible conditions could have prevailed: a) venodilation, as reflected by displacement of the MCFP–blood volume curve toward the volume axis (position 1, Figure 2); b) a lower point through changes in slope and volume axis intercept (Table 2).

In the anephric rats, ANP infusion decreased mean arterial and central venous pressures and blood volume and increased hematocrit (Table 3). However, these changes tended to be less than those observed in intact rats. After 30 minutes of vehicle infusion, mean arterial pressure was slightly decreased in the anephric rats, but no changes occurred in the other variables (Table 3).

Table 3. Effect of Rat ANP 99-126 on Blood Volume in Anephric Rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>ANP, 0.5 μg/kg per minute</th>
<th>Vehicle, 0.02 ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>ANP</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>100 ± 4</td>
<td>91 ± 3*</td>
</tr>
<tr>
<td>CVP (mm Hg)</td>
<td>−0.5 ± 0.3</td>
<td>−0.8 ± 0.3t</td>
</tr>
<tr>
<td>BV (ml/kg)</td>
<td>51.1 ± 2.5</td>
<td>49.5 ± 2.3t</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>51.1 ± 1.2</td>
<td>53.5 ± 1.2*</td>
</tr>
</tbody>
</table>

Values are means ± SEM. n = 10 and 8 in ANP and vehicle groups, respectively. Body weights were 334 ± 12 and 319 ± 9 g in the ANP and vehicle groups, respectively. MAP, mean arterial pressure; CVP, central venous pressure; BV, blood volume; Hct, hematocrit.

*p < 0.001, t p < 0.05.
Decreased MCFP resulting from decreased blood volume with and blood volume at "control" state. 

I. Decreased MCFP stressed circulatory volume. 

2. MCFP associated with decreased blood volume. 

3. Decreased MCFP resulting from decreased blood volume and venodilation. 

4. Increased MCFP resulting from venoconstriction no venodilation or venoconstriction. 

V'0: extrapolated unstressed circulatory volume. 

FIGURE 18 

An infusion of norepinephrine into intact rats was 4.7 ± 0.8 ml/kg as compared with 0.6 ± 0.1 ml/kg in the vehicle-infused rats. In the spinal-cord-transected rats ANP was infused for 30 minutes before measurements commenced and continued to be infused for an additional 40 minutes during determination of the MCFP-blood volume relationship. Assuming a diuretic action in the spinal-cord-transected rats similar to that in the intact rats, it is conceivable that urinary fluid loss could have at least partially accounted for the 8.0 and 6.5 ml/kg decreases in blood volume in the ANP- and norepinephrine-plus-ANP-infused groups, respectively (Table 2). Following decreases in intravascular volume and distending pressure, the capacitance system exhibits passive recoil due to viscoelastic properties (reverse stress-relaxation) that partially restores the filling pressure. 

Therefore, the observed decrease in vascular capacitance after ANP infusion may have partially resulted from decreased blood volume followed by passive venoconstriction (analogous to moving from position 2 to position 3 in Figure 2). 

It is also possible that ANP caused some active venoconstriction resulting from a direct effect of the peptide on venous smooth muscle or indirectly through reflexes or endogenous release of vasoconstrictor(s). Active venoconstriction can decrease blood volume by altering the precapillary-to-postcapillary resistance ratio favoring net filtration of intravascular fluid into the extravascular compartment. For instance, the previously observed decrease in blood volume following epinephrine infusion in anephric rats was attributable to a shift in extracellular fluid across the capillaries. 

The decrease in blood volume following norepinephrine infusion (Table 2) could also be partly explained by this mechanism of active venoconstriction and fluid shifts (position 4, Figure 2). Thus, an increase in post-capillary resistance could enhance the blood volume contraction resulting from ANP-induced diuresis by causing a further decrease in blood volume or, at least, by retarding blood restoration via absorption of interstitial fluid, which usually occurs following vascular volume depletion. That ANP may have caused some active venoconstriction is supported by the decreased blood volume following ANP administration in anephric rats (Table 3). Although this was a relatively small decrease (1.6 ml/kg), the direction of the blood volume change is consistent with our foregoing hypothesis. 

The results of this study confirm and extend several earlier observations. In their first report on the natriuretic effect of atrial extract, deBold et al. observed increased hematocrit and decreased blood pressure in rats. They attributed this hypotensive effect to fluid loss by urinary excretion. Later, Maack et al. found a reversible increase in hematocrit during continuous infusion of ANP 102-125 (auriculin A) in dogs despite fluid replacement and raised the possibility that ANP increased capillary permeability. In this present study we confirm that ANP infusion increased hematocrit and we extend the observation to include decreased blood volume. Although these findings do not exclude the possibility that ANP increases capillary permeability, since there is yet no evidence for such a permeability change, whereas there is evidence for venoconstriction, we hypothesize that the mechanism for the reversible loss of intravascular fluid is based on an alteration in the ratio of precapillary-to-postcapillary resistance. In a previous study we did not observe a decrease in blood volume following bolus injection of ANP; but this finding may be ascribed to a lesser diuresis and lack of a cardiovascular steady state following bolus injection.

Lappe et al. and Breuhaus et al. reported that ANP decreased central venous pressure in conscious, spontaneously hypertensive rats and sheep, respectively. Our results extend these observations to anesthetized rats. The previous authors suggested that the ANP-induced decrease in central venous pressure was caused by relaxation of capacitance vessels. However, the present results provide no evidence of venodilation, but rather, indicate that ANP infusion produces a state of venoconstriction. These results are more compatible with the hypothesis that the decrease in central venous pressure is caused by a diminished blood volume. In a later study, Lappe et al. mentioned unpublished data of a fall in central venous pressure in nephrectomized rats, suggesting that the response was not due to decreased plasma volume. Our results also indicate that ANP decreased central venous pressure in anephric rats; but in addition, they show a small decrease in blood volume and increase in hematocrit (Table 3). Thus, despite the lack of diuresis, these results indicate that ANP can cause a small decrease in blood volume, which we suggest results from increased post-capillary resistance. Perhaps part of the 0.3 mm Hg
decrease in central venous pressure in the anephric rats can be accounted for by reduced blood volume. However, this small decrease in blood volume may be indicative of an even more important factor under these conditions, namely, increased resistance to venous return. Models of the circulation predict that increases in venous resistance result in diminished venous return and right atrial pressure.17 Furthermore, a change in venous resistance has been previously suggested as an important mechanism mediating the alteration in venous return following isoproterenol administration.16 Therefore, from both theoretical and experimental evidence, the ANP-induced decrease in central venous pressure in anephric rats can be explained by increased resistance to venous return and decreased blood volume.

In conclusion, we postulate two mechanisms by which short-term ANP administration at pharmacological doses decreases venous return and lowers cardiac output: 1) decreased blood volume resulting from urinary fluid loss and 2) increased resistance to venous return. In renal-intact animals, in whom a significant diuretic effect occurs, the decreased blood volume may play a more dominant role. In anephric animals (or after bolus injection) in whom the decreased blood volume is less pronounced, increased resistance to venous return may be important.

Acknowledgments

We express our appreciation to M. Lynn DeBose, Nell Taylor, and Wanda Myers for excellent technical assistance and to Dr. Yong Wei Chien for his participation in these experiments. We also thank Gayle Birou for typing this manuscript.

References


Key Words • atrial natriuretic hormone • cardiac output • blood volume • mean circulatory filling pressure • circulatory capacity • unstressed volume • compliance • norepinephrine • spinal-cord-transected rats • anephric rats • venous capacitance
Atrial natriuretic peptide decreases circulatory capacitance in areflexic rats.
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doi: 10.1161/01.RES.59.3.291

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