Hemodynamic Responses to Atrial Natriuretic Factor in Nephrectomized Rabbits: Attenuation of the Circulatory Consequences of Acute Volume Expansion

Massimo Volpe, Filippo Vecchione, Alberto Cuocolo, Giuseppe Lembo, Silvana Pignalosa, Mario Condorelli, and Bruno Trimarco

We investigated the hemodynamic responses to three doses of atrial natriuretic factor [human atrial natriuretic factor-(99-126)] (ANF) in nephrectomized rabbits anesthetized with ketamine and acepromazine. The influence of the different doses of the peptide on the hemodynamic consequences produced by acute volume expansion (0.9% NaCl, 1.4 ml/kg/min for 60 minutes) was also studied. All three dosages of ANF (0.001, 0.01, and 0.2 µg/kg/min for 20 minutes) significantly reduced blood pressure. With the lowest dose, the hypotensive effect was associated with reduction in systemic vascular resistance and no significant change in heart rate, stroke volume, central venous pressure, and hematocrit. In contrast, the intermediate and high doses, which resulted in markedly higher plasma levels, caused a significant decrease in heart rate, central venous pressure, and stroke volume; a slight rise in hematocrit; and no change in systemic vascular resistance. Volume expansion produced by saline infusion in an additional group of nephrectomized rabbits increased central venous pressure and decreased hematocrit. When ANF infusion was associated to volume expansion, each dosage of ANF was able to reduce the rise in central venous pressure, while only the higher dosage attenuated the progressive fall in hematocrit caused by volume expansion. Plasma volume, measured at the end of volume expansion was lower in the group treated with the highest dose of ANF than in the control animals (28.2 ±9 vs. 35.1 ±3 ml/kg, p<0.05). We conclude that 1) ANF induces significant hemodynamic effects independently from its renal action. These effects vary qualitatively according to the circulating levels achieved with the different dosages. 2) The administration of the peptide attenuates the hemodynamic consequences of acute volume overload. (Circulation Research 1988;63:322-329)

The administration of atrial natriuretic factor (ANF) produces hemodynamic effects in various animal models1-7 and in man.8-12 In particular, ANF causes a slight but consistent fall in systemic arterial pressure, which is frequently associated with a decrease in stroke volume4-7,9,11-13 and with a rise in hematocrit.1,2,14 These responses cannot be totally accounted for by the fluid losses generated by the diuretic effect because both hypotension and hemoconcentration have been observed during ANF infusion in nephrectomized animals.15-17 Therefore, it has been hypothesized that the circulatory effects of the peptide are at least partially caused by an increased efflux of fluids from the intravascular space toward the interstitium.15,16 This latter hypothesis is supported by a recent report from Huxley and coworkers,18 who showed that ANF directly elevates capillary hydraulic conductivity in the frog mesenteric circulation. On the other hand, it has been shown that low doses of ANF are unable to modify blood pressure or hematocrit even in the presence of significant renal responses.19,20

In the present study, we evaluated the hemodynamic responses to ANF in nephrectomized rabbits to investigate the direct hemodynamic effects of ANF independently from its renal actions. Since it has been previously reported that the quality of the hemodynamic changes during ANF infusion is related to the dose administered,7 we tested different doses of the peptide. Finally, to assess the potential contribution of the hemodynamic effects
of ANF in the maintenance of body fluid homeostasis during acute volume perturbations, we also studied the influence of the peptide on the hemodynamic consequences of acute volume expansion in nephrectomized animals.

Materials and Methods

General Procedures

The study was performed in white, male New Zealand rabbits weighing between 2.5 and 2.8 kg anesthetized with intramuscular ketamine (60 mg/kg) and acepromazine (0.3 mg/kg). Supplemental doses of the two drugs were given periodically during the study. A temperature probe was placed in the rectum of the animals, which were on a warming blanket to maintain their body temperature constant at 37°C throughout the experiment. A midline cervical incision was performed to expose the carotid arteries, the jugular veins, and the trachea, which was then cannulated with polyethylene tubing. The right carotid artery was cannulated with a PE-60 catheter and connected to a Statham P23 db transducer (Gould, Cleveland, Ohio) and to a Harvard multichannel polygraph (South Natick, Massachusetts) for continuous monitoring of pulsatile blood pressure. Heart rate was recorded with a Harvard cardiotachometer triggered by the arterial pressure pulse. A thermodilution catheter was introduced through the right jugular vein with the proximal hole in the right atrium and the thermistor tip in the lumen of the pulmonary artery for measurement of right atrial pressure and cardiac output by thermodilution, respectively. Blood sampling was performed through the contralateral external jugular vein. For fluid and drug administration, two additional intravenous lines were prepared by percutaneous introduction of small polyethylene catheters through the main ear veins. Bilateral nephrectomy was performed by a dorsal approach.

Experimental Protocol

After surgery, the animals were allowed a 3-hour recovery period. Then, they were divided into two groups. In the first group of animals (n = 29), the hemodynamic effects of a 20-minute constant infusion of vehicle (0.9% NaCl) (number of tests = 21) or of three different dosages of ANF ([human atrial natriuretic factor-(99-126)], Bissendorf Peptide GmbH, Wedemark, FRG; 0.001 µg/kg/min, n = 10; 0.01 µg/kg/min, n = 13; 0.01 µg/kg/min, n = 12; 0.2 µg/kg/min, n = 10) were evaluated. In most of the animals, only one dosage of ANF and one administration of vehicle were tested in a randomized sequence. In only a few animals were the hemodynamic effects of three injections (ANF or vehicle) assessed, so that the total number of rabbits receiving the vehicle was 15, while nine, nine, and eight animals received 0.001, 0.01, and 0.2 µg/kg/min of ANF, respectively. In the animals receiving two doses of ANF, the lower dose was always given first.

A 30-minute recovery period was interposed between the different dosages. Such an interval was constantly sufficient to achieve hemodynamic values not different from the original baseline.

To demonstrate that steady-state hemodynamic responses were achieved within 20 minutes of infusion, in a separate group of rabbits, a single treatment (one dose of ANF or vehicle) was performed, the infusion was carried out for 60 minutes, and the hemodynamic measurements were performed at 10-minute intervals. Each treatment was performed in four rabbits.

In the second group of nephrectomized rabbits (n = 22), the influence of the peptide on the hemodynamic response to acute volume expansion was investigated. In these animals, after two 15-minute control periods during which a constant intravenous infusion of isotonic saline at a rate of 0.1 ml/min was maintained, volume expansion was produced by increasing the rate of saline infusion to 1.4 ml/kg/min (approximately 8% of body weight) by means of a Harvard infusion pump. This rate of saline infusion was continued for the remainder of the experiment (60 minutes). Six of these animals received saline alone, while the others were simultaneously infused with one of the dosages of ANF (0.001 µg/kg/min, n = 4; 0.01 µg/kg/min, n = 5; 0.2 µg/kg/min, n = 7). In all groups, the infusions were started after two 15-minute control periods during which the animals received only a constant infusion of 0.9% NaCl. At the end of each control period and at 10-minute intervals during the experimental periods, the following measurements were obtained: pulsatile blood pressure, heart rate, right atrial pressure, cardiac output and the derived parameters (stroke volume and systemic vascular resistance), and hematocrit. Venous blood samples (2 ml) for determination of plasma ANF levels were collected at the end of the second control period and at the end of ANF infusion or after 60 minutes of volume expansion.

The possibility that time-dependent changes in the hemodynamic variables could occur during the study was ruled out in pilot experiments showing that the experimental protocol adopted provided hemodynamic stability during a 3-hour observation period after the recovery from nephrectomy.

Plasma volume was measured by the radiiodinated (125I) serum albumin method (Sorin Biomedica, Saluggia, Italy). At the end of volume expansion, 1 µCi was injected as a bolus into the jugular vein of each animal and the catheter was flushed with saline. Exactly 10 minutes after the injection, 1 ml of blood was collected from the carotid catheter for determination of the plasma radioactivity in two 50-µl duplicate samples. Standard, whole blood, and plasma samples were counted for a minimum of 10,000 radioactive decays with a Packard Auto-Gamma 500. The background was counted for an equivalent time and subtracted from plasma, whole blood, and standard decays. Plasma volume was calculated by the following formula: plasma
### Table 1. Steady-State (20 Minutes) Hemodynamic Effects of Atrial Natriuretic Factor in Nephrectomized Rabbits

<table>
<thead>
<tr>
<th></th>
<th>ANF</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>0.001 μg/min</td>
<td>0.01 μg/min</td>
<td>0.2 μg/min</td>
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<tr>
<td></td>
<td></td>
<td>kg body wt</td>
<td>kg body wt</td>
<td>kg body wt</td>
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<td></td>
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<tr>
<td>MBP (mm Hg)</td>
<td></td>
<td>C</td>
<td>E</td>
<td>C</td>
<td>E</td>
<td>C</td>
</tr>
<tr>
<td>(n = 21)</td>
<td></td>
<td>86 ± 3</td>
<td>84 ± 3</td>
<td>89 ± 5</td>
<td>84 ± 5*</td>
<td>99 ± 5</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td></td>
<td>C</td>
<td>E</td>
<td>C</td>
<td>E</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>226 ± 7</td>
<td>227 ± 7</td>
<td>245 ± 10</td>
<td>246 ± 9</td>
<td>243 ± 8</td>
</tr>
<tr>
<td>SVI (ml/kg)</td>
<td></td>
<td>C</td>
<td>E</td>
<td>C</td>
<td>E</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.70 ± 0.04</td>
<td>0.73 ± 0.07</td>
<td>0.87 ± 0.1</td>
<td>0.75 ± 0.1*</td>
<td>0.80 ± 0.09</td>
</tr>
<tr>
<td>SVRI (HRU/kg)</td>
<td></td>
<td>C</td>
<td>E</td>
<td>C</td>
<td>E</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>78 ± 8</td>
<td>76 ± 7</td>
<td>70 ± 6</td>
<td>59 ± 6*</td>
<td>65 ± 9</td>
</tr>
<tr>
<td>CVP (mm Hg)</td>
<td></td>
<td>C</td>
<td>E</td>
<td>C</td>
<td>E</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.6 ± 1</td>
<td>9.8 ± 1</td>
<td>8.8 ± 1</td>
<td>8.3 ± 1</td>
<td>12.0 ± 1</td>
</tr>
<tr>
<td>Hct (%)</td>
<td></td>
<td>C</td>
<td>E</td>
<td>C</td>
<td>E</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27.5 ± 1</td>
<td>27.7 ± 1</td>
<td>26.1 ± 0.8</td>
<td>26.2 ± 1</td>
<td>26.0 ± 0.5</td>
</tr>
</tbody>
</table>

C, control; E, experimental; MBP, mean blood pressure; HR, heart rate; SVI, stroke volume index; SVRI, systemic vascular resistance index; CVP, central venous pressure; Hct, hematocrit.

*p<0.05 vs. control.
†p<0.001 vs. control.

Plasma volume, stroke volume, and systemic vascular resistance results are shown normalized to 1,000 g of body weight.

### Atrial Natriuretic Factor Assay

Plasma levels of ANF were determined by radioimmunoassay (RIA) according to the technique described by Epstein et al,21 Rabbit antiserum (RAS 8798, Peninsula Laboratories Europe, Merseyside, England), iodinated human ANF (2,000 Ci/mmol, Amersham, Arlington Heights, Illinois), and human atrial natriuretic factor-(99-126) (Bissendorf) were used as standards. A 100% cross-reactivity with the rat 28-residue peptide has been shown for the antibody used in our assay. In addition, rat ANF has a sequence identical to rabbit ANF. Plasma samples were extracted by using C-18 Sep-pak cartridges (Waters Instruments, Milford, Massachusetts). The peptide retained on C-18 was eluted by means of 80% aqueous acetonitrile. The eluates, lyophilized, were reconstituted in RIA buffer (phosphate buffer 0.1 M, pH 7.4). Bound/free separation was carried out by using 1.5% charcoal-dextran. Nonspecific binding was about 4%. Recoveries, determined for each plasma sample by adding a small amount of radiolabeled ANF, ranged from 59% to 85%. Intraassay and interassay coefficients of variation were 6.6% and 10.5%, respectively. The RIA sensitivity was 3 pg/tube.

### Statistical Analysis

Comparisons of the values obtained during ANF infusion versus baseline were performed by paired t test. Steady-state responses obtained in the different groups were compared by the rank sum test. Significance of the changes produced by volume expansion in the different variables was tested by one-way analysis of variance (ANOVA). A comparison of the responses obtained during volume expansion alone versus those measured with the simultaneous infusion of ANF was performed by comparing the values of the integrated areas of the response as obtained by planimetry.

### Results

#### Hemodynamic Effects of Different Doses of Atrial Natriuretic Factor in Nephrectomized Rabbits

The lowest dose of ANF tested in the present study (A; 0.001 μg/kg/min) raised immunoreactive plasma levels of the peptide from 22 ± 3 to 242 ± 88 pg/ml (p<0.05) after 20 minutes of infusion. The intermediate dose (B; 0.01 μg/kg/min) increased plasma ANF levels from 29 ± 7 to 2,271 ± 384 pg/ml (p<0.001), and finally, at the highest dose (C), the circulating levels of ANF rose from 32 ± 8 to 4,246 ± 976 pg/ml (p<0.001). Infusion of vehicle...
alone (NaCl 0.9%) did not modify plasma levels of ANF measured in control conditions (from 31 ± 10 to 32 ± 9 pg/ml; NS).

The hemodynamic effects produced by saline alone and by the three different doses of ANF are shown in Table 1. For each variable, the values obtained at 10 minutes of each experimental period (not shown) were comparable to those obtained at 20 minutes (shown), so that the latter closely approximated steady-state responses. This is confirmed by the observation that in the control group of animals receiving only one treatment for 60 minutes, the hemodynamic responses recorded after the 60-minute period were quite comparable to those measured at 20 minutes (Table 2). As shown in Table 1, vehicle administration did not modify significantly any of the measured variables, whereas ANF induced hemodynamic changes that achieved significance at the lowest level of infusion. In detail, all three doses of ANF reduced mean blood pressure and the hypotensive response tended to increase progressively as the dose administered was increased (see also Figure 1). However, the hemodynamic response underlying the blood pressure-lowering effect was qualitatively different in the three groups. In fact, the slight decrease in blood pressure induced by the lowest dose (A) was associated with a significant reduction in systemic vascular resistance, whereas the other hemodynamic variables did not change. The dose B of the peptide caused a comparable reduction in blood pressure (see also Figure 1), but this effect was accompanied by significant reductions in heart rate, stroke volume, and central venous pressure, while vascular resistance and hematocrit did not change. Finally, the hypotensive response induced by the highest dose of the peptide (C) was associated with significant reductions in heart rate, stroke volume, and central venous pressure and with a significant rise in hematocrit, whereas systemic vascular resistance did not change.

As shown in Figure 1, the blood pressure responses to ANF were statistically greater than that observed with vehicle alone. In addition, the hypotensive response to the highest rate of ANF infusion was more marked than those evoked with the doses A and B. Changes in heart rate, central venous pressure, and stroke volume achieved statistical significance only with the doses B and C as compared with vehicle alone. Finally, the change in vascular resistance observed with dose A (−10 ± 3 hybrid resistance units (HRU)/kg) was different from those observed with the higher rates of infusion of the peptide (dose B, +3.7 ± 3 HRU/kg; dose C, −5.5 ± 3 HRU/kg; both p<0.05 vs. A).

### Influence of Atrial Natriuretic Factor on Hemodynamic Effects of Volume Expansion in Nephrectomized Rabbits

The time-course of the main circulatory effects of volume expansion alone are shown in the left panel of Figure 2. During saline load, blood pressure showed a tendency to rise, although this phenomenon did not achieve statistical significance (F = 1.99). Central venous pressure showed a progressive increase (F = 2.41, p<0.05) and hematocrit fell (F = 3.22, p<0.05). Stroke volume index (not shown in Figure 2) tended to increase progressively during volume expansion (from 0.55 ± 0.1 ml/kg in control conditions to 0.87 ± 0.3 ml/kg after 60 minutes of saline load), although variance analysis did not confirm a significant change. Finally, heart rate did not change significantly.

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**Table 2.** Hemodynamic Effects of a 60-Minute ANF Infusion in Nephrectomized Rabbits

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n=5)</th>
<th>0.001 μg/min kg body wt (n=5)</th>
<th>0.01 μg/min kg body wt (n=5)</th>
<th>0.2 μg/min kg body wt (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>98 ± 5</td>
<td>99 ± 3</td>
<td>101 ± 6</td>
<td>100 ± 2</td>
</tr>
<tr>
<td>E</td>
<td>97 ± 4</td>
<td>95 ± 4*</td>
<td>94 ± 5†</td>
<td>90 ± 3†</td>
</tr>
<tr>
<td>SVI (ml/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.73 ± 0.05</td>
<td>0.77 ± 0.1</td>
<td>0.92 ± 0.2</td>
<td>0.78 ± 0.1</td>
</tr>
<tr>
<td>E</td>
<td>0.75 ± 0.06</td>
<td>0.86 ± 0.2</td>
<td>0.79 ± 0.2*</td>
<td>0.71 ± 0.1*</td>
</tr>
<tr>
<td>SVRi (HRU/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>76 ± 9</td>
<td>78 ± 9</td>
<td>66 ± 14</td>
<td>80 ± 10</td>
</tr>
<tr>
<td>E</td>
<td>74 ± 7</td>
<td>58 ± 11*</td>
<td>63 ± 11</td>
<td>75 ± 14</td>
</tr>
<tr>
<td>CVP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>10 ± 1</td>
<td>8.4 ± 7</td>
<td>11.5 ± 1.5</td>
<td>11.5 ± 0.5</td>
</tr>
<tr>
<td>E</td>
<td>9.8 ± 0.8</td>
<td>8.1 ± 1</td>
<td>9.6 ± 1*</td>
<td>9.3 ± 1*</td>
</tr>
</tbody>
</table>

C, control; E, experimental (60-minute infusion); MBP, mean blood pressure; SVI, stroke volume index; SVRi, systemic vascular resistance index; CVP, central venous pressure. *p<0.05 vs. control. †p<0.001 vs. control.
In the nephrectomized animals receiving graded rates of infusion of ANF during volume expansion (Figure 2), mean blood pressure tended to decrease in all three groups, but variance analysis again did not show statistical significance at any level of infusion. In contrast, all three doses of ANF were able to attenuate the volume expansion-induced rise in central venous pressure (all \( F \) values NS). This phenomenon was confirmed by the comparison of the mean integrated areas of rise in central venous pressure measured in the individuals receiving volume expansion alone (\( \Delta \text{CVP} = 22 \pm 13 \text{ mm Hg} \cdot \text{min} \)) with those obtained with the doses A (16.5 ± 9 mm Hg · min, \( p < 0.05 \)), B (8 ± 5 mm Hg · min, \( p < 0.05 \)), and C (11 ± 6 mm Hg · min, \( p < 0.05 \)). The fall in hematocrit caused by volume expansion was prevented only by the highest dose of ANF (\( F = 1.55 \), NS), while the intermediate dose (B) was able to attenuate the hemodilution (\( F = 2.70 \), \( p < 0.05 \)) (integrated areas of the phenomenon \( \Delta \text{HCT} = 15 \pm 6 \% / \text{min} \) in the animals receiving ANF vs. \( 21 \pm 4 \% / \text{min} \) in the controls, \( p < 0.05 \)).

In the group of animals receiving volume expansion alone, the initial plasma ANF levels (38 ± 10 pg/ml, \( n = 6 \)) rose to 234 ± 66 pg/ml (\( p < 0.05 \)) at the end of volume loading. In the group receiving the lowest dose of ANF (A) the venous levels of the peptide rose to 394 ± 76 pg/ml (\( n = 4 \)), at the end of volume expansion, while dose B raised plasma levels to 2,981 ± 608 pg/ml (\( n = 5 \)) and dose C to 4,198 ± 1,176 pg/ml (\( n = 7 \)) (the values obtained in A, B, and C during ANF infusion were significantly higher than the respective control values). Figure 3 shows individual and mean values of plasma volume measured in the four volume-expanded groups of animals (control, A, B, and C) at the end of volume expansion. Although some overlap among the different groups was noted, the animals infused with the highest dose of ANF (C) had a mean plasma volume (28.2 ± 0.9 ml/kg, \( n = 7 \)) significantly lower than those measured in the group not receiving ANF (35.1 ± 3 ml/kg, \( n = 6 \), \( p < 0.05 \)) or in the groups treated with dose A (34.1 ± 0.7 ml/kg, \( n = 4 \), \( p < 0.05 \)) and B (31.9 ± 0.5 ml/kg, \( n = 5 \), \( p < 0.05 \)). The mean values of plasma volume

\[ \text{MAP, mean arterial pressure; CVP, central venous pressure; Hct, hematocrit. Analysis of variance did not show significant changes in MBP. CVP increased significantly during VE alone (F=2.41, p<0.05), but not during ANF (all F values NS). Hct decreased significantly in VE (A and B), while F value in C was not significant (see text). Mean integrated area of hemodilution calculated by planimetry was significantly smaller in B than in VE (also see text).} \]
minute infusion of 0.2 μg/kg/min of ANF (a dose (authors' unpublished observations) that a 20-
rectomized animals. Furthermore, we have observed
markedly higher than those obtained in nonneph-
in our nephrectomized animals, ANF levels were
volume expansion within 10% of body weight pro-
coworkers20 performed in intact dogs, a low dose of
as well as in a recent study of Zimmerman and
recent studies 914-20 in man and intact
animals with the infusion of comparable doses of
ANF. It is particularly interesting that in our study
anephric rabbits. However, previous human and
animal studies23-25 showed that kidneys are largely
responsible for the clearance of atrial peptides, and
therefore, it is quite likely that bilateral nephrec-
tomy reduced ANF clearance in our experiments.
In fact, the circulating levels of ANF measured in
our study are considerably higher than those pro-
duced in the ANF-treated groups A and B did not
differ from those measured in the animals receiving
volume-expansion alone.

Discussion
The results obtained in the present study demon-
strate that ANF induces significant hemodynamic
responses in nephrectomized rabbits, and there-
fore, they indicate that the hemodynamic effects of
the peptide occur, at least in part, independently
from its renal actions. In addition, the hemody-
namic responses to ANF vary both qualitatively
and quantitatively according to the plasma levels of
ANF achieved.
To eliminate the possible interference of the renal
effects in the assessment of the direct hemodynamic
actions of the peptide, we performed the study in
anephric rabbits. However, previous human and
animal studies23-25 showed that kidneys are largely
responsible for the clearance of atrial peptides, and
therefore, it is quite likely that bilateral nephrec-
tomy reduced ANF clearance in our experiments.
In fact, the circulating levels of ANF measured in
our study are considerably higher than those pro-
duced in previous studies9,14,20 in man and intact
animals with the infusion of comparable doses of
ANF. It is particularly interesting that in our study
as well as in a recent study of Zimmerman and
coworkers20 performed in intact dogs, a low dose of
ANF (0.001 and 0.0025 μg/kg/min, respectively) or
volume expansion within 10% of body weight pro-
duced a similar rise in ANF blood levels. However,
in our nephrectomized animals, ANF levels were
markedly higher than those obtained in nonneph-
rectomized animals. Furthermore, we have observed
(authors' unpublished observations) that a 20-
minute infusion of 0.2 μg/kg/min of ANF (a dose
correspondent to the highest dose used in the preent
study) in intact rabbits (n=5) resulted in ANF
plasma levels of 3.025 ± 192 pg/ml, a value mark-
edly lower than that obtained in this study in the
nephrectomized animals. These observations may
provide indirect evidence in the whole animal that
kidneys play an important role in removing ANF
from the blood.
Although the plasma levels of ANF achieved
constant gly exceeded the physiological range, even
when extremely low rates of infusion were used,
our experiments show that different hemodynamic
changes occur in response to gradual increases in
the circulating levels of the peptide. In fact, all three
doses were able to significantly reduce blood pres-
sure, but a marked hypotension was produced with the
highest dose. In addition, the intrinsic hemody-
namic mechanisms underlying the blood pressure-
lowering effect varied according to the different
dosage of ANF.
At the lowest rate of infusion, which resulted in a
10-fold increase of the circulating levels, the hypo-
tensive action was mostly accounted for by a reduc-
tion in systemic vascular resistance, while central
venous pressure and stroke volume showed only a
slight decrease, and heart rate was unchanged. It
should be noted that other authors failed to observe
significant hemodynamic responses to ANF with the
administration of comparable doses in intact
dogs.20 However, as mentioned above, the ANF
circulating levels measured in that study were mark-
edly lower as compared with those obtained in our
anephric rabbits.
The hemodynamic adjustments evoked by the
higher dosages of the peptide were quite different
from those produced by the lowest dose. In fact,
with both the intermediate and the high dose, which
were associated with extremely elevated plasma
ANF immunoreactivity, the fall in blood pressure
was associated with a significant reduction in stroke
volume and heart rate and no significant change in
vascular resistance. The observation that with these
doses central venous pressure fell suggests that the
reduced stroke volume was in turn mediated by a
decrease in cardiac preload more than by a fall in
ventricular performance, as postulated in previous
studies.12,26 Finally, in this particular experimental
model, hematocrit was significantly increased only
by high rates of ANF infusion.
The finding that the lowest dose of ANF reduced
arterial pressure mostly through a decrease in vas-
cular resistance, whereas higher doses did not mod-
ify vascular tone, is in keeping with the previous
hypothesis that the peptide acts as a vasodilator in
vivo only at concentrations close to the normal
range.7 However, previous studies in the intact
animal demonstrated that atrial peptides produce a
relevant vasodilation in the kidney and no change or
even an increase in vascular resistance in other
vascular beds.13 Our observation that with the low
dose of ANF overall vascular resistance fell, despite
the absence of the kidneys, might speak against the hypothesis that ANF-induced vasodilation is largely accounted for by the response of the renal vasculature.27-29

The hemodynamic responses observed at very high circulating levels of ANF confirm recent findings in experimental animal models4-7,12,13 and in man9,11 and are consistent with the view that the reduction in stroke volume caused by ANF infusion is due to a decreased venous return to the heart.30 On the basis of the hemoconcentration reported by several investigators during ANF,1,14-17 it has been postulated that the reduced venous return is mediated, at least in part, by an increased eflux of fluids from capillaries with a resulting decrease in plasma volume.15 However, in our experiments, a significant rise in hematocrit, and possibly, a decrease in blood volume, was achieved only with the highest dose, which produced extremely high circulating levels of the peptide. At lower plasma ANF concentrations, only a tendency for hematocrit to increase was noted. It could be hypothesized that in this specific experimental model, characterized by a very low initial hematocrit, the ANF-induced hemoconcentration is somehow blunted. However, the observed reduction in central venous pressure seems to confirm increased resistance to venous return and/or enhanced capillary permeability during ANF, as previously postulated.15,17,18,31

The observation that the ANF-induced reduction in blood pressure was not associated with the expected reflex tachycardia and that, actually, heart rate significantly fell at the highest rates of infusion of the peptide despite the more profound hypotensive response, supports the hypothesis that ANF interferes with the autonomic control of circulation.35 In keeping with this view is a recent observation by Thoren et al.33 who showed that the arterial baroreflex-mediated increase in renal sympathetic nerve activity is reduced by ANF through the sensitization of receptors with vagal afferents.

In an attempt to investigate the potential contribution of the hemodynamic properties of ANF in the maintenance of body fluid homeostasis, independently from the renal action of the peptide, we also evaluated the influence of different doses of ANF on the hemodynamic consequences of acute volume expansion in nephrectomized rabbits.

The results obtained show that the low dosage of the peptide was already able to attenuate the rise in central venous pressure but not the hemodilution produced by acute saline load. In fact, this rate of infusion of ANF resulted in circulating levels only slightly higher than those produced by volume expansion per se in the nephrectomized animals. Therefore, the effectiveness of this dose in modulating the central venous pressure response to volume expansion was surprising, especially in view of the lack of a significant decrease of central venous pressure when this dose was given to the euvoletic animals. However, recent data obtained in our laboratory in the intact rabbit show that the hemodynamic effects of the peptide are potentiated in volume-expanded states (authors' unpublished observations). Thus, it could be hypothesized that the circulatory influence of subtle changes in plasma levels of ANF might be amplified during volume expansion. When plasma levels were further raised either with the intermediate or with the highest dose, the hemodynamic consequences of acute volume expansion (rise in central venous and blood pressure, hemoconcentration) were further attenuated. In addition, plasma volume measured at the end of volume expansion was significantly lower in the group treated with the high dose of ANF than in the animals receiving saline load alone. Although these latter observations do not permit us to conclude that physiological ANF secretion can effectively buffer the acute perturbations of intravascular volume, they lend further support to the current hypothesis13 that in volume-expanded states, increased plasma levels of ANF may contribute to maintain plasma volume within near-normal limits. Our results demonstrate that this can be accomplished at least in part by the circulatory effects of the peptide, such as a redistribution of fluids from the intravascular to the interstitial compartment, besides the well-known diuretic effect.

In conclusion, the present study demonstrates that ANF induces significant hemodynamic responses independent from its renal effect. These responses vary both qualitatively and quantitatively according to the plasma levels of ANF produced by the different dosages. Such levels were relatively high compared with observations in intact animals, thus confirming the important role of kidneys in the clearance of endogenous (and possibly exogenous) peptide. The hemodynamic responses associated with progressive increases in the circulating levels of ANF were able to antagonize to a different extent the hemodynamic consequences of acute volume expansion, even without the concurrent intervention of renal actions. While these data do not demonstrate that ANF plays a role in the homeostatic regulation of plasma volume, the observation that pharmacological doses of ANF can reduce venous return to the heart in the absence of kidneys suggests that ANF might be beneficial through its hemodynamic effects in the management of clinical conditions characterized by acute volume overload, even in patients with severe renal failure.

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Volpe et al

Circulatory Responses to ANF in Anephric Rabbits 329


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