Cardio-Renal-Endocrine Dynamics During Stepwise Infusion of Physiologic and Pharmacologic Concentrations of Atrial Natriuretic Factor in the Dog


Infusion of α-human-atrial natriuretic factor (α-h-ANF) into pentobarbitol anesthetized dogs (n = 10) at 0.0025, 0.005, 0.01, and 0.3 μg/kg/min was performed to differentiate the physiologic actions of atrial natriuretic factor from its pharmacologic actions. The lowest doses of atrial natriuretic factor infusion resulted in circulating levels that were previously produced by 0–10% saline volume expansion. At the lowest infusion rate, circulating ANF increased 31 ± 3 pg/ml, resulting in a significant increase in absolute sodium excretion, fractional excretion of sodium, and fractional excretion of lithium, and a significant decrease in urine osmolality. A greater change in circulating atrial natriuretic factor (96 ± 12 pg/ml) was required to significantly decrease right atrial pressure, cardiac output, and plasma renin activity, and to increase systemic vascular resistance and total and fractional excretion of potassium. The highest dose of atrial natriuretic factor infused was required to decrease arterial pressure and renal vascular resistance. The present study demonstrates that 1) atrial natriuretic factor is natriuretic and diuretic at physiologic concentrations; 2) at low concentrations, atrial natriuretic factor appears to decrease the whole kidney proximal tubular reabsorption of sodium and does not affect glomerular filtration rate; 3) a greater (but physiologic) change in circulating atrial natriuretic factor is required to significantly decrease cardiac output, cardiac filling pressure, and plasma renin activity than is required to significantly increase sodium excretion; and 4) a decrease in systemic arterial pressure and vascular resistance does not occur at physiologic concentrations of atrial natriuretic factor. (Circulation Research 1987;60:63-69)

Renal sodium excretion is mediated by multiple regulatory mechanisms. Previous studies have demonstrated that distension of cardiac atria results in prompt increases in renal sodium and water excretion. Recent investigations have reported the existence of the hormone atrial natriuretic factor (ANF), which is stored in secretory granules of cardiac atria. Intravenous administration of pharmacologic doses of ANF results in a marked natriuresis and diuresis, decrease in arterial pressure, and inhibition of the renin-angiotensin-aldosterone system.

We and others have recently demonstrated that physiologic maneuvers such as saline volume expansion and mineralocorticoid administration are associated with an increase in sodium excretion coincident with an increase in circulating ANF. To date, it remains unclear if intravenous infusions of ANF at doses that approximate concentrations achieved by physiologic maneuvers do indeed result in an increase in sodium excretion. Further, whether the threshold for the renal actions of ANF is lower than the threshold for arterial blood pressure regulation or for renin inhibition is equally unclear. The present study was, therefore, designed to test the following hypotheses: 1) ANF is natriuretic at physiologic doses; 2) ANF acutely decreases arterial pressure at physiologic doses; 3) ANF influences the renin-angiotensin-aldosterone system at physiologic doses; and 4) ANF affects cardiac hemodynamics and cardiac output at physiologic doses.

Materials and Methods

Studies were performed in 10 pentobarbital anesthetized (30 mg/kg iv) mongrel dogs (18–22 kg) of either sex. The femoral artery was cannulated for measurement of systemic arterial pressure and for blood sampling. Both femoral veins were cannulated; one port was used for infusion of inulin in saline at a rate of 1 ml/min to achieve a plasma concentration of 50 mg/dl, and the other port was used for infusion of α-H-ANF. The right external jugular vein was isolated, and a seven French balloon-tipped thermodilution pulmonary artery catheter (American Edwards Laboratory, Santa Ana, Calif.) was advanced into the pulmonary artery. A left flank incision was then made, and the left kidney was exposed. The ureter was cannulated with polyethylene tubing for urine collection. The renal artery was isolated in situ. An electromagnetic flow...
probe was placed on the renal artery and connected to a Carolina Instruments flowmeter (King, N.C.). The trachea was intubated using a 9.5 mm endotracheal tube, and the animal was mechanically ventilated (Harvard Apparatus, Millis, Mass.).

Following surgical preparation, the dog was suspended in a prone position and allowed to stabilize for 1 hour. Two 10-minute control clearances were performed prior to ANF infusion, during which time saline was infused at 1 ml/min. 

\[ \text{ANF dose (ug/kg/min)} \] was placed on the renal artery and connected to a 

\[ \text{MAP (mm Hg)} \]

\[ \text{RAP (mm Hg)} \]

\[ \text{PCWP (mm Hg)} \]

\[ \text{SVR (dyne-sec-cm}^{-2}) \]

\[ \text{MAP (mm Hg) - RAP (mm Hg)/CO(l/min)} \times 80 \]

Table 1. Effects of ANF Infusion on Cardiac Systemic Hemodynamics

<table>
<thead>
<tr>
<th>ANF dose (ug/kg/min)</th>
<th>RAP (mm Hg)</th>
<th>PCWP (mm Hg)</th>
<th>SVR (dyne-sec-cm}^{-2})</th>
<th>MAP (mm Hg)</th>
<th>CO (l/min)</th>
<th>HR (bpm)</th>
<th>Hct (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-1.3 ± 0.4</td>
<td>2.4 ± 0.5</td>
<td>2296 ± 180</td>
<td>120 ± 4</td>
<td>4.5 ± 0.4</td>
<td>108 ± 5</td>
<td>40 ± 1</td>
</tr>
<tr>
<td>0.0025</td>
<td>-1.6 ± 0.4</td>
<td>2.1 ± 0.6</td>
<td>2496 ± 233</td>
<td>120 ± 4</td>
<td>4.2 ± 0.4</td>
<td>106 ± 6</td>
<td>40 ± 1</td>
</tr>
<tr>
<td>0.005</td>
<td>-2.2 ± 0.5*</td>
<td>1.9 ± 0.6</td>
<td>2672 ± 227*</td>
<td>121 ± 4</td>
<td>3.9 ± 0.4*</td>
<td>106 ± 5</td>
<td>39 ± 1</td>
</tr>
<tr>
<td>0.01</td>
<td>-2.3 ± 0.5*</td>
<td>1.6 ± 0.6*</td>
<td>2895 ± 248*</td>
<td>120 ± 4</td>
<td>3.6 ± 0.3*</td>
<td>105 ± 5</td>
<td>40 ± 1</td>
</tr>
<tr>
<td>0.3</td>
<td>-2.2 ± 0.5*</td>
<td>1.2 ± 0.7*</td>
<td>3020 ± 261*</td>
<td>106 ± 4*</td>
<td>3.1 ± 0.3*</td>
<td>108 ± 7</td>
<td>42 ± 1*</td>
</tr>
<tr>
<td>Recovery</td>
<td>-2.5 ± 0.5*</td>
<td>1.9 ± 0.5</td>
<td>3633 ± 342*</td>
<td>120 ± 4</td>
<td>2.9 ± 0.3*</td>
<td>105 ± 5</td>
<td>40 ± 1</td>
</tr>
</tbody>
</table>

ANF, atrial natriuretic factor; RAP, right atrial pressure; PCWP, pulmonary capillary wedge pressure; SVR, systemic vascular resistance; MAP, mean arterial pressure; CO, cardiac output; HR, heart rate; Hct, hematocrit; *p < 0.05 compared with control.

Data from control clearances and from clearances for each dose of ANF was presented and expressed as mean ± SE. Comparisons with control values were analyzed by Dunnett’s paired t test following ANOVA for repeated measurements. Significance was achieved at p < 0.05.

Results

Hemodynamic Response to ANF Infusion

ANF infusion at 0.0025 μg/kg/min had no significant effect on RAP, PCWP, SVR, MAP, CO, or HR (see Table 1). When ANF infusion was increased to 0.005 μg/kg/min, RAP decreased from -1.3 ± 0.4 (control) to -2.2 ± 0.5 mm Hg (p < 0.05), SVR increased from 2296 ± 180 (control) to 2672 ± 227 dyne·sec·cm\(^{-2}\) (p < 0.05), and CO decreased from 4.5 ± 0.4 (control) to 3.9 ± 0.4 l/min (p < 0.05, Figure 1). An ANF infusion rate of 0.01 μg/kg/min was required to significantly decrease PCWP from 2.4 ± 0.5 (control) to 1.6 ± 0.6 mm Hg (p < 0.05), and an infusion rate of 0.3 μg/kg/min was required to...
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Cardio-Renal-Endocrine Response to ANF

Renal Response to ANF Infusion

ANF infusion at 0.0025 µg/kg/min increased single kidney urine flow (V) from 0.16 ± 0.02 (control) to 0.38 ± 0.08 ml/min (p < 0.05), fractional excretion of sodium (FE_{Na}) from 0.41 ± 0.11 (control) to 1.30 ± 0.33% (p < 0.05, Figure 1), absolute sodium excretion (U_{Na}V) from 22 ± 6 (control) to 66 ± 15 µeq/min (p < 0.05), and fractional excretion of lithium (FE_{Li}) from 21 ± 2 (control) to 32 ± 3% (p < 0.05) and decreased urine osmolality (U_{osm}) from 1,292 ± 136 (control) to 994 ± 137 mosm/kg H2O (p < 0.05) (see Table 2). Infusion of ANF at 0.005 µg/kg/min was required to increase absolute potassium excretion from 29 ± 3 (control) to 39 ± 4 µeq/min (p < 0.05) and fractional excretion of potassium from 20 ± 2 (control) to 30 ± 2% (p < 0.05). An ANF infusion dose of 0.3 µg/kg/min was required to decrease renal vascular resistance from 0.90 ± 0.08 (control) to 0.70 ± 0.06 mm Hg/ml·min⁻¹. During increasing doses of ANF, urine flow rate, FE_{Na}, U_{Na}V, and FE_{Li} continued to increase, and U_{osm} continued to decrease. During the recovery period, urine flow rate, FE_{Na}, and U_{Na}V decreased but remained above control values (p < 0.05), FE_{Li}, U_{K}V, and FE_{K} returned to baseline levels, and U_{osm} increased but remained depressed compared with control values (p < 0.05). Renal blood flow and GFR did not significantly change during ANF infusion.

Hormonal Response to ANF Infusion

Stepwise increases in ANF infusion increased circulating levels of ANF in a stepwise fashion (see Figure 1 and Table 3). Circulating ANF increased from 68 ± 8 (control) to 100 ± 8 pg/ml (p < 0.05), an increase of 31 ± 3 pg/ml, during infusion of ANF at 0.0025 µg/kg/min. At an infusion rate of 0.005 µg/kg/min, circulating ANF increased to 164 ± 18 pg/ml (p < 0.05, compared with control), an increase of 96 ± 12 pg/ml. An infusion rate of 0.01 µg/kg/min resulted in a further increase of circulating ANF to 282 ± 22 pg/ml (p < 0.05, compared with control), an absolute increase of 214 ± 20 pg/ml. At 0.3 µg/kg/min, circulating ANF increased to 519 ± 34 pg/ml (p < 0.05, compared with control), an increase of 451 ± 32 pg/ml, and during recovery, circulating ANF returned to baseline levels (70 ± 10 pg/ml). An infusion rate of 0.01 µg/kg/min resulted in a further increase of circulating ANF to 282 ± 22 pg/ml (p < 0.05, compared with control), an absolute increase of 214 ± 20 pg/ml. At 0.3 µg/kg/min, circulating ANF increased to 519 ± 34 pg/ml (p < 0.05, compared with control), an increase of 451 ± 32 pg/ml, and during recovery, circulating ANF returned to baseline levels (70 ± 10 pg/ml). An infusion rate of 0.01 µg/kg/min resulted in a further increase of circulating ANF to 282 ± 22 pg/ml (p < 0.05, compared with control), an absolute increase of 214 ± 20 pg/ml. At 0.3 µg/kg/min, circulating ANF increased to 519 ± 34 pg/ml (p < 0.05, compared with control), an increase of 451 ± 32 pg/ml, and during recovery, circulating ANF returned to baseline levels (70 ± 10 pg/ml). An infusion rate of 0.01 µg/kg/min resulted in a further increase of circulating ANF to 282 ± 22 pg/ml (p < 0.05, compared with control), an absolute increase of 214 ± 20 pg/ml. At 0.3 µg/kg/min, circulating ANF increased to 519 ± 34 pg/ml (p < 0.05, compared with control), an increase of 451 ± 32 pg/ml, and during recovery, circulating ANF returned to baseline levels (70 ± 10 pg/ml). An infusion rate of 0.01 µg/kg/min resulted in a further increase of circulating ANF to 282 ± 22 pg/ml (p < 0.05, compared with control), an absolute increase of 214 ± 20 pg/ml. At 0.3 µg/kg/min, circulating ANF increased to 519 ± 34 pg/ml (p < 0.05, compared with control), an increase of 451 ± 32 pg/ml, and during recovery, circulating ANF returned to baseline levels (70 ± 10 pg/ml).

Discussion

The present study demonstrates that atrial natriuretic factor has significant effects on renal sodium and water...
excretion, cardiac hemodynamics, and plasma renin activity at circulating levels that approximate those achieved during 0–10% saline volume expansion. Specifically, an increase of $31 \pm 3$ pg/ml of ANF is involved in the natriuresis of volume expansion. We have previously demonstrated that during 0–10% body weight saline volume expansion (VE), circulating ANF of 96 ± 12 pg/ml was required to decrease RAP and CO, in association with an increase in circulating ANF of 96 ± 12 pg/ml. The present finding that ANF infusion during volume expansion increases sodium excretion supports a physiologic natriuretic role for ANF in volume expansion. This interpretation is in agreement with studies by Schwab et al.28 Hirth et al demonstrated that removal of the right atrial appendage in rats attenuates the rise in ANF and the natriuresis associated with acute volume expansion produced by 25% body weight albumin infusion. Hirth et al demonstrated a blunted natriuresis following saline infusion in animals treated with ANF antibodies. The observation that the change in FENa and UNaV was greater during volume expansion than during ANF infusion, despite achieving similar circulating levels of ANF, demonstrate that ANF is only one factor involved in the natriuresis of volume expansion.

Recent studies by Goetz et al.29 demonstrate that balloon distension of the left atrium results in a significant increase in circulating ANF in both intact and cardiac-denervated dogs, but an increase in sodium excretion was only observed in the intact dogs. Goetz suggested that ANF may not be natriuretic at physiologic doses and that the primary stimulus for sodium excretion caused by atrial distension was mediated by a cardio-renal neuronal mechanism. It is possible that the cardiac-denervated dogs in his study had a significantly lower mean arterial pressure, which may have decreased the effect of circulating ANF on renal sodium excretion. Alternatively, an intact cardio-renal neuronal axis may be necessary for ANF to exert its natriuretic effect.

Intravenous infusion of ANF also resulted in a significant increase in fractional excretion of lithium. Several laboratories have established lithium as a marker for proximal sodium reabsorption. The absence of a change in glomerular filtration rate (GFR) in the present studies in association with increased fractional excretion of sodium and lithium supports a proximal tubular action of ANF at physiologic levels. An action of ANF on proximal tubule reabsorption has been controversial. The proximal tubule is essentially free of receptors for ANF. Micropuncture studies of superficial proximal tubules in the rat have failed to detect a decrease in proximal reabsorption. Baum has failed to detect a direct action of ANF on isolated perfused rabbit proximal tubules. In contrast, we have reported that ANF results in a decrease in whole kid-
nephron proximal tubule reabsorption as estimated by the lithium clearance technique in normal dogs,10 dogs with experimental heart failure,30 and humans with chronic congestive heart failure.31 Hammond et al32 also demonstrated a proximal action as documented by an ANF-mediated inhibition of sodium cotransport in vitro in rat brush border vesicles. Recently, Roy33 has reported that ANF inhibits proximal tubule reabsorption of juxtedudillary nephrons. Thus, the present studies extend these previous investigations and support a physiologic action of ANF on proximal reabsorption in the dog. The mechanism of this inhibition of proximal reabsorption may be indirect and mediated through increases in intrarenal hydraulic pressures.3435

The threshold for sodium excretion appears to be lower than that for potassium excretion. The mechanism by which ANF infusion increases potassium excretion is unclear. The increase in potassium excretion does not appear to be related to an increase in aldosterone since no change in aldosterone was observed. The most likely explanation is an increase in tubular flow rate as manifested by an increase in urine volume.

Urine osmolality decreased at the lowest dose of ANF infused and did not return to baseline during recovery. These findings suggest a decrease in interstitial oncoticity resulting in washout of fluid from the medulla. It is interesting to note that in the present study a decrease in right atrial pressure occurred at a lower dose of ANF than that required to increase hematocrit. This finding suggests that the fall in cardiac filling pressure is likely due to increased venous capacitance rather than transudation of fluid into the interstitial space. It is interesting that in the present study a decrease in right atrial pressure occurs at a lower circulating concentration of ANF than that required to increase hematocrit. This finding suggests that the fall in cardiac filling pressure with low dose ANF infusion is more likely due to an increase in venous capacitance than transudation of fluid to the interstitial space.

Physiologic doses of ANF resulted in inhibition of plasma renin activity. Because ANF is released by volume overload,12 it is possible that ANF may participate in the inhibition of renin caused by volume overload. The mechanism of this ANF inhibition of renin release may be secondary to enhanced delivery of sod-

### Table 2. Continued

<table>
<thead>
<tr>
<th>RVR (mm Hg/ml/min)</th>
<th>FEa (%)</th>
<th>U_{\text{ex}} (mosm/kg H_2O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.90 ± 0.08</td>
<td>1.29 ± 1.36</td>
<td></td>
</tr>
<tr>
<td>0.90 ± 0.05</td>
<td>32 ± 137</td>
<td></td>
</tr>
<tr>
<td>0.88 ± 0.05</td>
<td>43 ± 86</td>
<td></td>
</tr>
<tr>
<td>0.91 ± 0.07</td>
<td>50 ± 52</td>
<td></td>
</tr>
<tr>
<td>0.70 ± 0.05*</td>
<td>67 ± 18</td>
<td></td>
</tr>
<tr>
<td>1.01 ± 0.11</td>
<td>37 ± 8</td>
<td></td>
</tr>
</tbody>
</table>

ANF infusion resulted in decreases in arterial pressure and cardiac output at higher doses than those required for its renal actions, though at doses within the physiologic range. Such a response in cardiac hemodynamics is consistent with a decrease in venous return. During expansion of the intravascular space with saline, the peak increase in circulating ANF is associated with significant increases in cardiac filling pressure and cardiac output.12 Therefore, the integration of the known action of exogenously administered ANF and endogenous release induced by volume expansion may be to protect the heart from volume overload by decreasing venous return. Very high concentrations of ANF were required to decrease arterial pressure. The decrease in arterial pressure resulted from a marked decrease in cardiac filling pressure and cardiac output with an inability of systemic vascular resistance to maintain arterial pressure. These studies suggest that the acute physiologic action of ANF is primarily on the regulation of venous return and cardiac filling pressure and/or volume.

Although significant changes in sodium excretion occurred at a lower dose of ANF than that required to significantly change potassium excretion, renin secretion, cardiac output, or right atrial pressure, it is possible that the apparent lower threshold for sodium excretion is statistic rather than biologic since measurements of the latter variables are subject to greater variability than the measurement of sodium excretion.

ANF infusion results in increased systemic vascular resistance at physiologic levels. In vitro studies of ANF on arterial strips demonstrate a vasorelaxant effect of ANF.3839 The present study suggests that a decrease in cardiac output caused by ANF infusion results in arterial vasoconstriction, most likely by non-ANF neuronal and/or hormonal mechanisms that overcome the vasorelaxant actions of ANF.

The observation that hematocrit significantly increased at an ANF infusion rate of 0.3 μg/kg/min, despite replacement of urine and blood loss with saline, supports previous studies in anephric rats that similarly demonstrate an increase in hematocrit with high dose ANF infusion.40 These studies support the concept that high doses of ANF may increase transudation of fluid into the interstitial space. It is interesting that in the present study a decrease in right atrial pressure occurs at a lower circulating concentration of ANF than that required to increase hematocrit. This finding suggests that the fall in cardiac filling pressure with low dose ANF infusion is more likely due to an increase in venous capacitance than transudation of fluid to the interstitial space.
um to the macula densa. The lack of inhibition of aldosterone in this study may be due to the acute nature of the study.

In summary, the present studies demonstrate that exogenously administered atrial natriuretic factor, when infused at doses to mimic physiologic concentrations, results in a selective renal action manifested by an increase in sodium excretion and urine flow, a decrease in urine osmolality, and a decrease in whole kidney proximal tubule reabsorption. At higher physiologic concentrations, a cardiovascular action resulted in decreases in cardiac filling pressures and cardiac output, with an associated inhibition of renin. At pharmacologic concentrations, a decrease in arterial pressure and an increase in hematocrit were observed. These studies support a physiologic action of ANF on renal, endocrine, and cardiovascular function.

References


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**Key Words** • atrial natriuretic factor • sodium excretion • cardiac hemodynamics • renin • aldosterone
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