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 pentobarbital 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-ANF. The right external jugular vein was isolated, and a seven French balloon-tipped thermodynamic 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. α-H-ANF (α-atrial natriuretic polypeptide, human 28 a.a., Peninsula Laboratories, Inc., Belmont, Calif.) in saline was subsequently infused at 0.0025, 0.005, 0.01, and 0.3 μg/kg/min at a rate of 1 ml/min. Each dose was infused over 35 minutes, and two 10-minute clearances were performed during the last 20 minutes of ANF infusion for each dose. Saline was subsequently infused at 1 ml/min over 55 minutes, and a final 10-minute recovery clearance was performed starting at 45 minutes. After each clearance period, saline was infused to replace urinary output and volume loss from blood withdrawal.

During each clearance, the following hemodynamic data were collected: mean arterial blood pressure (MAP), right atrial pressure (RAP), pulmonary artery capillary wedge pressure (PCWP) (to assess left atrial pressure), heart rate (HR), renal blood flow (RBF), and cardiac output (CO). Cardiac output was measured by thermodilution using American Edwards Cardiac Output Model 9510-A computer. For each clearance, cardiac output was determined in triplicate and averaged.

During each clearance period, arterial blood was collected for hormone analysis, electrolyte determination, and hematocrit. Blood for hormone analysis was placed in EDTA tubes, immediately placed on ice, and centrifuged at 2,500 rpm at 3°C. Plasma was separated and stored at −20°C until assay. ANF was extracted by use of C18 Sep-Pak with a recovery of 86%. ANF was measured by radioimmunoassay to α-H-ANF. Interassay coefficient of variation was 9%; intraassay coefficient of variation was 6%. Cross-reactivity was assumed to be 100% with dog ANF because the amino acid sequences are identical. Plasma renin activity was determined by radioimmunoassay using the method of Haber et al. Aldosterone (immuno-aldosterone-iodine-125 Kit, Pantex D18, Santa Monica, Calif.) levels were determined after dichloromethane extraction by radioimmunoassay (95% recovery). Serum and urine sodium concentrations were determined during each clearance by use of ion-selective electrodes using a Beckman E2A analyzer (Brea, Calif.). Glomerular filtration rate was determined by the clearance of inulin. Plasma and urine inulin concentrations were measured by the anthrone method. Osmolality of urine and plasma was determined by a Wescor Model 5100 vapor pressure osmometer (Logan, Utah). Whole kidney proximal reabsorption of sodium was estimated by the lithium-clearance technique. This technique has been shown to be a reliable method for estimating whole kidney delivery of sodium from the proximal tubule since lithium is reabsorbed exclusively by the proximal tubule. The dogs were given 300 mg of lithium orally the night before each experiment. Lithium concentrations in the plasma and urine were measured by flame emission spectrophotometry (Instrumentation Laboratories, model 357, Lexington, Mass.). Systemic vascular resistance (SVR) was calculated as follows:

\[
SVR \text{ (dyne-sec-cm}^{-2}\text{)} = \frac{\text{MAP(mm Hg)} - \text{RAP(mm Hg)}}{\text{CO(l/min)}} \times 80
\]

Data from control clearances and from clearances for each dose of ANF infusion were meaned and expressed as mean ± SE. Comparisons with control values are 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). 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

<table>
<thead>
<tr>
<th>ANF dose (μg/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.
significantly decrease mean arterial pressure from 120 ± 4 (control) to 106 ± 4 mm Hg (p < 0.05) and to increase hematocrit from 40 ± 1 (control) to 42 ± 1% (p < 0.05). During the recovery period, RAP (-2.5 ± 0.5 mm Hg) and CO (2.9 ± 0.3 l/min) remained significantly decreased, SVR (3633 ± 342 dyne·sec·cm⁻²) remained significantly increased, and MAP (120 ± 4 mm Hg) and hematocrit (40 ± 1%) returned to control levels. Heart rate did not significantly change during ANF infusion.

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 (FE₂Na) from 0.41 ± 0.11 (control) to 1.30 ± 0.33% (p < 0.05, Figure 1), absolute sodium excretion (U₂Na·V) from 22 ± 6 (control) to 66 ± 15 μeq/min (p < 0.05), and fractional excretion of lithium (FE₂Li) from 21 ± 2 (control) to 32 ± 3% (p < 0.05) and decreased urine osmolality (U₂osm) from 1,292 ± 136 (control) to 994 ± 137 m osm/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, FE₂Na, U₂Na·V, and FE₂Li continued to increase, and U₂osm continued to decrease. During the recovery period, urine flow rate, FE₂Na, and U₂Na·V decreased but remained above control values (p < 0.05), FE₂Li, U₂V, and FE₂Li returned to baseline levels, and U₂osm 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 0.005 μg/kg/min was required to significantly decrease plasma renin activity from 3.6 ± 1.3 (control) to 2.2 ± 0.8 ng/ml/hr (p < 0.05). Increasing doses of ANF resulted in further decreases in plasma renin activity. During recovery, plasma renin activity (2.7 ± 0.7 ng/ml/hr) returned to baseline levels. Circulating levels of aldosterone did not change throughout the experimental protocol.

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 ± 3 pg/ml of ANF in circulating ANF increases sodium excretion and fractional excretion of lithium, urine osmolality. A greater increase in 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 of 31 ± 3 pg/ml of ANF increases sodium excretion caused by atrial distension was mediated by 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 physiologic natriuretic role for ANF in volume expansion. 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-

### Table 2. Effects of ANF Infusion on Renal Function

<table>
<thead>
<tr>
<th>ANF dose (μg/kg/min)</th>
<th>V (ml/min)</th>
<th>GFR (ml/min)</th>
<th>U_{Na}V (μg/ml/min)</th>
<th>FE_{Na} (%)</th>
<th>U_{K}V (μl/min)</th>
<th>FE_{K} (%)</th>
<th>RBF (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.16 ± 0.02</td>
<td>37.6 ± 1.4</td>
<td>22 ± 6</td>
<td>0.41 ± 0.11</td>
<td>29 ± 3</td>
<td>20 ± 2</td>
<td>147 ± 12</td>
</tr>
<tr>
<td>0.0025</td>
<td>0.38 ± 0.08*</td>
<td>34.5 ± 2.5</td>
<td>66 ± 15*</td>
<td>1.30 ± 0.33</td>
<td>32 ± 3</td>
<td>27 ± 4</td>
<td>144 ± 9</td>
</tr>
<tr>
<td>0.005</td>
<td>0.72 ± 0.15*</td>
<td>36.3 ± 2.9</td>
<td>131 ± 26*</td>
<td>2.61 ± 0.55</td>
<td>39 ± 4*</td>
<td>30 ± 2*</td>
<td>148 ± 7</td>
</tr>
<tr>
<td>0.01</td>
<td>0.94 ± 0.14*</td>
<td>31.7 ± 1.5</td>
<td>171 ± 26*</td>
<td>3.76 ± 0.54*</td>
<td>37 ± 2*</td>
<td>34 ± 2*</td>
<td>143 ± 9</td>
</tr>
<tr>
<td>0.3</td>
<td>2.66 ± 0.46*</td>
<td>38.2 ± 3.9</td>
<td>398 ± 50*</td>
<td>6.81 ± 0.60*</td>
<td>56 ± 6*</td>
<td>42 ± 4*</td>
<td>166 ± 11</td>
</tr>
<tr>
<td>Recovery</td>
<td>0.40 ± 0.06*</td>
<td>38.2 ± 4.0</td>
<td>70 ± 9*</td>
<td>1.31 ± 0.16*</td>
<td>29 ± 3</td>
<td>22 ± 3</td>
<td>136 ± 14</td>
</tr>
</tbody>
</table>

ANF, atrial natriuretic factor; V, urine flow; GFR, glomerular filtration rate; U_{Na}V, absolute sodium excretion; FE_{Na}, fractional excretion of sodium; U_{K}V, absolute potassium excretion; FE_{K}, fractional excretion of potassium; RBF, renal blood flow; RVR, renal vascular resistance. *p < 0.05 compared with control.
creatinine clearance in the dog. The mechanism of this inhibition of proximal reabsorption may be indirect and mediated through increases in intrarenal hydraulic pressures. 34 35 Previous studies have reported that in the pathophysiologic syndrome of congestive heart failure, 10 and humans with chronic congestive heart failure, 31 Hammond et al 12 also demonstrated a proximal action as documented by an ANF-mediated inhibition of sodium cotransport in vitro in rat brush border vesicles. Recently, Roy 33 has reported that ANF inhibits proximal tubule reabsorption of juxtamedullary 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. 36 39

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.

Urinary osmolality decreased at the lowest dose of ANF infused and did not return to baseline during recovery. These findings suggest a decrease in interstitial tonicity supporting medullary washout secondary to an increase in medullary blood flow as reported by Borenstein 36 employing atrial extracts, and they support the concept of a physiologic ANF action on medullary hemodynamics.

A decrease in renal vascular resistance was noted only at the highest dose of ANF infused, suggesting that this effect is pharmacologic or pathophysiologic. Previous studies have reported that in the pathophysiologic syndrome of congestive heart failure, ANF circulates at plasma concentrations achieved at the highest dose in the present study. 9 10 No change in total renal blood flow was observed. ANF infusion caused variable changes in GFR at the highest infusion rate, increasing in 5 dogs and decreasing in 5 dogs. This finding is in agreement with previous studies of stepwise renal artery infusion of ANF by Seymour et al. 37 However, some of our previous studies demonstrated that ANF infusion at 0.3 μg/kg/min over 45 minutes resulted in increased GFR. 10 Taken together, these observations suggest that ANF-mediated increases in GFR are not required for the natriuresis and that stepwise increases in ANF may blunt the effect of high doses of ANF on GFR.

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. 38 39 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. 10 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 to 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 sodi-
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.

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