Atrial Natriuretic Factor Release by Angiotensin II in the Conscious Rat

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SUMMARY Since it was previously reported that atrial natriuretic factor (ANF) may exert an inhibitory effect on renin release, the existence of an Angiotensin II (Ang II)–ANF feedback mechanism was investigated. Male rats were infused intraperitoneally for 7 days with either saline, a nonpressor dose of Ang II (200 ng/kg/min), or a pressor dose (800 ng/kg/min) of Ang II. Systolic blood pressure, plasma ANF, 24-hour urinary sodium excretion, urine volume, and water intake were measured. A significant increase in plasma ANF was observed in the group with a pressor response (blood pressure rose from 89.0 ± 3.9 to 136.7 ± 11.4 mm Hg; ANF rose from 36.8 ± 4.9 to 92.7 ± 17.7 pg/ml). There was no significant time effect on 24-hour sodium excretion, urine volume, and water intake in both Ang II–infused groups. In a second set of experiments, male rats were infused intravenously for 60 minutes with either saline, a nonpressor dose of Ang II (16 ng/kg/min), or a pressor dose (800 ng/kg/min) of Ang II. Left ventricular end-diastolic pressure, right atrial pressure, and mean arterial pressure were monitored. There was a significant increase in plasma ANF and left ventricular end-diastolic pressure only with the pressor dose (blood pressure rose from 85.0 ± 6.1 to 140.0 ± 5.5 mm Hg; ANF rose from 22.6 ± 6.0 to 108.3 ± 47.7 pg/ml; left ventricular end-diastolic pressure rose from 5.3 ± 5.7 to 20.8 ± 7.9 mm Hg). No significant modification of right atrial pressure was recorded. In a last set of experiments, atrial slices were incubated for 30 minutes with Ang II in increasing doses from $10^{-8}$ to $10^{-5}$ M without any modification in ANF release into the medium. Thus, ANF release induced by pressor doses of Ang II seems to be mediated by hemodynamic changes, particularly by an elevation of left ventricular end-diastolic pressure rather than right atrial pressure. (Hypertension 11: 502-508, 1988)

KEY WORDS • atrial natriuretic factor • angiotensin II • blood pressure • left ventricular end-diastolic pressure • right atrial pressure

Atrial natriuretic factor (ANF) is a potent natriuretic and vasoactive peptide localized in secretorylike atrial specific granules.\(^1\)\(^2\)\(^3\)\(^4\) Since ANF is released into the circulation in response to physiological stimuli, it may play a major role in body fluid volume and blood pressure (BP) homeostasis. The secretion of ANF into the circulation is now known to be regulated by conditions that lead to atrial distention.\(^1\)\(^2\)\(^3\)\(^4\) Furthermore, some experiments suggest that, unlike other neurohumoral volume regulation systems, this atrial distention does not promote the release of ANF through a reflex mechanism involving atrial receptors but rather by a direct action of increased atrial pressure.\(^1\)\(^2\)\(^3\) Steroids seem to have a direct effect on its synthesis and release.\(^6\)\(^7\)\(^8\)\(^9\)\(^10\) However, it is less clear whether ANF secretion induced by pressor agents, such as vasopressin, angiotensin II (Ang II), or phenylephrine,\(^6\) is due to hemodynamic changes (resulting in increased atrial pressure) or to direct receptor stimulation. The literature on the actions of several agonists on ANF release by incubated atrial slices is somewhat contradictory.\(^7\)\(^9\)\(^10\)

ANF and the renin-angiotensin-aldosterone system appear to interact in the control of body fluids. The peptide has an inhibitory effect on Ang II–induced aldosterone secretion by adrenal cells in vitro\(^11\)\(^12\) and in vivo,\(^12\) but its influence on plasma renin activity (PRA) is a controversial subject. Acute experiments on anesthetized animals with stimulated renin release have demonstrated that ANF administration depresses PRA and the renin secretory rate.\(^14\)\(^15\) Other evidence...
suggests that renin has to be prestimulated before it can be inhibited. 16

In the present experiments, we investigated whether there is a relationship between hemodynamic changes (more precisely in right and left atrial pressure) and Ang II-induced ANF release and whether a positive Ang II–ANF feedback mechanism can be operant.

**Materials and Methods**

**Chronic Experiment**

Male Sprague-Dawley rats (weight, 200–250 g; Charles River, St.-Constant, Quebec, Canada) were accommodated in individual metabolic cages for 48 hours before the experiment to acclimatize them to their new environment. After this initial period, they were divided into three groups (control, nonpressor, and pressor) and maintained in their metabolic cages on regular rat chow and tap water ad libitum for the duration of the experiment. The following basal measurements were recorded before drug administration: indirect systolic BP by means of a tail-cuff with the rats under light ether anesthesia, 24-hour urine volume, 24-hour urinary sodium excretion, water intake, and body weight. A blood sample (1.2–1.5 ml) was drawn from the jugular vein with the rats under sodium pentobarbital anesthesia (60 mg/kg i.p.) to measure ANF and hematocrit.

On Day 0, osmotic minipumps (Model 2001, Alza, Palo Alto, CA, USA), releasing either 0.9% NaCl or 200 or 800 ng/kg/min Ang II ([Ile 8 ]Ang II, Peninsula, Belmont, CA, USA), diluted in saline, were implanted (i.p. with the rats under ether anesthesia) in the control, nonpressor, and pressor groups, respectively.

Urine volume, urinary sodium excretion, and water intake were recorded daily; BP and body weight were recorded on Days −4, 1, 3, and 6. Blood samples were withdrawn, with the rats under sodium pentobarbital anesthesia (60 mg/kg i.p.) to measure ANF and hematocrit. Urinary sodium was assessed by flame photometry.

Seven days after the pumps were installed, the rats were killed by decapitation, blood was collected for measurement of PRA and plasma aldosterone, the hearts were excised and weighed, and right and left atria were dissected separately. PRA was quantified by flame photometry (with blood from donor rats). A 25-minute rest period was then allowed for redistribution of the blood volume and for possible readjustments of vascular tone. Infusions were given to the control, nonpressor, and pressor groups over 60 minutes through the femoral vein (at a rate of 0.8 ml/60 min) as either 0.9% NaCl or Ang II, 16 or 800 ng/kg/min (Ang II was diluted in saline). Blood was taken at 5 minutes (Ang II–induced MAP peak), at 30 minutes during the infusion, and 15 minutes after the infusion was terminated. The volume removed during the infusion was replaced with blood from donor rats. The pressures recorded after each blood withdrawal were used for the calculations. The blood samples were collected in glass tubes containing the same protease inhibitors as in the chronic experiment and immediately centrifuged at 3000 rpm at 4°C for 10 minutes. Plasma ANF was extracted by means of Vycor glass and measured by RIA. 21

**Incubation of Atrial Slices**

Male Sprague-Dawley rats (weight 200–250 g, Charles River) were killed by decapitation. The thoracic cage was opened, and the right and left atria were rapidly excised, pooled separately, and put into 20 ml of Krebs-Henseleit solution at 4°C. For each experiment, 20 rats were used.

Each right and left atrium was sliced in four parts on a plate kept on ice, and all slices from right or left atria were pooled and washed in 20 ml of fresh Krebs solution. They were then distributed in 20 flasks, each containing four slices from the right and four slices from the left atrium in 2 ml of fresh Krebs solution at 4°C, thus being further washed. They were finally transferred to 20 other flasks containing 3 ml of incubation medium, preincubated for 30 minutes at 37°C,
aerated with 95% O₂, 5% CO₂, and further incubated for 30 minutes at 37°C under continuous bubbling. In each experiment, a total of 20 flasks, each containing four right and four left randomly chosen atrial slices, were prepared.

The incubation medium consisted of a Krebs solution with the following composition (mM): NaCl, 123; KCl, 2.2; MgCl₂, 0.5; NaH₂PO₄, 0.4; CaCl₂, 1.3; NaHCO₃, 25.0; glucose, 5.6; pH, 7.4 and 299 mosm. The following proteases inhibitors were added to the incubation medium (final concentration): 10⁻⁶ M pepstatin; aprotinin, 100 KIU/ml; 10⁻⁶ M phenylmethylsulfonyl fluoride; 10⁻³ M dipyridyl; 2 × 10⁻⁵ M leupeptin; and 10⁻⁴ M captopril.

Ang II was diluted with incubation medium at 37°C and added to the preincubated atrial slices at four different doses ranging from 10⁻⁷ M to 10⁻³ M (n = 4 flasks for each dose). To the control group (n = 4), the same volume of incubation medium alone was added. A set of two experiments was performed, and the results were pooled (n = 8 for each dose).

At the end of the 30-minute incubation period, an aliquot of each flask was taken and kept at −70°C until assayed to measure the concentration of ANF in the media. The RIA procedures have been described elsewhere. An aliquot of each flask containing Ang II was also taken at the beginning (Time 0) and at the end (Time 30 minutes) of the incubation period to measure the percent degradation of total immunoreactive Ang II by RIA. The direct determination of the concentration of ANF in the incubation media was made by a modification of the technique of Oster et al.

The results of the in vivo experiments (chronic and acute experiments), expressed as means ± SEM, were evaluated by two-way analysis of variance with repeated measures on one factor (time) to globally test the time effect, the group effect, and the group interaction by time. The data on parameters that were measured at the same time as BP, did not change significantly in either group. Plasma ANF was increased significantly (p < 0.01) only in rats infused with a pressor dose of Ang II, presenting an elevation of about 150% by Day 2 and 100% by Day 4. There was a significant but poor correlation between systolic BP and plasma ANF concentration when values for all three groups were pooled (r = 0.30, p < 0.05).

There was no significant time effect on 24-hour urine volume, urinary sodium excretion, and water intake during the course of the chronic experiment (Table 1). Rats infused with the pressor dose of Ang II presented a significant, progressive loss of weight (p < 0.01) and a significant increase in hematocrit (p < 0.05) when compared with controls or recipients of the nonpressor dose (Table 2). Plasma aldosterone concentration, assessed at the end of the 7-day Ang II infusion period, was higher in the pressor than in the control or nonpressor groups (p < 0.05; Table 3). PRA tended to be lower in the pressor group, but the difference was not significant (see Table 3). Heart weight and atrial ANF content and concentration, measured at the end of the experiment, were unchanged (see Table 2).

### Acute Ang II Infusion

The effect of acute Ang II infusion on MAP is illustrated in Figure 3A. The i.v. administration of Ang II, after the minipumps were implanted but became significant only by Day 3 (p < 0.01). A maximum rise of about 50% over basal values occurred after 6 days of infusion. Rats implanted with minipumps releasing either 0.9% NaCl or Ang II, 200 ng/kg/min, did not show any significant elevations of BP. Heart rate, measured at the same time as BP, did not change significantly in either group. Plasma ANF was increased significantly (p < 0.01) only in rats infused with a pressor dose of Ang II, presenting an elevation of about 150% by Day 2 and 100% by Day 4. There was a significant but poor correlation between systolic BP and plasma ANF concentration when values for all three groups were pooled (r = 0.30, p < 0.05).

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### Results

#### Chronic Ang II Infusion

Figures 1 and 2 depict the effect of chronic Ang II infusion on systolic BP and plasma ANF. Administration of Ang II, 800 ng/kg/min, induced a progressive increase of systolic BP that was first observed 24 hours after the minipumps were implanted but became significant only by Day 3 (p < 0.01). A maximum rise of about 50% over basal values occurred after 6 days of infusion. Rats implanted with minipumps releasing either 0.9% NaCl or Ang II, 200 ng/kg/min, did not show any significant elevations of BP. Heart rate, measured at the same time as BP, did not change significantly in either group. Plasma ANF was increased significantly (p < 0.01) only in rats infused with a pressor dose of Ang II, presenting an elevation of about 150% by Day 2 and 100% by Day 4. There was a significant but poor correlation between systolic BP and plasma ANF concentration when values for all three groups were pooled (r = 0.30, p < 0.05).

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#### Acute Ang II Infusion

The effect of acute Ang II infusion on MAP is illustrated in Figure 3A. The i.v. administration of Ang II,
800 ng/kg/min, elicited a rapid increase in MAP that reached its maximum pressure peak of about 65% over basal values \((p < 0.01)\) within 5 minutes of infusion. This peak was followed by a decrease to a plateau level \(0.05\) than basal values (422 \(\pm\) 18 beats/min).

As shown in Figure 3D, rats given the pressor dose of Ang II presented, parallel to the elevation of MAP, a significant increase \((p < 0.05)\) in plasma ANF concentrations \(\times 600\%\) at 5 minutes and \(800\%\) at 30 minutes of infusion). The postinfusion ANF levels remained higher than basal values, but this difference was not significant. Like MAP, there was no significant change in plasma ANF concentrations in rats infused with either 0.9% NaCl or the nonpressor dose of Ang II. Plasma ANF was positively correlated with MAP \((r = 0.47, p < 0.001)\).

Figures 3B and 3C demonstrate the effects of Ang II infusion on LVEDP and CVP. Neither the pressor nor the nonpressor dose of Ang II had any significant influence on CVP. On the other hand, Ang II, 800 ng/kg/min, significantly \((p < 0.01)\) increased LVEDP over baseline values \((by about 300\% at 5 minutes and 150\% at 30 minutes of infusion)\). Like MAP and plasma ANF concentrations, postinfusion LVEDP remained slightly but not significantly higher than basal levels. There were no significant changes in LVEDP in rats infused with either 0.9% NaCl or the nonpressor dose of Ang II. Plasma ANF was positively correlated.

### Table 1. Sodium Excretion, Urine Volume, and Water Intake During Chronic Ang II Administration

<table>
<thead>
<tr>
<th>Variable</th>
<th>Basal</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UNaV (μEq/24 hr)</td>
<td>563 ± 80</td>
<td>274 ± 42</td>
<td>516 ± 115</td>
<td>291 ± 60</td>
<td>402 ± 73</td>
</tr>
<tr>
<td>UV (ml/24 hr)</td>
<td>6 ± 1</td>
<td>3 ± 0</td>
<td>3 ± 1</td>
<td>2 ± 1</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>WI (ml/24 hr)</td>
<td>25 ± 3</td>
<td>25 ± 3</td>
<td>25 ± 1</td>
<td>22 ± 3</td>
<td>25 ± 2</td>
</tr>
<tr>
<td>Nonpressor (n = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UNaV (μEq/24 hr)</td>
<td>677 ± 102</td>
<td>380 ± 91</td>
<td>731 ± 165</td>
<td>628 ± 162</td>
<td>808 ± 126</td>
</tr>
<tr>
<td>UV (ml/24 hr)</td>
<td>7 ± 1</td>
<td>7 ± 3</td>
<td>8 ± 3</td>
<td>5 ± 2</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>WI (ml/24 hr)</td>
<td>26 ± 3</td>
<td>34 ± 6</td>
<td>36 ± 5</td>
<td>28 ± 4</td>
<td>31 ± 6</td>
</tr>
<tr>
<td>Pressor (n = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UNaV (μEq/24 hr)</td>
<td>699 ± 134</td>
<td>1160 ± 375</td>
<td>788 ± 146</td>
<td>389 ± 88</td>
<td>839 ± 122</td>
</tr>
<tr>
<td>UV (ml/24 hr)</td>
<td>8 ± 2</td>
<td>9 ± 2</td>
<td>12 ± 3</td>
<td>9 ± 2</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>WI (ml/24 hr)</td>
<td>31 ± 3</td>
<td>28 ± 5</td>
<td>33 ± 5</td>
<td>33 ± 7</td>
<td>32 ± 6</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Nonpressor = Ang II, 200 ng/kg/min; pressor = Ang II, 800 ng/kg/min; UNaV = urinary sodium excretion; UV = urine volume; WI = water intake.

### Table 2. Effect of Chronic Infusion of Ang II on Hematocrit, Body and Heart Weights, and Atrial ANF Concentration

<table>
<thead>
<tr>
<th>Variable</th>
<th>Last day of measurement</th>
<th>24-hr period after infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 10)</td>
<td>Basal</td>
<td>1st</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>34 ± 1</td>
<td>33 ± 1</td>
</tr>
<tr>
<td>Body wt (g)</td>
<td>255 ± 7</td>
<td>258 ± 23</td>
</tr>
<tr>
<td>Heart wt (mg/100 g body wt)</td>
<td>305 ± 6</td>
<td>3.3 ± 0.5            (n = 6)</td>
</tr>
<tr>
<td>Left</td>
<td>3.3 ± 0.5</td>
<td>(n = 6)</td>
</tr>
<tr>
<td>Right</td>
<td>4.0 ± 1.0</td>
<td>(n = 6)</td>
</tr>
<tr>
<td>Nonpressor (n = 10)</td>
<td>Basal</td>
<td>1st</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>36 ± 1</td>
<td>34 ± 1            (n = 9)</td>
</tr>
<tr>
<td>Body wt (g)</td>
<td>263 ± 6</td>
<td>262 ± 10           (n = 9)</td>
</tr>
<tr>
<td>Heart wt (mg/100 g body wt)</td>
<td>323 ± 5</td>
<td>3.5 ± 0.5           (n = 9)</td>
</tr>
<tr>
<td>Left</td>
<td>3.5 ± 0.5</td>
<td>(n = 9)</td>
</tr>
<tr>
<td>Right</td>
<td>4.9 ± 0.8</td>
<td>(n = 9)</td>
</tr>
<tr>
<td>Pressor (n = 10)</td>
<td>Basal</td>
<td>1st</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>33 ± 1</td>
<td>38 ± 3*            (n = 9)</td>
</tr>
<tr>
<td>Body wt (g)</td>
<td>265 ± 5</td>
<td>224 ± 11           (n = 9)</td>
</tr>
<tr>
<td>Heart wt (mg/100 g body wt)</td>
<td>390 ± 12</td>
<td>3.1 ± 0.5           (n = 8)</td>
</tr>
<tr>
<td>Atrial ANF (μg/mg protein)</td>
<td></td>
<td>3.1 ± 0.5           (n = 8)</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Hct = hematocrit; nonpressor = Ang II, 200 ng/kg/min; pressor = Ang II, 800 ng/kg/min.

### Table 3. Effect of Chronic Infusion of Ang II on PRA and Plasma Aldosterone

<table>
<thead>
<tr>
<th>Group</th>
<th>PRA</th>
<th>PA (ng/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.57 ± 0.07</td>
<td>20.9 ± 3.1</td>
</tr>
<tr>
<td>Nonpressor</td>
<td>0.46 ± 0.37</td>
<td>27.2 ± 5.9</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Ang I = angiotensin I; PA = plasma aldosterone; nonpressor = Ang II, 200 ng/kg/min; pressor = Ang II, 800 ng/kg/min.

\(* p < 0.05, \dagger p < 0.01, \text{compared with basal and control values.}\)
Incubation of Atrial Slices

Incubation of atrial tissue for 30 minutes with increasing doses of Ang II ranging from $10^{-8}$ to $10^{-3}$ M did not modify ANF release into the medium. ANF concentration was rather constant within and between each experiment, with a control level of $75.7 \pm 10.7$ ng/ml (mean $\pm$ SEM; $n = 8$; $F_{4,35} = 0.05$). The mean percent degradation of total immunoreactive Ang II for each concentration was $44 \pm 6$ (SEM), $40 \pm 4$, $26 \pm 6$, and $17 \pm 5\%$, for $10^{-4}$, $10^{-3}$, $10^{-2}$, and $10^{-1}$ M concentrations, respectively.

with LVEDP ($r = 0.49$, $p < 0.001$) but not with CVP ($r = -0.10$, $p = \text{NS}$).

**Discussion**

Angiotensin is a powerful pressor agent that produces a prompt increase in total systemic vascular resistance and systemic arterial pressure, more likely as a result of systemic arteriolar vasoconstriction. With the rise in systemic arterial pressure, which represents an elevation of cardiac afterload, heart rate is slowed reflexively and end-diastolic pressure (or filling pressure) is enhanced.

The effects of angiotensin on the pulmonary vascular bed and CVP differ from one report to another. Doses that are adequate to elevate systemic pressure in anesthetized cats and dogs increase pulmonary arterial pressure only slightly or not at all. Since the pulmonary vessels in cats and dogs are relatively insensitive to angiotensin, the rise in pulmonary arterial pressure could be a passive phenomenon, because of the elevation of left atrial pressure. Results in rats not only differ from those obtained in other species, but also present much more variability. Following angiotensin infusion, blood flow to the lungs could in turn be increased or decreased or remain unchanged. In this context, our experiments are in disagreement with the data of Katsube et al., who reported an initial transient elevation of CVP with continuous i.v. infusion of Ang II in rats. However, the doses infused in their studies were much higher than the pressor amounts used in the present investigations. Thus, the hemodynamic effects of angiotensin could depend on the heart's ability to increase left ventricular work against pressure in order to prevent passive rises in the pulmonary vascular bed and right-sided heart pressures.

The nonpressor dose of Ang II used in our acute experiment failed to increase right atrial pressure, LVEDP, or ANF levels. However, when a pressor dose was administered, LVEDP and plasma ANF followed a parallel time course and both were positively correlated. Thus, Ang II infusion in the rat stimulates ANF release only when there is a rise in left ventricular pressure, even in the absence of a change in CVP. These results suggest that the hemodynamic alterations induced by Ang II may be responsible for ANF secretion and that afterload (increased LVEDP and, hence, left atrial pressure) may activate its release from the left atrium.

Other evidence also suggests that hemodynamic changes are necessary for ANF secretion upon administration of pressor agents. Experiments in our own laboratory have demonstrated that chronic infusion of subpressor doses of phenylephrine does not modify plasma ANF levels. Manning et al. have shown that a significant rise in plasma ANF concentrations can be produced by bolus administration of arginine vasopressin, phenylephrine, or Ang II, but not by nonpressor derivatives of arginine vasopressin. Katsube et al. have suggested that the observed changes in plasma ANF during treatment with pressor agents are more closely correlated with an increase in CVP. This assumption is based on the finding that acute continuous infusion of Ang II induces a sustained ele-
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... of LVEDP, whereas there is only a transient initial augmentation of both CVP and plasma ANF. However, CVP later decreases to subbasal levels, which could contribute to the finding that plasma ANF does not remain elevated despite the sustained increase in LVEDP. Nevertheless, it is possible that this reduction in CVP could only decrease circulating ANF by an amount equivalent to the basal concentration and would not offset completely the large initial increase in ANF observed during the infusion of Ang II. Therefore, the reason for the discrepancy of results between their study and ours is not obvious and may be related to the difference in the pressor dose used (a higher dose may initially deplete ANF stores), the use of different anesthetics (Katsube et al.30 used chloral hydrate), or other dissimilarities in experimental protocols. Thus, both atria could be involved in the ANF release induced by hemodynamic changes: an increase in preload (as in volume expansion or water immersion) may stimulate ANF release mainly from the right atrium, whereas an augmentation of afterload (as during elevated systemic arterial pressure) may evoke ANF secretion mainly from the left atrium. This latter hypothesis is also suggested by the finding that, in the hypertensive rat, it is mainly the left atrium that is found to have a lower atrial tissue ANF content.33,34

As in our acute experiment, the chronic i.p. infusion of a nonpressor dose of Ang II failed to produce significant increases in plasma ANF levels. This finding is consistent with the hypothesis that ANF release during the administration of a pressor agent is induced by an indirect effect, through heightened atrial pressure, rather than by direct receptor stimulation. However, the situation here is somewhat more complex than in the acute experiment, because of all the implications that the renin-angiotensin-aldosterone axis, which operates on a long-term basis, may have on body fluid regulation. Both angiotensin and aldosterone can cause changes in urine flow and sodium excretion that can affect extracellular fluid volume and, therefore, possibly ANF release. Indeed, escape from mineralocorticoid-induced renal sodium retention appears to be mediated largely by extracellular fluid volume expansion, a phenomenon in which ANF could play a role.35

The effects of angiotensin on urine flow and sodium excretion are dose-dependent. Small doses of angiotensin (producing only minimal or no pressor responses) elicit a decline in urine flow and sodium excretion attributed to renal vasoconstriction. With larger doses, the diuretic effects of increased arterial pressure may predominate (pressure diuresis). In our chronic experiment, neither nonpressor nor pressor doses of Ang II produced significant changes in urinary sodium excretion or urine volume. However, animals infused with the pressor dose showed a tendency to a slight natriuresis, as indicated by a level of significance of p less than 0.1 between the pressor dose and the two other ones for the first 24 hours after the start of the infusion. Moreover, the pressor-dose group presented a higher hematocrit and a lower body weight, suggesting a fluid loss.

In the present investigation, plasma aldosterone concentration following 7 days of chronic i.p. Ang II infusion was significantly increased only in the group receiving the pressor dose. The exogenous Ang II-induced steroidogenesis observed in the pressor group (see Table 3) and the salt-retaining effects of aldosterone could explain why the chronic administration of Ang II did not evoke a significant natriuretic and diuretic response in these animals.

Thus, there is little evidence that the increase in plasma ANF levels, noted in the present study during chronic infusion of the pressor dose of Ang II, is mediated by mineralocorticoid-induced extracellular fluid volume expansion (thus contributing to mineralocorticoid escape), since rats receiving the pressor dose did not show any signs of antidiuresis or antinatriuresis in the first two 24-hour postoperative periods preceding the first elevated plasma ANF measurement (Figure 2) and since rats given the pressor dose had a significant weight loss accompanied by an increase in hematocrit, which would instead be related to extracellular fluid volume depletion. Although salt intake was not assessed for a more accurate evaluation of water and sodium balance, the previous experimental observations cannot be easily interpreted as being associated with a positive sodium balance (as in aldosterone-induced renal sodium retention).

From our in vivo experiments, we can see that doses of Ang II that are unable to elevate blood pressure are also incapable of stimulating ANF release. However, atrial Ang II receptors may only be activated by doses of Ang II that increase blood pressure. Incubation of atrial slices with Ang II provides the opportunity to expose atrial tissue to high doses of Ang II without the effect of increased atrial pressure, thus making a disassociation between pressor doses of Ang II and hemodynamic parameters. Incubation of atrial slices with Ang II did not produce any effect on ANF release, suggesting that even with doses that increase blood pressure, a positive Ang II–ANF feedback mechanism is not operant.

Systemic arterial pressure was more closely correlated with plasma ANF in the acute than in the chronic experiment. Systemic arterial pressure elevation represents an afterload increase that, by raising LVEDP and, consequently, left atrial pressure, may stimulate ANF release. Since CVP was not measured during the chronic experiment, the possibility that changes in this parameter may have had an effect on ANF secretion cannot be ruled out. Also, the possibility that some other factor, such as force of contraction and heart rate, could influence ANF release may account for the level of correlation found in both acute and chronic experiments.

In summary, chronic administration of Ang II produced a substantial rise in ANF release that was not related to mineralocorticoid-induced extracellular fluid volume expansion but was associated with an elevation of systolic BP. Acute Ang II infusion stimulated ANF secretion only when there was a concomitant rise in left atrial pressure and even in the absence of a
modification in CVP. Incubation of atrial slices with high concentrations of Ang II did not modify ANF release. It is therefore suggested that the mechanism by which Ang II induces ANF release is through a hemodynamic mechanism rather than through a direct receptor stimulation. Furthermore, afterload rather than preload increases appear to be the stimulus for Ang II–induced ANF release, indicating that both atria could be independently implicated in the ANF secretion evoked by hemodynamic changes.

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