Angiotensin II–Induced Atrial Natriuretic Factor Release in Dogs Is Not Related to Hemodynamic Responses

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Angiotensin II (Ang II) and atrial natriuretic factor (ANF) appear to act as functional antagonists in the regulation of fluid and electrolyte homeostasis and blood pressure. To further define the relations between these hormones in vivo, we investigated the effect of low doses of Ang II (1–10 ng/kg/min) on plasma ANF levels. We also evaluated the influence of ANF release on the renal and hormonal responses to Ang II. Studies were performed in anesthetized and conscious instrumented dogs during sustained saline load and converting enzyme inhibition. In the anesthetized dogs, Ang II significantly increased plasma ANF levels and ANF arteriovenous difference without changing either atrial pressures or hematocrit. In both conscious and anesthetized dogs, ANF increases were not correlated with blood pressure responses to Ang II and did not occur in control groups when Ang II was replaced by vehicle. Ang II–induced sodium retention and stimulation of aldosterone production were attenuated, and renin suppression was enhanced in dogs having the largest changes in plasma ANF in response to converting enzyme inhibition or Ang II. These results demonstrate that in volume-replete dogs Ang II can promote ANF release independently of changes in atrial pressures or systemic hemodynamics, suggesting that Ang II may exert a significant modulatory effect on ANF secretion. The results also show significant relations between ANF and renal and adrenal responses to Ang II, which may suggest that, in turn, endogenous ANF modulates the effects of Ang II (Circulation Research 1990;67:774–779).

Atrial natriuretic factor (ANF) appears to act as a functional antagonist of the renin-angiotensin system in the control of fluid balance,1 because it has been shown to inhibit release of renin,2 aldosterone,3,4 and vasopressin5 and to inhibit thirst.6 In addition, ANF antagonizes angiotensin II (Ang II)–induced vasoconstriction both in vitro7 and in vivo.8

Large pressor doses of Ang II have been shown to promote ANF release in vivo.9–11 These pharmacological responses have been attributed to hemodynamic effects of Ang II leading to increased atrial stretch, which appears to be the major signal mediating ANF secretion.9–12 The design of those studies does not, however, permit one to exclude the possibility that more physiological levels of Ang II might modulate ANF secretion independently of its systemic hemodynamic effects.

The purpose of the present study was to evaluate the effects of low doses of Ang II on ANF secretion and on systemic hemodynamics in dogs. We also examined the relations between ANF, renal, and adrenal responses during Ang II infusion. To achieve relative stability of hormonal and renal parameters as well as to minimize the influence of changes in atrial wall stretch or endogenous Ang II, the study was performed during the steady-state phase of sustained volume expansion and converting enzyme inhibition.

Materials and Methods

The experiments were performed on female mongrel dogs, weighing between 18 and 22 kg, fed a standard diet. The protocol and the size of the experimental sample were approved by the Institutional Animal Care and Use Committee. In 12 dogs the experiments were performed during anesthesia induced with sodium pentobarbital (30 mg/kg i.v.) and maintained relatively constant by periodic administration of 5 mg/kg/hr. This anesthesia proto-
col does not alter hemodynamics over 8 hours of observation. The temperatures of the room and the animals were kept constant at 22° C and 37° C, respectively.

After placement of an endotracheal tube, a catheter was positioned into the abdominal aorta for continuous monitoring of pulsatile and mean arterial pressure, and a Swan-Ganz thermodilution catheter was placed in the pulmonary artery for continuous measurement of right atrial and pulmonary capillary wedge pressures. Cardiac output and the derived parameters were obtained from the average of three to five thermodilution curves. Heart rate was continuously recorded.

Blood samples for measurement of plasma levels of ANF, renin activity (PRA), aldosterone, and hematocrit were obtained from the arterial line, while blood for venous plasma ANF and cyclic GMP (cGMP) was obtained from a catheter positioned in the inferior vena cava. A Foley catheter was inserted into the bladder for urine collection.

To evaluate the possible influence of anesthesia, studies were also performed in a separate group of awake dogs (n=5) previously instrumented with vascular catheters routed subcutaneously to the dorsal neck area and exteriorized under aseptic conditions. These animals were studied by the same protocol while resting quietly in loose canvas sling supports.

During placement of catheters and throughout the study, the animals received an infusion of saline (0.3 ml/min) containing 50 μCi/ml [14C]inulin and 200 μCi/ml p-amino-[3H]hippuric acid for determination of the clearance of inulin and of PAH, respectively.

After three 15-minute baseline clearance periods, the animals received a rapid saline load (2.5% of body weight of 0.9% NaCl over 45 minutes); subsequently, three additional 15-minute clearances were performed. At the end of each period and throughout the experiment, urinary and blood losses were replaced with saline. After urine output appeared to be constant, endogenous formation of Ang II was inhibited by an intravenous dose of enalaprilat (0.15 mg/kg body wt) reported to produce immediate and sustained inhibition of angiotensin-converting enzyme (CEI) in plasma as well as in tissues. In our study, the mean blood pressure response to exogenous Ang I (400 ng i.v. bolus) was 23±3 mm Hg before CEI and 2±1 mm Hg (p<0.001) at the end of the study. CEI was performed to avoid the changes in endogenous Ang II formation that usually accompany infusions of Ang II.

After three additional collection periods, Ang II was administered as graded, constant infusion at rates of 1, 2.5, 5, and 10 ng/kg/min for 30 minutes each; two clearances were performed during each dose. Longer infusion of the same four doses of Ang II (60 minutes) produced responses similar to those observed at 30 minutes (data not shown). After the highest dose, a 45-minute recovery period was allowed. A time-control group (n=4) served to assess spontaneous variations occurring in each of the parameters during the course of an infusion of vehicle alone (0.9% NaCl). PRA was determined by radioimmunoassay of generated Ang I expressed as nanograms per milliliter per hour. Plasma aldosterone (nanograms per deciliter) was determined by radioimmunoassay (RLS, Carson, Calif.). Plasma cGMP was determined by radioimmunoassay after ethanol extraction of plasma with a commercial kit (Amersham, Arlington Heights, Ill.). Plasma immunoreactive ANF was measured by radioimmunoassay after extraction on C18 Sep-Pak cartridges (Waters, Millipore Corp., Milford, Mass.) as recently described except that bound and free peptide were separated by the addition of goat-antirabbit immunoglobulin G coupled to a solid support. The sensitivity of assay was 0.5 fmol/tube with a 50% inhibitory concentration of 8.1±0.4 fmol/tube.

Analysis of variance (two-way ANOVA) followed by Dunnett’s t test for selected comparisons was used for normally distributed data. Friedman’s test was used for data not normally distributed. Similarly, the unpaired t test and Mann-Whitney U test were used as appropriate to compare observations obtained in independent groups. Spearman rank correlation coefficients were used to assess relations between variables that did not have a normal distribution of data. All values are expressed as mean±SEM.

**Results**

Figure 1 shows the behavior of plasma ANF and hemodynamic parameters in anesthetized dogs. Volume expansion increased right atrial and pulmonary wedge pressures and both arterial and venous plasma ANFs, as well as the arteriovenous difference across the heart (from 4.1±1 to 6.3±2 fmol/ml). These changes were associated with the expected hemodilution. CEI did not significantly modify atrial pressures and plasma ANF levels. Detailed analysis showed that CEI indeed reduced arterial ANF levels in four dogs, while it did not change or increase ANF in the remaining four animals.

Graded infusion of Ang II caused progressive increases in blood pressure and systemic vascular resistance, which became significant at 2.5 ng/kg/min. Opposite changes in stroke volume occurred (Figure 1). Right and left atrial pressures and hematocrit were unchanged during Ang II infusion (Figure 1).

Despite this, both arterial and venous plasma ANF levels rose progressively (arterial: from 33.6±6 to 39.2±11, 55±20, 68.6±20, and 80.3±23 fmol/ml). There was also a significant increase in the arteriovenous difference of ANF (from 7.5±1 to 10.1±3.5, 21.5±11, 26.6±12, and 24±10 fmol/ml; F=6.07, p<0.05), suggesting increased secretion of the peptide. The magnitude of ANF response to a given Ang II dose was variable, ranging from 23% to 294% increase at the highest dose. The most marked increases in plasma ANF levels were recorded in the same four animals that showed a decrease in ANF levels after CEI. Most of the parameters returned toward baseline by 45 minutes of recovery.
No significant correlations \((p>0.05, \ n=28-32)\) were found between plasma ANF levels and systemic mean blood pressure \((r=-0.134)\), or between the changes in systemic mean blood pressure and arterial ANF during Ang II infusion \((r=0.173)\) (Figure 2). Similarly, during Ang II infusion, plasma ANF levels were not correlated with systolic \((r=-0.402)\), diastolic \((r=0.103)\), or mean blood pressure \((r=-0.134)\); right atrial pressure \((r=0.345)\); cardiac output \((r=-0.217)\); or pulmonary wedge pressure \((r=0.362)\).

The effects of Ang II or vehicle alone on hormonal and renal variables are summarized in Figure 3. Baseline values of each parameter were comparable in the experimental and control groups. Volume expansion and CEI caused the expected changes in PRA and plasma aldosterone, arterial ANF, urine flow rate, sodium excretion rate, and fractional excretion of sodium. Ang II infusion produced a dose-dependent increase in plasma aldosterone and reduction in PRA, in parallel with the rise in ANF. These changes were associated with significant and progressive reductions in sodium excretion rate and fractional excretion of sodium (Figure 3). The clearance of PAH/hematocrit fell from 284±29 to 227±30, 210±22, 197±27, and 207±31 ml/min, recovering to 233±30 ml/min, whereas clearance of inulin/PAH clearance of PAH gradually increased (49±3% to 59±3%, 61±2%, 67±2%, and 64±1%, recovering to 61±4%). The maintenance of CEI throughout the experiment is demonstrated by the observation that in the control group the increase in PRA and suppression of plasma aldosterone were still sustained 150 minutes after treatment with enalaprilat. ANF levels were stable in the time controls (Figure 3).

Among the effects of Ang II infusion on other parameters, it is noteworthy that plasma cGMP increased in parallel with arterial ANF levels (from 11.3±6 to 10.6±1, 13.3±1, 13.3±1, and 14.0±1 pmol/ml; \(p<0.05\)) and that the changes in these two parameters were correlated \((r=0.457, \ n=28, p<0.05)\).

The responses evoked by volume expansion, CEI, and Ang II in the conscious dogs were qualitatively similar to those observed in the anesthetized dogs. The ANF and plasma aldosterone responses to Ang II appeared to be less marked than in the anesthetized dogs. Nonetheless, Ang II induced a progressive increase in plasma ANF levels (17.3±2.8%, 31.8±7.7%, 37.2±12%, and 39.1±10% with the graded doses of Ang II) \((F=5.621, p<0.05).\) Also, in the awake dogs, the Ang II–induced changes in ANF and in systemic blood pressure were not significantly correlated \((r=0.184, \ NS).\) After discontinuation of Ang II infusion, plasma ANF levels returned toward baseline.

A significant positive correlation was found between the changes in arterial ANF levels and the

**Figure 1.** Effects of angiotensin II (Ang II) infusion on plasma atrial natriuretic factor (ANF) concentrations and on hemodynamic parameters in anesthetized dogs \((n=8).\) The horizontal bars indicate the periods of maintained volume expansion (saline) and converting enzyme inhibition (CEI). Significant values of F (Friedman's test) were obtained for arterial \((F=26.3, \ p<0.001)\) and venous \((F=18.9, p<0.01)\) ANF levels during Ang II infusion. Significant F values (analysis of variance) were also obtained for mean blood pressure (MBP) \((F=13.9, p<0.001)\), systemic vascular resistance (SVR) \((F=9.4, p<0.001)\), stroke volume (SV) \((F=8.1, p<0.001)\), and heart rate (HR) \((F=3.2, p<0.05)\). Right atrial pressure (RAP), pulmonary wedge pressure (PWP), and hematocrit (Hct) did not change significantly during Ang II infusion. *p<0.05 vs. CEI; †p<0.05 vs. pre-saline control.

**Figure 2.** Lack of correlation between the changes in systemic mean blood pressure (MBP) and the changes in arterial atrial natriuretic factor (ANF) levels during angiotensin II infusion in anesthetized dogs \((r=0.1473, \ NS).\)
corresponding changes in the sodium excretion rate $(r=0.404, n=32, p<0.05)$, whereas the changes in arterial ANF were inversely correlated with the corresponding changes in plasma aldosterone observed during ANF infusion $(r=-0.519, n=32, p<0.05)$.

Further analysis of the relations between ANF and the renal and adrenal responses to Ang II is presented in Figure 4. The animals were divided into two groups of four dogs each according to the response of plasma ANF levels to CEI. The first group included the dogs showing a decrease in arterial ANF levels in response to CEI, which also showed the greatest ANF increase for each dose of Ang II ("ANF responders"). The second group included the dogs in which CEI did not cause a reduction in plasma ANF levels and in which Ang II caused smaller ANF increases. Baseline values of ANF and of other parameters were not different in the two subgroups (see legend to Figure 4). Figure 4 shows that in the ANF responders, plasma aldosterone failed to increase in response to any dose of Ang II administered. This is in contrast to the marked plasma aldosterone response (to 300% and 500% of control) at the two highest rates of Ang II infusion in the dogs having smaller ANF responses. PRA fell significantly in both subgroups, but a much greater fall was associated with the largest ANF responses, since at 2.5 and 5 ng/kg/min, PRA fell to 35% and 20% of control (versus 80% and 55% of control in the less responsive dogs).

The sodium-retaining effects of Ang II also varied, although net sodium retention occurred in all animals. A dose-dependent fall in the sodium excretion rate with increasing doses of Ang II was observed only in the dogs having lesser ANF response, while in the ANF responders there was no further sodium retention beyond the first dose of ANF. At 5 and 10 ng/kg/min, the sodium excretion rate had fallen to only 75% of control in the ANF responders, while it was as low as 30% of control in the low ANF responders. This different pattern was observed despite a comparable rise in systemic blood and atrial pressures in the two subgroups (Figure 4).

**Discussion**

We examined the effect of low doses of Ang II on plasma ANF levels in volume-replete anesthetized or conscious dogs and demonstrated stimulation of ANF secretion, which could not be accounted for by hemodynamic responses to Ang II. The doses of Ang II used in our study, which should result in circulating levels within the physiological range, 17 were able to increase plasma ANF without changing atrial pressures, heart rate, or hematocrit. While blood pressure did increase, the rise in ANF was not correlated with the associated changes in systemic blood pressure. Our study suggests that the rise in plasma immunoreactive ANF induced by Ang II is due to an increase in its secretion rather than a decrease in its metabolic clearance rate, since the arteriovenous differences of ANF across the heart also increased during Ang II. Therefore, Ang II increases secretion of ANF by mechanisms other than its systemic hemodynamic actions, although it cannot be completely ruled out that the increase in systemic blood pressure may represent a contributory factor, especially at high doses of Ang II. 9–11 The results obtained in awake dogs, though less pronounced, were similar to those in anesthetized dogs, indicating that anesthesia per se was not responsible for Ang II–induced increase in plasma ANF.

A possible direct effect of Ang II on ANF release is suggested by previous studies. Thus, Ang II has been reported to stimulate ANF release from isolated atrial appendages, 18 and results consistent with
a direct secretory effect of Ang II have recently been obtained in our laboratory in superfused rat atrial minces. A direct action of Ang II may depend on increased tension in the myocyte, because Ang II prolongs the plateau phase of the action potential in atrial myocytes, thus increasing inward calcium fluxes and force of contraction. It is also possible that the stimulatory effect of Ang II on ANF release is amplified by volume expansion. In fact, it has been reported that intracerebroventricular administration of Ang II enhances ANF secretion induced by volume loading but does not affect basal plasma ANF levels.

The considerable variability in the ANF response to Ang II or to CEI suggests that multiple factors modulate ANF secretion. Although correlations do not imply causality, the analysis of the relations between the induced changes in ANF and in other parameters suggests that the Ang II–induced rise in endogenous ANF simultaneously modulates adrenal and renal responses to Ang II. Because ANF administration causes natriuresis and specifically antagonizes Ang II–induced aldosterone production, it may be hypothesized to be a counteracting effect of circulating ANF on the sodium-retaining and aldosterone-stimulating actions of Ang II. This is supported by the analysis based on the different responsiveness of the animals to manipulation of Ang II levels. Thus, it was only among the animals showing small changes in ANF release in response to Ang II that aldosterone rose as expected; those having marked ANF responses exhibited no increase in plasma aldosterone, even at the highest dose of Ang II. Similarly, animals having the most marked ANF responses had a much less sodium-retaining response to Ang II and also showed enhanced suppression of PRA. The pressor responses to Ang II were comparable in animals showing large or small ANF changes, suggesting that blood pressure differences did not account for different natriuretic or ANF responses in the two groups. Together with the parallel rise in plasma cGMP during Ang II infusion, these observations suggest that increases in plasma ANF within the physiological range may have biological relevance with respect to volume regulation.

In conclusion, our study demonstrates that Ang II–induced stimulation of ANF secretion occurs independently of the changes in atrial pressures and is not correlated with the increase in systemic arterial pressure. Our data also suggest that the Ang II–induced rise in plasma ANF levels modulates three major actions of Ang II, appearing to counteract the sodium-retaining effect and the increase in aldosterone production by Ang II and to potentiate the suppression of renin release. This finding may have particular relevance to pathological states in which increased levels of Ang II and ANF coexist.

Acknowledgments

The authors wish to thank Rose Aceto, Henrietta Manapat, Philip Karg, Ana Sjoberg, Carmen Chaves, Anna Christian, and Audrey Kauff for technical support and Linda Stackhouse for assistance in preparing the manuscript.

References


**Key Words** • renin-angiotensin-aldosterone system • kidney • body fluid homeostasis • hemodynamics
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Circ Res. 1990;67:774-779
doi: 10.1161/01.RES.67.3.774

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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