The Effect of Atrial Natriuretic Factor on Blood Pressure, Heart Rate, and Renal Functions in Conscious, Spontaneously Hypertensive Rats

Miklos Gellai, Robin E. DeWolf, Lewis B. Kinter, and Reinier Beeuwkes III

SUMMARY Atrial natriuretic factors, polypeptides released by atrial myocytes, may play a role in the control of blood pressure and the regulation of renal salt and water excretion. Our studies were designed to assess the role of a synthetic peptide, atriopeptin II, on blood pressure and heart rate, renal hemodynamics, and salt and water excretion in conscious, spontaneously hypertensive rats and in normotensive Wistar-Kyoto rats. Changes in mean arterial pressure and heart rate were recorded following intravenous bolus injections (0.1, 1.0, 10, 100 μg/kg) of atriopeptin II in 5 spontaneously hypertensive and 5 Wistar-Kyoto rats. In a second group of rats the peptide was infused for 90 minutes in two different doses: low dose, 1 μg/kg + 2 μg/kg/hr; and high dose, 10 μg/kg + 20 μg/kg/hr. Bolus injections of atriopeptin II resulted in dose-dependent decreases in mean arterial pressure in the hypertensive, but not in the normotensive, rats; heart rates remained unchanged. Blood pressure decreased gradually during the sustained infusion of both doses of atriopeptin II, with the spontaneously hypertensive strain showing increased sensitivity compared to the Wistar-Kyoto strain. Heart rate decreased in both strains during infusion of the high dose; the decrease was significant only in the hypertensive rats. The low dose of atriopeptin II increased the clearance of free water in both strains of rats; sodium excretion was increased only in the hypertensive rats. The high-dose atriopeptin II was associated with transient natriuresis, unaltered glomerular filtration rate, and decreased effective renal blood flow in both strains. These results 1) show increased sensitivity of spontaneously hypertensive rats to the hypotensive action of atrial natriuretic factor, 2) do not support the hypothesis that an increased glomerular filtration rate is a prerequisite to the natriuretic effect of atrial peptide, and 3) suggest that atriopeptin II may exert separate and dose-dependent effects on renal water and sodium excretion in conscious rats. (Circulation Research 1986;59:56-62)

Key Words • atrial natriuretic factor • atriopeptin II • blood pressure • natriuresis • conscious SHR rats • conscious WKY rats

The pioneering studies of deBold and associates have demonstrated that injection of extracts of rat atria into rats was associated with significant increases in the renal excretion of sodium and water. Since then many "atrial natriuretic factors" (ANFs) have been isolated, purified, and synthesized. All of the factors reported to date are peptides and share substantial structural homologies and biological activities. Atrial natriuretic factor is presumably released from atria under conditions of salt excess and/or volume expansion. However, the circulating peptide (and thus the presumed hormone) has not yet been identified.

Despite the impressive list of publications on the subject, understanding of the physiological and possible pathophysiological role of the atrial peptides is limited. That the atrial extracts and synthetic peptides increase the excretion of sodium in anesthetized animals and isolated kidney preparations has been confirmed by many investigators (reviewed by Napier and Blaine). In vitro studies show that some of the peptides relax vascular smooth muscle. Still other evidence indicates that ANF (or the lack of it) may play a role in maintenance of high blood pressure.

The aim of the current studies was to systematically examine the effect of a synthetic ANF on hemodynamic and renal function in adult genetically hypertensive (SHR) and normotensive (WKY) rats. The 23 amino acid peptide, atriopeptin II (APII), was used. This peptide was first purified and described by Currie and associates and was shown by the same authors to have natriuretic and vasorelaxant activities similar to those of atrial extracts in rats.

In order to avoid possible effects of anesthesia and postsurgical stress, the experiments were performed in conscious, chronically instrumented rats. The preparation allowed continuous recording of blood pressure, heart rate, collection of urine, infusion of drugs, and maintenance of fluid balance in conscious trained rats weeks or months after surgery. Studied under these rigorous conditions, APII administration was associated with reductions in blood pressure without reflex tachycardia. Separate and dose-dependent effects of APII on renal water and sodium handling were observed. In addition, APII increased renal salt excretion in the absence of changes in glomerular filtration rate and in spite of a significant decrease in renal blood flow.
Materials and Methods

Experiments were performed on young adult (16-20 weeks old) male, Okamoto strain, spontaneously hypertensive (SHR) and normotensive Wistar-Kyoto (WKY) rats (Charles River Breeding Laboratories, Wilmington, Mass.) The surgical preparation and the experimental set-up, which have been previously described, were slightly modified. Under ketamine (60 mg/kg, IM) and pentobarbital (20 mg/kg, IP), anesthesia catheters were implanted in the abdominal aorta and vena cava via the left femoral artery and vein. To facilitate replacement of excessive fluid loss, an additional medical-grade Tygon catheter was placed in the stomach at the left extremity of the greater curvature. All catheters were exteriorized behind the neck, filled with a 1:1 mixture of 50% dextrose and heparin (1000 U/ml), and plugged with a stainless steel pin. To allow for continuous collection of urine, a Silastic-covered stainless steel cannula was sewn in the urinary bladder, exteriorized through the ventral abdominal wall, and plugged with a Silastic-covered pin. No experiments were conducted until 6-7 days after surgery, by which time the rats had fully recovered and, as was shown in a previous study, renal functions had stabilized. The body weights of the rats at surgery and on the day of experiment were 281 ± 8.2 g and 288 ± 5.1 g, respectively (mean ± SEM).

The rats were accustomed to an experimental cage (Model ECU, Braintree Scientific, Inc., Braintree, Mass.) before surgery and during the recovery period. After surgery they were housed individually and had continuous access to food (Formula 500, Ralston Purina Co., St. Louis, Mo.) and water until the day of experiment.

For the experiments the rats were placed in the plastic experimental cage. The arterial catheter was attached to a Statham pressure transducer (Model P23G6) and recorder (Dynograph, Beckman Instruments) for monitoring arterial pressure and heart rate. The venous line was connected to a syringe for bolus injection, or to infusion pumps via miniaturized manifold for the continuous infusion of fluids and drugs. During the renal clearance studies, the stomach tube was attached to a syringe filled with tap water, and the bladder cannula was extended with polyethylene tubing to facilitate collection of urine in pretrained tubes. Body weight was recorded before and after the experiment; the final weight was corrected for fecal loss.

Atriopeptin II (APIII) was purchased from Peninsula Laboratories, Inc. (Belmont, Calif.). The purity of the peptide was checked by high-performance liquid chromatography (HPLC) and amino acid analysis (84% peptide). For storage, APIII was dissolved in 0.05 M acetic acid in a concentration of 2 mg/ml, divided into 50-μl aliquots, frozen, and stored at -20°C. For experiments, the aliquots were diluted in 5% dextrose to the required concentrations. The experiments were performed on WKY and SHR rats in a random order. Preliminary studies showed that there was no detectable difference in the biological activities of the "old" (in storage for more than 90 days) and fresh peptide.

Two types of experiments were performed. In the first experiment, bolus intravenous injections of atriopeptin II, in a volume of 50 μl/100 g body weight, were given at 10-minute intervals in the amounts of 0.1, 1, 10, and 100 μg/kg [SHR and WKY rats (n = 5)]. Changes in mean arterial pressure (MAP) and heart rate (HR) were monitored continuously.

In the second experiment performed in a different group, a bolus injection of atriopeptin II was followed by a 90-minute infusion. Two regimens were employed: a 1 μg/kg bolus followed by a 2 μg/kg/hr infusion [low dose (LD)]; and a 10 μg/kg bolus followed by a 20 μg/kg/hr infusion [high dose (HD)] [SHR and WKY rats (n = 5)]. In these experiments, blood pressure, heart rate, changes in renal hemodynamics, and water and electrolyte excretion were measured. The protocol is illustrated in Figure 1. Throughout the experiments, 0.9% NaCl containing 10% polyfructosan (Inutest; Laevosan — Gesellschaft, Linz-Donau, Austria) and 1% PAH (Merck, Sharp and Dohme, West Point, Pa.) was infused at a rate of 20 μl/min. The sodium content of the infusate was measured and amounted to 1 μEq/min/100 g for a 300 g rat. This rate of infusion was selected to replace basal fluid and electrolyte loss in normally hydrated rats. After equilibration for 60 minutes, control clearance periods were begun. At the end of the second 30-minute period, APIII was given as a bolus, followed by continuous infusion. Five minutes were allotted for washout of dead-space (approximately 300 μl) before urine collection was resumed for three further clearance measurements. Arterial blood samples of 150 μl were drawn, plasma was separated, and the cells were resuspended in sterile saline solution and returned to the rat. In most experiments, APIII infusion caused an increase in urine flow exceeding the rate of infusion (20 μl/min). When the excess loss reached 2 ml, it was replaced via the intragastric catheter with lukewarm water. The replacement was slow (1 minute), and had no effect on blood pressure and heart rate. Urine volume was measured gravimetrically.

**Figure 1. Schematic representation of the experimental protocol.**

![Experimental Protocol Diagram](image-url)
Spectrophotometric methods were used to determine the urinary and plasma concentration of polyfructosan and PAH. Osmolality was measured with a vapor pressure osmometer (model 1500C, Wescor, Logan, Utah); electrolytes and urea, by a multi-channel electrolyte and chemistry analyzer (IL System 508, Instr. Lab. Inc., Edison, N.J.). Glomerular filtration rate (GFR) was estimated from the renal clearance of polyfructosan, effective renal plasma flow (ERPF) from the clearance of p-aminohippurate. Clearance and excretion rates are expressed per 100 g body weight. Effective renal blood flow (ERBF) was calculated as ERPF/(1-hematocrit).

Statistical comparisons were made with the Dunnett multiple-range test. Values are presented as mean ± SEM. A p value of <0.05 was considered statistically significant.

Results

Bolus Injection of Atriopeptin II

Control values for mean aortic pressure (MAP) and heart rate in WKY and SHR rats were 123 ± 22 mm Hg and 405 ± 22 beats/min, and 179 ± 8 mm Hg and 449 ± 16 beats/min, respectively. Bolus injection of APII resulted in a dose-dependent decrease in MAP in SHR rats but had no observable hypotensive effect in WKY rats (Figure 2A). Maximum effect in SHR rats occurred at 2 minutes after injection; time of recovery was dose-dependent, requiring 5-7 minutes after the highest dose. Heart rate was not affected, although a trend toward increased levels was observed in the WKY rats (Figure 2B).

Continuous Infusion of Atriopeptin II

As shown in Table 1, careful replacement of fluid and electrolyte loss ensured the maintenance of body weight, hematocrit, plasma osmolality, and plasma electrolyte concentrations during the 3½-hour experimental procedure.

Mean aortic pressure (MAP) decreased in SHR rats during the infusion of low and high dose of APII (Figure 3B). In WKY rats only the administration of the high dose was associated with lower pressures (Figure 3A). Heart rate decreased during the infusion at high dose in both strains of rats; however, the decrease was significant only in the SHR group (Figures 3C and 3D). The changes in MAP and heart rate were gradual; the downward trend persisted to the end of the 90-minute infusion.

The effects of APII on urinary concentrating ability and renal excretory functions are shown in Figures 4, 5, and 6. Infusion of the low dose of the peptide resulted in a gradual increase in urinary flow with a concomitant decrease in urinary osmolality in both strains of rats (Figure 4). The changes associated with the infusion of the high dose were immediate; maximum rates of urinary flow were reached within 5 minutes following injection of bolus and start of infusion. However, the increase in urine flow was not sustained in the presence of high-dose infusion. By the end of the 90-minute infusion, the values were not significantly different from control (Figures 4A and 4B). The decrements in urine osmolality were larger in the WKY rats, despite higher control values (Figures 4C and 4D).

Control sodium excretion rates for the WKY and SHR groups were .69 ± .16 and 1.16 ± .37 μEq/min·100 g, respectively. Low-dose APII increased sodium excretion only in SHR rats (Figure 5B). The natriuretic effect associated with infusion of high dose paralleled the diuretic action in both strains. The large natriuresis, which was most pronounced in SHR rats during the first 30 minutes, diminished slowly during the remainder of the infusion period (Figures 5A and 5B). A small increase in potassium excretion (not statistically significant) was observed only in WKY rats, and only during the high-dose infusion of the peptide (Figure 5C). Urinary excretion of urea (not shown) remained unaltered in all experimental groups.

Low and high doses of atriopeptin II affected the clearance of free water and total osmoles differently (Figure 6). The increase in urine flow observed during the infusion of low doses of atriopeptin II (Figures 4A and 4B) was the result of selective increase in free water clearance (Figure 6A). Both osmolar and free water clearance increased with the administration of bolus or continuous infusion of atriopeptin II.
TABLE 1. Indices of Fluid Balance During Infusion of Atriopeptin II

<table>
<thead>
<tr>
<th></th>
<th>WKY (n = 5)</th>
<th>SHR (n = 5)</th>
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<tbody>
<tr>
<td></td>
<td>LD</td>
<td>HD</td>
</tr>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>288±9.8</td>
<td>288±10</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>43.5±0.7</td>
<td>44.0±0.5</td>
</tr>
<tr>
<td>P&lt;osm (mOsm/kg H2O)</td>
<td>292±1.6</td>
<td>292±1.7</td>
</tr>
<tr>
<td>PNa (mEq/l)</td>
<td>144±1.2</td>
<td>144±1.2</td>
</tr>
<tr>
<td>PK (mEq/l)</td>
<td>3.9±0.1</td>
<td>4.0±0.1</td>
</tr>
</tbody>
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Values are expressed as mean ± SEM. LD, low dose; HD, high dose; P<osm, plasma osmolality; PNa, plasma sodium concentration; PK, plasma potassium concentration. Where p values were not listed, final values were not significantly different from initial values.

of high doses (Figure 6). Again, the changes were transient; maximum effects were observed during the first 30 minutes.

Control values for GFR and ERBF were higher in WKY rats; GFR, 752 ± 82 (WKY) vs. 630 ± 80 (SHR); ERBF, 5994 ± 845 (WKY) vs. 4538 ± 575 (SHR); all values expressed in microliters per minute per 100 g body weight. Glomerular-filtration rates did not increase significantly in any of the experimental groups; a significant decrease was observed in the WKY rats during the second period of high-dose APII infusion (Figures 7A and 7B). Effective renal blood flow decreased in SHR and WKY rats; the changes were highly significant (p < 0.01) during the infusion of the high-dose APII (Figures 7C and 7D). Filtration fraction (GFR/ERPF) increased in all groups of rats; the increases were significant during high-dose infusion: 22 ± 1% to 33 ± 1% in WKY rats (p < 0.001), 22 ± 1% to 31 ± 1% in SHR rats (p < 0.001). Due to the fact that GFR did not change significantly in any of the groups studied, changes in fractional excretion of water and electrolytes mirrored those reported in absolute values.

Discussion

The experimental conditions in these studies differ markedly from those used in most other in vivo experi-
ments with ANF. First, the experiments were conducted in conscious, trained animals, completely recovered from surgery. Ample evidence indicates that anesthesia and surgery affect vascular smooth muscle tone,\(^{12}\) impair renal hemodynamics,\(^{9}\) and influence renal excretion of sodium and water.\(^ {13}\) Second, urinary losses were carefully replaced during the infusion of atriopeptin II; thus, changes in fluid and electrolyte homeostasis, resulting from increased excretion of salt and water, were prevented.

Under these conditions, injection of atriopeptin II at doses from 0.1 to 100 \(\mu\)g/kg lowered blood pressure in a dose-dependent fashion in SHR rats with no increase in heart rate. The same dose range was not associated with acute blood pressure lowering effects in normotensive WKY controls, suggesting an enhanced sensitivity of the SHR strain to the hypotensive action of APII. This observation was strengthened by the results of subsequent studies in which APII was infused for 90 minutes in two different doses. Although blood pressure decreased during the infusion of both low and high doses of APII in the SHR strain, only the infusion of high doses resulted in hypotensive response in WKY rats.

The absence of hypotensive activity following bolus injection of APII in WKY rats differs from published observations in normotensive rats.\(^ {1,14}\) However, those studies were performed with atrial extracts in anesthetized or acutely stressed animals in which plasma angiotensin II and catecholamine levels were presumably higher than in conscious, trained animals.\(^ {15}\) Camargo and associates proposed that atrial peptides may act as functional agonists or antagonists of angiotensin II or

**Figure 5.** Effect of atriopeptin II on sodium excretion (panels A & B) and potassium excretion (panels C & D) in WKY and SHR rats. Values for each clearance period are represented by the symbols and bars as mean ± SEM. Compared with mean control values: *, \(p < 0.05\).

**Figure 6.** Comparison of the effects of low- and high-dose APII infusion on free water clearance (panels A & B) and osmolar clearance (panels C & D) in WKY and SHR rats. Low-dose APII induced a gradual increase in \(C_{\text{H}_{2}O} (V-C_{\text{osm}})\) without changing \(C_{\text{osm}}\). The transient changes resulting from the infusion of high-dose APII parallel those illustrated in Figures 4 and 5. Symbols and bars represent mean ± SEM. Compared with control values: *, \(p < 0.05\).

**Figure 7.** Effect of atriopeptin II infusion on GFR (panels A & B) and ERBF (panels C & D) in WKY and SHR rats. Values are presented as percent of the averages of the two control periods (mean ± SEM). Compared with mean control values: *, \(p < 0.05\).
norepinephrine, depending on the concentration of these endogenous vasoconstrictors. Such functional selectivity could provide a possible explanation for the lack of blood-pressure-lowering activity of APII (bolus injections and low-dose infusion) in conscious trained WKY rats. Our results are in agreement with the findings of Garcia et al. that the SHR strain is more sensitive to the blood-pressure-lowering action of APII than WKY controls. The mechanisms underlying this difference are unclear but may represent different sensitivity of vascular and/or cardiac APII receptors in the SHR strain. Alternatively, the apparent increased sensitivity of SHR rats may be due to higher concentrations of endogenous atrial peptides. Finally, the different responsiveness to APII may be explained solely by the differences in the geometry of the vasculature (wall-to-lumen ratio) in the two strains of rats (see Folkow).

The decrease in blood pressure was associated with a gradual fall in heart rate with high-dose APII infusion in both strains of rats (Figures 3A and 3B). It was previously suggested that the hypotensive action of ANF is partly due to its effect on reflex responses controlled by chemo- and baroreceptors. Indeed, recently published studies show that the fall in blood pressure following acute administration of ANF is due to decreased cardiac output and not to the vasorelaxant activity of the peptides.

Our findings confirm the potent natriuretic response associated with administration of ANF in rats. The mechanism of this well-documented activity remains controversial, as discussed in a recent review by Maack et al. Proposed mechanisms include 1) increase in renal blood flow, 2) increase in GFR without an increase, or actual decrease in renal blood flow, and 3) direct tubular effect of APII. Careful examination of the literature shows that the magnitude of ANF-associated changes in renal hemodynamics depends on control conditions. Camargo and co-workers have shown that the isolated rat kidney that the increase in GFR with atrial extract injections was inversely proportional to the baseline GFR. Using purified APII, dose-dependent increases in renal blood flow were observed in the high-resistance autoperfused kidney preparation by Oshima et al. As summarized by Napier and Blaine, most investigators using intact, anesthetized animals, when basal GFR is presumably reduced, report an increase in GFR with no change in renal blood flow. Based on these observations, the results from our experiments in conscious, trained rats, in which GFR and renal blood flow are 20–30% higher than those in anesthetized rats might have been anticipated. In these rats, natriuresis associated with infusion of APII was accompanied by an actual decrease in ERBF, without a significant effect on GFR. This may indicate efferent arteriolar constriction (Figure 7). Our results do not confirm the recent observation by Hintze et al. in conscious dogs. Those authors reported that bolus injections of atriopeptin II and III were associated with dose-dependent increases in renal blood flow. However, others have shown that with sustained infusion of ANF in anesthetized and conscious dogs, the initial increase in renal blood flow is followed by an actual decrease. Thus, observations vary regarding the effect of atrial peptides on GFR and renal blood flow, but not on filtration fraction. The significant increase in filtration fraction observed with the infusion of a high dose of APII in our experiments is in agreement with published reports. What cannot be inferred from our data is whether the changes in excretory functions are the result of the increased filtration fraction. Finally, our experimental design does not allow us to address the importance of possible immediate (first 5 minutes of ANF infusion) changes in renal hemodynamics in the mediation of ANF-induced diuresis.

The significant features of the APII-induced changes in renal excretory functions were, first, the selective and transient effects on sodium excretion, and second, the differential and dose-dependent effects on free water and osmolar clearances. The selective stimulation of renal sodium excretion, without kaliuresis, is a seemingly unique diuretic response. Others have reported natriuresis with kaliuresis in rats, dogs, and monkeys. In man, synthetic human ANF was not associated with a significant increase in potassium excretion. The transient nature of the natriuretic response to APII infusion in our experiments was not due to depletion of body fluid volume of sodium content; depletion of a local pool also seems unlikely. The simplest explanation may be that changes in excretion of sodium and water paralleled the changes in renal perfusion pressure, which were decreasing during the infusion of high-dose APII in both strains of rats.

The diuretic response to low-dose infusion of APII in WKY rats is characterized by a selective increase in free water excretion (CH2O), without significant change in Cc, or UrV. A similar response was observed in SHR rats, although a small, but significant, increase in UrV occurred. Here, we would like to reemphasize the importance of maintaining fluid and electrolyte balance during the experimental period. As shown in Table 1, this was achieved in the WKY and SHR rats receiving the low dose of APII. Furthermore, in the WKY rats, total sodium excreted during the 150-minute experimental period was actually less than the amount infused: 154 μEq/100 g vs. 161 μEq/100 g. This, we believe, rules out any possibility of impaired vasopressin release due to changes in water balance. On the contrary, it suggests, but does not prove, inhibition of the vasopressin system by APII. That such inhibition occurs during hemorrhage and dehydration in rats was recently reported by Samson. In addition, APII inhibition of vasopressin-stimulated tubular water reabsorption cannot be excluded at this time. The profile of diuresis associated with the infusion of high-dose APII differs markedly from that observed during low-dose infusion and is similar to observations reported in anesthetized dogs. At high dose the increase in urine flow was associated with large increases in Cc and UrV. On the basis of these observations, we
propose that ANF exerts separate actions on tubular water and salt handling mechanisms. The effect on CH₂O is more sensitive than the effect on Cós, suggesting a possible interaction of the atrial peptides with the release and/or the end-organ action of vasopressin.

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