Atrial Natriuretic Factor Prevents NaCl-Sensitive Hypertension in Spontaneously Hypertensive Rats

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Our previous studies demonstrated that acute infusion of atrial natriuretic factor (ANF) produces an enhanced depressor response in NaCl-sensitive spontaneously hypertensive rats (SHR-S) fed a high (8%) NaCl diet compared with control SHR-S fed a normal (1%) NaCl diet and that dietary NaCl loading increases circulating ANF levels in Wistar-Kyoto (WKY) rats but not in SHR-S. The current study tested the hypotheses that 1) long-term infusion of ANF at a dose that elevates plasma ANF to levels comparable with those seen in high NaCl-fed WKY rats prevents the NaCl-induced exacerbation of hypertension in SHR-S and 2) ANF lowers blood pressure in this model by a sympatholytic effect. Male SHR-S received infusions of ANF (0.1 μg/hr) or vehicle intravenously via osmotic minipump for 3 weeks beginning immediately before initiation of 1% or 8% NaCl diets at age 7 weeks. Chronic ANF infusion prevented the increase in arterial pressure in response to a high NaCl diet in SHR-S but had no effect in 1% NaCl-fed SHR-S. Thus, the NaCl-sensitive component of hypertension in SHR-S was more sensitive to ANF than the non-NaCl-sensitive component. Plasma norepinephrine was significantly increased in ANF-treated, 8% NaCl-fed SHR-S compared with vehicle controls, suggesting that ANF did not prevent NaCl-sensitive hypertension by a sympatholytic effect. During ANF infusion, plasma ANF was increased by only 36% and 40% in the 1% and 8% NaCl groups, respectively, so that long-term infusion of exogenous ANF in a dose that resulted in plasma ANF levels well within the physiological range abolished the NaCl-induced exacerbation of hypertension in SHR-S. The data suggest that a deficiency in circulating endogenous ANF may play a role in NaCl-sensitive hypertension in this model. (Hypertension 1990;15:170-176)

Atrial natriuretic factor (ANF) has potent natriuretic, diuretic, vasodilator, sympatholytic, and renin- and aldosterone-suppressing activities and is involved in the regulation of volume and electrolyte balance and blood pressure. However, the pathophysiological role of ANF in the development or maintenance of systemic hypertension is unclear. Elimination of endogenous ANF by autoimmune immunization has been shown not to affect the development or the severity of hypertension in the spontaneously hypertensive rat (SHR). In contrast, it was recently reported that long-term blockade of endogenous ANF with monoclonal antibody to ANF accelerates the development and exacerbates the severity of hypertension in SHR-stroke prone and deoxycorticosterone acetate–salt rats. In neither study were the animals characterized with respect to sensitivity or resistance to the pressor effects of a high NaCl diet (NaCl-sensitivity or NaCl-resistance).

We have previously identified substrains of SHR that are NaCl-sensitive (SHR-S) or NaCl-resistant (SHR-R). The Wistar-Kyoto (WKY) rats that we used as normotensive controls for these experiments are NaCl-resistant. SHR-S fed high NaCl diets display increased blood pressure in association with increased peripheral vascular tone, as evidenced by an exaggerated depressor response to ganglion blockade. We recently demonstrated that acute infusion of ANF produced an enhanced depressor response in SHR-S fed a high NaCl diet compared with control SHR-S fed a normal NaCl diet and that dietary NaCl loading is associated with significantly increased circulating ANF levels in WKY rats but not in SHR-S. In view of the growing evidence...
that ANF inhibits both sympathetic outflow from the central nervous system and norepinephrine release from sympathetic nerve terminals in the periphery.\textsuperscript{6-8} we considered the possibility that the enhanced depressor effect of ANF in NaCl-supplemented SHR-S may be related to an increased sympatholytic action.

The current study was designed to test the hypotheses 1) that long-term intravenous infusion of ANF at a dose that elevates plasma ANF to levels comparable with those seen in high NaCl-fed WKY rats prevents the NaCl-induced exacerbation of hypertension in SHR-S and 2) that ANF lowers blood pressure in this model by a sympatholytic effect. Our results demonstrate that long-term infusion of ANF, at a dose that has no effect on blood pressure in SHR-S fed 1% NaCl and that elevates plasma ANF, to levels comparable with those seen in high NaCl-fed WKY rats, prevents the NaCl-induced exacerbation of hypertension in SHR-S. These findings suggest that a deficiency in endogenous ANF may play a role in NaCl-sensitive hypertension in this model. Replacement of the deficiency in endogenous ANF by long-term infusion of exogenous ANF elevated, rather than lowered, plasma norepinephrine levels, suggesting that ANF does not prevent NaCl-sensitive hypertension by a sympatholytic effect.

**Methods**

Male SHR-S were obtained from Taconic Farms (IBU3 colony, Germantown, New York) at 7 weeks of age. All rats were maintained four per cage at constant humidity (60±5%), temperature (24±1° C), and 12-hour light/dark cycle (6:00 AM–6:00 PM). Two days after arrival, osmotic minipumps (2002 mini-osmotic pump, Alza Corp., Palo Alto, California) filled with ANF (rat ANF-(1-28), 0.22 μg/μl in 0.1 M acetic acid; Bachem, Inc., Torrance, California) or 0.1 M acetic acid vehicle were implanted in the right ventricle and septum (LV+S). LV+S were weighed immediately.

**ANF measurement.** The current study was designed to test the hypotheses 1) that long-term intravenous infusion of ANF at a dose that elevates plasma ANF to levels comparable with those seen in high NaCl-fed WKY rats prevents the NaCl-induced exacerbation of hypertension in SHR-S and 2) that ANF lowers blood pressure in this model by a sympatholytic effect. Our results demonstrate that long-term infusion of ANF, at a dose that has no effect on blood pressure in SHR-S fed 1% NaCl and that elevates plasma ANF, to levels comparable with those seen in high NaCl-fed WKY rats, prevents the NaCl-induced exacerbation of hypertension in SHR-S. These findings suggest that a deficiency in endogenous ANF may play a role in NaCl-sensitive hypertension in this model. Replacement of the deficiency in endogenous ANF by long-term infusion of exogenous ANF elevated, rather than lowered, plasma norepinephrine levels, suggesting that ANF does not prevent NaCl-sensitive hypertension by a sympatholytic effect.

Eighteen days after initiation of the special diets, rats were anesthetized with ether. Polyethylene catheters (PE-10 fused to PE-50) filled with heparin-saline solution (50 units/ml) were implanted into the abdominal aorta through the right femoral artery for blood collection and measurement of arterial pressure. After catheter implantation, all rats were housed individually. Two days after implantation, tubing was connected with the femoral arterial catheter for blood sampling. At least 1 hour was allowed to pass before 0.7 ml blood was collected from conscious, unrestrained, resting animals for norepinephrine and epinephrine determination as an index of sympathoadrenal activity. The blood was placed in iced tubes containing 1.8 mg EDTA and 1.2 mg glutathione. Then 1.0 ml blood for ANF determination was collected in iced tubes containing 1.5 mg EDTA and 1 trypsin inhibitor unit of aprotinin. The blood withdrawn was immediately replaced with an equal volume of 0.9% saline. Plasma was separated by centrifugation at 4° C. Plasma samples were stored at -80° C until assay for norepinephrine, epinephrine, and ANF. Twenty-four hours later, mean arterial pressure and heart rate were recorded continuously through the arterial catheter via a Model CP-01 pressure transducer (Century Technology Co., Inglewood, California) coupled to a Grass Model 7 polygraph (Grass Instrs. Co., Quincy, Massachusetts) in conscious, unrestrained rats. After a stable mean arterial pressure was obtained, mean arterial pressure and heart rate were recorded.

The spent minipumps were removed and weighed to confirm that they were delivering their contents properly and that the infusate had not run out completely. The infusate samples were stored at -80° C until radioimmunoassay (RIA) for ANF.

To determine the time course of plasma ANF levels during intravenous infusion of exogenous peptide, minipumps containing ANF (0.44 μg/μl) were implanted into the right jugular vein under ether anesthesia. This concentration of ANF delivered a dose of 0.1 μg/peptide/hr. Two weeks later, the minipumps were changed under ether anesthesia and replaced with the same ANF or vehicle solution as during the first 2 weeks of infusion. Rats were then divided at random into four equal groups. Half were placed on an 8% NaCl diet (ICN Biochemicals, Purina Chow, Costa Mesa, California); the other half remained on the basal 1% NaCl diet (Ralston Purina diet 5001, St. Louis, Missouri). Food and water were available ad libitum throughout the study. Systolic blood pressure was measured twice weekly in conscious, prewarmed, restrained rats by the tail-cuff method with an electrothymographmanometer and physiograph recorder (Narco Bio-Systems, Houston, Texas). The median of five successive measurements was used as the estimate of blood pressure. Body weight was determined on the same day.

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To determine the time course of plasma ANF levels during intravenous infusion of exogenous peptide, minipumps containing ANF (0.44 μg/μl) were implanted into the right jugular vein, and catheters were placed in the right femoral artery as previously described in two rats. Blood (1 ml) was collected from the arterial catheter of conscious rats for ANF measurement 1, 3, 7, 10, and 14 days after minipump implantation. An equal volume of saline was infused to replace the blood loss.

We carried out an additional pilot study to determine the stability of ANF in 0.1 M acetic acid at body temperature in vitro. ANF was dissolved in 0.1 M acetic acid at a final concentration of 0.44 μg/μl. The ANF solution was placed in a vacuum oven at 37° C, and aliquots (50 μl) were removed after 1, 2, 3, 4, 5, 6, 8, 10, 12, and 14 days and stored at -80° C before ANF measurement.

After mean arterial pressure measurement, the rats were decapitated without prior anesthesia. The heart was removed and the atria and major vessels were dissected off by a circular incision. The right ventricular free wall was dissected from the left ventricle and septum (LV+S). LV+S were weighed immediately.

ANF concentration in plasma and 0.1 M acetic acid was measured by a modification of the RIA of Tanaka et al.\textsuperscript{18} and Eskay et al.\textsuperscript{19} Plasma for ANF measurement was prepared as described above, and 0.1 M acetic acid was added to each vial to stabilize the ANF concentration for the duration of the experiment.
determination was extracted with Sep-Pak C-18 cartridges (Waters Associates, Milford, Massachusetts) by the method of Eskay et al. Extracts were dried under vacuum and reconstituted in RIA buffer (see below). Rat ANF-(1-28) (Peninsula Labs., Belmont, California) was used as the reference standard. Rabbit anti-rat ANF-(99-126) antiserum was generously donated by Wyeth Laboratories (Philadelphia, Pennsylvania). During the assay, 10 µl standard (2-250 pg) or sample were incubated for 48 hours at 4°C with 100 µl (8,000 cpm) iodine-125-labeled rat ANF (DuPont/New England Nuclear Research Products, Boston, Massachusetts), 100 µl ANF antiserum, and 200 µl RIA buffer (50 mM potassium phosphate buffer, pH 7.4, containing 0.1% bovine serum albumin, 0.01% NaN₃, 0.1% Triton X-100, 50 µM phenylmethylsulfonyl fluoride, 50 mM NaCl, and 0.0005% aprotinin). Separation of bound from free tracer was done by adding 750 µl 20% polyethylene glycol-8000 and 75 µl 1.5% bovine gamma globulin to each assay tube and centrifuging for 1 hour at 2,200g. Recovery of ANF from plasma, as assessed by addition of unlabeled ANF-(8-33) to normal rat plasma, was 91 ±4%. Nonspecific binding of the tracer was 3%. The sensitivity of the ANF RIA was 3.3 pg/assay tube with 50% displacement at 33 pg/assay tube.

Plasma norepinephrine and epinephrine concentrations were measured by a modification of the radioenzymatic assay of Peuler and Johnson using the catecholamine radioenzymatic assay kit (The Upjohn Company, Kalamazoo, Michigan). The sensitivity of the assay was 2 pg for norepinephrine and epinephrine per 50 µl sample.

Statistics

Results were expressed as mean±SEM and analyzed by one-way and two-way analyses of variance followed by Newman-Keuls post hoc analysis. p<0.05 was considered significant.

Results

The 8% NaCl diets produced significant increases in systolic blood pressure (tail-cuff technique) (p<0.01, Figure 1, top panel) and mean arterial pressure (direct intra-arterial recording) (p<0.01, Figure 2, top panel) in vehicle-treated SHR-S compared with control vehicle-treated rats fed 1% NaCl. Long-term ANF infusion significantly lowered systolic blood pressure and mean arterial pressure in the 8% NaCl-fed rats (p<0.01) but had no effect on either parameter in the 1% NaCl-fed rats. In the ANF-treated SHR-S, there were no significant differences in systolic blood pressure and mean arterial pressure between the 1% and 8% NaCl groups after 15 and 21 days on the special diets, respectively, indicating that long-term ANF infusion completely prevented NaCl-sensitive hypertension in SHR-S. This effect was seen at doses of ANF that had no effect on blood pressure in SHR-S maintained on a 1% NaCl diet.
in these groups, there was no correlation between blood pressure and LV+S/BW ratio ($r=0.06, p>0.5$). In the 1% NaCl--fed rats, neither blood pressure nor LV+S/BW ratio was altered by ANF treatment.

Long-term ANF infusion produced slight but significant ($p<0.05$) increases in plasma ANF levels in both 1% and 8% NaCl groups (Figure 3, top left panel). After the ANF infusion, plasma ANF was increased by 36% and 40% in the 1% and 8% NaCl--fed NaCl-sensitive spontaneously hypertensive rats (SHR-S). **p<0.01 compared with vehicle controls fed same diet. #p<0.05, ##p<0.01 compared with respective 1% NaCl group.

The time course study revealed a fivefold elevation in plasma ANF levels compared with noninfused control levels on day 1 of infusion (Figure 3, bottom right panel). Plasma ANF fell to 2.5 times control levels by day 3, plateaued at roughly 2 times control levels by day 10, and remained at that level on day 14. Survival of ANF dissolved in 0.1 M acetic acid and incubated at 37° C in vitro was approximately 50% of starting concentrations at 1–2 weeks (Figure 3, bottom left panel). Further, the mean ANF concentration in the reservoirs of the spent minipumps was approximately 60% of the initial ANF concentration (Figure 3, top right panel). This decrement was proportional to the percent decrease in plasma ANF levels between days 1 and 14 of ANF infusion. Thus, hydrolysis of ANF in the pump reservoirs likely accounts for the fall in plasma ANF levels seen during the course of the infusion.

Long-term ANF infusion was associated with increases in plasma norepinephrine in both 1% and 8% NaCl groups, but the difference reached statistical significance in the 8% NaCl--fed rats only ($p<0.05$) (Figure 4, left panel). There was no difference in plasma epinephrine among the four experimental groups (Figure 4, right panel). Thus, there was no evidence that exogenous ANF had a sympatholytic effect in this model.

**Discussion**

In the current study, chronic intravenous infusion of ANF (0.1 μg/hr) via osmotic minipump into SHR-S beginning before initiation of a high NaCl diet prevented the NaCl-induced increase in arterial pressure but did not affect arterial pressure in control SHR-S fed a normal NaCl diet. Plasma levels of endogenous ANF were not significantly different between the high and normal NaCl groups, and the ANF infusions elevated plasma levels by only 40 and 36% in the high and normal NaCl groups, respectively, at 3 weeks. Thus, administration of exogenous ANF in a dose that elevated circulating peptide concentrations to a level well within the physiological range abolished the NaCl-sensitive component of hypertension in SHR-S without affecting the non-NaCl-sensitive component. This is the first demonstration that long-term elevation of circulating ANF within the physiological range can modulate NaCl-sensitive hypertension.

Our finding that exogenous ANF did not alter blood pressure in SHR-S fed a normal NaCl diet is consistent with the report of Kohzuki et al that infusing ANF at a dose of 150 μg/kg/day (roughly 1 μg/hr/rat) for 2 weeks beginning at age 6 weeks did not alter blood pressure in SHR fed a normal NaCl diet. This dose of peptide was roughly 10 times that used in the current study. Kohzuki et al did not report plasma ANF levels. Further, they found a transient (days 0–3) depressor effect of exogenous ANF in SHR that consumed 1% NaCl solution as drinking water; this was not sustained beyond day 3 of the regimen. Their rats did not show a pressor response to the high NaCl intake, however, so they did not assess the effects of ANF on a NaCl-sensitive model.
In contrast, a number of laboratories have reported a depressor effect of infused ANF in SHR on normal, low, and high NaCl diets. These studies differ from ours in a number of respects. First, the SHR examined in these previous studies either were not NaCl-sensitive or were not characterized with respect to NaCl sensitivity of blood pressure. Rats examined in these previous studies were older (12-15 weeks of age) than the rats used in the current study (7 weeks). Finally, the ANF used in these studies was either administered at higher doses or in different molecular form [ANF-(101-126); human ANF-(99-126), or atriopeptin III-(103-126)] than the ANF-(1-28) used in our study. These differences in experimental design and reagents could account for the apparent inconsistency between previous results and our own.

In the current study, we measured plasma catecholamine levels in ANF-infused and control rats to test the hypothesis that exogenous ANF prevents NaCl-sensitive hypertension via a sympatholytic effect. We have previously shown that SHR-S fed high NaCl diets display increased blood pressure in association with increased peripheral sympathetic nervous system activity and enhanced peripheral vascular tone, as evidenced by an exaggerated depressor response to ganglion blockade. Further, there is growing evidence that ANF is an inhibitory modulator of sympathetic outflow. ANF has been shown to inhibit norepinephrine release from
nerve terminals in the periphery and to reduce membrane excitability of neurons in a number of brain regions. Long-term ANF infusion has been reported to inhibit norepinephrine-induced hypertension in conscious rats. Thus, it might be expected that the enhanced depressor effect of ANF in NaCl-loaded SHR-S is related to an increase in sensitivity to its direct vasodilator action or an increased sympathetic action. However, our current observation that long-term infusion of ANF did not decrease plasma norepinephrine or epinephrine in either normal or high NaCl–fed SHR-S does not support this hypothesis. In fact, there was a tendency for plasma norepinephrine to increase after ANF administration in both experimental groups, and this increase was statistically significant in the 8% NaCl rats.

These data strongly suggest that ANF infusion did not prevent NaCl-sensitive hypertension via a sympatholytic effect. The mechanism of the ANF-related increase in circulating norepinephrine in the current study is uncertain but may reflect a reflex response to decreased blood pressure, cardiac output, or plasma volume.

In the current study, the high NaCl diet was associated with a significant increase in LV+S/BW ratio in both vehicle-treated and ANF-treated rats. There was a significant positive correlation between LV+S/BW ratio and mean arterial pressure in vehicle-treated but not ANF-treated rats. Thus, despite a significant decrease in blood pressure, ANF-treated 8% NaCl-fed rats had evidence of left ventricular hypertrophy comparable in magnitude to their vehicle-treated controls. The mechanism of the ANF-induced increase in LV+S/BW ratio was not specifically elucidated by the current study but might be related to the ANF-induced increase in sympathetic nervous system activity, which attained the level of statistical significance in the 8% NaCl–fed SHR-S only.

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References

- Atrial Natriuretic Factor


**KEY WORDS** • atrial natriuretic factor • sodium chloride • sodium-dependent hypertension • spontaneously hypertensive rats
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