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Blood Pressure and Renal Responses to Synthetic Rat Atrial Natriuretic Factor in Deoxycorticosterone Acetate-Salt Hypertension

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KOHZUKI, M., ABE, K., YASUJIMA, M., KASAI, Y., HIWATARI, M., KANAZAWA, M., SATO, M., OMATA, K., KUDO, K., TAKEUCHI, K. and YOSHINAGA, K. *Blood Pressure and Renal Responses to Synthetic Rat Atrial Natriuretic Factor in Deoxycorticosterone Acetate-Salt Hypertension.* Tohoku J. Exp. Med., 1989, **157** (4), 301-311 —— To assess possible roles of atrial natriuretic factor (ANF) in the regulation of blood pressure in deoxycorticosterone acetate (DOCA)-salt hypertensive rats, we performed two series of experiments. First, we studied acute hypotensive, and natriuretic and diuretic effects of ANF in pentobarbital-anesthetized DOCA-salt hypertensive rats and age-matched controls. A synthetic rat ANF was intravenously administered as a bolus at doses of 0.5, 2.5 and 5.0 μg/kg. In DOCA-salt rats, a significant decrease in mean arterial pressure was observed at a dose of 5.0 μg/kg, whereas at a dose of 2.5 μg/kg in control rats. On the other hand, the diuretic and natriuretic effects of ANF were observed at a dose of 2.5 μg/kg in DOCA-salt rats and 5.0 μg/kg in control rats. Second, we examined chronic effect of ANF on the development of hypertension in DOCA-salt rats. The DOCA-salt rats, given 1% NaCl solution for drinking, were continuously infused with ANF (15, 75 and 150 μg/kg/day) or vehicle (physiological saline) into the jugular vein by osmotic minipumps for up to 14 days. In DOCA-salt treated rats, ANF at doses of 75 and 150 μg/kg/day attenuated significantly the development of hypertension, although ANF at a dose of 15 μg/kg/day did not. The hypotensive effect of ANF was sustained throughout the experimental period and the effect of ANF at a dose of 150 μg/kg/day was more prominent than that of this peptide at a dose of 75 μg/kg/day. ANF did not induce any significant changes in urine volume, fluid intake and urinary excretion of sodium and potassium in DOCA-salt rats when compared to those in vehicle-infused DOCA-salt rats. These results indicate that DOCA-salt rats are more sensitive to ANF in diuretic and natriuretic effects, and less sensitive to ANF in hypotensive effect compared to control rats.

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Moreover, it is suggested that ANF can affect the regulation of blood pressure by its vascular effect in the development of hypertension in DOCA-salt rats. ——— atrial natriuretic factor (ANF); deoxycorticosterone acetate (DOCA); hypertension; sodium-water excretion; vascular smooth muscle

Atrial natriuretic factor (ANF), which is released from membrane-bound storage granules within atrial muscle cells (DeBold 1979, 1982) is a peptide which promotes vasodilation, natriuresis, diuresis (Flynn et al. 1983; Sonnenberg et al. 1983; Atlas et al. 1984; Kangawa and Matsuo 1984; Misono et al. 1984), and furthermore, inhibits aldosterone production in adrenal capsular cells (Atarashi et al. 1984; Maack et al. 1985; Needleman et al. 1985). Therefore, ANF is expected to play an important role in the regulation of blood pressure by its renal, vascular and adrenal effects. However, Sonnenberg et al. (1983) reported that atrial natriuretic activity is decreased in spontaneously hypertensive rats (SHR), while Hirata et al. (1984) showed that it is increased in Dahl salt-sensitive rats as compared to Dahl salt-resistant rats. Thus the involvement of ANF in the regulation of blood pressure in experimental models of hypertension is still controversial. Schiffrin and St-Louis (1987) reported that the density of vascular ANF binding sites is reduced and aortic responsiveness to ANF is decreased in deoxycorticosterone acetate (DOCA)-salt hypertensive rats, a model of volume-expanded hypertension.

Recently, in vivo studies, we have shown that chronic infusion of a non-hypotensive dose of synthetic ANF (150 μg/kg/day) attenuated the hypertensive effect of chronic infusion of norepinephrine (Yasujima et al. 1985), angiotensin II (Yasujima et al. 1986a) or vasopressin (Yasujima et al. 1986b) in conscious rats and that the antihypertensive effect was independent of renal effects of this peptide. In addition, we have also shown that chronic infusion of the same dose of a synthetic ANF did not affect the development of hypertension in young SHR on normal sodium diet or on sodium loading with 1% NaCl solutions in place of drinking water (Kohzuki et al. 1986).

In the present study, we have determined whether ANF could affect the development of hypertension in DOCA-salt rats, and to ascertain the sensitivity of these animals in the hypotensive, diuretic and natriuretic responses to ANF.

Materials and Methods

Experiment 1

Sprague-Dawley rats (Charles River Breeding Laboratory, Tokyo) weighing from 200 to 250 g were used. DOCA-salt hypertension was produced in rats that had previously undergone left nephrectomy under anesthesia with pentobarbital sodium (30 mg/kg, i.p.; Abott Laboratories, Tokyo). One week after the surgery, the rats were injected subcutaneously twice a week with deoxycorticosterone acetate in olive oil (Percoteni®, Ciba; 1.5 mg/100 g body weight/dose). They were housed in individual cages, with free access to commercial rat food (sodium, 0.24%; potassium, 0.69%; Oriental CMF, Oriental Yeast, Tokyo) and to 1% NaCl solution as a drinking water. Age-matched uninephrectomized

Sprague-Dawley rats that received twice a week subcutaneous injections with olive oil alone served as controls. Blood pressure was monitored at weekly intervals by tail-cuff method beginning on the day prior to the nephrectomy.

After hypertension had been entablished (4-5 weeks after the beginning of treatment with DOCA-salt), experiments were performed in DOCA-salt hypertensive rats ($n=6$) and controls ($n=6$). Anesthesia was done by an intraperitoneal injection of pentobarbital sodium (30 mg/kg) and the body temperatures were maintained at 37°C with an electric heater during the whole experimental procedure. A cannula (PE-10) was inserted into the left femoral vein for continuous infusion of physiological saline (0.05 ml/min) and for administration of ANF as a bolus, and another one into the left femoral artery for continuous measurement of mean arterial pressure (MAP). MAP was recorded by connecting the arterial catheter to a pressure transducer and a two channel recorder (Biophysiograph, 180 system, San-Ei, Tokyo). A midline abdominal incision was used to expose the ureter from the right kidney. The ureter was cannulated (PE-10) for urine collection. After operation, the infusion of a saline solution through the femoral venous catheter was started at a rate of 0.05 ml/min. After surgical preparation, the animals were allowed to stabilize for 40 min before starting the control measurements. Urine was collected at 15-min intervals. A synthetic rat ANF of 28-amino acids polypeptide was administered intravenously as a bolus at doses of 0.5, 2.5 and 5.0 μg/kg (dissolved in 0.1 ml of physiological saline), at an interval of 15 or 30 min. The maximum MAP responses were taken as the hypotensive effects of ANF. Urine samples for measurement of urine volume and urinary excretion of sodium and potassium were obtained every 15 min before and after administration of ANF.

Experiment 2

Sprague-Dawley rats (Charles River Breeding Laboratory, Tokyo) weighing from 200 to 250 g were used. Animals anesthetized with sodium pentobarbital (30 mg/kg, i.p.) were nephrectomized unilaterally through a flank incision and received subcutaneous silicone-rubber implants containing 100 mg/kg of DOCA made by the method of Ormsbee and Ryan (1973). All DOCA-treated rats were randomly housed in individual metabolic cages, with free access to commercial rat food (sodium, 0.24%; potassium, 0.69%; Oriental CMF, Oriental Yeast, Tokyo) and to 1% sodium chloride solution as a drinking water. They were maintained in a room controlled for humidity and temperature. Throughout the study, each rat was housed in a metabolic cage designed to prevent feces-urine contact (Model ST; Sugiyamagen, Tokyo). After 2-week-treatment, they were infused for up to 2 weeks with 15, 75 and 150 μg/kg/day of rat ANF or vehicle (physiological saline) (0.5 μl/hr) delivered via osmotic minipumps (Model 2002, Alzet®, Palo Alto, CA, USA) which were connected to the left jugular vein by means of a vascular catheter. Jugular cannula was tunnelled subcutaneously to the osmotic minipump which was implanted in the interscapular region of the rat's back.

Assuming that ANF did not degrade during the study, and that the pumps dispensed fluid at the specified rate of approximately 0.5 μl/hr, the highest infusion dose (150 μg/kg/day) was chosen to be sufficient to induce small but significant diuresis and natriuresis. This dosage did not affect blood pressure in a normotensive rat bioassay system (Yasujima et al. 1985). The stability of ANF in the osmotic minipump was examined by comparing the diuretic and natriuretic activities of freshly dissolved ANF with those in the ANF solution recovered from the minipump after 14 days of use in the rat. No difference in activities was observed.

Systolic blood pressure was determined daily during the control and infusion periods at the same time of the day by an indirect tail-cuff method without anesthesia (Pfeffer et al. 1971). The daily fluid intake, body weight, urine volume and urinary excretion of sodium and potassium were also checked.

Urine was collected into vessels at 4°C and was kept at −20°C until the assay. Urine volume was measured gravimetrically and expressed as μl/min (Experiment 1) or ml/day

(Experiment 2). Urinary sodium and potassium concentrations were measured with a flame photometer. In Experiment 2, blood for the measurements of plasma ANF concentration was obtained by decapitation at the termination of infusion period. The concentration of ANF was measured by the modified method reported previously (Kimura et al. 1986). Blood was collected into heparinized tubes and centrifuged at 4°C. Plasma samples were immediately mixed with 0.1 N HCl and kept at −20°C. Radioimmunoassay was performed after extraction of acidified plasma through C 18 Sep-Pak cartridges with 80% acetone-HCl (pH 1.5). Rat ANF antiserum (Mitsubishi Petrochemical Co., Ltd, Tokyo) cross-reacts completely with α-human ANF (100%), and atriopeptin III, but not significantly with anginine vasopressin, ACTH, angiotensin II, substance P, oxytocin and methionine-enkephalin. Fifth μl of synthetic α-rat ANF or extracted sample, the antiserum (final dilution, 1 : 90.000) and ^{125}I-human ANF (specific activity : 2,000 μCi/g, Amersham Japan Co., Tokyo), and 250 μl of the buffer were incubated in a non-equilibrium method. The lowest detectable level of ANF was 7 pg/tube and 50% inhibition of binding was 50 pg/tube. The recovery rate of added α-rat ANF (125 pg) was 74.2 ± 13.3 ($n = 15$) and inter- and intra-assay coefficients were 17.9% and 13.9%, respectively. The serial dilutions of the extracted plasma sample paralleled the standard curve.

Statistical analysis

All results were expressed as means ± S.E. The data were statistically analyzed in the following ways. In Experiment 1, the paired t-test was used to compare the parameters before and after ANF administration. In Experiment 2, statistical analysis of the data between groups was performed by two-way analysis of variance for repeated measurements. Statistically significant differences on each day were determined by unpaired t-test or Cochran-Cox test. A probability less than 0.05 was considered statistically significant in both experiments.

Results

Experiment 1

In DOCA-salt rats, basal MAP was not affected by the administration of ANF at doses of 0.5 and 2.5 μg/kg. MAP decreased significantly from 129.8 ± 3.2 mmHg to 102.5 ± 10.8 mmHg ($p < 0.05$) at a dose of 5.0 μg/kg (Table 1). In control animals, MAP was not affected at a dose of 0.5 μg/kg but decreased significantly from 115.0 ± 5.4 mmHg to 99.4 ± 7.0 mmHg ($p < 0.01$) at a dose of 2.5 μg/kg and from 112.8 ± 6.1 mmHg to 103.0 ± 5.4 mmHg ($p < 0.01$) at a dose of 5.0 μg/kg. Thus the hypotensive response to ANF in DOCA-salt rats was observed at a greater dose of ANF (5.0 μg/kg) than in controls. In both DOCA-salt rats and controls, MAP decreased in a dose-related manner. Significant changes in urine volume and urinary excretion of sodium were observed at a dose of 2.5 μg/kg in DOCA-salt rats and at a dose of 5.0 μg/kg in controls. Urinary excretion of potassium also increased at a dose of 5.0 μg/kg in both DOCA-salt rats and controls. The dose of ANF to produce the diuretic and natriuretic responses in DOCA-salt rats was smaller than the dose sufficient for a significant depressor response. In addition, the dose of ANF to induce diuresis and natriuresis was significantly less in DOCA-salt rats than that in control animals.

TABLE 1. *Acute effects of synthetic ANF on mean arterial pressure, urine volume, and urinary sodium and potassium excretion in DOCA-salt treated rats*

	ANF (μg/kg)					
	0.5		2.5		5.0	
	Before	After	Before	After	Before	After
MAP (mmHg)						
DOCA-salt ($n=6$)	128.8 $\pm$3.3	131.0 $\pm$4.2	129.0 $\pm$3.3	129.2 $\pm$3.5	129.8 $\pm$3.2	102.5* $\pm$10.8
Controls ($n=6$)	116.2 $\pm$5.0	115.2 $\pm$4.7	115.0 $\pm$5.4	99.4** $\pm$7.0	112.8 $\pm$6.1	103.0** $\pm$5.4
UV (μl/min)						
DOCA-salt ($n=6$)	14.0 $\pm$5.8	16.3 $\pm$4.9	14.0 $\pm$4.9	29.1* $\pm$6.1	14.7 $\pm$6.1	56.0* $\pm$5.7
Controls ($n=6$)	5.1 $\pm$0.8	4.9 $\pm$1.0	4.6 $\pm$0.8	5.0 $\pm$1.6	5.2 $\pm$1.0	21.5* $\pm$6.3
$U_{Na}V$ ($\times 10^{-3}$ mEq/min)						
DOCA-salt ($n=6$)	1.87 $\pm$1.04	1.99 $\pm$1.22	2.08 $\pm$0.73	3.80* $\pm$1.23	1.87 $\pm$1.06	7.31** $\pm$1.45
Controls ($n=6$)	0.46 $\pm$0.31	0.41 $\pm$0.35	0.35 $\pm$0.27	0.37 $\pm$0.41	0.40 $\pm$0.23	1.23* $\pm$0.47
U_KV ($\times 10^{-3}$ mEq/min)						
DOCA-salt ($n=6$)	1.02 $\pm$0.37	0.94 $\pm$0.33	1.05 $\pm$0.42	1.28 $\pm$0.17	0.94 $\pm$0.33	2.08** $\pm$0.33
Controls ($n=6$)	0.62 $\pm$0.16	0.55 $\pm$0.07	0.49 $\pm$0.13	0.52 $\pm$0.17	0.53 $\pm$0.07	1.18* $\pm$0.20

Results are expressed as means$\pm$S.E. The paired t-test was used to compare the parameters before and after administration of ANF. *$p<0.05$ and **$p<0.01$ compared to values before administration of ANF.

MAP, mean arterial pressure; UV, urine volume; $U_{Na}V$, urinary sodium excretion; U_KV, urinary potassium excretion; ANF, atrial natriuretic factor.

Experiment 2

Before long-term infusion of ANF or vehicle, body weight, systolic blood pressure, fluid intake, urine volume and urinary excretion of sodium and potassium were not significantly different among the groups (Table 2).

In DOCA-salt treated rats, ANF at a dose of 15 μg/kg/day did not affect the development of hypertension compared to the controls of vehicle alone (Fig. 1). On the contrary, ANF at doses of 75 and 150 μg/kg/day attenuated significantly the development of hypertension in DOCA-salt treated rats. On Day 1, tail systolic blood pressure in ANF-infused rats fell down to 154.4$\pm$6.4 mmHg ($p<0.05$) at a dose of 150 μg/kg/day and 158.0$\pm$4.2 mmHg ($p<0.01$) at a dose of 75 μg/kg/day, while it was 173.3$\pm$4.1 mmHg in the vehicle-infused group. The antihypertensive effect of ANF was sustained throughout the experimental period. On Day 14, tail systolic blood pressure in conscious rats infused with ANF at doses

TABLE 2. *Preinfusional data on body weight, systolic blood pressure, fluid intake, urine volume, and urinary sodium and potassium excretion in DOCA-salt treated rats*

	BW (g)	SBP (mmHg)	FI (ml/day)	UV (ml/day)	$U_{Na}V$ (mEq/day)	U_KV (mEq/day)
Vehicle ($n=6$)	259.5 ±3.2	159.3 ±4.0	58.2 ±4.5	43.7 ±2.8	9.05 ±0.98	2.89 ±0.20
ANF (15 μg/kg/day) ($n=6$)	263.3 ±3.7	164.0 ±7.9	55.3 ±3.7	38.6 ±4.4	8.29 ±0.78	2.83 ±0.09
ANF (75 μg/kg/day) ($n=6$)	265.0 ±4.9	165.0 ±3.1	63.5 ±4.2	38.5 ±4.3	8.27 ±0.86	2.72 ±0.26
ANF (150 μg/kg/day) ($n=6$)	253.4 ±4.5	164.2 ±5.4	54.2 ±3.1	36.4 ±6.3	8.16 ±0.92	3.05 ±0.27

Results are expressed as means±S.E. BW, body weight; SBP, systolic blood pressure; FI, fluid intake; UV, urine volume; $U_{Na}V$, urinary sodium excretion; U_KV, urinary potassium excretion; ANF, atrial natriuretic factor.

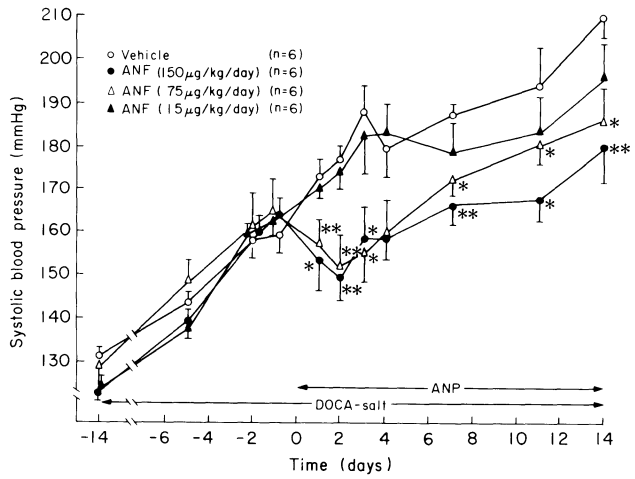

Fig. 1. Chronic effect of synthetic rat atrial natriuretic factor (ANF) on systolic blood pressure in DOCA-salt treated rats. Results are expressed as means± S.E. Analysis of variance for repeated measurements revealed a significant change in systolic blood pressure in rats given 75 μg/kg/day of ANF (△) ($p<0.05$) and 150 μg/kg/day of ANF (●) ($p<0.01$) compared to those in rats given vehicle (○). Statistically significant differences on each day were determined by unpaired t-test or Cochran-Cox test. $^*p<0.05$, $^{**}p<0.01$ compared to values in rats given vehicle alone.

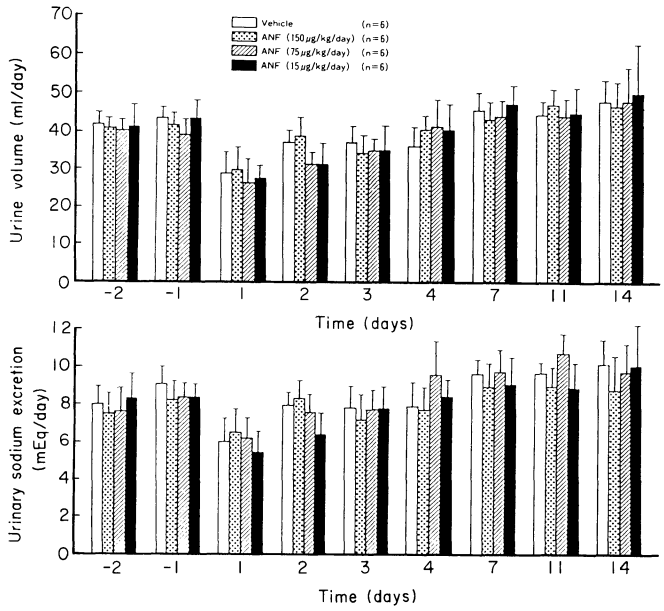

Fig. 2. Chronic effect of synthetic rat atrial natriuretic factor (ANF) at doses of 150 (▩), 75 (▨) and 15 (■) μg/kg/day on urine volume (top panel) and urinary sodium excretion (bottom panel) in DOCA-salt treated rats. (□) indicates values in vehicle-infused rats as controls. Results are expressed as means±S.E.

of 150, 75 and 15 μg/kg/day were 180.3±7.2 mmHg ($p<0.01$), 182.0±9.4 mmHg ($p<0.05$) and 197.7±7.1 mmHg (n.s.), respectively, compared to 212.3±3.4 mmHg in the vehicle-infused group, and the effect of ANF at a dose of 150 μg/kg/day was more prominent than that at a dose of 75 μg/kg/day ($p<0.05$).

On the other hand, ANF at doses of 15, 75 and 150 μg/kg/day did not induce any significant changes in fluid intake, urine volume and urinary excretion of sodium and potassium in DOCA-salt treated rats (Fig. 2). In addition, ANF did not alter the body weight, which was 270.7±3.8 g in ANF-infused rats at a dose of 150 μg/kg/day, 283.5±10.5 g at a dose of 75 μg/kg/day and 279.0±7.4 g at a dose of 15 μg/kg/day, and 274.2±7.9 g in control rats. The chronic ANF infusion at a dose of 150 μg/kg/day for 14 days induced a 22,918 pg/ml increase in the absolute value of circulating level of ANF compared to that of vehicle-infused rats, a 3,800 pg/ml increase at a dose of 75 μg/kg/day, and a 844 pg/ml increase at a dose of 15 μg/kg/day.

Discussion

The present study demonstrated that there was a significant difference in the sensitivity of diuretic, natriuretic and hypotensive responses to ANF between DOCA-salt rats and control rats. In addition, it was also indicated that ANF

attenuated the development of hypertension in DOCA-salt treated rats, which was independent of renal effects of this peptide. Therefore it is suggested that ANF can regulate blood pressure by its vascular effects.

Recent studies have suggested that elevation of atrial pressure or distension of atrial walls stimulate the release of ANF (Dietz 1984; Lang et al. 1985). Ackermann and Irizawa (1984) indicated that the synthesis of atrial granules is enhanced in DOCA-salt hypertensive rats. In contrast, DeBold (1979) reported that the granules in atrial cardiocytes are decreased in DOCA-salt rats. According to Sugimoto et al. (1986), depletion of atrial ANF and elevation of circulating ANF were found in the DOCA-salt hypertensive rats. They also demonstrated that some DOCA-salt rats had relatively low blood pressure and high plasma/atria ratio of ANF, suggesting that elevation of blood pressure may have been attenuated in these rats by an excessive increase in the release of ANF from the atria for some undefined reasons. Taken together with the present results, it seems likely that the increased synthesis and release, which occur in response to expansion of extracellular fluid volume or to elevation of blood pressure, may modify the development of hypertension in DOCA-salt rats.

Our previous study showed that in young SHR a significant decrease in mean arterial pressure (MAP) was observed during the infusion of ANF at a dose of 1.0 μg/kg, and diuretic and natriuretic effects of ANF were observed at a dose of 2.5 μg/kg (Kasai et al. 1986). The DOCA-salt model of rats differs from the young SHR in that a significant decrease in MAP was observed at a dose of 5.0 μg/kg in the former, whereas the results of diuretic and natriuretic response to ANF were similar. In DOCA-salt treated rats, moreover, ANP at doses of 75 and 150 μg/kg/day attenuated significantly the development of hypertension throughout the experimental period. In our previous study (Kohzuki et al. 1986), on the contrary, ANP at a dose of 150 μg/kg/day attenuated only transiently the development of hypertension in young SHR on high salt diet and was not affected in young SHR on normal sodium diet. It is surprising that the antihypertensive effect of ANF is much apparant not in SHR but in DOCA-salt rats which is less sensitive to acute antihypertensive effect of ANF. There may be a difference in the downward regulation to vascular receptor of ANF between these two types of hypertensive models of rat. It is also postulated that there may be a difference in the response to anesthesia between the two since the stability and bioavailability of ANF was not changed during the infusion as confirmed in the preliminary experiments.

Elevation of blood pressure in DOCA-salt treated rats has been known to be triggered by expansion of extracellular fluid volume (Haack et al. 1977), and associated with elevation of central venous pressure (Miller et al. 1979). Therefore, ANF may play a more powerful role in DOCA-salt rats than in control rats. In the present study, it is of interest to note that ANF had no modulatory effect on urine volume, fluid intake, urinary sodium and potassium excretion, or body

weight in not only control rats but also DOCA-salt rats which have an increase in body fluid. Therefore it may be assumed that the hypotensive response in DOCA-salt rats may not be due to a circulatory volume contraction via its renal effects, but due to its direct vascular effects. That the hypotensive effect of ANF appears to be related to a direct vasodilation-effect is not surprizing because recent studies have reported that similar effect of ANF was observed in conscious rats made hypertensive by chronic infusion of norepinephrine (Yasujima et al. 1985), angiotensin II (Yasujima et al. 1986a) or vasopressin (Yasujima et al. 1986b), or in conscious two-kidney, one clip hypertensive rats (Garcia et al. 1985), or conscious young SHR (Kohzuki et al. 1986).

The doses of ANF employed in chronic experiments of the present study resulted in higher concentration of ANF in plasma compared to the levels in vehicle-infused DOCA-salt hypertensive rats. At the dose of 15 μg/kg/day of ANF, which was ineffective in attenuating the development of hypertension in this model of rats, the mean plasma levels of ANF were significantly higher, but these levels of ANF were still within physiological ranges reported previously (Schiffrin and St-Louis 1987). On the contrary, higher doses (75 and 150 μg/kg/day) were significantly hypotensive in DOCA-salt hypertensive rats. However, the attained levels of ANF seem to be pharmacological. Therefore, it is still uncertain whether physiologically circulating levels of ANF play a significant role in the regulation of blood pressure in this model of rats.

In summary, the present results indicate that ANF may play possible roles in the development of hypertension in DOCA-salt treated rats, and that SHR and DOCA-salt hypertensive rats are different in the blood pressure response to ANF. Further studies will be needed to elucidate the role of ANF in the regulation of blood pressure.

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References

1) Ackermann, U. & Irizawa, T.G. (1984) Synthesis and renal activity of rat atrial granules depend on extracellular volume. *Am. J. Physiol.*, **247**, R750-R752.
2) Atarashi, K., Mulrow, P.J., Franco-Saenz, R., Snajdar, R. & Rapp, J. (1984) Inhibition of aldosterone by an atrial extract. *Science*, **224**, 992-994.
3) Atlas, S.A., Kleinert, H.D., Camargo, M.J., Januszewicz, A., Sealey, J.E., Laragh, J.H., Schilling, J.W., Lewicki, J.A., Johnson, L.K. & Maack, T. (1984) Purification, sequencing and synthesis of natriuretic and vasoactive rat atrial peptide. *Nature*, **309**, 717-719.
4) DeBold, A.J. (1979) Heart atria granularity : Effects of changes in water electrolyte balance. *Proc. Soc. Exp. Biol. Med.*, **161**, 508-511.

5) DeBold, A.J. (1982) Tissue fractionation studies on the relationship between water-electrolyte, atrial natriuretic factor and specific atrial granules. *Can. J. Physiol. Pharmacol.*, **60**, 324-330.
6) Dietz, J.R. (1984) Release of natriuretic factor from rat heart-lung preparation by atrial distension. *Am. J. Physiol.*, **247**, R1093-R1096.
7) Flynn, T.G., DeBold, M.L. & DeBold, A.J. (1983) The amino acid sequence of an atrial peptide with potent diuretic and natriuretic properties. *Biochem. Biophys. Res. Commun.*, **117**, 859-865.
8) Garcia, R., Thibault, G., Gutkowska, J., Hamet, P., Cantin, M. & Genest, J. (1985) Effect of chronic infusion of synthetic atrial natriuretic factor (ANF 8-33) in conscious two-kidney, one-clip hypertensive rats. *Proc. Soc. Exp. Biol. Med.*, **178**, 155-159.
9) Haack, D., Mohring, J., Mohring, B., Petri, M. & Hackenthal, E. (1977) Comparative study on development of corticosterone and DOCA hypertension in rats. *Am. J. Physiol.*, **233**, F403-F411.
10) Hirata, Y., Ganguli, M., Tobian, L. & Iwai, J. (1984) Dahl S rats have increased natriuretic factor in atria but are markedly hyporesponsive to it. *Hypertension*, **6**, Suppl. I, I-148-I-155.
11) Kangawa, K. & Matsuo, H. (1984) Purification and complete amino acid sequence of α-human atrial natriuretic polypeptide (α-hANP). *Biochem. Biophys. Res. Commun.*, **118**, 131-139.
12) Kasai, Y., Abe, K., Yasujima, M., Tanno, M., Kohzuki, M., Yoshinaga, K. & Saso, S. (1986) The augmented hypotensive response to a synthetic atrial natriuretic factor in spontaneously hypertensive rats. *J. Hypertension*, **4**, Suppl. 3, S325-S327.
13) Kimura, T., Abe, K., Ota, K., Omata, K., Shoji, M., Kudo, K., Matsui, K., Inoue, M., Yasujima, M. & Yoshinaga, K. (1986) Effects of acute water load, hypertonic saline infusion and furosemide administration on atrial natriuretic peptide and vasopressin release in humans. *J. Clin. Endocrinol. Metab.*, **62**, 1003-1010.
14) Kohzuki, M., Abe, K., Yasujima, M., Tanno, M., Kasai, Y., Sato, M., Omata, K., Kudo, K., Takeuchi, K. & Yoshinaga, K. (1986) Chronic effect of a synthetic atrial natriuretic factor on the development of hypertension in young spontaneously hypertensive rats. *J. Hypertension*, **4**, Suppl. 3, 487-489.
15) Lang, R.E., Tholken, H., Ganten, D., Luft, F.C., Ruskoaho, H. & Unger, T. (1985) Atrial natriuretic factor: A circulating hormone stimulated by volume loading. *Nature*, **314**, 264-266.
16) Maack, T., Camargo, M.J.F., Kleinert, H.D., Laragh, J.H. & Atlas, S.A. (1985) Atrial natriuretic factor: Structure and functional properties. *Kidney Int.*, **27**, 607-615.
17) Miller, A.W., Bohr, D.F., Schork, A.M. & Terris, J.M. (1979) Hemodynamic responses to DOCA in young pigs. *Hypertension*, **1**, 591-597.
18) Misono, K.S., Fukumi, H., Grammer, R.T. & Inagami, T. (1984) Rat atrial natriuretic factor: Complete amino acid sequence and disulfide linkage essential for biological activity. *Biochem. Biophys. Res. Commun.*, **119**, 524-529.
19) Needleman, P., Adams, S.P., Cole, B.R., Currie, M., Geller, D.M., Michener, M.L., Saper, C.B., Schwartz, D. & Standaert, D.G. (1985) Atriopeptins as cardiac hormones. *Hypertension*, **7**, 469-482.
20) Ormsbee, H.S. & Ryan, C.F. (1973) Production of hypertension with deoxycorticosterone acetate-impregnated silicone rubber implants. *J. Pharm. Sci.*, **62**, 255-257.
21) Pfeffer, J.M., Pfeffer, M.A. & Frohlich, E.D. (1971) Validity of an indirect tail-cuff method for determining systolic arterial pressure in unanesthetized normotensive and spontaneously hypertensive rats. *J. Lab. Clin. Med.*, **78**, 957-962.
22) Schiffrin, E.L. & St-Louis, J. (1987) Decreased density of vascular receptors for atrial natriuretic peptide in DOCA-salt hypertensive rats. *Hypertension*, **9**, 504-512.
23) Sonnenberg, H., Milojevic, S., Chong, C.K. & Veress, A.T. (1983) Atrial natriuretic

factor: Reduced cardiac content in spontaneously hypertensive rats. *Hypertension*, **5**, 672-675.

24) Sugimoto, T., Ishii, M., Hirata, Y., Matsuoka, A., Toshimori, T., Masuda, H., Kangawa, K. & Matsuo, H. (1986) Increased release of atrial natriuretic polypeptide in rats with DOCA-salt hypertension. *Life Sci.*, **38**, 1351-1358.

25) Yasujima, M., Abe, K., Kohzuki, M., Tanno, M., Kasai, Y., Sato, M., Omata, K., Kudo, K., Tsunoda, K., Takeuchi, K., Yoshinaga, K. & Inagami, T. (1985) Atrial natriuretic factor inhibits the hypertension induced by chronic infusion of norepinephrine in conscious rats. *Circ. Res.*, **57**, 470-474.

26) Yasujima, M., Abe, K., Kohzuki, M., Tanno, M., Kasai, Y., Sato, M., Omata, K., Kudo, K., Takeuchi, K., Hiwatari, M., Kimura, T., Yoshinaga, K. & Inagami, T. (1986a) Effect of atrial natriuretic factor on angiotensinII-induced hypertension in rats. *Hypertension*, **8**, 748-753.

27) Yasujima, M., Abe, K., Kohzuki, M., Tanno, M., Kasai, Y., Sato, M., Omata, K., Kudo, K., Takeuchi, K. & Yoshinaga, K. (1986b) Antihypertensive effect of atrial natriuretic factor in vasopressin-infused rats. *Jpn. Circ. J.*, **50**, 1185-1190.