Effects of Chronically Administered Atrial Natriuretic Factor in Aldosterone-Infused Hypertensive Rats

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To assess the pathophysiological role of atrial natriuretic factors in mineralocorticoid hypertension, we studied the effects of chronic infusion of synthetic atrial natriuretic factor on blood pressure and sodium-water excretion in rats with aldosterone salt-induced hypertension. Administration of synthetic atrial natriuretic factor (150 $\mu\text{g/kg/day}$) to rats made hypertensive by 7-day infusion of aldosterone (100 $\mu\text{g/kg/day}$) and sodium loading with 1% NaCl as drinking water returned the blood pressure to control levels, and the anti-hypertensive effect was not associated with any changes in urine volume and urinary sodium excretion. These results indicate that atrial natriuretic factors may be involved in the regulation of blood pressure in mineralocorticoid hypertension, independent of the renal effects of these substances.

WE have recently reported that chronic infusion of a nonhypotensive dose (150 $\mu\text{g/kg/day}$) of synthetic atrial natriuretic factor (ANF) attenuated the hypertensive effect of sustained administration of norepinephrine¹ or angiotensin II,² and the antihypertensive effect was not associated with any significant changes in urine volume and urinary sodium excretion. Therefore, we suggest that ANF may be involved in the regulation of blood pressure by modulating vasopressor effects of norepinephrine or angiotensin II, independent of the renal effects of these substances. However, we have failed to demonstrate that chronic infusion of the same dose of synthetic ANF affects the development

of hypertension in spontaneously hypertensive rats of Okamoto-Aoki strain on normal sodium diets.³

To assess possible roles of ANF in the regulation of blood pressure in mineralocorticoid hypertension in rats, we studied the effects of chronic infusion of a synthetic rat ANF on blood pressure and sodium-water excretion in rats made hypertensive by the chronic administration of aldosterone plus sodium loading with 1% NaCl solution as a drinking water.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 150 to 250g were used. All rats were maintained in a humidity- and temperature-controlled room. Throughout the study, each rat was housed in a metabolic cage designed to prevent feces-urine contact (Model ST; Sugiyamagen, Tokyo, Japan).

Key words:

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TABLE I EFFECTS OF ATRIAL NATRIURETIC FACTOR IN ALDOSTERONE-INFUSED RATS

	Control	Day		
		8	9	10
SBP (mmHg)				
ALDO + Vehicle (n = 7)	142.1 ± 2.4	141.7 ± 4.0	143.1 ± 3.0	147.3 ± 3.9
ALDO + ANF (n = 7)	142.0 ± 1.9	139.2 ± 2.9	134.0 ± 2.0*	136.2 ± 4.3*
Water intake (ml/day)				
ALDO + Vehicle (n = 7)	17.3 ± 1.7	21.6 ± 0.9	27.0 ± 3.5	25.0 ± 1.8
ALDO + ANF (n = 7)	21.4 ± 3.1	32.4 ± 3.0	27.0 ± 2.7	25.2 ± 2.6
Urine Volume (ml/day)				
ALDO + Vehicle (n = 7)	12.1 ± 0.8	14.1 ± 1.0	16.8 ± 5.6	14.1 ± 1.1
ALDO + ANF (n = 7)	12.0 ± 2.7	10.4 ± 1.5	15.6 ± 1.8	13.6 ± 1.2
U _{Na} V (ml/day)				
ALDO + Vehicle (n = 7)	3.10 ± 1.27	2.91 ± 1.19	4.38 ± 0.28	3.56 ± 0.25
ALDO + ANF (n = 7)	3.59 ± 0.58	2.83 ± 0.38	4.25 ± 0.36	4.09 ± 0.25

Results are mean ± SEM. Analysis of variance for repeated measurements revealed a significant change in SBP in aldosterone-infused rats given ANF compared to that in aldosterone-infused rats given vehicle on sodium loading ($p < 0.01$). * $p < 0.05$ and * $p < 0.01$ compared to values in aldosterone-infused rats given vehicle. Abbreviations: SBP = systolic blood pressure; U_{Na}V = urinary sodium excretion; ALDO = aldosterone.

The rats were fed on a regular diet (Oriental CMF, 0.24% of sodium, 0.69% of potassium, Oriental Yeast, Tokyo, Japan) and sodium-loaded with 1% NaCl as drinking water. Studies were performed after a 7-day period of acclimatization to the housing, feeding and drinking conditions.

Synthetic ANF was administered for up to 3 days to rats made hypertensive by the chronic infusion of aldosterone at a rate of 100 µg/kg/day and sodium loading with 1% NaCl as drinking water for 7 days. Synthetic ANF (Protein Research Foundation, Osaka, Japan) at a rate of 150 µg/kg/day, dissolved in physiological saline or vehicle, was delivered by an osmotic minipump (Alzet, Palo Alto, CA, USA) into the jugular vein. With the rat under pentobarbitone sodium (Abbott Laboratories, Tokyo, Japan) anesthesia, the osmotic minipump was implanted in the interscapular region of the rat's back and a vascular catheter (PE-60) was settled subcutaneously to connect the pump and the jugular vein. We chose the subdepressor dose of ANF (150 µg/kg/day) as described previously and confirmed the stability of ANF in the osmotic minipump.¹

D-aldosterone (Wako Pure Chemical Industries Ltd., Sendai, Japan) was dissolved in poly-

ethylene glycol, and the aldosterone was infused intraperitoneally by another osmotic minipump. Assuming that aldosterone did not degrade during the study, the pump dispensed fluid at the specified rate of approximately 0.5 µl/hr. The infusion dose was set to induce and maintain a distinct elevation of the blood pressure.

Systolic blood pressure in the rats was recorded daily by an indirect tail cuff method without anesthesia.⁴ Daily fluid intake, urine volume and urinary sodium excretion were determined.

All results were expressed as mean ± SEM. 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 isolated by the unpaired t-test between the groups.

RESULTS

The systolic blood pressure of aldosterone-infused rats on sodium loading with 1% NaCl as drinking water began to rise significantly on the 3rd day of the infusion and reached its maximum in 6 days which was sustained up to the end of the experiment.

When ANF was administered to rats made

hypertensive by a 7-day infusion of aldosterone and sodium loading, systolic blood pressure fell to 136.2 ± 4.3 mmHg on Day 10 as shown in Table I. This value was significantly lower ($p < 0.01$) than the value of systolic blood pressure obtained when vehicle was infused in aldosterone-infused rats on sodium loading (147.3 ± 3.9 mmHg on Day 10). The antihypertensive effect of ANF was sustained for the following 3 days, but it was not associated with significant changes in urine volume and urinary sodium excretion (Table I).

DISCUSSION

In the present study, we demonstrated that chronic infusion of a subdepressor dose of synthetic ANF abolished the hypertensive effect of chronic administration of aldosterone and sodium loading and returned the blood pressure to control levels. This attenuation in the blood pressure elevation induced by chronic infusion of aldosterone and sodium loading, was not accompanied by diuresis or natriuresis, suggesting, as in our previous reports,^{1,2,5} that the reduction of blood pressure is not secondary to the renal effects of this peptide. It is also of interest to note that chronically administered ANF did not induce any changes in fluid intake, body weight, urine volume and urinary sodium excretion in aldosterone-salt treated rat, although chronic administration of aldosterone and sodium loading caused a significant elevation of blood pressure due to the volume expansion associated with sodium retention.

To our knowledge, the hypotensive effect of the subdepressor dose of ANF in rats made hypertensive by chronic infusion of aldosterone and sodium loading has not been previously reported, whereas it has been shown that the hypotensive effect was observed in two-kidney one-clip⁶ or one-kidney one-clip rats⁷ during chronic infusion of ANF.

The pathogenesis of hypertension in the aldosterone-salt model has been attributed to volume expansion by sodium retention. However, recent studies have shown that several factors may be involved in the regulation of blood pressure at the chronic stage of hypertension induced by aldosterone-salt.⁸ Therefore, the mechanism of hypertension remains to be clarified.

The mechanism(s) by which ANF blocks the hypertension caused by chronic infusion of

aldosterone and sodium loading cannot be explained by the present experiment. Although the substance has natriuretic and diuretic actions, we could not find any significant changes in urine volume and urinary sodium excretion in aldosterone-salt treated rats during the infusion of ANF. This suggests that the hypotensive effect of ANF is not due to the loss of water and sodium.

Garcia et al⁶ suggested that the hypotensive response of ANF observed in two-kidney, one clip rats could be secondary to an inhibitory effect on renin release. However, it is unlikely that an inhibitory effect of ANF on renin release is related to the hypotensive effect in aldosterone-salt model, since the suppressed renin release is one of the most characteristic findings in this model.

Recently, Koike et al⁹ showed in conscious spontaneously hypertensive rats that the acute hypotensive effect of ANF appeared to result from vasodilation. In addition, it has been suggested that the hypotensive response to chronic infusion of ANF at the same dose range or less, used in the present study may be, in part, due to vasodilation in experimental models of hypertension in conscious rats.^{6,7,10} Therefore it is possible that ANF interferes with the vascular effects of aldosterone and sodium. However, the exact mechanism of the effect of ANF on vascular smooth muscle has not been fully studied.

In conclusion, the present finding, that chronic infusion of a subdepressor dose of ANF attenuates the hypertension caused by aldosterone and sodium, suggests that ANF may be involved in the regulation of blood pressure in mineralocorticoid hypertension.

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