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Augmented Expression of Cardiac Atrial Natriuretic Peptide System in Hypertensive Rats

The present study was aimed at investigating the regulation of atrial natriuretic peptide (ANP) system in association with either enhanced or attenuated activity of the renin-angiotensin system (RAS). The cardiac tissue mRNA and peptide levels of ANP were measured in rats with two-kidney, one clip (2K1C) or deoxycorticosterone acetate (DOCA)-salt hypertension. Plasma renin concentration was increased in 2K1C hypertension along with increases of renin mRNA and protein contents in the clipped kidney. On the contrary, it was suppressed in DOCA-salt hypertension along with decreases of renin mRNA and protein contents in the remaining kidney. The plasma ANP concentration was similarly increased in both models of hypertension. The cardiac tissue ANP contents were not significantly changed, but the tissue ANP mRNA levels were upregulated in the hypertrophied heart in these two models of hypertension. It is suggested that the cardiac ANP system is transcriptionally enhanced by cardiac hypertrophy associated with hypertension, independent of the systemic RAS.

Key Words: *Atrial natriuretic factor; Renin-angiotensin system; Hypertension; Hypertrophy*

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INTRODUCTION

Atrial natriuretic peptide (ANP) is produced mainly in the cardiac atria (1). It has been variously suggested to act as an endogenous antagonist to the renin-angiotensin system (RAS). It inhibits renin secretion, suppresses aldosterone release, and attenuates the vasoconstrictor effect of angiotensin (Ang) II (2, 3).

The direct effect of RAS on ANP system has been also suggested. Infusion of subpressor doses of Ang II increases ANP secretion (4) or Ang II stimulates ANP gene expression in cultured cardiomyocytes (5). In addition, cardiac ANP gene expression is enhanced during the activation of systemic RAS by clipping the unilateral renal artery (6). Although these findings suggest a role for an enhanced activity of RAS to regulate ANP system, it has not been established to what extent an attenuated activity of RAS modifies ANP system.

The present study was aimed at investigating the interaction of ANP system with RAS. To enhance or diminish the activity of RAS, two-kidney, one clip (2K1C) or deoxycorticosterone acetate (DOCA)-salt hypertension was induced in rats, and the expression of renal renin and cardiac ANP genes was determined.

MATERIALS AND METHODS

Animal preparation

Experiments were done using male Sprague-Dawley rats weighing 150-200 g. They were kept in accordance with the Institutional Guidelines for Experimental Animal Care and Use. 2K1C hypertension was induced by partial constriction of the left renal artery with a silver clip, having an internal diameter of 0.2 mm under ketamine anesthesia. The contralateral kidney was left untouched. Animals subjected to sham-operation served as control.

To develop DOCA-salt hypertension, one week after the left unilateral nephrectomy, rats were subcutaneously implanted with silastic DOCA (Sigma; St. Louis, MO, U.S.A.; 200 mg/kg body weight) strip. They were then supplied with 1% saline for drinking water. The control group was treated the same as the experimental group except that DOCA strip was not implanted.

Four weeks after inducing hypertension, systolic blood pressure was measured by means of a tail-cuff method without anesthesia. The rats were then decapitated, and their trunk blood was collected to determine renin and

ANP in the plasma. Kidneys and hearts were rapidly removed, frozen in liquid nitrogen, and stored at -70℃ until use.

Radioimmunoassay of renin and ANP

The renal tissue homogenates were prepared as described previously (7). The concentrations of renin in the plasma and cortical tissue homogenates were determined by the amounts of Ang I generated in the presence of excess renin substrate using radioimmunoassay (RIA) kit (New England Nuclear; Boston, MA, U.S.A.).

The plasma was extracted with Sep-Pak C_{18} cartridges (Waters Associates; Milford, MA, U.S.A.). The atria or ventricles were boiled in 0.1 N acetic acid for 10 min, homogenized, centrifuged, and the supernatant was collected. Concentrations of ANP in the plasma and tissue homogenates were determined by RIA (Research & Diagnostic Antibodies; Berkely, CA, U.S.A.).

RNA extraction and Northern blot analysis

Total RNAs were isolated from the kidney, atrium, and ventricle using Ultraspec™ RNA isolation system (Biotecx Laboratories; Houston, TX, U.S.A.) according to the single-step method of Chomczynski and Sacchi (8). The RNA samples were resolved by electrophoresis through 8% formaldehyde-1.4% agarose denaturing gel buffered with 20 mM 3-N-morpholinopropane sulfonic acid and 1 mM ethylenediaminetetraacetic acid, pH 7.4. They were then transferred overnight from gels to nylon membrane (Boehringer Mannheim Biochemicals; Mannheim, Germany) in 20×SSC (1×SSC contains 150 mM NaCl and 15 mM sodium citrate), and baked at 80℃ for 2 hr under vacuum.

To determine renin mRNA, ^{32}P-labeled RNA transcribed from plasmid containing cDNA sequence for renin gene, linearized with *Bam*H1, was used. The probe used to detect ANP mRNA was 600 bp *Eco*R1 insert of rat ANP cDNA generated with Prime-a Gene® labeling system (Promega; Madison, WI, U.S.A.) in the presence of [^{32}P]-dCTP. The filters were prepared as previously described (9), and exposed to X-ray films (O-MAT™, Kodak). The mRNA levels for renin and ANP were determined by densitometric analysis of Northern blot autoradiograms, which were normalized with 18S rRNA.

Data were expressed as means±SEM. For statistical analysis of differences between the groups, unpaired t test was used.

RESULTS

Blood pressure and cardiac tissue weights

Systolic blood pressure and cardiac tissue weights are summarized in Table 1. Four weeks after inducing hypertension, ventricles but not atria were significantly greater in 2K1C hypertensive rats than in their control. Both ventricles and atria were significantly greater in DOCA-salt hypertensive rats compared with those in their control.

Renin

Table 2 shows the renin levels in the plasma and renal tissue. Renin mRNA data are also shown in Fig. 1 and

Table 1. Characteristics of 2K1C and DOCA-salt rats

	2K1C		DOCA-salt	
	Control	Hypertensive	Control	Hypertensive
SBP (mmHg)	113±5	186±19*	121±3	217±11*
Atria (μg/g BW)	223±9	236±11	154±6	233±9*
Ventricles (μg/g BW)	3637±55	4117±95*	2974±83	3981±47*

Tissues were blotted dry and weighed.
SBP, systolic blood pressure; BW, body weight; n=9, number of rats in each group; *$p<0.05$, compared with control

Table 2. Plasma renin concentration (PRC) and renal renin contents (RRC) in 2K1C and DOCA-salt rats

		Control	Hypertensive
2K1C	PRC (ng/mL/hr)	38.0±3.7	74.7±10.4*
	RRC (μg/mg/hr)-Clipped	7.56±2.69	15.47±1.15*
	-Contralateral	10.03±0.63	2.50±0.25*
DOCA-salt	PRC (ng/mL/hr)	7.1±0.5	3.1±0.3*
	RRC (μg/mg/hr)	1.48±0.11	0.23±0.07*

n=6, number of rats in each group; *$p<0.05$, compared with control

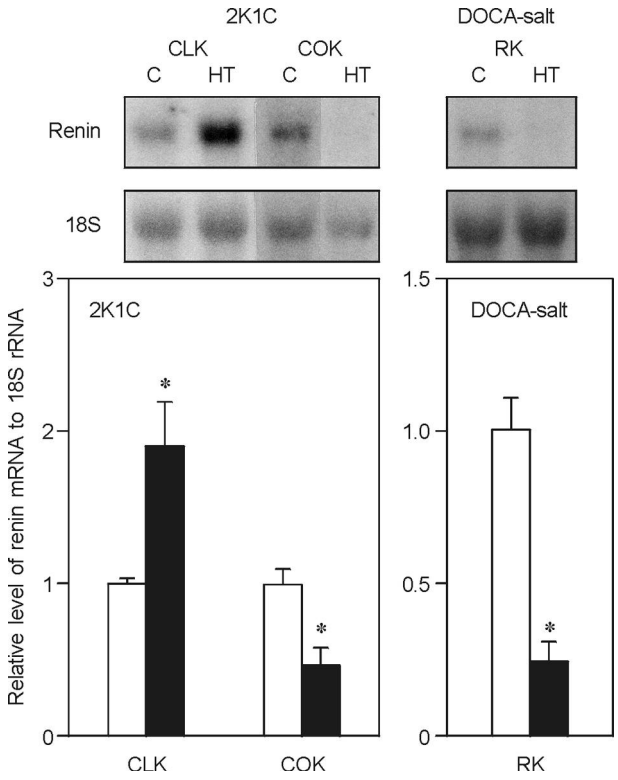

Fig. 1. Autoradiograms show Northern blot analysis for renin mRNA and 18S rRNA in clipped (CLK) and contralateral kidneys (COK) of 2K1C and remaining kidney (RK) of DOCA-salt rats at four weeks after inducing hypertension. The size of rat renin mRNA was approximately 1.6 kb. Bar graphs represent relative renin mRNA levels, which were measured with an Image Analyzer and normalized to the signal for 18S rRNA. n=3-6. *p<0.05, Control (C, open bar) vs 2K1C hypertensive group (HT, closed bar).

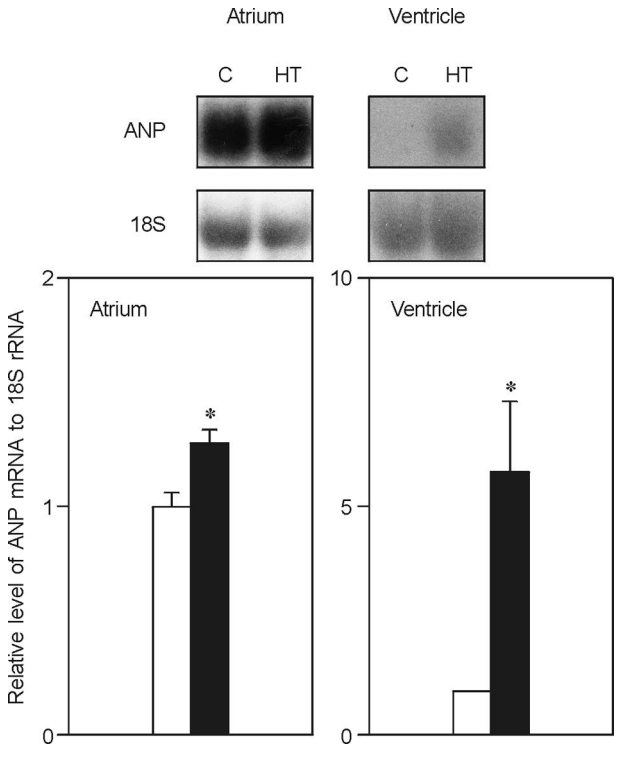

Fig. 2. Autoradiograms show Northern blot analysis of total RNA (5 and 20 μg, respectively) extracted from atrium and ventricle of 2K1C rats at four weeks after renal artery clipping to measure ANP mRNA and 18s rRNA. Bar graphs represent relative ANP mRNA levels. n=6. *p<0.05, Control (C, open bar) vs 2K1C hypertensive group (HT, closed bar).

2. Plasma renin concentration (PRC) was significantly higher in 2K1C hypertensive rats, and lower in DOCA-salt hypertensive rats than in their respective controls (Table 2). In 2K1C hypertensive rats, renin mRNA and protein contents in the clipped kidney were significantly increased, whereas those in the contralateral kidney were decreased (Table 2, Fig. 1). In DOCA-salt hypertensive rats, renin mRNA and protein contents in the remaining kidney were significantly decreased (Table 2, Fig. 1).

ANP

Plasma ANP levels were similarly increased in both 2K1C and DOCA-salt hypertensive rats compared with those in their corresponding controls (Table 3). Atrial and ventricular ANP contents were not significantly changed either in 2K1C or DOCA-salt rats (Table 3). However, atrial and ventricular ANP mRNA levels were significantly increased both in 2K1C (Fig. 2) and DOCA-salt hypertensive rats (Fig. 3).

DISCUSSION

Hypertension induced by constricting the unilateral renal artery was associated with increases of renin mRNA

Table 3. Plasma and cardiac tissue ANP levels in 2K1C and DOCA-salt rats

	2K1C		DOCA-salt	
	Control	Hypertensive	Control	Hypertensive
Plasma (pg/mL)	268±38	436±57*	259±23	409±60*
Atria (ng/mg)	125.6±7.9	123.8±12.2	132.9±7.0	136.1±15.6
Ventricles (pg/mg)	1.75±0.17	1.81±0.40	4.57±0.53	5.01±0.31

n=6, number of rats in each group; *p<0.05, compared with control

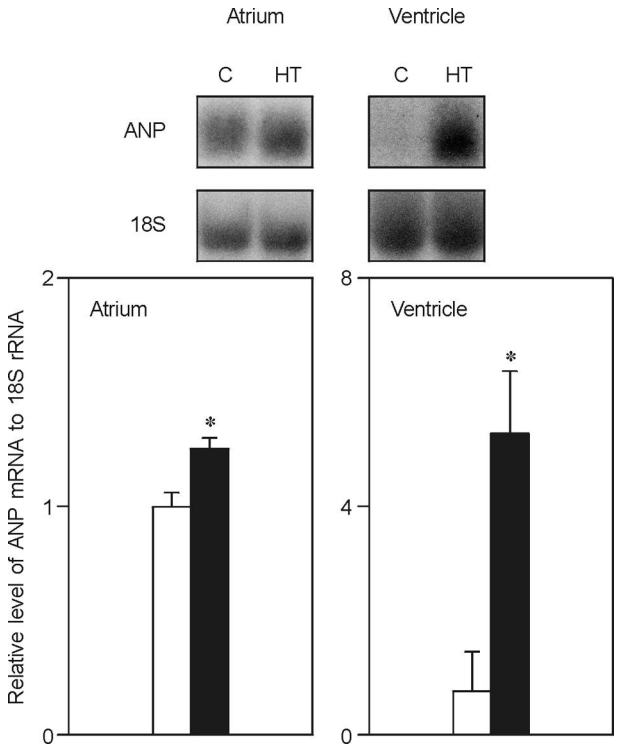

Fig. 3. Autoradiograms show Northern blot analysis of total RNA (5 and 20 μg, respectively) extracted from atrium and ventricle of DOCA-salt rats at four weeks after DOCA implantation and drinking of 1% NaCl to measure ANP mRNA and 18s rRNA. Bar graphs represent relative ANP mRNA levels. n=6. *$p<0.05$, Control (C, open bar) vs DOCA-salt hypertensive group (HT, closed bar).

and protein levels in the clipped kidney, and hence, of circulating renin. In contrast, renin mRNA and protein levels in the contralateral kidney were markedly decreased. The suppressed renin in the contralateral kidney has been attributed primarily to elevated levels of circulating Ang II and/or the resultant increases in arterial pressure (10, 11). On the other hand, DOCA-salt hypertension has been characterized by extracellular volume expansion and suppressed renin (12, 13). We also observed decreased renin content and renin gene expression with concomitant changes in PRC.

However, the plasma ANP was similarly increased in 2K1C and DOCA-salt hypertension. Since the increased plasma ANP level was accompanied by increases of cardiac tissue ANP mRNA levels in both models of hypertension, an increased synthesis is suggested. This may be in line with previous observations which showed an increased atrial specific granule formation in DOCA-salt rats (14, 15). However, neither atrial nor ventricular ANP content was significantly altered by the hypertension in the present study. Sato et al. (16) also failed to observe any significant alterations in cardiac ANP contents despite the increased plasma levels in DOCA-salt hypertension. No increase of tissue peptides in the presence of the increased tissue mRNA may indicate a stimulated release, so that the newly synthesized ANP would not be piled up as such.

The mechanism by which cardiac ANP gene expression is stimulated in hypertension has not been established. It has been suggested that Ang II plays a role in the stimulation of ANP gene expression, i.e., Ang converting enzyme inhibitors or Ang receptor antagonists blunted the gene expression and promoted regression of ventricular hypertrophy during unilateral renal artery clipping (6, 17, 18). In contrast, the stimulation of RAS by loop diuretic furosemide or calcium antagonist amlodipine did not influence or decrease cardiac ANP mRNA levels (6). Taken together, an increase of systemic renin and Ang II levels per se has no direct effect on cardiac ANP gene expression.

On the other hand, ANP mRNA was increased in dexamethasone-induced hypertrophied ventricles, and in the ventricles of spontaneously hypertensive rats (19, 20). In addition, ventricular ANP is synthesized and secreted into the circulation in proportion to its degree of hypertrophy (17). A significant correlation has been also noted between the degree of ventricular hypertrophy and plasma ANP levels (19, 21, 22). The prolonged stenosis of unilateral renal artery results in severe hypertension and cardiac hypertrophy (23). We also observed cardiac hypertrophy in both models of hypertension. It is likely that the cardiac hypertrophy associated with hypertension represents a major factor stimulating ANP gene expression. The augmented synthesis and release of ANP may then serve to counteract the high blood pressure.

In summary, our study demonstrated an altered regulation of ANP system in both 2K1C and DOCA-salt hypertension, in which plasma ANP levels were similarly increased along with augmented ANP gene expression in the hypertrophied heart. It is suggested that the cardiac hypertrophy associated with hypertension is causally related with the enhanced cardiac ANP gene expression, independent of the activity of systemic RAS.

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