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Enhanced Expression of Aldosterone Synthase and Adrenomedullin in Left and Right Ventricular Hypertrophy in Rats

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The pathophysiological implications of aldosterone and adrenomedullin in the cardiac ventricular hypertrophy were examined. Male Sprague-Dawley rats were treated with deoxycorticosterone acetate (DOCA)-salt and monocrotaline (MCT) to selectively elicit left and right ventricular (LV, RV) hypertrophy, respectively. The mRNA expression of aldosterone synthase and adrenomedullin in LV and RV was determined by reverse transcription-polymerase chain reaction. The expression of aldosterone synthase and adrenomedullin was increased in LV, while not altered significantly in RV of DOCA-salt-treated rats. On the contrary, the expression was not significantly altered in LV, but increased in RV of MCT-treated rats. The enhanced expression of aldosterone synthase may be causally related with the development of ventricular hypertrophy, and the increased expression of adrenomedullin may act as a counter-regulatory mechanism.

Key Words: Ventricular hypertrophy, Aldosterone synthase, Adrenomedullin

INTRODUCTION

Renin-angiotensin system has been implicated in the ventricular hypertrophy and remodeling (Baker et al, 1990; Wollert & Drexler, 1999; Park et al, 2001). Several clinical and experimental studies indicate that aldosterone, one of the downstream effectors of renin-angiotensin system, may modulate cardiovascular function. A peripheral infusion of aldosterone in rats causes cardiac hypertrophy and fibrosis without increasing the blood pressure (Young et al, 1995). The excess aldosterone is associated with both increased left ventricular wall thickness and mass in hypertensive patients with primary aldosteronism (Rossi et al, 1997). Aldosterone has been recently known to be de novo synthesized in the heart in rats (Silvestre et al, 1998; Takeda et al, 2000). Therefore, the local aldosterone may also affect cardiac function and hypertrophy. The regulation of its synthesis in various pathophysiological states remains to be determined.

Adrenomedullin has been originally demonstrated to cause a potent and long-lasting hypotensive effect in anesthetized rats (Kitamura et al, 1993). Its pathophysiological role in various cardiovascular diseases has also been suggested. The ventricular levels of adrenomedullin mRNA and peptides are increased in association with volume-overloaded cardiac hypertrophy (Nishikimi et al, 1997). Moreover, adrenomedullin attenuates the stimulated increases of aldosterone concentrations in plasma and adrenal gland (Yamaguchi et al, 1996; Charles et al, 2001). Taken together, adrenomedullin may not only be a physiological inhibitor of aldosterone secretion, but also one of counter-regulatory mechanisms against hypertrophy. The regulation of adrenomedullin has not been examined in the ventricular hypertrophy.

The present study was aimed to determine the pathophysiological implications of aldosterone and adrenomedullin in the ventricular hypertrophy. We treated rats with deoxycorticosterone acetate (DOCA)-salt and monocrotaline (MCT) to selectively elicit left and right ventricular (LV, RV) hypertrophy, respectively, and determined the mRNA expression of aldosterone synthase and adrenomedullin in the ventricle.

METHODS

Left and right ventricular hypertrophy

Male Sprague-Dawley rats were used (250–300 g). The experimental procedure was carried out in accordance with the *Institutional Guidelines for the Care and Use of Laboratory Animals*. To induce LV hypertrophy, DOCA-salt hypertension was induced by subcutaneous implantation of silicone rubber containing DOCA (200 mg/kg), one week after unilateral nephrectomy. The rats were then supplied with 0.9% saline to drink. The control group without DOCA implantation was also unilaterally nephrectomized and supplied with saline to drink. To induce a selective RV hypertrophy, rats were given a single subcutaneous in-

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ABBREVIATIONS: DOCA, deoxycorticosterone acetate; MCT, monocrotaline; LV, left ventricle; RV, right ventricle; RT-PCR, reverse transcription-polymerase chain reaction; ACE, angiotensin converting enzyme.

Table 1. Oligonucleotide sequences used in PCR amplification

Gene	Primer sequence	References
ACE (389 bp)		
Sense	5'-GCCTCCCCAACAAGACTGCCA-3'	Passier et al, 1995
Antisense	5'-CCACATGTCTCCCCAGCAGATG-3'	
AS (314 bp)		
Sense	5'-TACAGGTTTTCCTCTACTCG-3'	Yoshiyu et al, 1996
Antisense	5'-AGATGCAAGACTAGTTAAAATC-3'	
AM (568 bp)		
Sense	5'-TGCCACCCGCACCTATAACC-3'	Owada et al, 1997
Antisense	5'-GAAGCTGGTTTCCATCGCCC-3'	
β-Actin (423 bp)		
Sense	5'-GACTACCTCATGAAGATCCTGACC-3'	Abassi et al, 1998
Antisense	5'-TGATCTTCATGGTGCTAGGAGCC-3'	

ACE: angiotensin converting enzyme, AS: aldosterone synthase, AM: adrenomedullin.

jection of MCT (60 mg/kg) which is known to cause a pulmonary vascular damage leading to pulmonary hypertension and eventually RV hypertrophy and failure (Honda et al, 1992). Control rats were injected with the solvent (phosphate-buffered saline, pH adjusted to 7.4 with 0.5 N HCl). Four weeks later, the heart was removed in each model under ether anesthesia.

RNA extraction & RT-PCR

The heart was divided into RV and LV, in which the ventricular septum was included in LV. They were rapidly frozen in liquid nitrogen and kept at $-70^{\circ}C$ until total RNA isolation. Total RNA was extracted according to the method of Chomczynski & Sacchi (1987), with a slight modification. The extract was stored at $-70^{\circ}C$ as a suspension in 70% ethanol. RNA was spectrophotometrically quantified by measuring absorbance at 280 mm.

The nucleotide sequences of the primers are presented in Table 1. Total RNA (20 μg) was treated with oligo (dT) primers, and the first strand cDNA was synthesized using Moloney murine leukemia virus reverse transcriptase (Promega; Madison, WI, USA) in a 50 μL of reaction volume for 90 min at 37°C. PCR cycles were performed in DNA thermal cycler (PTC-100, M.J. Research; Watertown, MA, USA) with the following profile: for ACE (35 cycles), denaturation 120 sec at 95°C, annealing 2 min at 65°C, and extension 3 min at 72°C; for aldosterone synthase (35 cycles), denaturation 45 sec at 94°C, annealing 45 sec at 56°C, and extension 1 min at 72°C; and for β-actin (25 cycles), denaturation 45 sec at 94°C, annealing 45 sec at 56°C, and extension 90 sec at 72°C. At the end of PCR, one-tenth of the reaction mixture was resolved on 1% agarose gel containing 0.5 μg/mL of ethidium bromide.

Polaroid film was scanned using Epson (GT-9500) scanner with a resolution of 72 DPI. The resulting image was analyzed using Image Analysis Program (NIH; Bethesda, MD, USA). The scale of each band was expressed by multiplying the mean density and the total area of the band.

Drugs and statistical analysis

Drugs were purchased from Sigma Chemical Company (St. Louis, MO, USA), unless stated otherwise. All data are presented as mean±SEM. To compare the data between the groups, unpaired t-test was used.

Table 2. Left and right ventricular weights in DOCA-salt and MCT-treated rats

	DOCA-salt		MCT	
	Control	Exp	Control	Exp
Left (mg/kg)	0.95±0.04	1.60±0.05*	1.03±0.04	0.94±0.04
Right (mg/kg)	0.22±0.04	0.23±0.03	0.25±0.01	0.32±0.02*

Data are mean±SEM (n=6 each). *$P<0.01$, compared with control.

RESULTS

Table 2 shows weights of LV and RV of different groups of rats. Experimental groups of DOCA-salt-treated rats showed selective LV hypertrophy, whereas MCT-treated rats showed selective RV hypertrophy. Fig. 1 shows the ventricular mRNA expression of ACE, aldosterone synthase, and adrenomedullin in DOCA-salt-treated rats. The expression of all these genes was significantly increased in LV, while not significantly altered in RV. Fig. 2 shows the ventricular mRNA expression of ACE, aldosterone synthase, and adrenomedullin in MCT-treated rats. They were significantly increased in RV, while not significantly altered in LV.

DISCUSSION

Previous studies indicated a role played by renin-angiotensin system in developing ventricular hypertrophy. ACE inhibitors and angiotensin II receptor antagonists caused a significant regression of LV hypertrophy in spontaneously hypertensive rats (Kim et al, 1996), and prevented the cardiac hypertrophy induced by pressure or volume overload (Baker et al, 1990; Ruzicka & Leenen, 1995). In MCT-

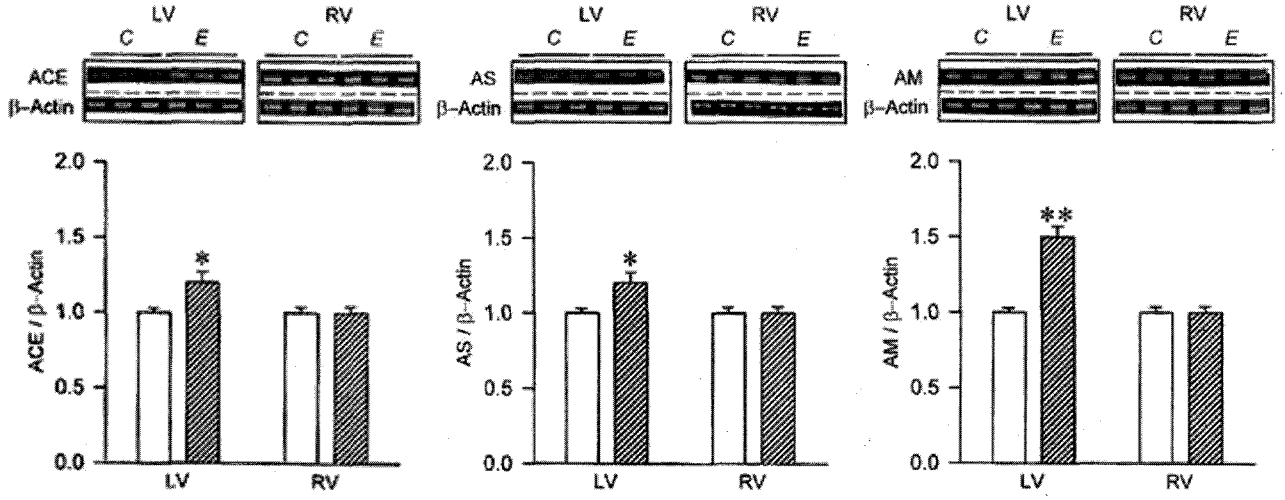

Fig. 1. Representative fluorographs and densitometric analysis of angiotensin converting enzyme (ACE), aldosterone synthase (AS), and adrenomedullin (AM) mRNA expression in LV and RV in DOCA-salt-treated rats. The open column represents the control, and the hatched column depicts the experimental (Numbers of rats were 6 each). *P<0.05, **P<0.01; compared with the control.

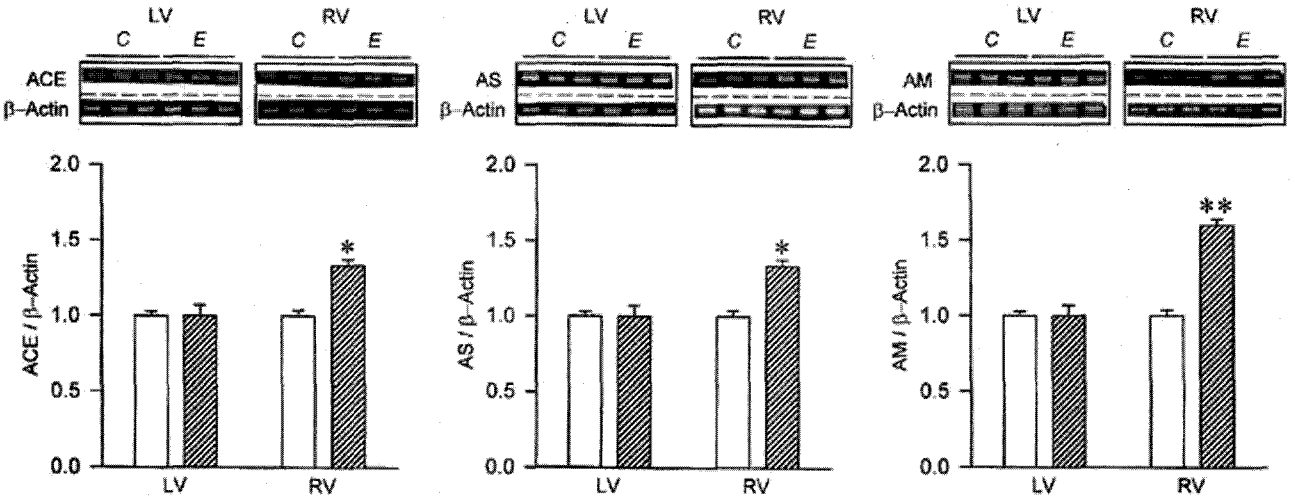

Fig. 2. Representative fluorographs and densitometric analysis of angiotensin converting enzyme (ACE), aldosterone synthase (AS), and adrenomedullin (AM) mRNA expression in LV and RV in MCT-treated rats. The open column represents the control, and the hatched column depicts the experimental (Numbers of rats were 6 each). *P<0.05, **P<0.01; compared with the control.

treated rats, enalapril reduced RV hypertrophy and cardiac failure, while pulmonary hypertension was not improved (Ishikawa et al, 1995). The present study demonstrated that tissue levels of ACE mRNA were specifically increased in LV and in RV following treatment with DOCA-salt and MCT, respectively, along with LV and RV hypertrophy. An activated local renin-angiotensin system may be causally related with ventricular hypertrophy.

The expression of aldosterone synthase was specifically increased in LV and in RV. Aldosterone is one of the downstream effectors of renin-angiotensin system. It has been known to increase LV wall thickness and mass in primary aldosteronism (Rossi et al, 1997). It has been also shown in genetically hypertensive rats to induce myocardial hypertrophy and spironolactone prevents cardiac hypertrophy (Takeda et al, 2000). The enhanced expression of aldosterone synthase may be causally related with the development of ventricular hypertrophy.

The expression of adrenomedullin mRNA was also specifically increased in LV and RV. It has been shown that the plasma concentration of adrenomedullin positively correlates with blood pressure in patients with primary aldosteronism (Kato et al, 1995). Adrenomedullin may participate in counteracting high blood pressure. However, plasma adrenomedullin concentrations do not always correlate with blood pressure or plasma renin activity (Sumimoto et al, 1997). On the contrary, the plasma adrenomedullin concentration correlates well with LV weight in Dahl salt-sensitive rats on a high-salt diet (Shimokubo et al, 1996). Furthermore, the ventricular mRNA and peptides of adrenomedullin are increased in various rat models of cardiac hypertrophy, such as spontaneous hypertension

(Shimokubo et al, 1995a; Inatsu et al, 1996) and Dahl salt-sensitive hypertension (Shimokubo et al, 1996). The concentrations of adrenomedullin immunoreactivity in MCT-induced pulmonary hypertension were significantly higher in RV and plasma (Shimokubo et al, 1995b). These findings suggest a paracrine/autocrine role of adrenomedullin synthesized in the ventricle.

Adrenomedullin has been demonstrated to suppress mesangial cell mitogenesis (Chini et al, 1995). In vascular smooth muscle cells, it also has an antiproliferative effect in a paracrine fashion (Kano et al, 1996). In addition, it inhibits the increase of aldosterone concentrations in both plasma and adrenal gland of rats fed a sodium-deficient diet (Yamaguchi et al, 1996), markedly suppresses angiotensin-II-stimulated aldosterone production (Andreis et al, 1997), and antagonizes the AII-induced rise of plasma aldosterone (Charles et al, 2001). A more recent study also demonstrated that adrenomedullin gene delivery attenuates renal damage and cardiac hypertrophy in Goldblatt hypertensive rats (Wang et al, 2001). Taken together, adrenomedullin may ameliorate the cardiac hypertrophy, not only through suppressing mitogenesis and cell proliferation but also through inhibiting the synthesis of aldosterone. Cardiac adrenomedullin may have a protective role in systemic or pulmonary hypertension.

In summary, the present study demonstrated that local tissue expression of aldosterone synthase and adrenomedullin mRNA was specifically increased in the hypertrophied ventricle. The increased aldosterone synthesis may be related with the development of ventricular hypertrophy, and the enhanced adrenomedullin formation may act as one of the counter-regulatory mechanisms against the hypertrophy.

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