Elevated cardiac tissue level of aldosterone and mineralocorticoid receptor in diastolic heart failure: beneficial effects of mineralocorticoid receptor blocker

Tomohito Ohtani,1,2 Miho Ohta,3 Kazuhiro Yamamoto,1 Toshiaki Mano,1,2 Yasushi Sakata,1 Mayu Nishio,1,2 Yasuharu Takeda,1,2 Junichi Yoshida,1,2 Takeshi Miwa,2 Mitsuhiko Okamoto,4 Tohru Masuyama,5 Yasuki Nonaka,6 and Masatsugu Hori1

1Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Suita, Japan; 2Genome Information Research Center, Osaka University, Suita, Japan; 3Laboratory of Nutrition, Koshien College, Nishinomiya, Japan; 4Department of Molecular Physiological Chemistry, Osaka University Graduate School of Medicine and 5Cardiovascular Division, Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Japan; and 6College of Nutrition, Koshien University, Takarazuka, Japan

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HIGH-ACTIVITY DIET, and mineralocorticoid receptor in diastolic heart failure: beneficial effects of mineralocorticoid receptor blocker

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Am J Physiol Regul Integr Comp Physiol 292: R946–R954, 2007. First published October 5, 2006; doi:10.1152/ajpregu.00402.2006.—Cardiac aldosterone levels have not been evaluated in diastolic heart failure (DHF), and its roles in this type of heart failure remain unclear. This study aimed to detect cardiac aldosterone by use of a liquid chromatographic-mass spectrometric method and to assess the effects of mineralocorticoid receptor blockade on hypertension. Dahl salt-sensitive rats fed 8% NaCl diet from 7 wk (hypertensive DHF model) were divided at 13 wk into three groups: those treated with subdepressor doses of eplerenone (12.5 or 40 mg/kg) and a group given a normal diet (normotensive DHF model). Male Dahl salt-sensitive rats (Japan SLC, Shizuoka, Japan) fed 8% NaCl from age 7 wk were used as a hypertensive DHF model (5). Male Dahl salt-sensitive rats fed 0.3% NaCl throughout the study protocol were normotensive and served as age-matched control. Dahl salt-sensitive rats fed 0.3% NaCl diet served as controls. Cardiac aldosterone levels in the hypertensive DHF rats increased, whereas those in the control rats were unchanged. Treatment with eplerenone attenuated ventricular hypertrophy and fibrosis, improved diastolic function, and decreased cardiac tissue aldosterone concentration.

METHODS

This study conforms with the guiding principles of Osaka University Graduate School of Medicine with regard to animal care and use, and was approved by the institutional ethics committee. Study animals. Male Dahl salt-sensitive rats (Japan SLC, Shizuoka, Japan) fed 8% NaCl from age 7 wk were used as a hypertensive DHF model (5). Male Dahl salt-sensitive rats fed 0.3% NaCl throughout the study protocol were normotensive and served as age-matched control. The data were collected at age 21 wk, when this DHF model develops.

A few groups, to our knowledge, succeeded in the detection of cardiac aldosterone using radioimmunoassay (6, 11, 29, 30). However, values of cardiac aldosterone levels are widely spread among the studies (7), and this is likely because its concentration in cardiac tissue is very low. In addition, two clinical studies (16, 33) suggested an increase in cardiac aldosterone levels in heart failure, but they raised contradictory mechanisms. Thus the cardiac aldosterone system in heart failure remains controversial.

Heart failure with normal or minimally impaired systolic function is attributed to diastolic dysfunction (44) and is termed diastolic heart failure (DHF). Presently, evidence about its pathophysiology and a reliable therapeutic strategy are lacking. Our laboratory developed an animal model of hypertensive DHF (5) and demonstrated a crucial role of an ANG II-mediated pathway in this type of heart failure (24, 41). Because aldosterone is likely a critical mediator of ANG II-induced tissue injury (20), we hypothesized that aldosterone plays an important role in the progression of DHF as well as systolic heart failure.

First, we aimed to detect aldosterone and to assess aldosterone synthase activity in the cardiac tissues of hypertensive DHF rats. We used the coupling of reverse-phase C18 cartridge and normal-phase HPLC for purification and liquid chromatography-mass spectrometry (LC-MS) for direct structural analysis of aldosterone with greater specificity and sensitivity (3, 21). To enhance the identification potential, the MS-MS approach was applied. Second, we assessed effects of mineralocorticoid receptor blockade on DHF independent of blood pressure lowering.

MINERALOCORTICOID RECEPTOR is present in cardiac tissues (14). Aldosterone promotes collagen synthesis and myocyte hypertrophy (27, 35), and a growing body of evidence, including randomized clinical trials, suggests that aldosterone plays a crucial role in the pathophysiology of heart failure with systolic dysfunction (22, 23).

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pulmonary congestion with increased left ventricular (LV) filling pressure (5). In the following three studies, different rats were used.

**Study 1: detection of cardiac steroids.** The rats were anesthetized with ketamine HCl (50 mg/kg) and xylazine HCl (10 mg/kg). The hearts were harvested, put on ice, and perfused through the aorta with an ice-cold NaCl 0.9% buffer to wash out plasma components. The LV myocardium was immediately weighed, placed in liquid nitrogen, and stored at −80°C for the measurement of aldosterone.

Our preliminary study demonstrated that the cardiac aldosterone level was too low to be detectable in the LV tissue of each rat (data not published), and the LV tissue homogenates obtained from four or five hearts were required to obtain one value of the cardiac aldosterone level. The LV tissues of 15 control rats were randomly divided into three groups (one group contained LV tissues from five rats) and homogenized together in each group for the aldosterone measurement. Thus the cardiac aldosterone level for control rats could be measured in three homogenate samples. The LV tissues of 19 DHF rats were randomly divided into four groups (one group contained LV tissues from four rats, and the other three groups contained those from five rats). Thus the cardiac aldosterone level for DHF rats could be measured in four homogenate samples.

The LV tissues were homogenized in 30 ml of ice-cold lysis buffer containing 50 mM Tris-HCl (pH 7.98) and 1 mM EDTA (pH 7.2). Equal volume of chloroform-methanol (2:1) was added to the homogenate, and 800 pmol of dexamethasone was added as an internal standard. The homogenate was vortexed for 5 min and centrifuged for 10 min at 2,000 rpm (3,500 g). The organic phase was recovered, and these extraction procedures were repeated three times with the addition of fresh chloroform to the homogenate. The organic phase fractions were combined and dried under N2. Steroids were extracted with the use of Sep-Pack C18 cartridges (Waters, Milford, MA). Sep-Pack-extracted samples were applied to normal-phase HPLC under the following conditions: column, YMC A-002; UV detection, 240 nm; flow rate, 1.0 ml/min; column temperature, 40°C; mobile phase, 97% dichloromethane-methanol-water (97:2.7:0.3, vol/vol/vol). The samples were divided into fractions and dried. A fraction with a retention time of 4–25 min was used for the detection of 11-deoxycorticosterone, corticosterone, 11-dehydrocorticosterone, and aldosterone.

Fractionated samples were dissolved in 25% acetonitrile and subjected to LC-MS system (Finnigan LCQ ion trap mass spectrometer with an APCI source; Thermo Electron) with MS-MS approach to measure aldosterone. The LV myocardium was immediately placed in liquid nitrogen and stored at −80°C for the measurement of mRNA and protein levels. The LV myocardium was immediately placed in liquid nitrogen and stored at −80°C for the measurement of mRNA and protein levels. The LV myocardium was immediately placed in liquid nitrogen and stored at −80°C for the measurement of mRNA and protein levels. The LV myocardium was immediately placed in liquid nitrogen and stored at −80°C for the measurement of mRNA and protein levels.

**Study 2: aldosterone synthase activity assessment.** After anesthesia, the hearts were harvested from the control and DHF rats (n = 5, respectively), put on ice, and perfused through the aorta with an ice-cold NaCl 0.9% buffer to wash out plasma components. Aldosterone synthase activity was assessed by modifying the previously described method (31). The LV myocardium was immediately weighed and minced with scissors and a Teflon homogenizer in 1 ml of DMEM (Invitrogen, Carlsbad, CA) on ice. LV myocardial cells were suspended in 2.0 ml DMEM containing 10% charcoal-stripped FBS (HyClone, Logan, UT), antibacterial agent (penicillin G and streptomycin), and 0.1 μM of 11-[2,3H]deoxycorticosterone (specific activity, 50 Ci/mmol; American Radiolabeled Chemicals, St. Louis, MO) and cultured in a cell culture device. After incubation for 24 h at 37°C, media were collected, extracted with chloroform-methanol (2:1), and evaporated in siliconized tubes to which unlabelled standards (aldosterone and corticosterone; Sigma) dissolved in ethanol were added. After extraction with Sep-Pak C18 cartridge, extracts were spotted onto a C18 reverse-phase TLC plate with fluoroscent indicator and presorbant (LKC18F; Whatman, Middlesex, UK) and then developed in 60% methanol. The radioactive products from 11-[2,3H]deoxycorticosterone incubations were visualized by autoradiography with the use of a Fuji imaging plate and a BAS1500 system after 24-h exposure at room temperature. The unlabelled standards were visualized under UV light. LV myocardial homogenate with 1 pmol of [2,3H]aldosterone (specific activity of 50 Ci/mmol; MP Biomedicals, Irvine, CA) was used as control. In our preliminary assay, the detection limit of aldosterone and corticosterone was smaller than 60 fmol (3 nCi).

**Study 3: effects of eplerenone.** The DHF model rats were randomly divided at age 13 wk [compensatory hypertrophic stage (5)] into 3 groups (n = 6, respectively); rats treated with eplerenone at 12.5 or 40 mg·kg−1·day−1 in the food and untreated rats. Six age-matched control rats were used. The doses of eplerenone were selected according to our preliminary study in which these doses did not lower blood pressure in this model. Systolic blood pressure was measured with a tail cuff system (BP-98A; Softron, Tokyo, Japan).

**Hemodynamic studies.** The rats were anesthetized with ketamine HCl (50 mg/kg) and xylazine HCl (10 mg/kg), and echocardiographic and LV pressure recordings were obtained to determine LV geometry, LV ejection fraction, LV end-diastolic pressure, time constant of LV relaxation, and myocardial stiffness constant (41).

**Tissue sampling and pathophysiological studies.** After the hemodynamic study and adequate anesthesia, blood was sampled from the right carotid artery for measurement of plasma aldosterone level (37), the lung and the heart were harvested, and the lung and the LV were weighed. The LV weight corrected for tibial length was determined as LV mass index. The LV myocardium was immediately placed in liquid nitrogen and stored at −80°C for the measurement of mRNA and protein levels. Samples for immunohistochemistry were embedded in Tissue-Tek OCT compound (Sakura Finetechnical, Tokyo, Japan) and frozen on dry ice. The rest of the LV was fixed with a phosphate-buffered 10% formalin solution for 48 h. The specimens were embedded in paraffin, and 2-μm-thick transverse sections were stained with Azan Mallory stain to evaluate the percent area of fibrosis (5).

**Quantification of gene expression.** Real-time quantitative PCR with Prism 7700 sequence detector (Perkin-Elmer, Foster City, CA) was conducted to measure mRNA levels of type I and III collagen, angiotensin-converting enzyme (ACE), atrial natriuretic peptide (ANP), monocyte chemotactic protein-1, and angiotensin II receptor type 1. Samples for immunohistochemistry were embedded in tissue-Tek OCT compound (Sakura Finetechnical, Tokyo, Japan) and frozen on dry ice. The rest of the LV was fixed with a phosphate-buffered 10% formalin solution for 48 h. The specimens were embedded in paraffin, and 2-μm-thick transverse sections were stained with Azan Mallory stain to evaluate the percent area of fibrosis (5).

**Study 5: Tissue ANG II measurement.** Frozen heart was homogenized on ice in 0.9% saline-0.1 mol/l HCl containing 0.1 mol/l aprotinin, and the ANG II level was measured by radioimmunoassay using two antibodies specific for ANG II (SRL, Tokyo, Japan) and as previously described (40). With the use of the Lowry protein assay with BSA as a standard (Bio-Rad protein assay reagent; Bio-Rad Laboratories, Hercules, CA),
the total protein content of an aliquot of the homogenate was determined.

**Western blot analysis.** ACE and mineralocorticoid receptor protein levels in the LV were assessed with antibody to ACE (1:2,000 dilution, Santa Cruz Biotechnology, Santa Cruz, CA) and mineralocorticoid receptor (H-300, sc-11412, 1:3,000 dilution; Santa Cruz Biotechnology) as previously described (26).

**Immunohistochemistry.** Cryostat transverse sections were stained with rabbit polyclonal rat type I collagen antisemur (1:2,000 dilution; LSL, Tokyo, Japan), rabbit polyclonal rat type III collagen antisemur (1:500 dilution; Chemicon, Temecula, CA), mouse anti-rat macrophage monoclonal antibody (1:50 dilution; Ki-M2R, BMA Biomedicals, Augst, Switzerland), or mouse monoclonal anti-4-hydroxy-2-nonenal (HNE) antibody (1:50 dilution; NOF Medical Department, Tokyo, Japan) as previously described (19, 41).

**Statistical analysis.** Results are expressed as means ± SE. Differences among groups were assessed by one-factor ANOVA with Tukey’s analysis. P < 0.05 was considered statistically significant.

## RESULTS

**Cardiac aldosterone system (studies 1, 2, and 3).** Aldosterone produces a protonated molecular ion ([M+H]+) at m/z 361 and a characteristic fragment ion at m/z 343 ([M+H]+−H2O) in LC-MS spectrum at the identical retention time to the standard. The molecular ion at m/z 361 was further analyzed by selected reaction monitoring in MS-MS in this study and confirmed to produce aldosterone-specific fragment at m/z 343. Aldosterone was detected in all four homogenate LV samples of the DHF rats, and its value was 0.18 ± 0.08 pmol/g; however, aldosterone was detected in only one of the three homogenate samples of the control rats (Fig. 1).

Aldosterone precursor, 11-deoxycorticosterone (at m/z 331), was detected in both the control (8.0 ± 1.5 pmol/g) and DHF (9.3 ± 3.1 pmol/g) rats, and there was no significant difference. Corticosterone (at m/z 347) was present at much higher levels compared with aldosterone in the control (188 ± 36 pmol/g) and DHF (161 ± 57 pmol/g) rats, but there was no significant difference between the two groups. The tissue level of inactive 11-dehydrocorticosterone (at m/z 345) that is converted from corticosterone by 11β-HSD2 was low in the LV tissue without any significant difference between the two groups (control: 9.8 ± 2.6 pmol/g, DHF: 6.3 ± 0.7 pmol/g).

The in situ production of either aldosterone or corticosterone from 11-deoxycorticosterone was not detected in the LV tissue of the control and DHF rats (Fig. 2). To confirm the absence of aldosterone and corticosterone, the unlabeled standards were visualized under UV light. The mRNA of CYP11B2 was not detected and that of CYP11B1 was expressed at only minimal levels in the LV tissue of the control and DHF rats. We confirmed that the aldosterone and corticosterone synthesis in the adrenal gland was assessable using the same method (Fig. 2).

Corticosterone is converted to inactive 11-dehydrocorticosterone by 11β-HSD2 and vice versa by 11β-HSD1. The mRNA level of 11β-HSD2 was also expressed at only minimal levels in the LV tissue of the control and DHF rats and that of 11β-HSD1 was 1.7-fold increased in the DHF rats compared with the control rats (P = 0.050).

Cardiac levels of mineralocorticoid receptor were significantly higher in the DHF rats than in the control rats (Fig. 3). Plasma aldosterone level did not increase in the DHF rats compared with the control rats (Table 1).

**Effects of eplerenone on hemodynamics, diastolic function, and LV structural characteristics (study 3).** The untreated DHF rats showed signs of overt heart failure such as tachypnea, labored respiration, and loss of activity, and LV end-diastolic pressure and lung weight were significantly increased, indicating the presence of pulmonary congestion due to congestive heart failure (5). The untreated rats developed increases in LV mass index, area of fibrosis, myocardial stiffness constant, and time constant of LV relaxation without changes in LV endocardial fractional shortening and LV end-diastolic dimension compared with the control rats. LV ejection fraction was higher in the untreated rats than in the control rats. This may be well explained by the fact that ejection fraction overestimates myocardial systolic function in hypertrophied hearts. LV midwall fractional shortening was calculated to avoid the overestimation of myocardial systolic function (5), and there was no significant difference between the control and untreated rats. These characteristics are compatible with those of DHF.

Administration of eplerenone at 12.5 or 40 mg·kg⁻¹·day⁻¹ prevented increases in LV end-diastolic pressure and lung weight without reduction of systolic blood pressure. The pre-

**Fig. 1.** Representative mass spectra of aldosterone (aldo) fractions in the left ventricular (LV) samples of the control rats (left) and the diastolic heart failure (DHF) rats (right). Aldosterone produces a protonated molecular ion ([M+H]+) at m/z 361 and a characteristic fragment ion at m/z 343 ([M+H]+−H2O). Both were observed in the DHF rats (arrows) but not in most of the control rats.
vention of hemodynamic deterioration was accompanied by eplerenone-induced decreases in myocardial stiffness constant and the time constant of LV relaxation. The improvement of LV diastolic function was associated with reduction of LV mass index and area of fibrosis. The reduction of the area of fibrosis was associated with decreases in mRNA and protein levels of type I and III collagen (Figs. 4 and 5). The effects of eplerenone on LV diastolic function and structure showed dose dependency.

In the untreated rats, the production of ACE, ANP, and MCP-1 was enhanced compared with the control rats (Fig. 6). Cardiac ANG II levels were higher in the untreated rats than in the control rats (528 ± 63 vs. 346 ± 24 fg/mg protein; P < 0.05). Immunohistochemical study revealed increases in macrophage infiltration and HNE generation (Fig. 7), a marker of reactive oxygen species production (41), in the untreated rats. Administration of eplerenone dose dependently attenuated the macrophage infiltration and decreased the HNE staining with concomitant decreases in the production of ACE and ANP. Cardiac ANG II levels significantly decreased in the eplerenone-treated rats (12.5 mg·kg⁻¹·day⁻¹ of eplerenone: 273 ± 15 fg/mg protein, 40 mg·kg⁻¹·day⁻¹ of eplerenone: 234 ± 12 fg/mg protein; P < 0.05 vs. untreated rats). The mRNA level of 11β-HSD1 decreased by 42% with the administration of eplerenone at 12.5 mg·kg⁻¹·day⁻¹ and by 49% with the administration of eplerenone at 40 mg·kg⁻¹·day⁻¹ (P < 0.05 vs. untreated rats). Gene expression of MCP-1 did not significantly change in the eplerenone-treated rats.

Plasma aldosterone level and cardiac mineralocorticoid receptor level did not change with the administration of eplerenone at 12.5 mg·kg⁻¹·day⁻¹ but significantly increased at 40 mg·kg⁻¹·day⁻¹, reflecting negative feedback effects (Table 1, Fig. 3).

DISCUSSION

A few groups, to our knowledge, succeeded in the detection of cardiac aldosterone using radioimmunoassay (6, 11, 29, 30); however, values of cardiac aldosterone levels are widely spread among the studies (7). We speculated that this is because of the very low concentration of aldosterone in cardiac tissue. The LC-MS-MS method enables direct structural analysis of steroid products, with greater specificity and sensitivity (3, 21). With the use of this method, this study confirmed and expanded the previous findings by demonstrating elevation of cardiac aldosterone levels in DHF rats. The tissue mineralocorticoid receptor level was also elevated in DHF rats.
Table 1. *Hemodynamic and structural parameters of each group*

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<td>Plasma aldosterone level, pmol/ml</td>
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Values are means ± SE. LV, left ventricle. *P < 0.05 vs. control group. †P < 0.05 vs. untreated group. ‡P < 0.05 vs. eplerenone 12.5 mg·kg⁻¹·day⁻¹ group.

The mechanism to increase cardiac aldosterone is controversial. One clinical study suggested an enhanced production of aldosterone in the cardiac tissue in heart failure (16), and another study suggested an enhanced transcardiac extraction of aldosterone (33). Aldosterone is produced from 11-deoxycorticosterone by a successive reaction, and the intermediate is corticosterone. This reaction is mediated by CYP11B2. This study demonstrated the absence of in situ production of aldosterone and corticosterone from 11-deoxycorticosterone in the LV of the control and DHF rats (Fig. 2). The band for aldosterone was slightly light, even in the adrenal gland; however, that for corticosterone was dense. In the cardiac tissue, the bands for corticosterone and aldosterone were undetectable, and gene expression of CYP11B2 was not detected in the LV tissue of the control and DHF rats. Thus cardiac aldosterone production may be very small, even if present. Our results are partly compatible with some studies (4, 6, 7, 18, 43) but not with others (11, 12, 32, 42), and the difference in study results are partly compatible with some studies (4, 6, 7, 18, 43) and not with others (11, 12, 32, 42). The difference in study results may partly explain the inconsistency. However, the present results at least suggest that there are mechanisms to increase cardiac aldosterone levels without enhancement of aldosterone synthase activity in DHF. Previous experimental and clinical studies showed that the elevation of the cardiac aldosterone level was associated with the increased plasma aldosterone level (7, 30, 33). However, the present study demonstrated an increase in cardiac aldosterone level without an elevation of plasma aldosterone level in DHF. In addition, the treatment of the DHF rats with eplerenone increased plasma aldosterone level (Table 1) but attenuated cardiac aldosterone accumulation (data not shown). Thus the present study suggests the presence of an independently regulated cardiac aldosterone system, and the activation of mineralocorticoid receptor-mediated signaling pathway may be partly responsible for the elevation of cardiac aldosterone level. One possible mechanism of the increase in cardiac aldosterone level is an enhanced transcardiac extraction of aldosterone, as a previous clinical study pointed out in patients with systolic heart failure (33); however, the in vivo blood sampling of the coronary sinus in small animals is technically difficult. Because of this technical limitation, we could not address this issue. The mechanisms for the elevation of cardiac aldosterone level in DHF need to be clarified in future studies.

Nagata et al. (18) showed the upregulation of 11β-HSD1 mRNA and the minimally detectable level of 11β-HSD2 mRNA in the LV myocardium of Dahl salt-sensitive rats fed a high-salt diet, which is compatible with our results. On the basis of these results, they speculated local corticosterone excess and attributed the beneficial effects of eplerenone to

Fig. 4. mRNA levels of collagen I (left) and III (right). Values are means ± SE. *P < 0.05 vs. control. †P < 0.05 vs. untreated.
blockade of glucocorticoid-activated mineralocorticoid receptors because both glucocorticoid and mineralocorticoid have affinity to mineralocorticoid receptor (2). However, they did not assess the cardiac corticosterone level. The present study showed that the tissue concentration of corticosterone in the LV myocardium was 1,000 times as high as that of aldosterone but was not different between the control and DHF rats. Although 11β-HSD1 mRNA was increased in the DHF rats, LV tissue level of 11-dehydrocorticosterone that is converted to corticosterone by 11β-HSD1 was not different between the control and DHF rats and was much lower than that of corticosterone. The CYP11B1 mRNA level in the LV myocardium was extremely low and ~0.1% of that in the adrenal gland (data not shown), and the production of corticosterone from 11-deoxycorticosterone was not detected (Fig. 2). These results do not support local excessive production of corticosterone in the myocardium of the DHF rats compared with the control rats; however, the upregulation of mineralocorticoid receptor may play central roles in the pathogenesis of DHF through stimulation by the abundant corticosterone rather than the scarce aldosterone. Although the cardiac aldosterone level increased in DHF, it was much lower than with corticosterone.

Fig. 5. Photomicrographs of Azan Mallory staining (A) and immunohistochemical staining of collagens type I (B) and type III (C) of the left ventricle of a rat from each group.

Fig. 6. A: mRNA levels of angiotensin-converting enzyme (ACE). B: representative Western blot analysis of ACE and its summary data. C: mRNA levels of atrial natriuretic peptide (ANP). D: mRNA levels of monocyte chemoattractant protein-1 (MCP-1). Values are means ± SE. *P < 0.05 vs. control. †P < 0.05 vs. untreated.
The pathophysiological significance of the increased local aldosterone level in DHF remains unclear, and future studies are required to clarify a principal agonist to mineralocorticoid receptor in the LV myocardium of DHF.

Previous studies (12, 18, 22, 23) demonstrated beneficial effects of mineralocorticoid receptor blockade in systolic heart failure with systolic dysfunction and LV dilatation. There are several medications to improve the prognosis of patients with systolic heart failure, including spironolactone, and one of principal mechanisms of the effective pharmacological interventions is the prevention or reversal of LV dilatation (8, 34, 36). However, DHF is not associated with LV dilatation, and the reverse LV remodeling is not expected in the treatment of DHF. Although ACE inhibitor is effective in both systolic heart failure and DHF model rats, the mechanisms to provide beneficial effects are not consistent in these two types of heart failure (25, 38). Masson et al. (15) showed beneficial effects of eplerenone in rats with mild to moderate diastolic dysfunction but not with hemodynamic deterioration. Mottram et al. (17) suggested benefits of spironolactone on diastolic function in DHF patients by demonstrating that spironolactone tended to decrease left atrial area. This study expanded the previous findings by demonstrating that eplerenone attenuated LV diastolic dysfunction and structural alterations and prevented the transition to hemodynamic deterioration independent of blood pressure lowering in the hypertensive DHF model without LV dilatation. Eplerenone slightly, although not significantly, reduced blood pressure in the DHF rats (Table 1). A much larger decrease in blood pressure provided by a calcium antagonist did not attenuate LV fibrosis in the same model (19). Thus such slight changes in blood pressure cannot explain the eplerenone-induced changes in LV structure and function.

Aldosterone is an important upstream and downstream local effector of renin-angiotensin system in failing hearts (9). The administration of eplerenone decreased ACE expression in the LV tissue of the DHF model rats in this study, and the eplerenone-induced suppression of the ACE production was associated with decreased cardiac ANG II levels. This result is partly consistent with a previous study (1), and our study expanded the previous study by demonstrating that the suppression of ACE and ANG II production was independent of antihypertensive effects of eplerenone. Administration of eplerenone also attenuated reactive oxygen species generation and macrophage infiltration in the LV tissue in this study, which is compatible with recent animal studies using a systolic heart failure model (12, 13, 18). The beneficial effects of cardiac mineralocorticoid receptor blockade in DHF may be at least partly attributed to suppression of these factors. Although the administration of eplerenone decreased mRNA levels of 11β-HSD1 and ANP, the gene expression of MCP-1 did not change with the eplerenone administration in this study. MCP-1 has been suggested to contribute to inflammatory changes and pathogenesis of heart failure (28); however, our previous study also showed that the effective treatment of DHF with ACE inhibitor and angiotensin receptor blocker was not associated with a decrease in MCP-1 mRNA (41). The present and previous studies suggest that the therapeutic effects of the blockade of renin-angiotensin-aldosterone system in DHF are not necessarily provided through the suppression of the MCP-1 production.

In conclusion, the myocardial aldosterone level increased in the untreated DHF rats. However, its value was extremely low compared with corticosterone, and no evidence for enhancement of intrinsic myocardial aldosterone production was found. Myocardial mineralocorticoid receptor level significantly increased in the untreated DHF rats, and blockade of mineralocorticoid receptor with eplerenone prevented the transition to overt DHF in association with the attenuation of structural alteration and diastolic dysfunction independent of blood pressure lowering. The upregulation of myocardial mineralocorticoid receptor may be one of key determinants of the pathogenesis of DHF, and mineralocorticoid receptor antagonism is likely an effective therapeutic regimen of DHF.

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