ANGIOTENSIN II ANTAGONIST, TCV-116, PREVENTS CARDIAC HYPERTROPHY AND FIBROSIS IN SPONTANEOUSLY HYPERTENSIVE RAT BY SUPPRESSING SYMPATHETIC NERVE ACTIVITY

TAKASHI MATSUDA
First Department of Internal Medicine, , Nara Medical University
Received August 21, 1998

Abstract: I investigated effects of AII receptor antagosist, (TCV-116) on suppression of cardiac hypertrophy and fibrosis via sympathetic nerve activity in the spontaneously hypertensive rat (SHR). Method: SHR was administered orally AII receptor antagonist, (TCV-116), angiotensin converting enzyme inhibitor, (cilazapril), β-receptor blocker (metoprolol), and arterial vasodilator, (hydralazine) for 16 or 24 weeks.
Pathophysiological study was performed to evaluate cardiac hypertrophy and fibrosis. Plasma concentration of catecholamines and density of β-adrenoceptors in cardiac myocyte were evaluated to determine sympathetic nervous system activity. Result: AII antagonist and ACE inhibitor prevented cardiac hypertrophy and development of myocardial fibrosis to a greater extent than other agents tested. While β-adrenergic blocker also suppressed myocardial hypertrophy and fibrosis, its effects were weaker than those of AII antagonist and ACE inhibitor. Bmax of myocardial adrenergic receptor was significantly higher in the groups administered with AII antagonist, ACE inhibitor, and β-adrenergic blocker as compared with hydralazine treated and untreated groups. Bmax of myocardial adrenergic receptor and plasma concentration of catecholamine level did not differ among groups receiving AII antagonist, ACE inhibitors and β-adrenergic blocker. Conclusion: A II antagonist and ACE inhibitor suppressed activity of renin-angiotesin system (RAS) and that of sympathetic nervous system, and AII antagonist and ACE inhibitor may be more effective than β-adrenergic blocker or hydralazine in preventing cardiac hypertrophy and fibrosis in SHR. (奈医誌. J. Nara Med. Ass. **49**, 373～383, 1998)

Key words: Angiotensin II antagosist, β-adrenergic receptor, cardiac fibrosis, cardiac hypertrophy, spontaneously hypertensive rat
Acknowledgement: The author is sincerely grateful to Prof. K. Dohi for pertinent advice and encouragement, and to Prof. Y. Hiasa and Prof. T. Nakashima for critically reading the manuscript.

INTRODUCTION

Several studies suggest an involvement of humoral factors in hypertesive cardiac hypertrophy observed in experimental animals and in humans[1-6]. Adrenergic factors and rein-angiotensin system (RAS) are important in development of cardiac hypertrophy and fibrosis[7-10]. Angiotensin II (AII) has been shown to act as a growth-promoting factor on cardiac myocytes in experimental animals and humans[11-13]. Agents that inhibit RAS such as AII antagonist and angiotensin coverting enzyme (ACE) inhibitors have been shown to prevent

cardiac hypertrophy and fibrosis[14-17]. It has been reported previously that AII antagonist TCV-116 and ACE inhibitor each prevented hypertrophy and fibrosis of myocardium via AII receptor in the spontaneously hypertensive rat (SHR)[18]. Several studies report that catecholamines can induce cardiac hypertrophy and fibrosis[19-21]. Although it is obvious that AII antagonist and ACE inhibitor can prevent cardiac hypertrophy and fibrosis via suppression of AII activity, it is unclear whether these agents can prevent cardiac hypertrophy and cardiac fibrosis also via supperssion of sympathetic nerve activity as compared with the case of β-adrenergic blocker or hydralazine. The present study investigated the effect of prolonged administration of various antihypertensive agents on suppression of cardiac hypertrophy and fibrosis via sympathetic nerve activity in the spontaneously hypertensive rat (SHR). I evaluated AII receptor antagonist (TCV-116), ACE inhibitor (cilazapril), β-receptor blocker (metoprolol), and arterial vasodilators (hydralazine). To determine which type of antihypertensive treatments is the most effective in preventing cardiac hypertrophy and fibrosis, pathophysiological studies were performed. Level of plasma catecholamines was measured, and density of β-adrenoceptors was measured in cardiac myocytes to determine suppressive effect on sympathetic nervous system activity after drug administration for 16 and 24 weeks.

MATERIAL and METHODS

The investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985). A total of 120 male SHR was used. Each drug was dissolved in distilled water and admisistered orally for 16 or 24 weeks. One hundred and twenty rats were divided into five groups of 24 animals each. Group A received TCV-116, an AII receptor antagonist, 1 mg/kg/day. Group C received 5 mg/kg/day cilazapril, orally. Group M received metoprolol, 64 mg/kg/day. Group H received 10 mg/kg/day hydralazine, and Group N received no tratment. Body weight was determined and systolic blood pressure was measured in each group at 16 and 24 weeks by tail-cuff method. At 16 weeks and 24 weeks, 12 SHR in each group were anesthetized by the inhalation of ether and intraperitoneal injection of pentobarbital sodium (40 mg/kg). The abdomen was opened, the abdominal aorta was punctured with a 21 G needle, and 6 ml of blood was collected into tubes that contained 0.05 ml heparin sodium. The chest was then opened and the heart was removed. Each heart was washed vigorously with ice-cold saline to wash out blood, and examined histopathologically. Plasma concentrations of epinephrine and norepinephrine were measured by high performance liquid chromatography (HPLC). Density of β-adrenergic receptors in myocardial membrane were determined by radioligand binding assay. Detailed methods are described below.

Histopathological analysis

Left ventricle was separated from atrium and fatty tissues, and sliced in 2 mm thickness at the half point of long axis of left ventricle. Staining with hematoxylin and eosin staining and Masson-trichrome stain were perpormed on sections of 10 % formalin-fixed, paraffin-embedded tissue blocks from left ventricle of each SHR. Diameter of cardiac myocytes and percent area of myocardial fibrosis in left ventricle were measured with a color image processor (SPICCA II, Nippon Avionics, Tokyo, Japan). Transverse diameter of cardiomyocytes was

measured in left ventricular free wall by the method of Takemori et al.[22] Fifteen different points were selected at random in a cross-section of myocardium stained with Masson-trichrome. Diameter of 20 cardiomyocytes was measured at each point. To determine percent area of myocardial fibrosis, five fields were selected at random from one cross-sectional cut of left ventricle. Percent area of myocardial fibrosis was determined as stated above.

Assay of *β-adrenergic receptor assay*

1) Preparation of membrane

Membrane isolated from cardiac muscle of SHR was prepared by the method described by Van et al. [23] In brief, each ventricle was homogenized in ice-cold 5 mM of Tris-HCl buffer (pH 7.4) that contained 1 mM $MgCl_2$, 0.25 M sucrose, and 1 mM phenylmethyl sulfonyl fluoride (PMSF) with a homogenizer (Nihonseiki, Japan). Homogenate was centrifuged at 400 g for 10 min. Supernatant was then centrifuged at 105,000 g for 40 min at 4℃. Resting pellet was resuspended in 7 ml of ice-cold 50 mM Tris-HCl buffer (pH 7.4) that contained 10 mM $MgCl_2$ and 1 mM PMSF, and was recentrifuged 105,000 g for 20 min at 4℃. Final pellet was resuspended in a medium containing 10 mM $MgCl_2$, 50 mM Tris HCl (pH 7.4) and 1 mM PMSF to give a protein concentration between 1.0 and 3.0 mg per ml. Protein concentration was measured according to the method of Lowry et al. [24]

2) Radioligand binding assay

Density of β-adrenergic receptors (Bmax) was evaluated by radioligand binding assay described by Williams et al. [25] A homogenate of cardiac membrane was incubated with ^{3}H-dihydroalprenolol (DHA) (Dupont, USA) at concentrations of 0.25 to 6 nM in a final volume of 200 μl at 37℃ for 10 min. Each incubation was terminated by addition of 15 ml of cold buffer (1 ml/sec for 15 sec) followed by rapid vacuum filtration through glass fiber filters (Whatman International, Maidstone, UK) Total radioactivity of membrane-bound ^{3}H-DHA was measured by a ligand scintillation counting method[26]. Non-specific binding was derermined by performing parallel assays in the presence of 1 mM propranolol[26]. Specific binding to β-adrenergic receptors was determined by subtracting radioactivity of non-specific binding from total binding. Data on specific binding were analysed by Scatchard plots to calculate Bmax, which represents density of β-adrenergic receptors[26].

Measurement of plasma concentrations of catecholamines

Plasma concentrations of epinephrine and norepinephrine were measured by the method described by Kissinger et al. [27,28] Blood was drawn into heparinized tubes on ice for analysis by high performance liquid chromatography (HPLC). Blood was immediately centrifuged, and plasma was removed. It was then stored frozen at-70℃ until assayed. HPLC analysis was performed to determine concentration of plasma catecholamines as follows. Ten mg alumina were added to glass tubes, and 1.5 ml of sample plasma and 5 pmoles of dihydroxybenzylamine (DHBA) as internal standard were added to each tube. pH was adjusted to 8.7 with 400 μl of 2 M-Tris EDTA. Tubes were shaken for 15 min at 4℃ for adsorption of catecholamines to alumina, then centrifuged at 1,500 rpm for 1 min. Next, supernatant was removed and alumina was washed three times with 1 ml of 2 % Tris-EDTA (pH 8.1). Catecholamines were desorbed from alumina with 100 μl of 0.1 M acetic acid containing 0.1 mM sodium metabisulfite. After

desorption of the catecholamines from alumina, samples were centrifuged at 1,500 rpm for 1 min, and 50 μl of supernatant were injected into column with an injector (U 6 K injector, Waters, USA). Catecholamines were separated by cation exchange chromatography (m-Bondapack C_{18} column, Waters, Milord, USA), and passed through a detector cell with a graphite paste electrode and a KCl reference electrode. Potential between electrodes was kept constant at (+0.60 V). When separated catecholamines were oxidized during exposure to an electric field, current was produced. Flow of current was measured with an electric controller unit (460 electrochemical detector, Waters, Milord, USA). Current flow chromatograms were recorded (M 740 data module, Waters, Milord, USA). Concentrations of norepinephrine, epinephrine, and internal standard were determined by measuring peak heights on chromatogram.

Statistical analysis

Results are expressed as mean±SD. Comparisons among three or more groups were made by one-way ANOVA followed by Dunnetts' modified *t*-test. A level of $P<0.05$ was considered statistically significant.

RESULTS

Blood pressure

At 16 weeks of age, blood pressure was 98±6.8 mmHg in group A, 100±11.3 mmHg in group C, 107±5.8 mmHg in group M, 104±5.8 mmHg in group H, and 149±4.6 mmHg in group N, respectively. Blood pressure of groups A, C, M and H group was significantly lower than that in group N. No difference was observed in this respect among groups A, C, M and H (Fig. 1).

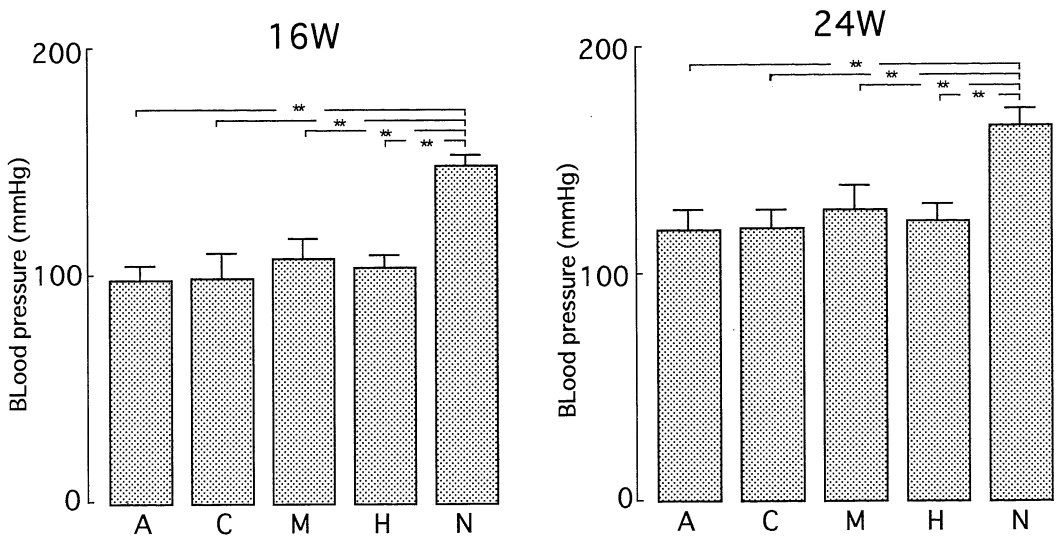

Fig. 1. Blood pressure in SHR at 16 weeks and 24 weeks. A ; AII antagonist, C ; cilazapril, M ; metprolol, H ; hydralazine, N ; no treatment. **P<0.01

At 24 weeks of age, blood pressure was 116±9.0 mmHg in group A, 117±8.3 mmHg in group C, 118±11.2 mmHg in group M, 121±7.3 mmHg in group H, and 161±6.9 mmHg in group N. Blood pressure of groups A, C, M and H was significantly lower than that in group N. No difference was observed among groups A, C, M and H (Fig. 1).

Diameter of Cardiac myocyte

At 16 weeks of age, diameter of cardiac myocyte was 13.53±0.78 μm in group A, 13.07±0.39 μm in group C, 13.93±0.36 μm in group M, 18.71±1.26 μm in group H, and 19.48±1.52 μm in group N, respectively. Diameters of cardiac myocytes in groups H and N significantly exceeded those of myocytes in groups A, C and M (Fig. 2). At 24 weeks of age, Diameter of cardiac myocyte was 15.63±2.12 μm in group A, 14.32±1.41 μm in group C, 15.72±0.53 μm in group M, 22.60±1.10 μm in group H, and 22.46±1.08 μm in group N. Diameters of cardiac myocytes in groups H and N significantly exceeded those in other groups. Diameters of cardiac myocyte in groups A and C were significantly less than those of group M. Cardiac myocyte diameter did not differ between group A and C (Fig. 2).

Percent area of myocardial fibrosis

At 16 weeks of age, percent area of myocardial fibrosis was 3.24±0.41 % in group A, 2.74±0.44 % in group C, 5.89±1.64 % in group M, 6.34±0.46 % in group H, and 6.84±0.46 % in group N. Percent area of myocardial fibrosis in groups A and C was significantly smaller than

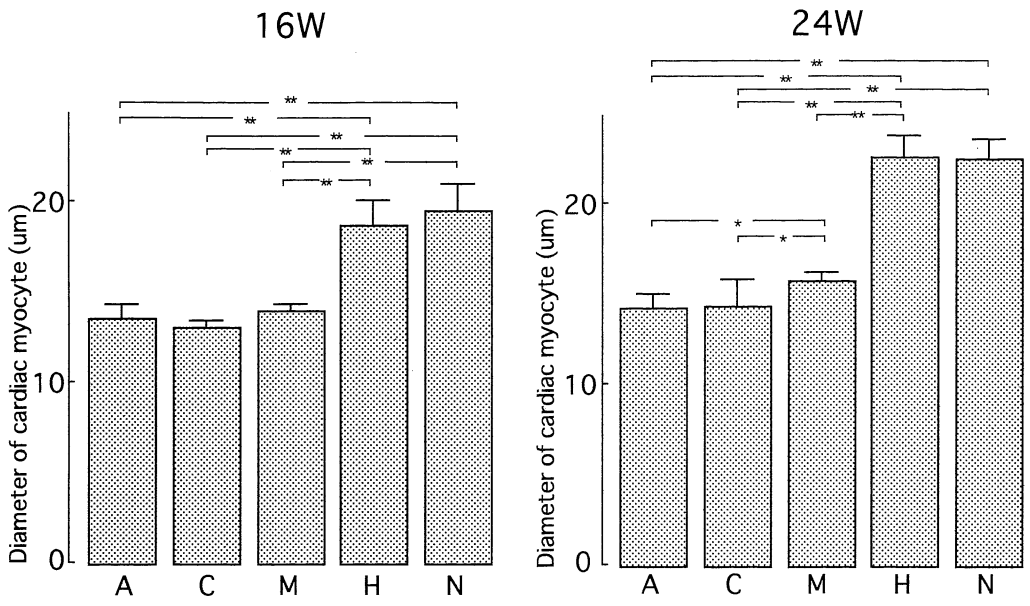

Fig. 2. Diameter of cardiac myocyte in SHR at 16 weeks and 24 weeks. A ; AII antagonist, C ; cilazapril, M ; metoprolol, H ; hydralazine, N ; no treatment. *P<0.05, **P<0.01

that in other groups (Fig. 3). At 24 weeks of age, percent area of myocardial fibrosis was 3.65 ±0.55 % in group A, 3.61±1.22 % in group C, 5.68±0.45 % in group M, 10.18±1.36 % in group H, and 11.81±2.52 % in group N. Percent area of myocardial fibrosis in group A and C was significantly smaller than that in other groups. Percent area of myocardial fibrosis was smaller in group M than that in groups H or N (Fig. 3).

Plasma concentrations of catecholamines

At 16 weeks of age, plasma concentration of epinephrine was 3.25±0.91 ng/ml in group A, 3.13±1.38 ng/ml in group C, 2.50±0.96 ng/ml in group M, 2.27±0.67 ng/ml in group H, and 2.91±0.18 ng/ml in group N. At 24 weeks of age, plasma concentration of epinephrine was 2.35±0.86 ng/ml in group A, 1.75±1.39 ng/ml in group C, 2.88±0.98 ng/ml in group M, 3.08 ±0.59 ng/ml in group H, and 2.29±0.88 ng/ml in group N. There were no significant differences in plasma concentration of epinephrine between any group. There was also no significant difference between groups as to plasma concentration of epinephrine at 16 weeks or 24 weeks (Fig. 4). At 16 weeks of age, plasma concentration of norepinephrine was 0.7±0.31 ng/ml in group A, 0.61±0.09 ng/ml in group C, 0.53±0.15 ng/ml in group M, 1.4±0.46 ng/ml in group H, and 0.76±0.16 ng/ml in group N. Plasma concentration of norepinephrine in group H sigsificantly exceeded that in groups A, C or M (Fig. 5). At 24 weeks of age, plasma concentration of norepinephrine was 0.65±0.28 ng/ml in group A, 0.36±0.24 ng/ml in group C, 0.55± 0.14 ng/ml in group M, 0.92±0.22 ng/ml in group H, and 0.82±0.53 ng/ml in group N. Level

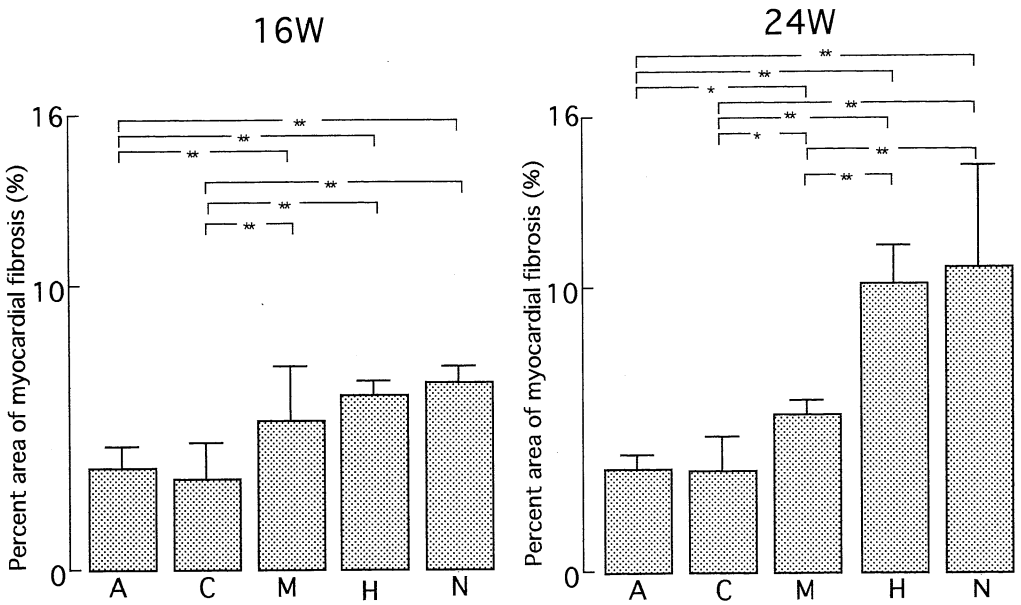

Fig. 3. Percent area of myocardial fibrosis in SHR at 16 weeks and 24 weeks. A ; AII antagonist, C ; cilazapril, M ; metoprolol, H ; hydralazine, N ; no treatment. *P< 0.05, **P<0.01

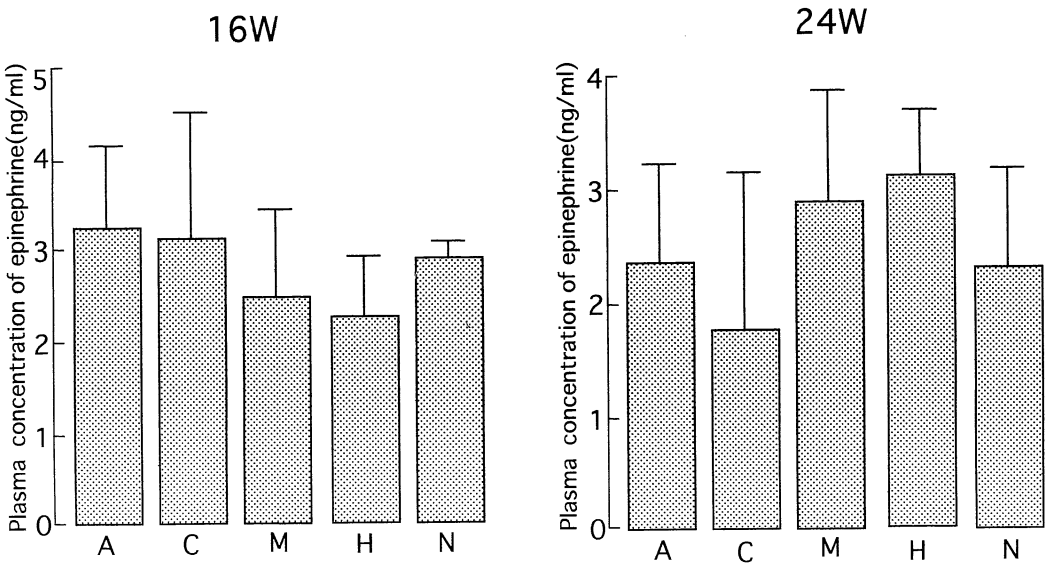

Fig. 4. Plasma concentration of epinephrine in SHR at 16 weeks and 24 weeks. A ; AII antagonist, C ; cilazapril, M ; metoprolol, H ; hydralazine, N ; no treatment.

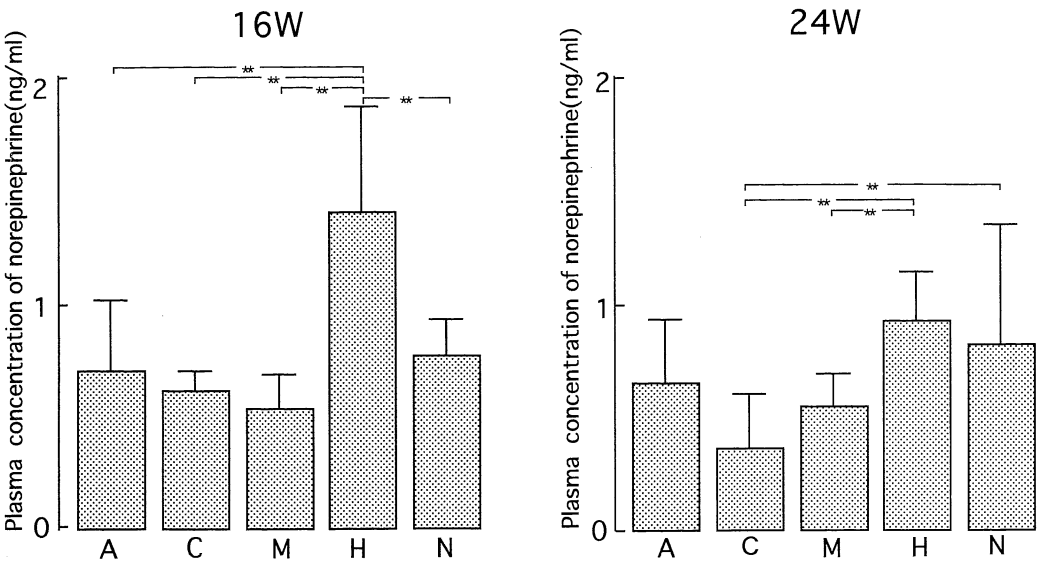

Fig. 5. Plasma concentration of norepinephrine in SHR at 16 weeks and 24 weeks. A ; AII antagonist, C ; cilazapril, M ; metoprolol, H ; hydralazine, N ; no treatment. **P< 0.01

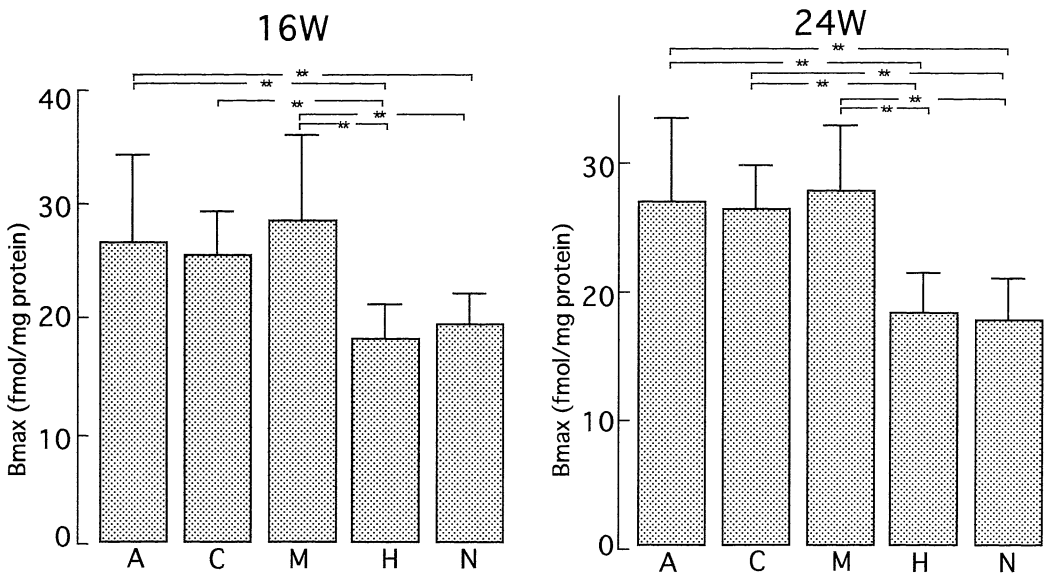

Fig. 6. Density of β-adrererergic receptor(Bmax)in SHR at 16 weeks and 24 weeks. A ; AII antagonist, C ; cilazapril, M ; metoprolol, H ; hydralazine, N ; no treatment. **P< 0.01

was higher in group H than in groups A, C or M. Plasma concentration of norepinephrine in group C was lower than that of group N (Fig. 5).

Density of β-adrenergic receptors

At 16 weeks of age, density of β-adrenergic receptors (Bmax) was 26.34±8.17 fmol/mg protein in group A, 25.44±4.04 fmol/mg protein in proup C, 28.4±8.17 fmol/mg protein in group M, 17.88±2.99 fmol/mg protein in group H, and 19.2±3.05 fmol/mg protein in group N. Density of β-adrenergic receptor in groups A and M significantly exceeded that in groups H and N (Fig. 6). At 24 weeks, density of β-adrenergic receptor was 26.55±6.81 fmol/mg protein in group A, 25.95±3.66 fmol/mg protein in group C, 27.43±5.06 fmol/mg protein in group M, 17.92±3.37 fmol/mg protein in group H, and 17.39±3.58 fmol/mg protein in group N. Density of β-adrenergic receptor in groups A, C and M was higher than that of other groups (Fig. 6).

DISCUSSION

Diameter of cardiac myocyte

Several studies have reported that hypertension and pressure afterload induce cardiac hypertrophy[2]. However, other factors also have effects on development of cardiac hypertrophy. Sympathetic nervous system and adrenergic factors are reportedly important in development of cardiac hypertrophy[1,5,29]. Cardiac hypertrophy can be induced in animals by administration of norepinephrine[9]. Stanton et al. reported that isoproterenol induces cardiomegaly in

rats in the presence of a plasma concentration of isoproterenol that did not increase blood pressure[20]. RAS is also important in cardiac hypertrophy[11]. In this study, diameter of cardiac myocyte in all astihypertensive drug treatment groups except for hydralazine, was less than that of untreated group. I demonstrated that AII antagonist and ACE inhibitor prevented cardiac hypertrophy more effectively than did β-adrenergic blocker in SHR. Hydralazine had no effect on prevention of cardiac hypertrophy compared with control group, despite its effect on normalizing blood pressure. There was no difference between AII antagonist and ACE inhibitor regarding the effect on prevention of cardiac hypertrophy. Plasma concentration of norepinephrine in hydralazine group significantly exceeded that of other groups. These findings suggest that endogenous catecholamines induce cardiac hypertrophy. Both AII antagonint and ACE inhibitor prevented cardiac hypertrophy more effectively than β-adrenergic blocker. No difference in plasma catecholamine levels was observed between groups given AII antagonist, ACE inhibitor, or β-adrenergic blocker. These results suggest that AII antagonist and ACE inhibitor each inhibited sympathetic nervous system activity and RAS in the observed prevention of cardiac hypertrophy.

Myocardial fibrosis

RAS is involved in development of cardiac fibrosis. AII, as well as TGF-β, in involved in production of collagen[11,13]. AII induces cardiac fibrosis by stimulating synthesis of collagen[11,13]. Several studies have shown that Angiotensin II acts directly on cultured fibrocytes to activate their proliferation[11,13]. In this study, development of myocardial fibrosis was inhibited to a greater extent by AII antagonist or the ACE inhibitor. While metoprolol, β-adrenergic blocker, also suppressed developmet of myocardial fibrosis, its effect was weaker than that of either AII antagonist or ACE inhibitor. Plasma concentrations of catecholamine did not differ significantly among the groups administered with AII antagonist, ACE inhibitor or β-adrenergic blocker. Plasma level of catecholamine seemed to have a relatively weaker effect on development of myocardial fibrosis than RAS. Since AII antagonist and ACE inhibitor each prevented development of myocardial fibrosis to a greater extent than β-adrenergic blocker, RAS may exert a stronger effect on progression of myocardial fibrosis in SHR. In addition to RAS-inhibiting activity, AII antagonist and ACE inhibitor seem to have a sympathetic nervous system-inhibiting activity as effective as β-adrenergic blocker. Thus, the most potent effect of AII antagonist and ACE inhibitor in suppressing cardiac cardiac fibrosis may be through the combined effect of the agents to the two systems.

β-adrenergic receptor assay and catecholamine

Measurement of plasma concentration of catecholamine and Bmax of myocardial adrenergic receptor are useful in evaluating activity of sympathetic nervous system[30]. Activation of sympathetic nervous system involves an increase in plasma concentratin of catecholamine with a down-regulation of myocardial adrenergic receptor[31]. Bristow et al. reported that density of β-adrenergic receptors was decreased when sympathetic nervous system was activated[32]. I tested the hypothesis that inhibition of sympathetic nerve activity by antihypertensive drug would reduce elevated plasma concentration of catecholamine and restore down-regulated β-adrenoceptor of myocardium, and prevent development of cardiac hypertrophy and fibrosis. I

found Bmax of myocardial adrenergic receptor to be significantly higher in groups of SHRs given AII antagonist, ACE inhibitor, or β-adrenergic blocker than those given hydralazine or no treatment. There were no significant inter-group differences regarding plasma concentration of epinephrine. Plasma concentration of norepinephrine in hydralazine group significantly exceeded that of A, C or M groups. Bmax of myocardial adrenergic receptor and plasma concentration of catecholamine did not differ significantly among proups receiving AII antagonist, ACE inhibitors, or β-adrenergic blocker. This suggests that administration of AII antagonist or ACE inhibitor suppresses sympathetic nerve activity to an extent simillar to that of a β-adrenergic blocker in SHR.

REFERENCES

1) **Ganguly, P K., Lee, SL,, Beamish, R. E.** and **Dhalla, N. S.**: Altered sympathetic system and adrenoceptors during the development of cardiac hypertrophy. Am. Heart. J. **118** : 520-525, 1989.
2) **Morgan, H. E., Gordon, E. E., Kita, Y., Chua, B. H. L., Russo, L. A., Peterson, C. J., Mcdermotto, P. J.** and **Watoson, P. A.**: Biochemical mechanism of cardiac hypertrophy. Annu. Rev. Physiol. **49** : 543-553, 1987.
3) **Pfeffer, J. M., Pfeffer, M. A., Mirsky, I.** and **Braunwald, E.**: Prevention of the development of heart failure and the regression of cardiac hypertrophy by captopril in the spontaneously hypertensive rat. Eur. Heart. J. **4** : 143-148, 1983.
4) **Sen, S.**: Regression of cardiac hypertrophy : experimental animal model. Am. J. Med. **75** : (suppl 6 A) : 87-93, 1983.
5) **Sen, S.** and **Trazi.**: Regression of myocardial hypertrophy and influence of adrenergic system. Am. J. Physiol. **244** : H 97-101, 1983.
6) **Turker, R. K., Page, I. H.** and **Bumpus, F. M.**: Antagonists of angiotensin II. in angiotensin edited by Page IH, Bumpus FM. Springer Verlag, New York, p 162-169, 1974.
7) **Dunn, F. G., Oigman, W., Ventura, H. O.,Messeri, F. H., Korbin, I.** and **Frolich, E. D.**: Enarapril improves systemic and renal hemodynamics and allows regression of left ventricular mass in essential hypertension. Am. J. Cardiol. **53** : 1044-1049, 1984.
8) **Laks, M. M.** and **Morady, F.**: Norepinephrine-the myocardial hypertrophy hormone? Am. Heart. J. **91** : 674-675, 1976.
9) **Laks, M. M., Morady, F.** and **Swan, H. J. C.**: Myocardial hypertrophy produced by chronic infusion of sub-hypertensive doses of norepinephrine in the dog. Chest. **64** : 75-78, 1973.
10) **Tazari, R. C., Sen, S., Saragoca, M.** and **Khahirallah, P. A.**: The multifactorical role of catecholamines in hypertensive cardiac hypertrophy. Eur. Heart. J. **3** (**SupplA**) : 110, 1982.
11) **Baker, K. M.** and **Aceto, J. F.**: Angiotensin II stimulation of protein synthesis and cell growth in chick heart cells. Am. Heart. J. **259** : H 610-H 618, 1990.
12) **Devereux, R. B., Pickering, T. G., Cody, R. J.** and **Laragh, J. H.**: Relation of renin-angiotensin system activity to left ventricular hypertrophy and function in experimental and human hypertension. J. Clin. Hypertens. **3** : 87-103, 1987.
13) **Katoh, Y., Komuro, I., Shibasaki, Y.** and **Yamaguchi, H.**: Angiotensin II induces hypertrophy and oncogene expression in cultured cardiac myocytes. Circulation. **80** (suppl II) : II-450, 1989.
14) **Kojima, M., Shiojima, I., Yamazaki, T., Komuro, I., Yunzeng, Z., Ying, Y., Mizuno, T.** and **Ueki, K.**: Angiotensin II receptor antagonist TCV-116 induces regression of hypertensive left ventricular hypertrophy in vivo and inhibits the intracellular signaling pathway of stretch-mediated cardiomyocyte hypertro-

phy in vitro. Circulation. **89** : 2204-2211, 1994.

15) **Lintz, W., Schaper, J., Wrener, G.** and **Albus, U.** : Raminipril prevents left ventricular hypertrophy with myocardial fibrosis without blood pressure reduction : a one-year study in rats. Br. J. Pharmacol. **107** : 970-975, 1992.

16) **Makino, N., Matsui, H., Matsumoto, K., Hata, T.** and **Yanaga, T.** : Effect of angiotensin converting enzyme inhibitor on regression in cardiac hypertrophy. Molecular. Cell. Biochem. **119** : 23-28, 1993.

17) **Mizuno, K., Tani, M., Hashimoto, S., Nimura, S., Sanada, H., Watanabe, H., Ohtsuki, M.** and **Fukuchi, S.** : Effect of Losartan, a nonpeptide angiotensin II receptor antagonist, on cardiac hypertrophy and the tissue angiotensin II content in spontaneously hypertensive rats. Life. Science. **51** : 367-374, 1992.

18) **Kagoshima, T., Masuda, J,, Sutani, T., Sakaguchi, Y.,Tsuchihashi, M., Tsuruta, S., Iwano, M., Dohi, K., Nakarura, Y., Konishi, N.** and **Hiasa, Y.** : Angiotensin II receptor antagonist, TCV-116, prevests myocardial hypertrophy in spontaneously hypertensive rats. Blood. Pressure. **3** : 89-93, 1994.

19) **Benjamin, I. J., Jalil, J. E., Tan, L. B., Cho, K., Weber, K. T.** and **Clark, W. A.** : Isoproterenol induced myocardial fibrosis in relation to myocyte necrosis. Circ. Res. **65** : 657-669, 1989.

20) **Stanton, H. C., Brenner, G.** and **Mayfield, E. D.** : Studies on isoproterenol-induced cardiomegaly in rats. Am. Heart. J. **77** : 72-80, 1969.

21) **Tsuruta, S., Sutani, T., Masuda, J., Sakaguchi, Y., Tsuchihashi, M., Hasimoto, T., Nakarura, Y.** and **Dohi, K.** : Mechanism of cardiac involvement in the WPN/Kob rat. J. Mol. Cell. Cardiol. **29** : 247-253, 1997.

22) **Takemori, E., Hasegawa, Y., Katahira, J., Nakao, K.** and **Ikutani, T.** : Effect of benazepril hydrochloride on cardiac hypertrophy in spontaneously hypertensive rats. Drug. Res. **41** : 612-615, 1991.

23) **Van, A. E., Burch, R. M., Knickelben, R. G., Hungerford, E. J., Webb, J. G.** and **Poe, S. L.** : Isolation of sealed vesicles highly enriched with sarcolemma markers from canine ventricle. Biochem. Biophys. **602** : 131-143, 1980.

24) **Lowry, O. H., Rosenbrough, N. J., Farr, A. L.** and **Randall, R. J.** : Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193** : 265-275, 1951.

25) **Williams, L. T., Synderman, R.** and **Lefkowitz, R. J.** : Identification of β-adrenergic blockers in human lymphocytes by $(-)^3$H-alprenolol binding. J. Clin. Invest. **57** : 149-155, 1976.

26) **Scathhard, G.** : The attractions of proteins for small molecules and ions. Ann. NY. Acad. Sci. **251** : 660-672, 1949.

27) **Kissinger, P. T.** : Amperometric and coulometric detectors for high performance, liquid chromatography. Ann. Chem. **49** : 447-456, 1977.

28) **Kissinger, P. T., Bruntlett, C. S.** and **Shoup, R. E.** : Neurochemical applications of liquid chromatography with electromechanical detection. Life. Science. **28** : 455-465, 1981.

29) **Kuo, Y. J.** and **Keeton, T. K. E.** : Captopril increases norepinephrine spillover rate in conscious spontaneously hypertensive rats. J. Pharmacol. Exp. Ther. **258** : 223-231, 1991.

30) **Townend, J., Virk, S. J. S., Qiang, F. X., Lawson, N., Bain, R. J.** and **Davies, M. K.** : Lymphocyte adrenoceptor upregulation and improved cardiac response to adrenergic stimulation following converting enzyme inhibition in congestive heart failure. Eur. Heart. J. **14** : 243-250, 1993.

31) **Gilbert, E. M., Sandoval, A., Larrabee, P., Renlund, D. G., Oconnell, J. B.** and **Bristow, M. R.** : Effect of lisinopril on cardiac adrenergic drive and myocardial β-receptor density in heart failure. Circulation. **78** : (suppl II) II-576, 1988.

32) **Bristow, M. R., Ginsburg, R.** and **Minobe, W.** : Decreasing catecholamine sensitivity and β-adrenergic receptor density in failing human hearts. N. Engl. J. Med. **307** : 205-211, 1982.