Original Article

Effects of TCV-116 on Endothelin-1 and PDGF A-Chain Expression in Angiotensin II-Induced Hypertensive Rats

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Angiotensin II (Ang II) has been shown to stimulate cardiac growth and collagen synthesis in cultured vascular smooth muscle cells and to increase fibroblast proliferation. Chronic infusion with Ang II increases blood pressure and activates growth mechanisms to produce hypertrophy of the heart. This study investigated the effects of an Ang II type 1 receptor antagonist, TCV-116, on preproendothelin-1 (preproET-1), ET_A receptor and platelet-derived growth factor (PDGF) A-chain expression in the left ventricle of Wistar-Kyoto rats treated for 2 weeks with Ang II (200 ng · kg⁻¹ · min⁻¹), and the relation of these effects to myocardial remodeling. Rats given Ang II alone (ANGII-V) were compared with rats also receiving TCV-116 (ANGII-TCV). In both groups, blood pressure was similar and significantly higher than in control rats. The preproET-1, ET_A receptor and PDGF A-chain expressions in the left ventricle were significantly increased in ANGII-V compared with control rats, and were significantly suppressed in ANGII-TCV compared with ANGII-V rats. ANGII-V rats showed a significant increase of the type I collagen expression, wall-to-lumen ratio, perivascular fibrosis, and myocardial fibrosis, with all these parameters being significantly improved by TCV-116. Myocardial remodeling in Ang II-induced hypertensive rats was significantly ameliorated by a subpressor dose of TCV-116, which may have been due to a decrease in ET-1 and PDGF A-chain expression in the left ventricle. (*Hypertens Res* 2001; 24: 55-64)

Key Words: angiotensin II type 1 receptor antagonist, endothelin, gene expression, PDGF

Introduction

Left ventricular hypertrophy is a strong predictor, independent of blood pressure or other risk factors, of cardiac and cerebrovascular morbidity and mortality among middle-age and elderly women and men in the general population, among patients with diverse forms of heart disease including hypertension, and among catheterized patients with and without coronary artery disease (1). On the other hand, angiotensin II (Ang II) induces the early

molecular signals of cardiac growth, and stimulates cell growth, myofibrillogenesis, and induction of fetal genes in isolated cardiomyocyte preparations (2, 3). Two main Ang II receptor subtypes, Ang II type 1 (AT₁) and Ang II type 2 (AT₂), have been identified, and AT₁ receptors are believed to mediate most of the cardiovascular actions of Ang II (4-6). AT₂ receptors are also involved in maintaining the systemic blood pressure and responsiveness of the cardiovascular system to Ang II (7), whereas chronic administration of subpressor doses of Ang II leads to a slowly developing hypertension, which resembles two-kid-

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ney, one-clip (2K1C) Goldblatt renovascular hypertension (8). However, the mechanisms responsible for the progressive nature of Ang-II induced hypertension are multifarious and incompletely understood.

Endothelin-1 (ET-1) is a potent vasoconstrictor peptide isolated from the supernatants of cultured endothelial cells, and induces cardiac hypertrophy and injury to cardiac myocytes, in addition to its potent positive inotropic and chronotropic actions (9–12). The vasoconstrictor actions of ET-1 appear to be mediated through two receptor subtypes, the endothelin A (ET_A) and B (ET_B) receptors (13). In addition to the systemic effects of ET-1, it has been demonstrated that activation of the ET_A receptor has direct effects on myocyte contractile function, protein expression, and electrophysiology (14).

Platelet-derived growth factor (PDGF) is a potent mitogen for vascular smooth muscle cells that has been implicated in the pathogenesis of atherosclerosis (15). The active PDGF molecule consists of a dimer formed by two homologous A- or B-chains, which gives the three possible isoforms, PDGF-AA, PDGF-AB, and PDGF-BB (16). The first demonstration of the effects of PDGF on vasomotion was reported by Berk *et al.* (15), who showed that PDGF induced endothelium-independent vasoconstriction of isolated rat aorta in a concentration-dependent manner. However, it is still uncertain whether ET-1 and PDGF A-chain contribute to myocardial remodeling in Ang II-induced hypertension, or whether the beneficial effects of AT₁ receptor antagonists on myocardial remodeling are associated with direct local changes in the gene expression of ET-1 and PDGF A-chain mRNA in the left ventricle (LV). To investigate the role of Ang II in the local regulation of ET-1 and PDGF A-chain, we examined the effects of an AT₁ receptor antagonist, TCV-116, on preproET-1, ET_A receptor, and PDGF A-chain expression in the LV, as well as the relation of these effects to myocardial remodeling, including type I collagen expression, in Ang II-induced hypertensive rats.

Methods

Animal Model and Experimental Design

All procedures were in accordance with institutional guidelines for animal research. Experiments were performed on 8-week-old male normotensive Wistar-Kyoto rats (Oriental Bioservice Kanto Inc., Ibaraki, Japan). The rats were randomly divided into 3 groups: 1) a saline-infused group (control group, CON, $n=8$), 2) an Ang II-infused group (ANGII-V, $n=8$), and 3) an Ang II-infused and TCV-116-treated group (ANGII-TCV, $n=8$). In all three groups, the rats were anesthetized lightly with ether, and an osmotic minipump (model 2ML2, Alzet Corp., Palo Alto, USA) containing Ang II dissolved in saline or saline alone (CON) was implanted subcutane-

ously. Ang II was continuously infused at 200 ng/kg min for 14 days. TCV-116 (1 mg/kg per day, subdepressor dose, Takeda Chemical Industries, Ltd., Osaka, Japan) was administered orally in a volume of 2 ml/kg as a gum arabic suspension. The body weight of the rats was determined before and at the end of treatment. Systolic blood pressure (SBP) and heart rate were measured in conscious rats by the tail-cuff method (model MK-1100, Muromachi Kikai, Tokyo, Japan) before treatment and at 1-week intervals thereafter (protocol 1). The numbers of animals and the experimental designs of protocols 2 and 3 were identical to those for protocol 1, above. Rats were housed at a constant temperature ($25\pm1^{\circ}\text{C}$), and were fed standard laboratory rat chow (0.4% sodium content) with free access to drinking water.

Protocol 1: Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

To evaluate the time course of gene expression, an osmotic minipump containing Ang II dissolved in saline was implanted subcutaneously for 7 days ($n=8$). After 7 or 14 days of treatment, the rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and decapitated, and their hearts immediately excised. The LV was carefully separated from the atria and right ventricle, weighed, immediately frozen in liquid nitrogen, and stored at -80°C until extraction of total RNA. Total RNA was prepared as described previously (17). The RT-PCR was performed by the standard method with 1 μg of total RNA. First-strand cDNA was synthesized with random primers and Molony murine leukemia virus reverse transcriptase (Promega, Madison, USA). PCR amplification was then performed with synthetic gene-specific primers for preproET-1 (upstream primer, 5'-CTAGGTCTAAGCG ATCCTTG-3'; downstream primer, 5'-TTCTGGTCTCT GTAGAGTTC-3'; product length, 319 bp) (18), ET_A receptor (upstream primer, 5'-GTGTTTAAGCTGTTGGC GGG-3'; downstream primer, 5'-CGAGGTCATGAGG CTTTTGG-3'; product length, 780 bp) (19), PDGF A-chain (upstream primer, 5'-AAGCATGTGCCG GAGA AGCG-3'; downstream primer, 5'-TCCTCTAACCTCA CCTGGAC-3'; product length, 305 bp) (20), and type I collagen (upstream primer, 5'-TGTTCTGGTTCTCAG GGTAG-3'; downstream primer, 5'-TTGTCGTAGCAG GGTCTTTC-3'; product length, 254 bp) (21), using a DNA PCR kit (Perkin Elmer, Norwalk, USA) for 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and elongation at 72°C for 1 min. Parallel amplification of rat glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) was performed for reference with primers as described previously (22). Reaction conditions were optimized to obtain reproducible and reliable amplification within the logarithmic phase of the reaction, as determined by preliminary experiments. The reaction was

linear to 35 cycles with use of the ethidium bromide detection method. PCR products were separated by electrophoresis on a 2% agarose gel containing ethidium bromide and were visualized by ultraviolet-induced fluorescence. The intensity of each band was quantified using a densitometer. The resulting densities of the preproET-1, ET_A receptor, PDGF A-chain and type I collagen bands were expressed relative to the corresponding densities of the GAPDH bands from the same RNA sample (23-26).

Protocol 2: Western Blot Analysis

LV was homogenized (25% wt/vol) in 10 mmol/l HEPES buffer, pH 7.4, containing 320 mmol/l sucrose, 1 mmol/l EDTA, 1 mmol/l DTT, 10 μ g/ml leupeptin, and 2 μ g/ml aprotinin at 0°C to 4°C with a polytron homogenizer. Homogenate was centrifuged at $1,000\times g$ for 5 min at 4°C and the resulting supernatant was used as a post-nuclear fraction. Protein concentrations were determined with bovine serum albumin as a standard protein (27). The post-nuclear fraction (ET_A receptor, PDGF A-chain, and type I collagen: 50 μ g of protein) of sample was subjected to SDS-PAGE using 10% gels (28). The proteins in the gels were transferred electrophoretically to PVDF sheets for 1 h at 2 mA/cm² as described (29). The sheets were immunoblotted with an anti-ET_A receptor, PDGF A-chain, and type I collagen antibody (Immuno-Biological Lab., Santa Cruz, USA) in a buffer containing 10 mmol/l Tris/HCl, pH 7.5, 100 mmol/l NaCl, 0.1% Tween 20, and 5% skim milk followed by peroxidase-conjugated anti-rabbit and anti-goat IgG (Amersham Life Sciences Inc.) (29). The ET_A receptor, PDGF A-chain, and type I collagen proteins transferred to the sheets were detected using an ECL immunoblotting detection system (Amersham Life Sciences Inc.). The amount of protein was quantified using a densitometer in a linear range and expressed as a relative percentage of that in non-treated rats (25).

Protocol 3: Histologic Examination and Evaluation of Cardiovascular Structural Changes

Histological examination was performed as described in detail previously (23-26, 30). Briefly, excised hearts were perfused with physiological saline solution containing adenosine 10 μ g/kg and nitroglycerin 10 μ g/kg and then with 6% formaldehyde solution *via* retrograde infusion into the ascending aorta at a pressure of 90 mmHg. The LV was separated from the right ventricle, the atria, and the great vessels, and cut into five pieces perpendicular to the long axis. For light microscopy, 1.5- μ m-thick sections were cut using a microtome (type ROM-380; Yamato Kohki, Saitama, Japan). Paraffin slices from each heart were mounted on glass slides and stained with hematoxylin-eosin and Masson's trichrome stains. All histopathological sections

of each animal were examined using a 3CCD color video camera (model DXC-930, Sony, Tokyo, Japan) mounted on a standard microscope (BHS-F, Olympus, Tokyo, Japan). Drawings of the limits of the vessels were made on the screen of a multiscan color computer display (model CPD-17SF7, Sony) and then digitized with a two-dimensional analysis system (Mac SCOPE, Mitani Corp., Fukui, Japan) connected to a Macintosh computer system (Power Macintosh G4, Apple Computer Inc., Cupertino, USA). Histopathological findings of the myocardium and coronary arterioles were examined. We always measured the capillary density and cross-sectional surface area in the endocardium of the posterior portion of the left ventricular free wall. In this part of the heart, shrinkage was minimal and orientation of the myocardial fibers was similar from one heart to another. We analyzed five sites from each ventricle in all rats. To assess thickening of the coronary arterial wall and perivascular fibrosis, the transsectional images of the area of the total small arteriolar lumen $\leq 10^4 \mu\text{m}^2$ were studied. The inner border of the lumen and the outer border of the tunica media were traced in each arterial image with hematoxylin-eosin staining at $\times 100$ to $\times 400$ magnification, and the areas encircled by the tracings were calculated. In quantification, nonround vessels resulting from oblique transsection or branching were excluded, and only round vessels were studied. The wall-to-lumen ratio (the area of the vessel wall divided by the area of the total blood vessel lumen) was determined. The area of fibrosis immediately surrounding blood vessels was calculated, and perivascular fibrosis was determined as the ratio of the area of fibrosis surrounding the vessel wall to the total area of the vessel. To assess the area of myocardial fibrosis, the area of pathological collagen deposition was measured in the microscopic field of each Masson's trichrome-stained section. The ratio of the total area of fibrosis within the left ventricular myocardium to the total area of the left ventricular myocardium in each heart was calculated and was used for analysis. The histopathological analysis of the sections from each rat was carried out by an operator who was blinded to the treatment groups.

Statistical Analysis

All results are expressed as the mean $\pm$ SEM. The mean values were compared among the three groups using ANOVA followed by the Bonferroni test. Differences of $p < 0.05$ were considered statistically significant. Calculations, including those of derived values, and statistical tests were performed using the appropriate software (Stat View-J 4.5, Abacus Concepts Inc., Berkeley, USA) and a Power Macintosh computer system (G4, Apple Computer Inc.).

Fig. 1. A typical gel electrophoresis of RT-PCR of left ventricular preproET-1 mRNA, ET_A receptor mRNA, platelet-derived growth factor (PDGF) A-chain mRNA, type I collagen mRNA, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. Lane 1 represents control rats (CON), lane 2 angiotensin II-induced hypertensive rats treated with the vehicle for 7 days, lane 3 angiotensin II-induced hypertensive rats treated with the vehicle for 14 days (ANGII-V), and lane 4 angiotensin II-induced hypertensive rats treated with TCV-116 (ANGII-TCV).

Results

Systemic Hemodynamics, Body Weight, and Left Ventricular Weight

The SBP values in ANGII-V and ANGII-TCV rats were similar, and were significantly higher than the SBP in CON rats (ANGII-V: 173 ± 4 and ANGII-TCV: 170 ± 4 vs. CON: 135 ± 3 mmHg; $p < 0.01$, respectively). Heart rate was similar in CON and ANGII-V rats (323 ± 11 vs. 329 ± 12 bpm), and was not changed by the administration of TCV-116 (342 ± 10 bpm vs. ANGII-V). Body weight was also similar among the three groups (CON, 260 ± 6 ; ANGII-V, 252 ± 8 ; ANGII-TCV, 250 ± 9 g). The left ventricular weight was significantly greater in ANGII-V than in CON rats (2.53 ± 0.05 vs. 2.02 ± 0.03 mg/g, $p < 0.01$), and was significantly less in ANGII-TCV than in ANGII-V rats (2.23 ± 0.04 vs. ANGII-V, $p < 0.01$).

RT-PCR for Left Ventricular PreproET-1, ET_A Receptor, PDGF A-Chain, and Type I Collagen mRNA Expression

With regard to the time course of gene expression, preproET-1, ET_A receptor, PDGF A-chain, and type I collagen mRNA levels increased after Ang II infusion (Figs. 1, 2A, B, 3A, B). The level of preproET-1 mRNA in the LV was significantly increased in ANGII-V com-

Fig. 2. Effects of chronic TCV-116 treatment on preproET-1 (A) and ET_A receptor (B) mRNA expression. Total RNA was assayed by RT-PCR with gene-specific primers for preproET-1, ET_A receptor, and GAPDH. The two panels represent the intensity ratio of preproET-1 (A) and ET_A receptor (B) to GAPDH. Values are expressed as means $\pm$ SEM. Group 1 represents control rats (CON), group 2 angiotensin II-induced hypertensive rats treated with the vehicle for 7 days, group 3 angiotensin II-induced hypertensive rats treated with the vehicle for 14 days (ANGII-V), and group 4 angiotensin II-induced hypertensive rats treated with TCV-116 (ANGII-TCV). $n = 8$ per group. ^{*} $p < 0.05$, ^{**} $p < 0.01$ vs. group 1. [†] $p < 0.05$, ^{††} $p < 0.01$ vs. group 3.

pared with CON rats, and significantly decreased in ANGII-TCV compared with ANGII-V rats (Figs. 1, 2A). Expression of ET_A receptor mRNA was significantly increased in ANGII-V compared with CON rats and significantly decreased in ANGII-TCV compared with ANGII-V rats (Figs. 1, 2B). The PDGF A-chain mRNA level was significantly greater in ANGII-V than in CON rats, and was significantly less in ANGII-TCV than in ANGII-V rats (Figs. 1, 3A). In addition, the type I collagen mRNA level was significantly greater in ANGII-V than in CON rats, and was significantly less in ANGII-TCV than in ANGII-V rats (Figs. 1, 3B).

Fig. 3. Effects of chronic TCV-116 treatment on PDGF A-chain (A) and type I collagen (B) mRNA expression. Total RNA was assayed by RT-PCR with gene-specific primers for PDGF A-chain, type I collagen, and GAPDH. The two panels represent the intensity ratio of PDGF A-chain (A) and type I collagen (B) to GAPDH. Values are expressed as the means $\pm$ SEM. Group 1 represents control rats (CON), group 2 angiotensin II-induced hypertensive rats treated with the vehicle for 7 days, group 3 angiotensin II-induced hypertensive rats treated with the vehicle for 14 days (ANGII-V), and group 4 angiotensin II-induced hypertensive rats treated with TCV-116 (ANGII-TCV). $n=8$ per group. * $p<0.05$, ** $p<0.01$ vs. group 1. $^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.01$ vs. group 3.

Western Blot Analysis for ET_A Receptor, PDGF A-Chain, and Type I Collagen Protein Expression in the LV

Western blot data are depicted in Figs. 4–6. The ET_A receptor protein mass in the LV was significantly increased in ANGII-V compared with CON rats, and significantly decreased in ANGII-TCV compared with ANGII-V rats (Fig. 4A, B). The PDGF A-chain protein mass of the LV was significantly greater in ANGII-V than in CON rats, and was significantly less in ANGII-TCV than in ANGII-V rats (Fig. 5A, B). Moreover, type I collagen protein level was significantly greater in ANGII-V than CON

Fig. 4. Effect of chronic TCV-116 treatment on ET_A receptor protein expression level. (A) A typical Western blot of ET_A receptor in the LV of ANGII rats treated with TCV-116. Western blot analyses were performed using anti-ET_A receptor antibodies. Lane 1 represents control rats (CON), lane 2 angiotensin II-induced hypertensive rats treated with the vehicle (ANGII-V), and lane 3 angiotensin II-induced hypertensive rats treated with TCV-116 (ANGII-TCV). (B) Group data depicting relative optical densities of left ventricular anti-ET_A receptor protein bands in the study groups. Values are expressed as the means $\pm$ SEM. $n=8$ per group. * $p<0.05$ vs. CON; $^{\dagger}p<0.05$ vs. ANGII-V.

rats, and was significantly less in ANGII-TCV than in ANGII-V rats (Fig. 6A, B).

Myocardial Remodeling

The wall-to-lumen ratio was significantly greater in ANGII-V than CON rats, but was significantly decreased by TCV-116 treatment (Figs. 7, 8A). The degree of perivascular fibrosis was significantly greater in ANGII-V than in CON rats, and was also significantly decreased by TCV-116 treatment (Figs. 7, 8B). Myocardial fibrosis was significantly greater in ANGII-V than in CON rats, but it was significantly less in ANGII-TCV than in ANGII-V rats (Fig. 8C).

Discussion

The present study demonstrated that the production of preproET-1, ET_A receptor and PDGF A-chain expression were increased in the LV of ANGII-V rats. In addition, with regard to the time course of gene expression, these mRNA levels increased after Ang II infusion. These up-regulated preproET-1, ET_A receptor and PDGF A-chain expressions were significantly decreased by the Ang II

Fig. 5. Effect of chronic TCV-116 treatment on PDGF A-chain protein expression level. (A) A typical Western blot of PDGF A-chain in the LV of ANGII rats treated with TCV-116. Western blot analyses were performed using anti-PDGF A-chain antibodies. Lane 1 represents control rats (CON), lane 2 angiotensin II-induced hypertensive rats treated with the vehicle (ANGII-V), and lane 3 angiotensin II-induced hypertensive rats treated with TCV-116 (ANGII-TCV). (B) Group data depicting relative optical densities of left ventricular anti-PDGF A-chain protein bands in the study groups. Values are expressed as the means $\pm$ SEM. $n=8$ per group. * $p<0.05$ vs. CON; † $p<0.05$ vs. ANGII-V.

Fig. 6. Effect of chronic TCV-116 treatment on type I collagen protein expression level. (A) A typical Western blot of type I collagen in the LV of ANGII rats treated with TCV-116. Western blot analyses were performed using anti-type I collagen antibodies. Lane 1 represents control rats (CON), lane 2 angiotensin II-induced hypertensive rats treated with the vehicle (ANGII-V), and lane 3 angiotensin II-induced hypertensive rats treated with TCV-116 (ANGII-TCV). (B) Group data depicting relative optical densities of left ventricular anti-type I collagen protein bands in the study groups. Values are expressed as the means $\pm$ SEM. $n=8$ per group. * $p<0.05$ vs. CON; † $p<0.05$ vs. ANGII-V.

Fig. 7. Micrographs of coronary arterioles with Masson's trichrome staining in the control rats (CON: left), angiotensin II-induced hypertensive rats treated with the vehicle (ANGII-V: middle), and angiotensin II-induced hypertensive rats treated with TCV-116 (ANGII-TCV: right).

Fig. 8. Effects of TCV-116 on myocardial remodeling in angiotensin II-induced hypertensive rats. Wall-to-lumen ratio (A), perivascular fibrosis (B), and myocardial fibrosis (C) were measured histopathologically. Values are expressed as the means $\pm$ SEM. ANGII-TCV, angiotensin II-induced hypertensive rats treated with TCV-116; ANGII-V, angiotensin II-induced hypertensive rats treated with the vehicle; CON, control. $n=8$ per group. * $p<0.05$, ** $p<0.01$ vs. CON. †† $p<0.01$ vs. ANGII-V.

type 1 receptor antagonist, TCV-116. Moreover, after 2-weeks of treatment, TCV-116 effectively improved left ventricular hypertrophy and cardiovascular structural changes, and decreased type I collagen expression. These results suggest that chronic Ang II type 1 receptor blockade improves the myocardial remodeling and ameliorates

the effects of left ventricular fibrosis factor. Therefore, it is strongly suggested that endogenous preproET-1, ET_A receptor and PDGF A-chain play an important role in the deterioration of myocardial remodeling.

Previous studies on cultured endothelial cells and vascular smooth muscle cells have shown that Ang II increases preproET-1 mRNA and ET-1 protein (31-34). Chua *et al.* (31) demonstrated that Ang II stimulated preproET-1 mRNA in a dose- and time-dependent manner in cultured endothelial cells derived from rat hearts, and that ET may play a role in modulating gene expression in Ang II-mediated cardiac hypertrophy. Imai *et al.* (32) suggested that preproET-1 mRNA expression was induced by Ang II in cultured bovine carotid artery endothelial cells. Emori *et al.* (33) showed that Ang II stimulated the release of ET-1, and that this release involved receptor-mediated mobilization of intracellular calcium and activation of protein kinase C in cultured bovine endothelial cells. Furthermore, Sung *et al.* (34) demonstrated that Ang II-mediated vascular smooth muscle cell proliferation occurs *via* the effects of the AT₁ receptor, and that Ang II-induced ET production may contribute to the proliferative response in these cells. These results show that ET-1 can in fact influence the vasoconstrictive action of Ang II. And indeed, in an *in vivo* study, Ishiye *et al.* (35) suggested that endogenous Ang II may contribute, *via* the AT₁ receptor, to the development of volume overload cardiac hypertrophy, and that endogenous ET-1 may mediate some of the hypertrophic effects of Ang II. In addition, Maeso *et al.* (36) demonstrated that endogenous Ang II, through the AT₁ receptors, plays a mediatory role in the contractile effect of endothelium vasoconstrictor factor, since the AT₁ receptor antagonist losartan attenuated the contractile response to ET in aortic rings from spontaneously hypertensive rats. Furthermore, Moreau *et al.* (37) demonstrated that vascular hypertrophy and the increase in blood pressure induced by Ang II *in vivo* was mediated at least in part by an increased production of endogenous ET, which activated ET_A receptors to produce the observed changes in the vascular structure of cerebral and mesenteric circulation. On the other hand, d'Uscio *et al.* (38) recently reported that the AT₁ receptor antagonist losartan prevented the increase in tissue ET-1 levels in the aortas of Wistar-Kyoto rats treated for 2 weeks with Ang II. However, it is not known whether preproET-1 or ET_A receptor mRNA expression is increased by Ang II *in vivo*. In this study, we have demonstrated that the production of preproET-1 and the expression of ET_A receptor mRNA were increased, and that the AT₁ receptor antagonist TCV-116 decreased these gene expressions in the LV of Ang II-induced hypertensive rats. These results suggest that the AT₁ receptor may directly modulate cardiovascular ET-1 production in the LV of Ang II-induced hypertensive rats (39).

With regard to cardiac cell proliferation and intramyocardial perivascular fibrosis, McEwan *et al.* (40) concluded that Ang II differentially regulates cell proliferation in the myocardium. Ang II directly increases proliferation of medial vascular smooth muscle cells and adventitial and interstitial fibrosis associated with intramyocardial blood vessels in atria and ventricles for the heart *in vivo*. In addition, these effects are mediated by the AT₁ receptor and are independent of elevated aldosterone and arterial blood pressure. These observations suggest an important role for losartan in reducing hyperplasia of intramyocardial coronary arterioles. These results have important implications not only in renin-dependent hypertension but also in chronic cardiac failure, in which the circulating Ang II levels are elevated.

Whereas, different contributions of ET_A and ET_B receptor in the pathogenesis, Matsumura *et al.* (41) demonstrated that selective ET_A receptor blockade efficiently overcame the development of hypertension, vascular hypertrophy, and renal injury in deoxycorticosterone acetate (DOCA)-salt hypertensive rats. On the other hand, ET_A and ET_B receptors have been shown to make different contributions to the pathogenesis. Matsumura *et al.* (41) demonstrated that selective ET_A receptor blockade efficiently overcame the development of hypertension, vascular hypertrophy, and renal injury in deoxycorticosterone acetate (DOCA)-salt hypertensive rats, whereas selective ET_B receptor blockade led to a deterioration in the pathogenesis of DOCA-salt hypertension. These results suggest that ET_A receptor-mediated actions play an important role in the pathogenesis of DOCA-salt-induced hypertension, and ET_B receptor-mediated actions protect against vascular and renal injuries in this model of hypertension.

PDGF was one of the first mitogens identified in cultured smooth muscle cells, and it has been postulated that PDGF induces smooth muscle proliferation in atherosclerosis (42). Libby *et al.* (43) concluded that smooth-muscle cells from diseased human arteries can induce mitogenic activity, some of which resembles the mitogenic activity of PDGF, and that these cells express the gene for the PDGF A-chain selectively. Rekhter and Gordon (44) suggested that PDGF A and other factors that are particularly associated with vascularized regions may be involved in the regulation of mesenchymal cell proliferation in human atherosclerotic plaques. Furthermore, Negoro *et al.* (45) demonstrated that PDGF A-chain mRNA levels are increased in hypertrophic vascular smooth muscle cells of spontaneously hypertensive rats *in vivo*, and that PDGF A-chain gene expression in these cells is associated with levels of both blood pressure and vascular hypertrophy in spontaneously hypertensive rats. In the present study, we have demonstrated that Ang II stimulates PDGF A-chain mRNA expression in the LV and induces myocardial remodeling in Ang II-induced hypertensive rats *in vivo*.

Naftilan *et al.* (46) demonstrated that the potent vasoconstrictor Ang II could induce the expression of the proto-oncogene *c-myc* along with the gene encoding the PDGF A-chain in cultured rat vascular smooth muscle cells. Wang *et al.* (47) demonstrated that Ang II infusion induces medial hypertrophy in the rat aorta and structural remodeling of the arteriole without measurable changes in media volume and that the hypertrophic growth response of the aortic wall to Ang II infusion is accompanied by both PDGF-A messages and protein expression. Furthermore, Parker *et al.* (48) indicated that the hypertrophic response of arterial vascular smooth muscle cells to Ang II infusion was mediated through an increase in arterial blood pressure, and that PDGF-A expression, which may be a mediator of the hypertrophic response, is also associated with the elevation in pressure rather than a direct effect of Ang II. Moreover, Fukuda *et al.* (49) indicated that the long-form of the PDGF A-chain contributes to the exaggerated growth of vascular smooth muscle cell from spontaneously hypertensive rats. In contrast, PDGF- α receptor (PDGF- α R) expression is substantially more elevated in the cultured vascular smooth muscle cells of spontaneously hypertensive rats than in those of Wistar-Kyoto rats. To evaluate the role of PDGF- α R in vascular remodeling and hypertension, Kitami *et al.* (50) investigated the underlying molecular mechanism and suggested that differential expression of members of the CCAAT-enhancer binding proteins (C/EBP) family, mainly C/EBP- δ and possibly C/EBP- β , are responsible for the strain-specific gene transcription of PDGF- α R in vascular smooth muscle cells. These results suggest that PDGF-A may contribute at least in part to the pathogenesis of atherosclerosis and the mediation of extensive myocardial remodeling *in vivo*.

In contrast, mitogen-activated protein kinases (MAPK) are a ubiquitous group of protein serine/threonine kinases and are important mediators of the signal transduction pathway, which is responsible for cell growth and proliferation. Extracellular signal-regulated kinases (ERK) and *c-jun* NH₂-terminal kinases (JNK) are the main subgroups of the MAPK family. An *in vitro* study has shown that ERK are rapidly activated by various growth factors and vasoactive hormones, such as Ang II (51). Recently, Yano *et al.* (52) demonstrated that both cardiac ERK and JNK are activated by Ang II-induced hypertensive rats, that activator protein-1 (AP-1) activation in Ang II-induced cardiac hypertrophy might be due in part to JNK activation, and that JNK might play some role in the Ang II-induced cardiac hypertrophic response *in vivo*. Moreover, Hamaguchi *et al.* (53) showed that Ang II-induced hypertension caused the activation of glomerular ERK and JNK, leading to the activation of AP-1, and suggested that ERK and JNK signaling cascades, *via* the activation of AP-1, might be implicated in the development of hypertension-induced glomerular injury. These

results suggest that the activation of ERK and JNK signaling cascades may have been at least partially responsible for the development of myocardial remodeling in the present study.

In conclusion, we examined the effects of long-term treatment with the AT₁ receptor antagonist TCV-116 on preproET-1, ET_A receptor, and PDGF A-chain expression in the LV, as well as the relation of these effects to cardiovascular structural changes, including type I collagen mRNA expression, in Ang II-induced hypertensive rats. The results demonstrated that upregulation of preproET-1, ET_A receptor, and PDGF A-chain expression in ANGII was significantly decreased by TCV-116 treatment. Myocardial remodeling in Ang II-induced hypertensive rats was significantly ameliorated by a subdepressor dose of TCV-116, possibly due to a decrease in ET-1 and PDGF A-chain expression in the LV.

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