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Celiprolol inhibits mitogen-activated protein kinase and endothelin-1 and transforming growth factor-β_1 gene in rats

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Abstract

We evaluated the cardioprotective effects of long-term treatment with celiprolol (for 5 weeks), a specific β_1-adrenoceptor antagonist with a weak β_2-adrenoceptor agonist action, on endothelin-1 and transforming growth factor (TGF)-β_1 expression and cardiovascular remodeling in deoxycorticosterone acetate (DOCA)-salt hypertensive rats. Upregulated preproendothelin-1, endothelin ET_A receptor, TGF-β_1, c-*fos*, and type I collagen expression and extracellular signal-regulated kinase activities were suppressed by celiprolol. Celiprolol effectively inhibited vascular lesion formation such as medial thickness and perivascular fibrosis. These observations suggested that extracellular signal-regulated kinase and c-*fos* gene pathway may contribute to the cardiovascular remodeling of DOCA rats, and that cardioprotective effects of celiprolol on cardiovascular remodeling may be mediated, at least in part, by suppressed expression of endothelin-1 and TGF-β_1.
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Keywords: β-Adrenoceptor antagonist; Endothelin-1; Signal transduction; TGF-β_1 (transforming growth factor-β_1)

1. Introduction

Left ventricular hypertrophy is a strong independent risk factor for development of ischemic heart disease, arrhythmia, ventricular dysfunction, and congestive heart failure (Levy et al., 1989). In hypertensive heart disease, myocytes hypertrophy and interstitial components undergo hyperplasia, hypertrophy and remodeling (Weber and Brilla, 1991). In addition, various vascular abnormalities result in a reduction in the maximal cross-sectional area of the coronary microvasculature. These abnormalities include inadequate vascular growth in response to increasing muscle mass, changes in vessel wall composition, vascular remodeling, and endothelial dysfunction. Extracellular signal-regulated kinases (ERK) composed of p42ERK and p44ERK, are one main subgroup of mitogen-activated protein kinases and are important mediators of the signal-transduction pathway responsible for cell differentiation and growth (Davis, 1993). Recent evidence indicates that ERKs in cultured neonatal rat cardiac myocytes are rapidly activated by various extracellular stimuli, such as growth factors and other mitogens, and play a key role in cell growth and the regulation of various gene expressions. Moreover, the c-*fos* gene is the most frequently studied member of the cellular immediate–early genes and, has been associated with cellular proliferation, differentiation, and hypertrophy (Verma and Sassone-Corsi, 1987). Therefore, the c-*fos* gene may play a critical role in myocardial signal transduction.

Endothelin-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 (Yanagisawa et al., 1988). The vasoconstrictor action of endothelin-1 appears to be mediated through two receptor subtypes, the endothelin ET_A and ET_B receptors (Sokolovsky, 1992). Endothelin-1 has direct effects on contractile function, protein synthesis, and electrophysiological events in cardiac myocytes, and these effects are mediated primarily by the endothelin ET_A receptors (Yanagisawa et al., 1988). Indeed, Barton et al. (1998) suggested that aortic tissue endothlelin-1 content acts as a local mediator of vascular dysfunction and aortic hypertrophy, and that endothelin ET_A receptor antagonism may have therapeutic potential for lowering vascular endothelin-1

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content, improving endothelial function, and preventing structural changes in the vascular hypertrophy. Furthermore, Moreau et al. (1997) demonstrated that vascular hypertrophy and the increase in blood pressure induced by angiotensin II in vivo was mediated at least in part by an increased production of endogenous endothelin, which activated endothelin ET_A receptors to produce the observed changes in vascular structure of cerebral and mesenteric circulation. On the other hand, transforming growth factor-β (TGF-β) is a multifunctional cytokine with fibrogenic and hemodynamic properties (Border and Nobel, 1994). Although all three isoforms of TGF-β (TGF-β_1, TGF-β_2, and TGF-β_3) are present in the heart, the level of the type 1 isoform seems to be particularly related to the development of left ventricular hypertrophy (Li and Brooks, 1997). In addition, left ventricular hypertrophy of stroke-prone spontaneously hypertensive rats was associated with the increase in TGF-β_1 mRNA levels (Kim et al., 1996). Moreover, upregulation of TGF-β_1 has been reported to be associated with cardiovascular alterations in hypertensive patients (Porreca et al., 1997). Therefore, endothelin-1 and TGF-β_1 may be produced and exert important roles in the cardiovascular remodeling.

β-adrenoceptor antagonists are widely used as effective antihypertensive agents, and recently we (Kobayashi et al., 2001c) and other investigators (Kakoki et al., 1999) have demonstrated that a β-adrenoceptor antagonist, celiprolol, exert their vasodilatory action through the nitric oxide pathway and that treatment with this agent may protect against endothelial injury in hypertension. However, it is still unclear whether the beneficial effects of β-adrenoceptor antagonists on coronary microvascular remodeling is associated with direct local left ventricular gene expression of endothelin-1 and TGF-β_1 mRNA in deoxycorticosterone acetate (DOCA)-salt hypertensive rats. To elucidate the potential cardioprotective effects of β-adrenoceptor antagonists in left ventricular hypertrophy, we evaluated the effects of long-term treatment with celiprolol, a specific β_1-adrenoceptor antagonist with a weak β_2-agonist action, on preproendothelin-1, endothelin ET_A receptor, TGF-β_1, and c-*fos* expression in the left ventricle and evaluated the relationships between these effects and coronary microvascular remodeling, and p42/p44ERK activity in DOCA-salt hypertensive rats.

2. Methods

2.1. Animal models and experimental designs

All procedures were in accordance with institutional guidelines for animal research. Twenty-four male normotensive Wistar rats (Oriental Bioservice Kanto, Ibaragi, Japan) aged 6 weeks were used, and DOCA-salt hypertension was induced in 16 rats as described previously (Kobayashi et al., 2001c). Eight rats received weekly subcutaneous injections of DOCA (30 mg/kg) after right nephrectomy and were given 1% saline as drinking water (DOCA−vehicle, $n=8$). The remaining eight DOCA-salt rats were treated with celiprolol (Nippon Shinyaku Co. Ltd., Kyoto, Japan) in their drinking water for 5 weeks, a fresh drug solution being prepared daily (DOCA-celiprolol, $n=8$). The drug was given at an average dose of 10 mg/kg/day, which was adjusted weekly to the drinking habits of the animals. The 10 mg/kg/day of celiprolol was not expected to influence blood pressure in rats, according to our preliminary data (data not shown). Age-matched sham-operated rats ($n=8$) served as a control group. Systolic blood pressure was measured using the tail-cuff method (Muromachi Kikai, model MK-1100, Tokyo, Japan) before the operation and at 1-week intervals thereafter. Rats were housed at a constant temperature (25 ± 1 °C), and fed a standard laboratory rat chow (0.4% sodium content).

2.2. Reverse transcription-polymerase chain reaction (RT-PCR) for preproendothelin-1, endothelin ET_A receptor, TGF-β_1, c-fos, and type I collagen mRNA expression in the left ventricle

After 5 weeks of treatment, the rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and decapitated, and the heart was immediately excised. The left ventricle was carefully separated from the atria and right ventricle, weighted, immediately frozen in liquid nitrogen, and stored at −80 °C until extraction of total RNA. The RT-PCR was performed by standard methods with 1 μg of total RNA. First-strand cDNA was synthesized with random primers and Molony murine leukemia virus reverse transcriptase (Promega, Madison, WI, USA). PCR amplification was then performed with synthetic gene-specific primers for preproendothelin-1 (sense primer, 5′-CTA GGT CTA AGC GAT CCT TG-3′; antisense primer, 5′-TTC TGG TCT CTG TAG AGT TC-3′) (Sakurai et al., 1991), endothelin ET_A receptor (sense primer, 5′-GTG TTT AAG CTG TTG GCG GG-3′; antisense primer, 5′-CGA GGT CAT GAG GCT TTT GG-3′) (Terada et al., 1992), TGF-β_1 (sense primer, 5′-AAT ACG TCA GAC ATT CGG GAA GCA-3′; antisense primer, 5′-GTC AAT GTA CAG CTG CCG TAC ACA-3′) (Qian et al., 1990), c-*fos* (sense primer, 5′-GGG ACA GCC TTT CCT ACT ACC ATT-3′; antisense primer, 5′-CGC AAA AGT CCT GTG TGT TGA-3′) (Lu et al., 2001), and type I collagen (sense primer, 5′-TGT TCG TGG TTC TCA GGG TAG-3′; antisense primer, 5′-TTG TCG TAG CAG GGT TCT TTC-3′) (Nicoletti et al., 1995), using a DNA PCR kit (Perkin Elmer, Norwalk, CT, 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 used as housekeeping gene. 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 preproendothelin-1, endothelin ET_A receptor, TGF-β_1, c-*fos*, and type I collagen bands were expressed relative to the corresponding densities of the GAPDH bands from the same RNA sample (Kobayashi et al., 2000, 2001c,d).

2.3. Western blot analysis for endothelin ET_A receptor, TGF-β_1, and type I collagen protein expression in the left ventricle

The left ventricles were carefully removed, weighted, and immediately frozen in liquid nitrogen, and then 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 dithiothreital, 10 μg/ml leupeptin, and 2 μg/ml aprotinin at 0–4 °C with a polytron homogenizer. The homogenate was centrifuged at 1000 × *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. The post-nuclear fraction (endothelin ET_A receptor, TGF-β_1, and type I collagen: 50 μg of protein) of sample was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using 10–13% gels. The proteins in the gels were transferred electrophoretically to polyvinylidene difluoride (PVDF) membranes (Amersham Pharmacia Biotechnology, Arlington Heights, IL, USA) for 1 h at 2 mA/cm^2. The membranes were immunoblotted with an anti-endothelin ET_A receptor, anti-TGF-β_1, and type I collagen antibody (Immuno-Biological Lab, Santa Cruz) 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 goat anti-rabbit and anti-goat immunoglobulin (Amersham Pharmacia Biotechnology). The endothelin ET_A receptor, TGF-β_1, and type I collagen proteins transferred to the membranes were detected using the enhanced chemiluminescence (ECL) immunoblotting detection system (Amersham Pharmacia Biotechnology). The amount of protein was quantified using a densitometer in a linear range and expressed as percent relative to that in non-treated rat (Kobayashi et al., 1999, 2001b,c).

2.4. Western blot analysis for phospho-p42/p44ERK activity in the left ventricle

Left ventricular ERK activity was measured as described in detail previously (Kobayashi et al., 2001a). Briefly, by using rabbit polyclonal phospho-specific ERK antibody (New England Biolabs) and anti-total ERK antibody (Santa Cruz) recognizing tyrosine-phosphorylated forms (active forms) of p44ERK and p42ERK, we measured left ventricular phosphorylated ERK proteins with Western blot analysis. Left ventricular protein extracts were boiled for 5 min in Laemmli sample buffer, then electrophoresed on an SDS-PAGE using 13% gels, and the separated proteins were electrophoretically transferred to Hybond-PVDF membranes. Complete protein transfer to the membrane was verified by staining the gels with Coomassie blue. The membrane was incubated with phospho-specific ERK antibody for 1 h at room temperature, washed four times with Tris-buffered saline containing 0.1% Tween 20 (TBS-T), and then incubated with horseradish peroxidase-conjugated donkey anti-rabbit immunoglobulin (Amersham Pharmacia Biotechnology).

2.5. Histologic examination and evaluation of coronary microvascular remodeling

Histological examination was as described in detail previously (Kobayashi et al., 1999, 2000, 2001a,–d). Briefly, the excised hearts were perfused with physiological saline solution containing adenosine 10 μg/kg and nitroglycerin 10 μg/kg and then with 6% formaldehyde solution via retrograde infusion into the ascending aorta at a pressure of 90 mm Hg. The left ventricle 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-μm-thick sections were cut (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 (Sony, model DXC-930, Tokyo, Japan) mounted on a standard microscope (Olympus, BHS-F, Tokyo, Japan). Drawings of the limits of the vessels were made on the screen of a multiscan color computer display (Sony, model CPD-17SF7, Tokyo, Japan) and then digitized with a two-dimensional analysis system (Mac SCOPE, Mitani Cooperation, Fukui, Japan) connected with a Macintosh computer system (Power Macintosh G3, Apple Computer, Cupertino, CA, 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 fibres 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 $\leq 10^4$ μm^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 × 100 to × 400 magnification, and the areas encircled by the tracings were calculated. On quantification, non-round 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. Histopathology on the sections from each rat was carried out by an operator who was blinded to the treatment groups.

2.6. Statistical analysis

All results are expressed as mean ± S.E.M. The mean values were compared among the three groups using analysis of variance (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, Berkeley, CA, USA) and a Power Macintosh computer system (G4, Apple Computer, Cupertino, CA, USA).

3. Results

3.1. Systemic hemodynamics, body weight, and left ventricular weight

Time-related changes in systolic blood pressure among three groups are shown in Fig. 1. At 5 weeks of celiprolol treatment, systolic blood pressure in DOCA–vehicle and DOCA–celiprolol rats was similar and was significantly higher than that in sham-operated rats (187 ± 5 and 184 ± 6 versus 129 ± 3 mm Hg, sham-operated rats, $P < 0.01$, respectively). Heart rate was similar in sham-operated rats and DOCA–vehicle rats (394 ± 15, 390 ± 17 bpm), and was not changed by the administration of celiprolol (385 ± 16 bpm). Body weight was also similar among the three groups. The left ventricular mass of the DOCA–vehicle rats was

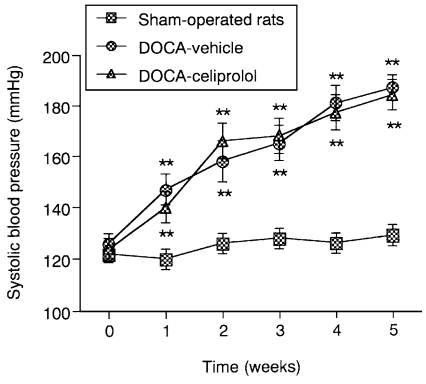

Fig. 1. Time-related changes in systolic blood pressure in the sham-operated rats, DOCA–vehicle, and DOCA–celiprolol groups. DOCA–vehicle, deoxycorticosterone acetate (DOCA)-salt hypertensive rats treated with vehicle; DOCA–celiprolol, DOCA-salt hypertensive rats treated with celiprolol. Values are expressed as means ± S.E.M. $n = 8$ per group. $^{**}P < 0.01$ vs. sham-operated rats.

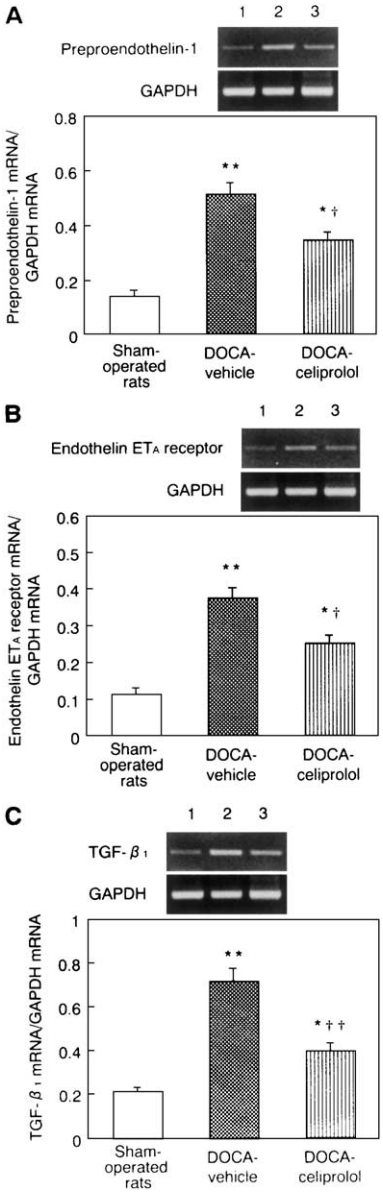

Fig. 2. Effects of chronic celiprolol treatment on preproendothelin-1 (A), endothelin ET_A receptor (B), and TGF-β_1 (C) mRNA expression. Top panels are representative typical gel electrophoresis of RT-PCR of left ventricular preproendothelin-1 mRNA, endothelin ET_A receptor mRNA, TGF-β_1 mRNA, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. Bottom panels show the mean densities of the preproendothelin-1 (A), endothelin ET_A receptor (B), and TGF-β_1 (C) bands in relation to the bands obtained for GAPDH. Lane 1 refers to the sham-operated rats, lane 2 to deoxycorticosterone acetate (DOCA)-salt hypertensive rats treated with vehicle (DOCA–vehicle), and lane 3 to DOCA-salt hypertensive rats treated with celiprolol (DOCA–celiprolol). The values are mean ± S.E.M. from eight RNA samples of eight separate rats. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. sham-operated rats, $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ vs. DOCA–vehicle.

significantly increased compared to that of sham-operated rats using body weight-corrected values (2.83 ± 0.07 versus 1.93 ± 0.04 mg/g, $P<0.01$), and significantly decreased in the DOCA–celiprolol (2.46 ± 0.08 mg/g, $P<0.01$) compared with the DOCA–vehicle rats after 5 weeks treatment with celiprolol.

3.2. Preproendothelin-1, endothelin ET_A receptor, TGF-β_1, c-fos, and type I collagen mRNA expression in the left ventricle

Left ventricular preproendothelin-1 and endothelin ET_A receptor mRNA levels were 3.6-fold and 3.4-fold ($P<0.01$, respectively) higher in DOCA–vehicle than in sham-operated rats, and 33%, and 32% ($P<0.05$, respectively) lower in DOCA–celiprolol than in DOCA–vehicle rats (Fig. 2A,B). The levels of TGF-β_1 mRNA in the left ventricle were 3.4-fold ($P<0.01$) higher in DOCA-V than in sham-operated rats, and 45% ($P<0.01$) lower in DOCA–celiprolol than in DOCA–vehicle rats (Fig. 2C). Left ventricular c-*fos* mRNA

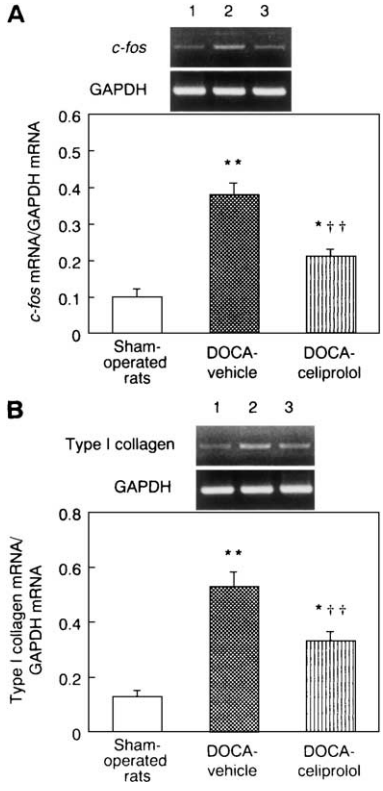

Fig. 3. Effects of chronic celiprolol treatment on c-*fos* (A) and type I collagen (B) mRNA expression. Top panels are representative typical gel electrophoresis of RT-PCR of left ventricular c-*fos* mRNA, type I collagen mRNA, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. Bottom panels show the mean densities of the c-*fos* (A) and type I collagen (B) bands in relation to the bands obtained for GAPDH. Lane 1 refers to the sham-operated rats, lane 2 to deoxycorticosterone acetate (DOCA)-salt hypertensive rats treated with vehicle (DOCA–vehicle), and lane 3 to DOCA-salt hypertensive rats treated with celiprolol (DOCA–celiprolol). The values are mean ± S.E.M. from eight RNA samples of eight separate rats. *$P<0.05$, **$P<0.01$ vs. sham-operated rats, ††$P<0.01$ vs. DOCA–vehicle.

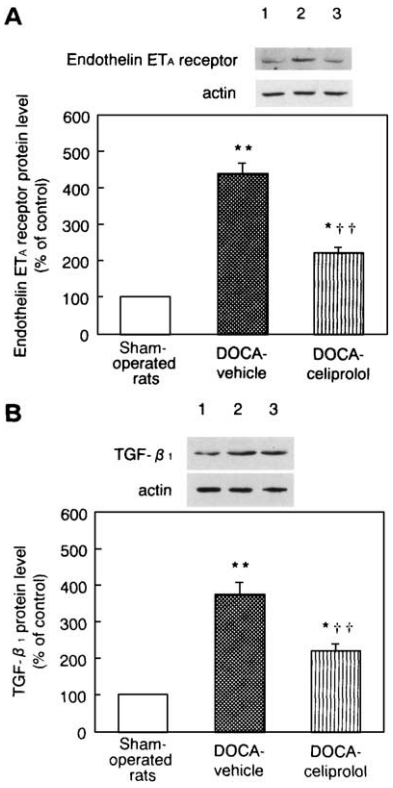

Fig. 4. Effects of chronic celiprolol treatment on endothelin ET_A receptor (A) and TGF-β_1 (B) protein levels. Top panels are representative typical Western blot of endothelin ET_A receptor and TGF-β_1 in the left ventricle. Bottom panels show percent of control of left ventricular endothelin ET_A receptor and TGF-β_1 protein levels. Lane 1 refers to the sham-operated rats, lane 2 to deoxycorticosterone acetate (DOCA)-salt hypertensive rats treated with vehicle (DOCA–vehicle), and lane 3 to DOCA-salt hypertensive rats treated with celiprolol (DOCA–celiprolol). The values are mean ± S.E.M. from eight protein samples of eight separate rats. *$P<0.05$, **$P<0.01$ vs. sham-operated rats, ††$P<0.01$ vs. DOCA-vehicle.

levels were 3.8-fold ($P<0.01$) larger in DOCA–vehicle rats than in sham-operated rats, and 45% ($P<0.01$) lower in DOCA–celiprolol than in DOCA-vehicle rats (Fig. 3A). The levels of type I collagen mRNA were 4.1-fold ($P<0.01$) larger in DOCA-V than in sham-operated rats, and 38% ($P<0.01$) lower in DOCA–celiprolol than in DOCA–vehicle rats (Fig. 3B). The values are mean ± S.E.M. from eight RNA samples of eight separate rats.

3.3. Endothelin ET_A receptor, TGF-β_1, and type I collagen protein expression in the left ventricle

Left ventricular endothelin ET_A receptor protein levels were 4.4-fold ($P<0.01$) higher in DOCA–vehicle than in sham-operated rats, and were 49% ($P<0.01$) lower in DOCA–celiprolol than in DOCA–vehicle rats (Fig. 4A). The levels of TGF-β_1 protein in the left ventricle were 3.7-fold ($P<0.01$) higher in DOCA–vehicle than in sham-operated rats, and were 41% ($P<0.01$) lower in DOCA–celiprolol than in DOCA–vehicle rats (Fig. 4B). Left ventricular type I collagen protein levels were 4.9-fold

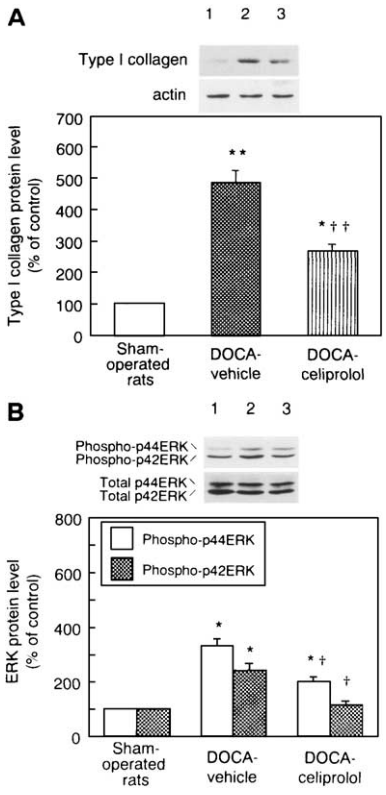

Fig. 5. Effects of chronic celiprolol treatment on type I collagen protein levels (A) and phospho-p42/p44ERK activity (B). Top panels are representative typical Western blot of type I collagen, phospho and total p42/p44ERK in the left ventricle. Bottom panels show percent of control of left ventricular type I collagen protein levels and phospho-p42/p44ERK activity. Lane 1 refers to the sham-operated rats, lane 2 to deoxycorticosterone acetate (DOCA)-salt hypertensive rats treated with vehicle (DOCA–vehicle), and lane 3 to DOCA-salt hypertensive rats treated with celiprolol (DOCA–celiprolol). The values are mean ± S.E.M. from eight protein samples of eight separate rats. *$P<0.05$, **$P<0.01$ vs. sham-operated rats, †$P<0.05$, ††$P<0.01$ vs. DOCA–vehicle.

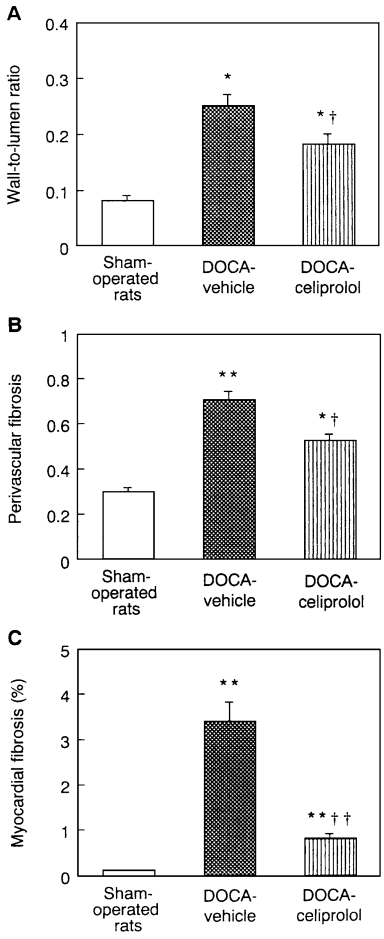

Fig. 7. Effect of celiprolol on (A) wall-to-lumen ratio, (B) perivascular fibrosis, and (C) myocardial fibrosis in deoxycorticosterone acetate (DOCA)-salt hypertensive rats. DOCA–vehicle, deoxycorticosterone acetate (DOCA)-salt hypertensive rats treated with vehicle; DOCA–celiprolol, DOCA-salt hypertensive rats treated with celiprolol. Values are expressed as means ± S.E.M. $n=8$ per group. *$P<0.05$, **$P<0.01$ vs. sham-operated rats. †$P<0.05$, ††$P<0.01$ vs. DOCA–vehicle.

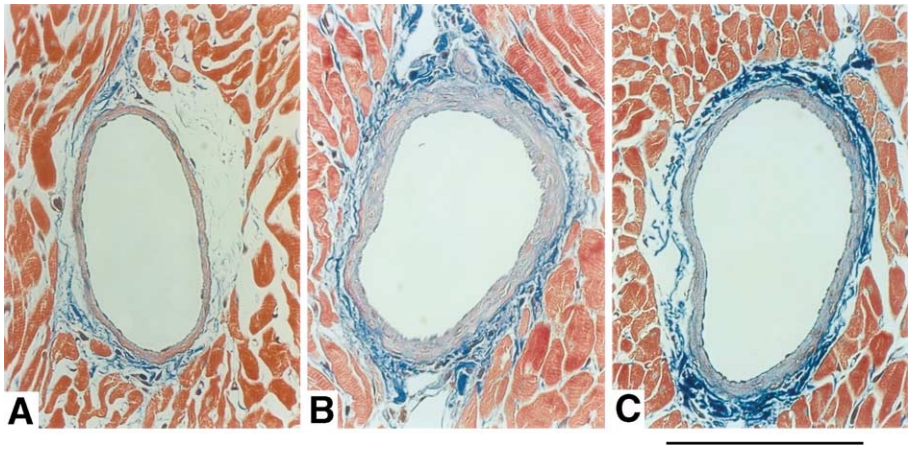

Fig. 6. Micrographs of small coronary arteries with Masson's trichrome stain for sham-operated rats (A), deoxycorticosterone acetate (DOCA)-salt hypertensive rats treated with vehicle (B), and DOCA-salt hypertensive rats treated with celiprolol (C). A bar indicates 100 μm.

($P<0.01$) larger in DOCA–vehicle rats than in sham-operated rats, and 45% ($P<0.01$) lower in DOCA–celiprolol than in DOCA–vehicle rats (Fig. 5A). The values are mean ± S.E.M. from eight protein samples of eight separate rats.

3.4. Left ventricular phospho-p42/p44ERK activity in the left ventricle

As shown by Western blot analysis in Fig. 5B, left ventricular phospho-p44ERK and phospho-p42ERK levels were significantly higher in DOCA–vehicle than in sham-operated rats, and were significantly lower in DOCA–celiprolol than in DOCA–vehicle rats.

3.5. Coronary microvascular remodeling

Micrographs of small coronary arteries with Masson's trichrome stain among three groups are shown in Fig. 6. The wall-to-lumen ratio was significantly increased in DOCA–vehicle compared with that of sham-operated rats, and was significantly decreased by celiprolol treatment (Fig. 7A). The perivascular fibrosis was significantly greater in DOCA–vehicle than in sham–operated rats, and was also significantly decreased by celiprolol treatment (Fig. 7B). Compared with that in sham-operated rats, myocardial fibrosis was significantly greater in DOCA–vehicle rats, and was significantly less in DOCA-celiprolol than in DOCA–vehicle rats (Fig. 7C).

4. Discussion

The present study demonstrated that preproendothelin-1, endothelin ET_A receptor, TGF-β_1, and c-*fos* expressions are increased and phospho-p42/p44ERK activities are upregulated in the left ventricle of DOCA-salt hypertensive rats. The chronic administration of a β-adrenoceptor antagonists, celiprolol, decreased preproendothelin-1, endothelin ET_A receptor, TGF-β_1, and c-*fos* expressions. In addition, after 5 weeks of treatment, celiprolol effectively improved left ventricular hypertrophy and coronary microvascular remodeling, and inhibited left ventricular p42/p44ERK phosphorylation. Therefore, p42/p44ERK and c-*fos* gene pathway may contribute to the cardiovascular remodeling of DOCA rats, and that effects of celiprolol on cardiovascular remodeling may be at least in part mediated by suppressed the expression of endothelin-1 and TGF-β_1 in the left ventricle.

We have demonstrated in the present study that upregulated preproendothelin-1 and endothelin ET_A receptor expressions are suppressed by celiprolol. Recently, several investigators have indicated that some β-adrenoceptor antagonists inhibit the endothelin-1. Regarding the clinical studies, in the report by Krum et al. (1996), there was a significant reduction in circulating endothelin-1 levels in patients with congestive heart failure treated with carvedilol, and was a significant correlation between the symptom severity and hemodynamic improvement with the reduction in plasma endothelin-1 levels. In middle-aged men with essential hypertension, antihypertensive treatment with the β-adrenoceptor antagonists atenolol over 22 weeks gave a significant decrease in plasma levels of endothelin-1, and the reduction in endothelin-1 levels was, however, not correlated to the reduction in blood pressure (Seljeflot et al., 1999). With regard to the experimental studies, Garlichs et al. (1999) suggest that the propranolol, metoprolol and celiprolol reduce the basal and stimulated release of preproendothelin-1 mRNA expression in cultured human endothelial cells. It is interesting to note that the β_1-adrenoceptor antagonists/β_2-agonist celiprolol had the strongest inhibitory effect on endothelin-1 release, followed by the β_1-selective adrenoceptor antagonists metoprolol, a relative weak inhibitory effect was observed for the β_1/β_2 unselective adrenoceptor antagonists propranolol. Brehm et al. (2000) showed that nebivolol inhibited the proliferation of human coronary smooth muscle cell, and during nebivolol incubation preproendothelin-1 mRNA production was reduced, with a subsequent decrease in endothelin-1 secretion. These results suggest that the effects of β-adrenoceptor antagonists on these reductions of endothelin-1 may at least partially explain the efficacy of β-adrenoceptor antagonists in the treatment of diseases such as advanced heart failure, essential hypertension as well as acute coronary syndromes.

In the present study, TGF-β_1 mRNA expression was increased in DOCA-salt hypertensive rats, and was decreased by celiprolol treatment. Boluyt et al. (1995) showed that chronic isoproterenol infusion elicits alterations in cardiac gene expression of TGF-β_1 mRNA that are consistent with the development of myocyte hypertrophy and interstitial fibrosis, and that upregulated levels of TGF-β_1 mRNA by infused isoproterenol were suppressed by propranolol. In addition, Brehm et al. (2000) reported that nebivolol inhibited accelerated human coronary smooth muscle cell proliferation, and during nebivolol incubation, TGF-β_1 mRNA production was reduced. These results have suggested that TGF-β_1 has an important role in cardiac hypertrophy formation and contributes to coronary microvascular remodeling and may play a role in the myocardial response to hypertrophic stimuli.

The present study has demonstrated that p42/p44ERK phosphorylations are upregulated in the left ventricle of DOCA-salt rats, and that celiprolol effectively improves cardiac hypertrophy and cardiovascular remodeling, and inhibits p42/p44ERK activity. In DOCA-salt hypertensive rat model, Kubo et al. (2000) has shown that endothelium removal-induced ERK activation is enhanced in aorta strips, and thus suggested that vascular structural remodeling function may be enhanced. In addition, because the endothelin receptor antagonist BQ123 inhibited the ERK activation, they concluded that the enhancement of ERK activation might be result at least in part from enhanced function of the vascular endothelin system in this model. Indeed, in the present study, preproendothelin-1 and endothelin ET_A recep-

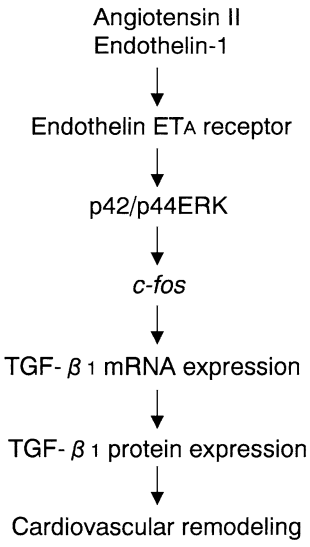

Fig. 8. Schematic chart of signal transduction pathways in DOCA-salt hypertensive rats.

tor expressions are upregulated in the left ventricle. Therefore, these results suggested that the enhanced reactivity to endothelin system might contribute to the enhanced left ventricular phospho-p42/p44 ERK activity in DOCA-salt hypertensive rats. On the other hand, few studies have evaluated that β-adrenoceptor antagonists inhibits phospho-p42/p44ERK activities. However, β-adrenergic agonists have been found to activate the ERK pathway (Daaka et al., 1997). These results suggested that phospho-p42/p44 ERK activity may contribute to the coronary microvascular remodeling of DOCA rats, and that protective effects of celiprolol on remodeling may be related to inhibition of ERK pathway.

The signal mechanisms of increased TGF-β_1 mRNA via the p42/p44ERK and c-*fos* mRNA pathway are as follows (Fig. 8). In DOCA-salt hypertensive rats, the development and maintenance of hypertension have been believed to be independent of the circulating renin–angiotensin system, because the plasma renin level in this model is lower than that in normotensive control rats (Wada et al., 1995). It is well recognized that there are two kinds of the renin–angiotensin system, circulating and local, and particular interest to evaluate the local renin–angiotensin system under the condition of low renin hypertensive animal models. Recently, we have demonstrated that gene expressions of angiotensin-converting enzyme and angiotensin II type I receptor mRNA are upregulated in the local left ventricle of DOCA-salt hypertensive rats (Hara et al., 2001). In addition, angiotensin II-mediated growth effects in target cells such as vascular smooth muscle cells and cardiac myocytes require the rapid activation of several mitogen-activated protein kinases including the p42/p44ERK (Weber et al., 1994). Indeed, in DOCA salt rats, chronic hypertension caused by a high-salt diet was followed by sustained activation of left ventricular p42/p44ERK, suggesting the involvement of p42/p44ERK in hypertensive vascular disease (Kim et al., 1997). In addition, angiotensin II-initiated activation of p70S6 kinase requires both ERK cascade and phosphatidylinositol 3-kinase (PI3K)/Akt cascade that bifurcate at the point of epidermal growth factor receptor (EGFR)-dependent Ras activation (Eguchi et al., 1999). Moreover, ERK is involved in the increased activator protein-1 (c-*fos* and c-*jun* complex) DNA binding activity, and the promoter region of TGF-β_1 gene contains c-*fos* responsive element (Hamaguchi et al., 1999). Therefore, the activated p42/p44ERK in turn increases c-*fos* gene binding activity, leading to an increase in TGF-β_1 mRNA.

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