Original Article

Upregulation of Interleukin-8/CXCL8 in Vascular Smooth Muscle Cells from Spontaneously Hypertensive Rats

Hyo Young KIM[1)], Young Jin KANG[2)], In Hwan SONG[3)], Hyung Chul CHOI[2)], and Hee Sun KIM[1)]

Chemokines promote vascular inflammation and play a pathogenic role in the development and maintenance of hypertension. In the present study, the expression of the chemokine interleukin-8/CXCL8 (IL-8/CXCL8) was investigated in cultured vascular smooth muscle cells (VSMC) obtained from the thoracic aorta of spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY). IL-8/CXCL8 expression in thoracic aorta tissue and VSMC in SHR were significantly higher than in WKY. However, the expression of CXCR1 mRNA in VSMC from WKY was higher than that in VSMC from SHR. Angiotensin II (Ang II) induced a higher level of IL-8/CXCL8 mRNA expression in VSMC from SHR than in VSMC from WKY. The time course of Ang II–induced IL-8/CXCL8 expression in VSMC from SHR correlated with those of Ang II–induced CXCL1 and Ang II type 1 (AT1) receptor expression, and the expression of IL-8/CXCL8 by Ang II was inhibited by the AT1 receptor antagonist losartan. The effect of Ang II on IL-8/CXCL8 expression was not dependent on nuclear factor-κB (NF-κB) activation, but was mediated by an extracellular signal–regulated kinase (ERK) signaling pathway. Although Ang II directly induced IL-8/CXCL8 expression, expression of Ang II–induced IL-8/CXCL8 decreased in VSMC transfected with heme oxygenase-1. These results suggest that IL-8/CXCL8 plays an important role in the pathogenesis of Ang II–induced hypertension and vascular lesions in SHR. (*Hypertens Res* 2008; 31: 515–523)

***Key Words*: hypertension, vascular smooth muscle cell, interleukin-8/CXCL8, angiotensin II**

Introduction

Hypertension is associated with vascular inflammatory responses and is a major risk factor for the development of atherosclerosis (*1*), but the underlying molecular and cellular mechanisms are unclear. Infiltration of monocytes/macrophages and proliferation of vascular smooth muscle cells (VSMC) and endothelial cells in arterial walls are mediated by chemokines (*2–4*). Excess production of chemokines from diverse immune cells can have detrimental effects that can result in an inflammatory environment. Chronic monocyte-mediated inflammation in arterial walls is observed in hypertensive patients and experimental animals (*5–7*). Inflammatory cell infiltration and oxidative stress in vascular walls contribute to the pathogenesis of hypertension in experimental animals (*1*, *6*, *8*), and the suppression of inflammatory cell infiltration has been shown to ameliorate hypertension in experimental animals (*9*). Thus, controlling chemokine production is important for regulating inflammatory reactions in

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hypertensive arterial walls.

MCP-1/CCL2 and CCR2 expression and activation are increased in the arterial walls of hypertensive animals, and the MCP-1/CCL2 and CCR2 pathway is involved in inflammatory aspects of vascular injury in hypertension (*4*, *5*, *10*, *11*). Most of the previous studies investigating the inflammatory aspects of hypertensive vascular injury have focused exclusively on the role of MCP-1/CCL2 as an inflammatory factor in hypertensive vascular wall cells. The CC chemokine MCP-1/CCL2 primarily activates monocytes, whereas the CXC chemokine interleukin-8/CXCL8 (IL-8/CXCL8) tends to preferentially activate neutrophils. However, IL-8/CXCL8 is known to play an important role in monocyte migration into the subendothelial space in the early phase of atherosclerosis (*12*). Therefore, we hypothesized that IL-8/CXCL8 might also be involved in the pathogenesis and maintenance of hypertensive vascular wall formation in hypertension. The aims of this study were to investigate the expression of IL-8/CXCL8 in VSMC from spontaneously hypertensive rats (SHR) compared to Wistar-Kyoto rat (WKY), and to analyze the mechanism of angiotensin II (Ang II)–induced IL-8/CXCL8 expression in VSMC from SHR.

Methods

Materials

Trizol reagent for total RNA isolation was purchased from Invitrogen (Carlsbad, USA). Dulbecco's phosphate-buffered saline (PBS), Dulbecco's modified Eagle's medium (DMEM), penicillin-streptomycin and fetal bovine serum (FBS) were purchased from Gibco/BRL (Life Technologies, Gaithersburg, USA). 15d-PGJ_2 was purchased from Biomol (Plymouth Meeting, USA). *Escherichia coli* lipopolysaccharide (LPS) (O111:B4) and PD123,319 were obtained from Sigma Chemical Co. (St. Louis, USA). Angiotensin II (Ang II) was obtained from Calbiochem (San Diego, USA). Losartan was obtained from MSD (Delaware, USA). Mitogen activated protein kinase (MAPK) inhibitors, 2′-amino-3′ methoxyflavone (PD98059) and (E)3-[(4-methylphenyl)sulfonyl]-2-propenenitrile (Bay 11-7082), were purchased from Calbiochem. Nitrocellulose transfer membrane was obtained from Schleicher & Schuell Bioscience (Dassel, Germany). Oligonucleotide primers for polymerase chain reaction (PCR) of IL-8/CXCL8, CXCR1, Ang II type 1 (AT1) receptor, Ang II type 2 (AT2) receptor, and β-actin were synthesized by Bionics (Seoul, Korea). The LightCycler FastStart DNA SYBR Green I Mix was obtained from Roche (Mannheim, Germany). Phospho-ERK, anti-rabbit nuclear factor-κB (NF-κB) p65 (Cat No. #3034), and γ-tubulin antibodies were obtained from Cell Signaling Technology (Danvers, USA). All other reagents were pure-grade commercial preparations.

Rats

Specific pathogen-free male inbred WKY and SHR, 20 to 27 weeks of age, were purchased from Japan SLC Inc. (Shizuoka, Japan). All experimental animals received autoclaved food and bedding to minimize exposure to viral or microbial pathogens. The rats were cared for in accordance with the Guide for the Care and Use of Experimental Animals of Yeungnam Medical Center.

Cell Culture

VSMC were obtained by an explant method from the thoracic aortas of 20- to 27-week-old male WKY and SHR (Japan SLC) as described in Griendling *et al*. (*13*). VSMC were cultured in DMEM supplemented with 10% FBS and penicillin-streptomycin. Cells were detached with 0.25% trypsin/EDTA and seeded into 75-cm^2 tissue culture flasks at a density of 10^5 cells/mL. All experiments were conducted at cell passage 3 to 5. Prior to stimulation, 95% confluent VSMC were serum-starved overnight by incubation in DMEM with 0.1% FBS. Cell cultures were incubated at 37°C and 5% CO_2 in the presence or absence of stimuli for the indicated times.

Immunohistochemistry

To detect α-smooth muscle actin in VSMC, the cells were stained using anti–α-smooth muscle actin antibody. Cells were cultured on coverslips for 3 days and fixed in −20°C methanol for 5 min. Nonspecific activity was blocked by incubating cells in 3% BSA for 20 min; cells were then incubated in primary anti–α-smooth muscle actin antibody (1:100 dilution; Abcam, Cambridge, USA) for 30 min and secondary fluorescein-5-isothiocyanate–conjugated rabbit anti–mouse IgG antibody (1:50; Abcam) for 30 min. The cells were counterstained with 5 μg/mL propidium iodide for 5 min after preincubation in 50 μg/mg RNase for 20 min.

To observe IL-8/CXCL8 expression in the thoracic aorta wall, transverse sections of formalin-fixed, paraffin-embedded thoracic aorta were placed on coated slides. Sections were autoclaved for antigen retrieval, and endogenous peroxidase was blocked with 3% H_2O_2. After blocking with normal blocking serum for 30 min at room temperature (RT), sections were incubated with anti–IL-8 antibody (1:50; BD Biosciences, San Jose, USA) for 1 h 30 min at RT and horseradish peroxidase–conjugated rabbit anti–mouse IgG antibody for 30 min at RT. To visualize the signal, sections were developed with the diaminobenzidine (DAB) substrate-chromogen system for 3 min at RT.

Preparation of Total RNA and Real-Time Polymerase Chain Reaction

Total RNA was extracted using Trizol reagent according to the manufacturer's instructions. The quantity of total RNA

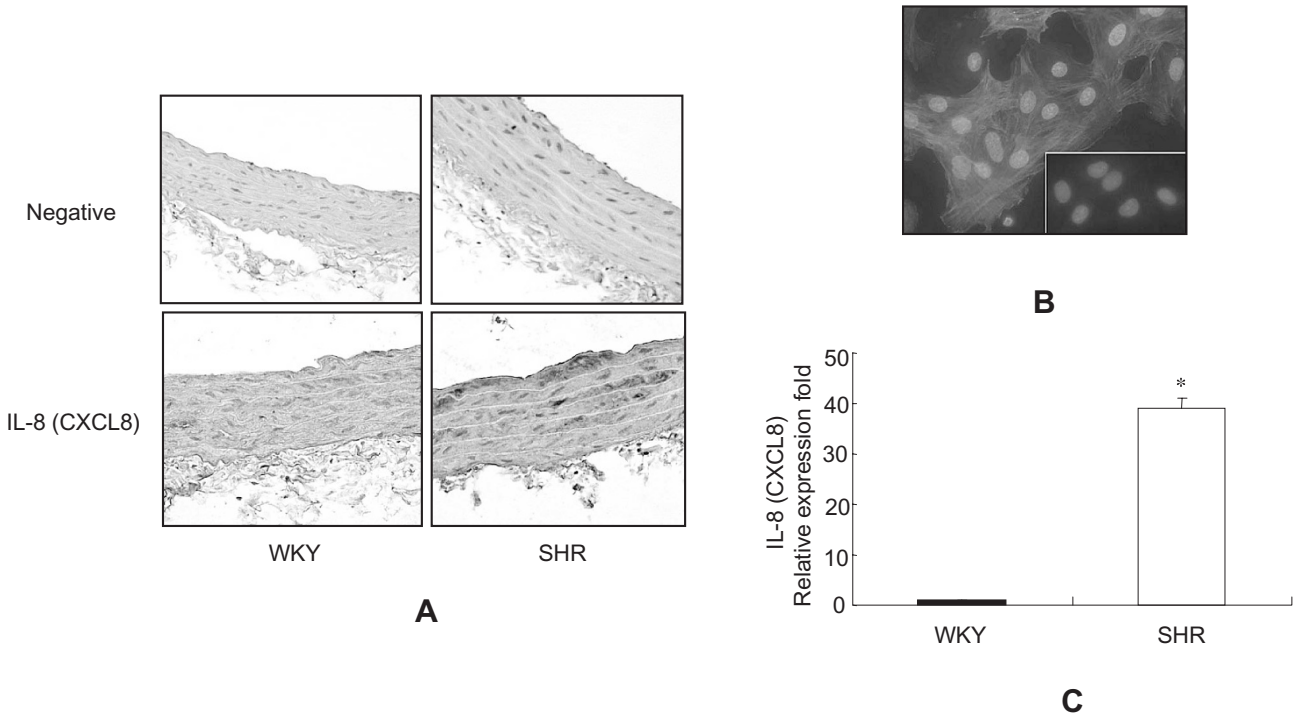

Fig. 1. *Expression of IL-8/CXCL8 in the endothelium and intima region of vascular smooth muscle in thoracic aorta tissues and IL-8/CXCL8 mRNA in vascular smooth muscle cells (VSMC) from SHR and WKY. A: Immunohistochemistry for IL-8/CXCL8, original magnification: ×200. B: Immunostaining for α-smooth muscle actin in cultured rat arterial smooth muscle cells and peritoneal macrophages as a control (in the box). Smooth muscle cells show a positive reaction in a fibriform pattern. Nuclei were counterstained with propidium iodide. Magnification: ×200. C: Real-time PCR for IL-8/CXCL8 mRNA expression in VSMC from WKY and SHR. Bars represent the means±SEM from three independent experiments. *$p < 0.05$ vs. IL-8/CXCL8 expression in VSMC from WKY.*

obtained was determined by measuring the optical density (OD) at 260 and 280 nm.

Real-time PCR for IL-8/CXCL8, CXCR1, AT1 receptor and AT2 receptor in VSMC was performed using a LightCycler (Roche). RNA was reverse transcribed to cDNA from 1 μg of total RNA, and then subjected to real-time PCR. PCR was performed in triplicate in a total volume of 20 μL of LightCycler FastStart DNA SYBR Green I mix (Roche) containing primer and 2 μL of cDNA. PCR amplification was preceded by incubation of the mixture at 95°C for 10 min, and the amplification step consisted of 45 cycles of denaturation (10 s at 95°C), annealing (5 s at the primer-appropriate temperature), and extension (10 s at 72°C), with fluorescence detection at 72°C after each cycle. After the final cycle, melting point analyses of all samples were performed over the range of 65 to 95°C with continuous fluorescence detection. β-Actin expression levels were used for sample normalization. Results for each gene are expressed as the relative expression level compared with β-actin. The primers were as follows. For IL-8/CXCL8 (365 bp): sense, 5′-gaagatagattg caccga-3′; antisense, 5′-catagcctc tcacacatttc-3′; for CXCR1 (518 bp): sense, 5′-aatttggaaatatca cccgaa-3′; antisense, 5′-gactgttccagaacggtatgg-3′; for AT1 receptor (445 bp): sense, 5′-cacctatgtaagatcgcttc-3′; antisense, 5′-gcacaatcgccataa ttatcc-3′; for AT2 receptor (65 bp): sense, 5′-ccgtgaccaagt cttgaagatg-3′; antisense, 5′-agggaagccagcaaatgatg-3′; and for β-actin (101 bp): sense, 5′-tactgccctggctcctagca-3′; antisense, 5′-tggacagtgaggccaggatag-3′. The levels of IL-8/CXCL8, CXCR1, AT1 receptor, and AT2 receptor mRNA were determined by comparing experimental levels to the standard curves and are expressed as fold of the relative expression.

Electrophoretic Mobility Shift Assay

Nuclear extracts were prepared as previously described (*14*). Cells were washed three times with cold PBS, then scraped and harvested by centrifugation. Cell pellets were resuspended and incubated on ice for 15 min in 400 μL of hypotonic buffer A (10 mmol/L HEPES, 10 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 0.5 mmol/L DTT, 0.1 mmol/L PMSF, 10 μg/mL pepstatin, 10 μg/mL leupeptin, 10 μg/mL autipain, and 10 μg/mL aprotinin). Nonidet P-40 was then added to a final concentration of 2.5%, and the cells were vortexed for 10 s. Nuclei were separated from the cytosol by centrifugation

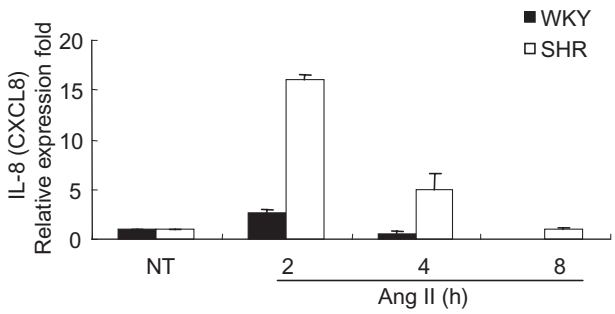

Fig. 2. *Angiotensin II (Ang II) directly induces IL-8/CXCL8 mRNA expression in vascular smooth muscle cells (VSMC) from both SHR and WKY, and the expression of Ang II–induced IL-8 mRNA in VSMC from SHR is stronger than that in VSMC from WKY. VSMC were untreated or treated with Ang II (0.1 μmol/L) for 2, 4, or 8 h, and the total RNA was analyzed by real-time PCR. Bars represent the means±SEM from three independent experiments.*

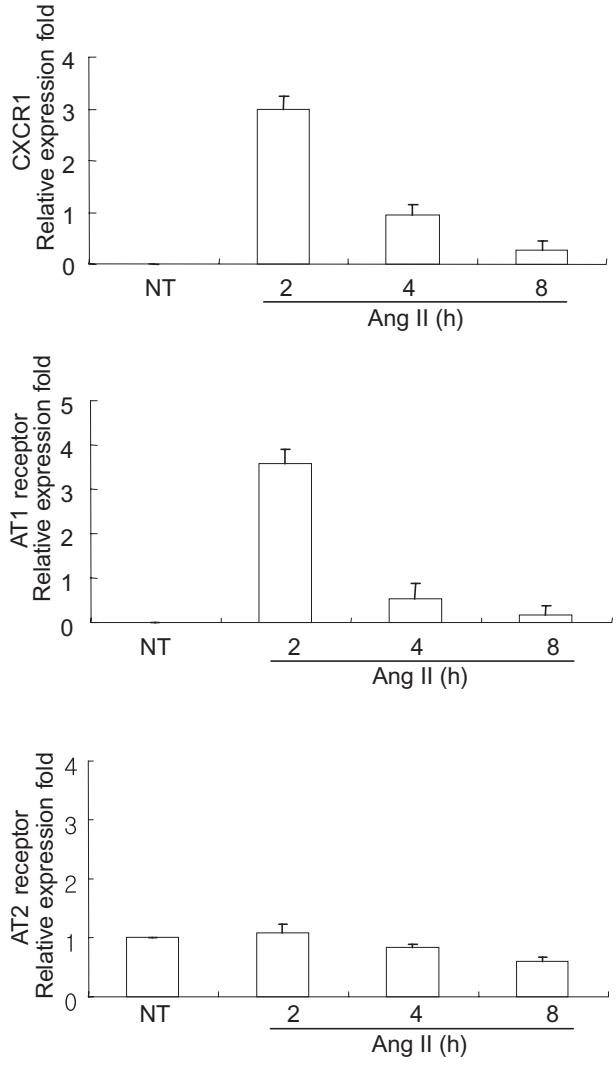

Fig. 3. *Time courses of angiotensin II (Ang II)–induced CXCR1, AT1 and AT2 receptor expression in vascular smooth muscle cells (VSMC) from SHR. VSMC were untreated or treated with Ang II (0.1 μmol/L) for 2, 4, or 8 h, and the total RNA was analyzed by real-time PCR. Bars represent the means±SEM from three independent experiments.*

at 12,000×*g* for 15 s. Pellets were resuspended in 40 μL of hypotonic buffer C (20 mmol/L HEPES, 25% glycerol, 0.4 mol/L NaCl, 1 mmol/L EDTA, 1 mmol/L EGTA, 0.5 mmol/L DTT, 0.1 mmol/L PMSF, 10 μg/mL pepstatin, 10 μg/mL leupeptin, 10 μg/mL autipain, and 10 μg/mL aprotinin). Samples were sonicated at level 3–4 for 2–3 s, then centrifuged for 10 min at 4°C. The nuclear protein concentration was measured using the Bradford assay (Bio-Rad, Richmond, USA). A consensus sequence for the NF-κB DNA binding site (5′-AGTTGAGGGGACTTTAGGC-3′) (*sc*-2505; Santa Cruz Biotechnology, Santa Cruz, USA) was labeled with [α-^{32}P]dCTP using a random-primed DNA labeling kit (Roche). The mutant NF-κB binding sequence was identical to *sc*-2505 except for a G→C substitution in the NF-κB DNA binding motif (*sc*-2511; Santa Cruz Biotechnology). The labeled DNA was purified over a S-200 HR column (Pharmacia, Piscataway, USA) to remove unbound nucleotides. Nuclear protein extracts were incubated at room temperature for 20 min with approximately 50,000 cpm of labeled oligonucleotide suspended in binding buffer (200 mmol/L HEPES, 500 mmol/L KCl, 10 mmol/L EDTA, 50% glycerol, 10 mmol/L DTT, 1 mg/mL BSA, 1 μg/μL poly[dI-dC]). Following this incubation, samples were resolved on 4% polyacrylamide gels at 140V and exposed to film.

Western Blotting

Total lysates were prepared in PRO-PREP buffer (iNtRON, Seoul, Korea). Protein concentrations were determined by a Bradford assay (Bio-Rad) using bovine serum albumin as a standard. Thirty-microgram protein samples were separated on 10% SDS-polyacrylamide gels, and then transferred to nitrocellulose membranes. The membranes were soaked in 5% nonfat dried milk in TBST (10 mmol/L Tris-HCl pH 7.5, 150 mmol/L NaCl and 0.05% Tween-20) for 1 h and then incubated for 16–18 h with primary antibodies against phospho-ERK1/2 and γ-tubulin at 4°C. Membranes were washed three times with TBST for 10 min and then incubated with horseradish peroxidase-conjugated secondary antibody for 1 h at 4°C. The membranes were rinsed three times with TBST for 10 min and antigen-antibody complex was detected using an enhanced chemiluminescence detection system (LAS-3000; Fujifilm, Tokyo, Japan).

Heme Oxygenase-1 Transfection

The rat heme oxygenase (HO)-1 expression plasmid contains

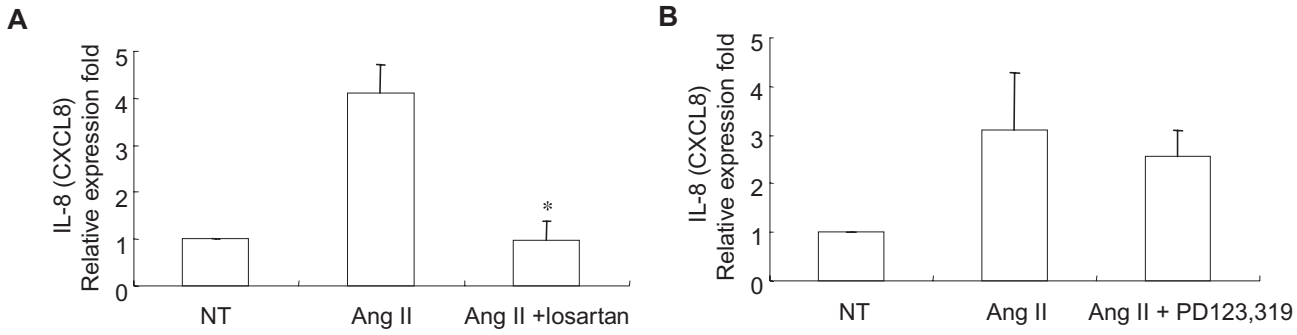

Fig. 4. *Expression of angiotensin II (Ang II)–induced IL-8/CXCL8 mRNA was inhibited by the AT1 receptor antagonist losartan in vascular smooth muscle cells (VSMC) from SHR. VSMC were untreated or treated with Ang II (0.1 μmol/L) with or without losartan (A, 10 μmol/L) or PD123,319 (B, 10 μmol/L) for 2 h, and the total RNA was analyzed by real-time PCR. Bars represent the means±SEM from three independent experiments. *$p < 0.05$ vs. VSMC of SHR treated with Ang II alone.*

a 1.0 kb murine HO-1 cDNA subcloned into a mammalian expression vector, pcDNA3. The cells were grown in Ham's F12 medium (Invitrogen, Gaithersburg, USA) supplemented with 10% FBS (Hyclone, Logan, USA) (Ham's F12–10% FBS), 25 IU/mL penicillin, and 25 μg/mL streptomycin (Mediatech, Herndon, USA). The cells were cultured at 5% CO_2 and 37°C in a humidified incubator.

Statistical Analysis

Data are expressed as the means±SEM. Results were analyzed by Wilcoxon signed-rank test. Values of $p<0.05$ were considered statistically significant.

Results

IL-8/CXCL8 Expression in VSMC from SHR and WKY

Immunostaining revealed strong expression of IL-8/CXCL8 protein in the endothelium and vascular smooth muscle region of thoracic aortic tissues from SHR compared with those from WKY (Fig. 1A). We also examined the expression patterns of IL-8/CXCL8 mRNA in VSMC from SHR and WKY. VSMC were isolated from thoracic aortas and cultured on plastic dishes through early passages (3 to 5), and it was confirmed that they contained α-smooth muscle actin (Fig. 1B). The expression of IL-8/CXCL8 mRNA was stronger in VSMC from SHR than from in those from WKY (Fig. 1C).

Ang II Induces IL-8/CXCL8 mRNA Expression in VSMC from SHR

To investigate whether Ang II can directly increase IL-8/CXCL8 mRNA expression, serum-starved VSMC were treated with or without Ang II (0.1 μmol/L) for 0 to 8 h. The expression of Ang II–induced IL-8/CXCL8 mRNA in VSMC from SHR was maximal at 2 h after treatment, and then gradually declined until 8 h. The expression levels of Ang II–induced IL-8/CXCL8 were remarkably higher in VSMC from SHR than in VSMC from WKY. In VSMC from WKY, although Ang II had induced IL-8/CXCL8 mRNA expression at 2 h, IL-8/CXCL8 mRNA was not detected at 4 h (Fig. 2).

To gain further mechanistic insights into the signaling mechanisms responsible for Ang II–induced IL-8 expression in VSMC from SHR, we first examined the time courses of Ang II–induced CXCR1, AT1 and AT2 receptor mRNA. The expression patterns of CXCR1 and AT1 receptor mRNAs were almost the same as that of Ang II–induced IL-8/CXCL8, but the expression pattern of AT2 was not (Fig. 3). Next, to determine whether Ang II–induced IL-8/CXCL8 mRNA expression is mediated by Ang II subtype receptors, VSMC from SHR were treated with Ang II in the presence or absence of the AT1 receptor antagonist losartan (10 μmol/L) or the AT2 receptor antagonist PD123,319 (10 μmol/L). Losartan inhibited the expression of Ang II–induced IL-8/CXCL8 mRNA remarkably, but PD123,319 did not inhibit Ang II–induced IL-8 expression (Fig. 4). This result suggests that the induction of IL-8/CXCL8 mRNA by Ang II is mediated through AT1 receptors in VSMC from SHR.

Expression of Ang II–Induced IL-8/CXCL8 Is Not Dependent on NF-κB Activation, but Is Related to the ERK Pathway

The role of NF-κB activation on Ang II–induced IL-8/CXCL8 expression was also investigated. Bay-11-7082 selectively blocks the phosphorylation of IκBα, thereby preventing the activation and nuclear translocation of NF-κB. After VSMC were treated with Ang II in the presence or absence of Bay-11-7082 (10 μmol/L) for 2 h, real-time PCR was performed. Bay-11-7082 did not block the expression of Ang II–induced IL-8/CXCL8 mRNA (Fig. 5A). To further confirm the association between NF-κB activity and IL-8/

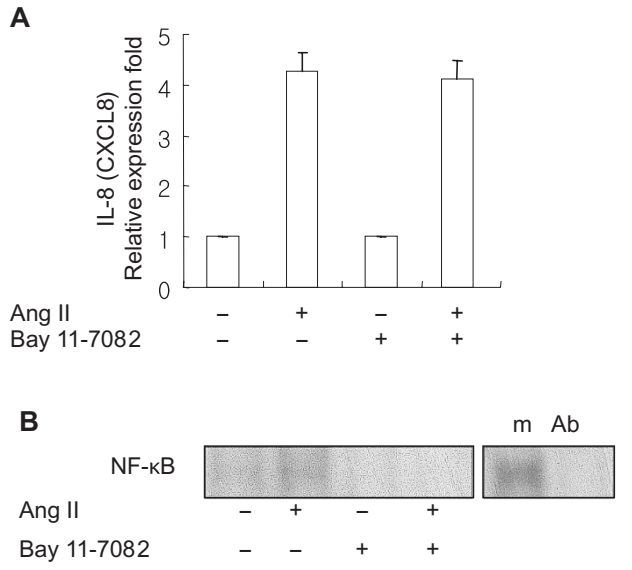

Fig. 5. *Action of angiotensin II (Ang II) on the expression of IL-8/CXCL8 is not dependent on NF-κB activation. A: Vascular smooth muscle cells (VSMC) were untreated or treated with Ang II (0.1 μmol/L) in the absence or presence of Bay-11-7082 (10 μmol/L) for 2 h. Total RNAs were prepared, and real-time PCRs were performed. Bars represent the means±SEM from three independent experiments. B: Specific binding activity of NF-κB from nuclear extracts was assessed by electrophoretic mobility shift assay (EMSA). Part of the nuclear extract was incubated with a 100-fold excess of mutant probe (m), or with 2 μg of anti NF-κB Ig (Ab) before EMSA. The data shown are representative of three independent experiments.*

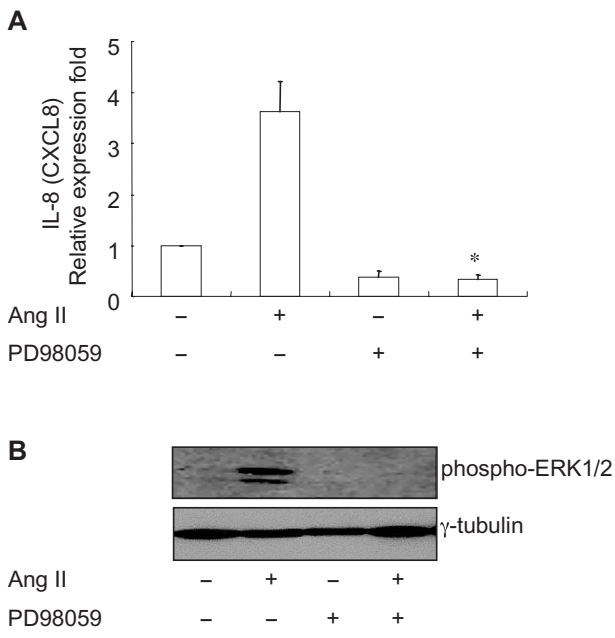

Fig. 6. *MAP kinase is involved in the action of angiotensin II (Ang II) on IL-8/CXCL8 expression. A: Vascular smooth muscle cells (VSMC) were untreated (NT) or pretreated with PD98059 (ERK inhibitor, 10 μmol/L) for 30 min. Cells were then untreated or treated with Ang II (0.1 μmol/L) for 2 h. After total mRNAs were isolated, real-time PCR was performed. B: Cell lysates were separated on 10% SDS-polyacrylamide gels and then immunoblotted with phospho-ERK1/2 antibodies. Data shown are representative of three independent experiments. *$p < 0.05$ vs. VSMC of SHR treated with Ang II alone.*

CXCL8 expression, electrophoretic mobility shift assay (EMSA) for NF-κB binding activity was performed. A higher level of NF-κB activity was detected in cells treated with Ang II compared to those in untreated cells and cells treated with Bay-11-7082 (Fig. 5B). We therefore concluded that Ang II–induced IL-8/CXCL8 expression is not mediated by NF-κB activation.

We next investigated whether a MAPK signaling pathway is involved in Ang II–induced IL-8/CXCL8 expression. After pretreatment with the ERK1/2 inhibitor PD98059 (10 μmol/L) for 0.5 h, VSMC were treated with Ang II for 2 h. Real-time PCR was then performed. As further confirmation of these results, we investigated the phosphorylation of ERK1/2 in cells treated with Ang II. PD98059 decreased the expression of Ang II–induced IL-8/CXCL8 mRNA (Fig. 6A), and ERK1/2 phosphorylation was detected in cells treated with Ang II (Fig. 6B). These results suggest IL-8/CXCL8 expression induced by Ang II is mediated through the ERK signaling pathway.

HO-1 Inhibits the Expression of IL-8 in VSMC from SHR

HO-1, an antioxidant, has an antihypertensive effect; therefore, we examined the effect of HO-1 on the expression of IL-8 in VSMC from SHR. After HO-1 was transfected into VSMC from SHR, HO-1–transfected and untransfected VSMC were treated with Ang II for 2 h, and real-time PCR was performed. The expression of Ang II–induced IL-8/CXCL8 in HO-1–transfected VSMC was decreased compared to the expression in untransfected VSMC (Fig. 7).

Discussion

Vascular lesions are spread by chemokine-triggered leukocyte accumulation *via* enhanced firm adhesion and chemotaxis. Although plasma levels of chemokines are significantly elevated in hypertensive patients, the role that chemokines may play in the pathogenesis and maintenance in hypertensive subjects is not yet clearly understood. Moreover, most chemokine studies associated with hypertension have focused

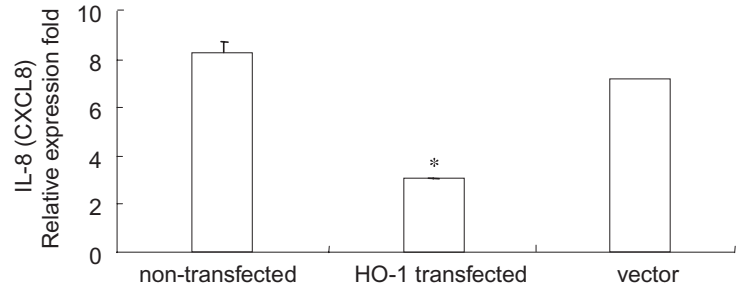

Fig. 7. *Expression of angiotensin II (Ang II)–induced IL-8/CXCL8 mRNA was decreased in HO-1–transfected vascular smooth muscle cells (VSMC) from SHR. After HO-1 was transfected into VSMC from SHR, untransfected VSMC, HO-1–transfected VSMC, and VSMC transfected with vector pcDNA3 alone were treated with Ang II for 2 h, and real-time PCR was performed. Bars represent the means ±SEM from three independent experiments. *$p < 0.05$ vs. HO-1–untransfected VSMC of SHR.*

on MCP-1/CCL2 and its receptor CCR2.

IL-8/CXCL8, a potent chemoattractant for neutrophils, is produced by a variety of cell types, including endothelial cells, monocytes/macrophages, and VSMC (*15*). Along with MCP-1/CCL2, IL-8/CXCL8 plays an important role in the pathogenesis of atherosclerosis, and elevated levels of IL-8/CXCL8 are associated with an increased risk of future coronary artery disease (*16*). Buemi *et al*. (*17*) suggested that IL-8/CXCL8 may directly enhance membrane permeability to Ca^{2+}, thus inducing vasoconstriction in the smooth muscle cells of patients with essential hypertension. In the present study, we examined the differential expression of IL-8/CXCL8 in VSMC from SHR and WKY. The expression of IL-8/CXCL8 was significantly upregulated in aortic tissues and VSMC of hypertensive rats, and Ang II directly induced IL-8/CXCL8 expression in VSMC from both SHR and WKY.

In studies in the role of MCP-1/CCL2 in hypertensive animals (*5*, *6*, *11*, *18*), the expression and activation of CCR2, the MCP-1/CCL2 receptor, has also been shown to be enhanced in the arterial walls of hypertensive models. Ishibashi *et al*. (*5*) suggested that CCR2 expression in monocytes has a critical role in vascular inflammation and remodeling in Ang II–induced hypertension. However, in our study, although the expression of IL-8/CXCL8 mRNA in VSMC from SHR was remarkably stronger than that in VSMC from WKY, expression of CXCR1, an IL-8/CXCL8 receptor, was lower in VSMC from SHR compared to those from WKY (data not shown). SHR have an altered leukocyte distribution in various tissues because of a reduction in the capacity of the leukocytes to adhere to capillary walls under endogenous inflammatory stimuli (*19*). This result might be attributed to alterations in chemokine receptors in hypertensive vascular injuries. Each of the chemokine receptors is thought to have a different, complex role in vascular inflammation depending on the cell types in the hypertensive vascular models.

The vasoconstrictive role of Ang II is well-known; it directly induces oxidative stress in the vasculature and stimulates the release of various substances that mediate arterial wall inflammation (*20*, *21*). The effect of Ang II on MCP-1/CCL2 and CCR2 expression in rat VSMC (*8*), and the activity of MCP-1/CCL2 and CCR2 in hypertension in the presence of an activated renin-angiotensin system have been well elucidated (*5*). Although Ang II–induced RANTES(CCL5) expression is mediated through AT2 receptor (*22*), it is generally thought that Ang II exerts its inflammatory effects through AT1 receptor. Thus, to clarify whether Ang II can directly affect IL-8/CXCL8 expression through AT receptors in VSMC from SHR, we incubated VSMC with Ang II, and observed a significant upregulation of IL-8/CXCL8 expression that was mediated through AT1 receptor. Therefore, the expression of IL-8/CXCL8, as well as MCP-1/CCL2, in VSMC may have an important role in Ang II–induced hypertension. Ang II activates NF-κB in human and porcine VSMC, cultured mesangial cells, mononuclear cells, glomerular endothelial cells and rat adrenal pheochromocytoma cell lines (*23–25*), and this NF-κB activation is mediated through AT1 and AT2 receptors in rat glomerular endothelial cells and adrenal pheochromocytoma cell lines (*25*). Moreover, one report demonstrated that AT2 receptor plays a role in Ang II–induced NF-κB activation (*26*). In the present study, we also detected increases of NF-κB activation in cells treated with Ang II. However, Ang II–induced IL-8/CXCL8 expression was not dependent on NF-κB activation in VSMC. In previous studies, Ang II was shown to activate MAP kinases in VSMC (*10*, *27*, *28*). Similarly, in the present work, the expression of IL-8/CXCL8 by Ang II was mediated by ERK1/2 MAP kinase activation.

Induction of HO-1 has been reported to lower blood pressure in several hypertensive experimental models (*29–32*). Quan *et al*. (*33*) suggested that overexpression of HO-1 is able to significantly attenuate oxidative damage induced by Ang II. NADPH oxidase plays a central role in LPS-induced IL-8/CXCL8 expression in endothelial cells or in reactive oxygen species–dependent IL-8/CXCL8 expression in airways (*34*, *35*). Ang II has been known to activate membrane-bound NADPH/NADPH oxidase and generates superoxide anion in VSMC. Thus, we examined the effect of HO-1 on IL-8/CXCL8 expression in VSMC from SHR. HO-1–transfected

VSMC showed decreased IL-8/CXCL8 expression compared to untransfected VSMC (data not shown). Also, Ang II–induced IL-8/CXCL8 expression was decreased in HO-1 transfected VSMC. Taken together with the results discussed above, this result suggests that IL-8/CXCL8 expression in VSMC is related to the development and maintenance of hypertensive vascular lesions in SHR or in Ang II–induced hypertension.

There have been no direct investigations into the expression of IL-8/CXCL8 in VSMC from SHR and WKY or the mechanism of action for Ang II–induced IL-8/CXCL8 expression in VSMC from SHR. Our results suggest a close association between elevated IL-8/CXCL8 expression and hypertension. Therefore, decreased IL-8/CXCL8 levels may contribute to the amelioration of Ang II–induced hypertension.

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