Up-Regulation of Angiotensin II Type 2 Receptor in Rat Thoracic Aorta by Pressure-Overload

Katsutoshi Yayama, Miyuki Horii, Hiromi Hiyoshi, Masaoki Takano, Hiroshi Okamoto, Satomi Kagota, and Masaru Kunitomo

Department of Pharmacology, Faculty of Pharmaceutical Sciences and High Technology Research Center, Kobe Gakuen University, Kobe, Japan (K.Y., M.H., H.H., M.T., H.O.); and Department of Pharmacology, Faculty of Pharmaceutical Sciences, Mukogawa Women’s University, Nisinomiya, Japan (S.K., M.K.)

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ABSTRACT
We have examined whether expression of angiotensin II (Ang II) type 1 (AT₁) and/or type 2 (AT₂) receptors are changed in thoracic aorta under pressure-overload by abdominal aortic banding in rats and determined whether their changes are accompanied by alteration in contractile response of thoracic aorta to Ang II. AT₂ receptor mRNA levels determined by reverse transcription-polymerase chain reaction or quantitative real-time polymerase chain reaction were increased by about 300% in aortas 4, 7, 14, and 28 days after banding without changes in AT₁ receptor mRNA levels. Contractile response of aortic rings to Ang II was decreased in thoracic aortas 7 days after banding not only abolished the up-regulation of AT₂ receptor mRNA in aortas but also recovered their Ang II responsiveness between sham and pressure-loaded rings. Either losartan (1 mg/kg/day i.p.) or candesartan (2 mg/kg/day p.o.) for 7 days after banding not only abolished the up-regulation of AT₂ receptor mRNA in aortas but also recovered their Ang II responsiveness. Basal cGMP levels were 2 times higher in pressure-loaded than in sham rings; both levels were not affected by Ang II (10⁻⁷ M; 5 min), but greatly decreased by L-NAME (10⁻⁴ M, 30 min). These results suggest that pressure-overload induces the up-regulation of AT₂ receptor expression in aortas via AT₁ receptor and thereby negatively modulates the vasoconstrictor sensitivity to Ang II, probably mediated by the mechanisms independent of the nitric oxide-cGMP system.

Angiotensin II (Ang II), the most active component of the renin-angiotensin system, is a multifunctional hormone that plays an important role in the cardiovascular physiology and pathology (de Gasparo et al., 2000). Ang II production in the proximity to its receptors on the target cells constitutes the local renin-angiotensin system, which regulates the cardiovascular functions in both autocrine and paracrine manners. The actions of Ang II are primarily mediated by two receptors, Ang II type 1 (AT₁) and type 2 (AT₂). The activation of the AT₁ receptor mediates vasoconstriction, proliferation of vascular smooth muscle cell, and production of extracellular matrix proteins by vascular smooth muscle cells (de Gasparo et al., 2000). In contrast, the AT₂ receptor has been considered to mediate vasodilation, antiproliferation, and proapoptosis in the vasculature, presumably mediated by the activation of the nitric oxide (NO) system via bradykinin production (de Gasparo et al., 2000).

Although a small number of AT₂ receptors are present in the vessels, the physiological actions of Ang II via AT₂ receptors have been difficult to determine, because the AT₂ subtype has a low degree of expression compared with the AT₁ subtype (Viswanathan et al., 1991). Nevertheless, most previous studies using normotensive or hypertensive animals (Scheuer and Perrone, 1993; Siragy and Carey, 1999), as well as knockout or transgenic mice for the AT₂ receptor gene (Hein et al., 1995; Ichiki et al., 1995; Akishita et al., 1999; Tsutsumi et al., 1999), have demonstrated that the AT₂ receptor mediates a depressor response to Ang II. Recently, it became evident that the AT₂ receptor levels in the vascula-

ABBREVIATIONS. Ang II, angiotensin II; AT₁, angiotensin II type 1; AT₂, angiotensin II type 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NO, nitric oxide; L-NAME, Nω-nitro-L-arginine methyl ester; IBMX, isobutylmethylxanthine; PRC, plasma renin concentration; PRA, plasma renin activity; RT-PCR, reverse transcription-polymerase chain reaction; SHR, spontaneously hypertensive rat; PD123319, (1-[[4-(dimethylamino)-3-methylphenyl]methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-6-carboxylic acid difluoroacetate).
tured were increased under some pathological conditions, such as hypertension and vascular injury (Otsuka et al., 1998; Hutchinson et al., 1999; Touyz et al., 1999). This evidence suggests that the up-regulation of vascular AT2 receptors under pathological conditions is one of the compensatory mechanisms of vessels counteracting the AT1-mediated contractile response to Ang II to protect vessels from the mechanical overload. To test this hypothesis, we used a rat model of pressure-induced left ventricular hypertrophy (Doggrell and Brown, 1998) produced by suprarenal abdominal aortic coarctation (banding) to determine how the increased transmural pressure influences the vascular AT2 receptor expression and thereby changes the contractile response to Ang II.

Materials and Methods

Materials. The following materials were purchased from commercial sources: Ang II, bradykinin, and ibutabitan were from Peptide Institute, Inc. (Osaka, Japan); acetylcholine chloride, L-phenylephrine hydrochloride, and N2,N2-dimethylarginine methyl ester hydrochloride (l-NAME) were from Nacalai Tesque (Kyoto, Japan); PD123319 and isobutylmethylxanthine (IBMX) were from Sigma-Aldrich (St. Louis, MO), and 125I-angiotensin I was from PerkinElmer Life Sciences (Japan). Losartan and candesartan were kindly supplied by Merck (Whitehouse Station, NJ) and Takeda Pharmaceutical Co. (Osaka, Japan), respectively.

Animals and Operation. All animal experiments were performed according to the guidelines of the Kobe Gakuin University Experimental Animal Care and Use Committee. Male Wistar rats (4 weeks old; Japan SLC, Hamamatsu, Japan) were divided into three groups: 1) untreated, 2) sham-operated, and 3) pressure-overloaded rats. Pressure-overload was produced by abdominal aortic banding, which has been primarily used as a model of cardiac hypertrophy (Doggrell and Brown, 1998). Briefly, animals were anesthetized with sodium pentobarbital (50 mg/kg i.p.), and the aorta was exposed through a midline abdominal incision. For the banding model, a blunt 22-gauge needle was placed adjacent to the abdominal aorta between the renal arteries just below the renal bifurcations, and a ligature was tightened around the aorta and adjacent needle. The sham procedure for control rats included injection of the same amount of anesthetic, in incision of approximately the same size, and a sham procedure for control rats included injection of the same saline.

Losartan and candesartan were administered orally at a dose of 1 mg/kg once a day for 7 days. Candesartan was suspended in 10% gum arabic and administered orally at a dose of 2 mg/kg once a day for 7 days. Losartan was dissolved in saline and administered i.p. at a dose of 1 mg/kg once a day for 7 days. Candesartan was dissolved in 10% gum arabic and administered orally at a dose of 2 mg/kg once a day for 7 days.

Blood Pressure Measurement. The patency of the aortic banding was assessed by measuring the blood pressure of carotid artery under pentobarbital anesthesia (50 mg/kg i.p. pentobarbital sodium). To measure the blood pressure, the left carotid artery was cannulated with a PE-10 polyethylene catheter, and pulse waveforms were monitored by a polygraph system (Nihon Kohden, Tokyo, Japan).

Assays of Plasma Renin Concentration (PRC) and Plasma Renin Activity (PRA). Blood was collected from the abdominal aorta into a syringe containing 1/10 volume of 3.8% sodium citrate under ether anesthesia. After centrifugation at 800g for 15 min, plasma samples were collected and stored at −90°C until assays. PRC or PRA in plasma samples were determined by radioimmunoassay of Ang I liberated in the presence or absence of plasma from bilaterally nephrectomized rats, respectively, as described previously (Ohtani et al., 1989).

Analysis of AT1 and AT2 Receptor mRNAs by Reverse Transcription-Polymerase Chain Reaction (RT-PCR). Animals were sacrificed under ether anesthesia 4, 7, 14, and 28 days after aortic banding or sham operation. Age-matched untreated rats were also sacrificed as a control. The excised thoracic aorta (approximately 10 mg) was stripped of adventitia and then homogenized in acid guanidinium thiocyanate-phenol-chloroform-isoamyl alcohol (GTC) as previously (Yayama et al., 2003). To detect AT1 and AT2 receptor mRNAs, we used two methods: one was RT-PCR followed by Southern blotting with respective specific probes, and second was quantitative real-time RT-PCR. Reverse transcription was performed in a reaction volume of 4 µl containing 0.3 µg of RNA, 1.55 µl of dNTP (10 mM), 0.8 µl of MgCl2 (20 mM), 0.4 µl of 10× buffer (500 mM KCl in 0.1 mol/l Tris-HCl, pH 8.3), 0.05 µl of 10 unit enzyme (Moloney murine leukemia virus reverse transcriptase), and 0.2 µl of reverse primer (50 pmol/µl). The mixture was incubated at 42°C for 15 min, 95°C for 5 min, and then 4°C for 5 min to allow synthesis of the first-strand cDNA. The cDNA was amplified in a 20-µl reaction mixture containing 10 pmol of the forward primer, 1.6 µl of 10× buffer, 4 µl of MgCl2 (25 mM), and 0.5 units of Taq DNA polymerase at 95°C for 9 min, at 94°C for 1 min, and annealing at 54°C (AT1 receptor), 56°C (AT2 receptor) or 60°C (glyceraldehyde-3-phosphate dehydrogenase; GAPDH) for 2 min, and then extension at 72°C for 3 min followed by at 72°C for 10 min. The amplification cycles were 35 for the AT1 receptor, 40 for the AT2 receptor and 25 for GAPDH. After amplification, the PCR products were electrophoresed on a 1.5% agarose gel, and then denatured, neutralized, and transferred onto a nylon membrane by capillary blotting and cross-linked by UV irradiation. The transferred membranes were detected by Southern blot hybridization with 32P-labeled cDNA probes specific for each DNA. The blot signals were detected using a Fujix Bio Imaging analyzer BAS 2000 (FujiFilm, Tokyo, Japan). The forward and reverse primers for the AT2 receptor were 5′-CACCACCTGCAATATTTCAG-3′ and 5′-GCCAATCGCACCATAATTCCTC-3′, for the AT1 receptor 5′-CTGACCTGAGACATGTTCGA-3′ and 5′-GGT-GTCATTGCTCCAAAGAACGAGTA-3′, and for GAPDH 5′-GTGCGCAAGGATGGTCATGTCTTCTAGGTTCTTTCCAAATG-3′ and 5′-CAACATCGAGAGATGGTGAACTTATTC-3′, respectively.

For quantitative measurement of the AT2 receptor mRNA, 0.3-µg RNA samples from the thoracic aorta were reverse-transcribed using a reverse primer (5′-ATACCATCAGGTGACGAAAT-3′) as described above. The cDNA products in 2 µl were mixed with 18 µl of TaqMan master mixture (2 µl of 10× buffer, 2.8 µl of MgCl2 (20 mM), 2 µl of reverse primer (3 µM), 2 µl of forward primer (3 µM), 2 µl of TaqMan probe (2 µM), 0.4 µl of 10 µl dUTP, 0.1 µl of uracil N-glycosylase (1 unit/µl), and 0.1 µl of Taq DNA polymerase (5 unit/µl)). PCR was carried out in an ABI Prism 7700 system (Applied Biosystems, Tokyo, Japan) under the following conditions: 95°C for 15 sec, 94°C for 15 sec, and 72°C for 1 min. For GAPDH, the forward and reverse primers and probes were used: forward primer, 5′-CCCCTGACCAAGTCCTTTGAGAT-3′; reverse primer, 5′-ATACCATCAGGTGACGAAAT-3′; TaqMan probe, 5′-FAM-TGGCACCTTGCTGGCTTTCC-CAGCATCAAAGGTGGAGGAAT-3′, for GAPDH, the following primers and probes were used: forward primer, 5′-CGGCTCAGGGCTTTGAGAT-3′; reverse primer, 5′-ATACCATCAGGTGACGAAAT-3′; TaqMan probe, 5′-FAM-TGGCACCTTGCTGGCTTTCCAGCATCAAAGGTGGAGGAAT-3′, for GAPDH, the following primers and probes were used: forward primer, 5′-CGGCTCAGGGCTTTGAGAT-3′; reverse primer, 5′-ATACCATCAGGTGACGAAAT-3′; TaqMan probe, 5′-FAM-TGGCACCTTGCTGGCTTTCCAGCATCAAAGGTGGAGGAAT-3′, for GAPDH, the following primers and probes were used: forward primer, 5′-CGGCTCAGGGCTTTGAGAT-3′; reverse primer, 5′-ATACCATCAGGTGACGAAAT-3′; TaqMan probe, 5′-FAM-TGGCACCTTGCTGGCTTTCCAGCATCAAAGGTGGAGGAAT-3′, for GAPDH, the following primers and probes were used: forward primer, 5′-CGGCTCAGGGCTTTGAGAT-3′; reverse primer, 5′-ATACCATCAGGTGACGAAAT-3′; TaqMan probe, 5′-FAM-TGGCACCTTGCTGGCTTTCCAGCATCAAAGGTGGAGGAAT-3′, for GAPDH, the following primers and probes were used: forward primer, 5′-CGGCTCAGGGCTTTGAGAT-3′; reverse primer, 5′-ATACCATCAGGTGACGAAAT-3′; TaqMan probe, 5′-FAM-TGGCACCTTGCTGGCTTTCCAGCATCAAAGGTGGAGGAAT-3′, for GAPDH, the following primers and probes were used: forward primer, 5′-CGGCTCAGGGCTTTGAGAT-3′; reverse primer, 5′-ATACCATCAGGTGACGAAAT-3′; TaqMan probe, 5′-FAM-TGGCACCTTGCTGGCTTTCCAGCATCAAAGGTGGAGGAAT-3′, for GAPDH, the following primers and probes were used: forward primer, 5′-CGGCTCAGGGCTTTGAGAT-3′; reverse primer, 5′-ATACCATCAGGTGACGAAAT-3′; TaqMan probe, 5′-FAM-TGGCACCTTGCTGGCTTTCCAGCATCAAAGGTGGAGGAAT-3′, for GAPDH, the following primers and probes were used: forward primer, 5′-CGGCTCAGGGCTTTGAGAT-3′; reverse primer, 5′-ATACCATCAGGTGACGAAAT-3′; TaqMan probe, 5′-FAM-TGGCACCTTGCTGGCTTTCCAGCATCAAAGGTGGAGGAAT-3′, for GAPDH, the following primers and probes were used: forward primer, 5′-CGGCTCAGGGCTTTGAGAT-3′; reverse primer, 5′-ATACCATCAGGTGACGAAAT-3′; TaqMan probe, 5′-FAM-TGGCACCTTGCTGGCTTTCCAGCATCAAAGGTGGAGGAAT-3′.
were allowed to equilibrate for 90 min before the start of the experiments. Isometric tension change was measured with a force displacement transducer (model t-7; NEC San-Ei, Tokyo, Japan) coupled to a dual-channel chart recorder (model 8K21; NEC San-Ei). After reaching equilibrium, cumulative concentration-response curves were constructed for Ang II (10⁻⁸–10⁻⁷ M) in each ring. In some experiments, various agents, such as AT₂ receptor antagonist PD123319 (1 × 10⁻⁶ M), bradykinin type 2 (B₂) receptor antagonist icatibant (1 × 10⁻⁶ M), and l-NAME (1 × 10⁻⁴ M), were added 30 min before the cumulative addition of Ang II. The contractile responses obtained were expressed as a percentage of the maximal constriction evoked by 40 mM KCl. No significant changes in the contractile response to 40 mM KCl were observed in aortic rings from rats 1 week after banding compared with those from age-matched untreated rats. Denudation of the endothelium was confirmed pharmacologically by the disappearance of the 1 × 10⁻⁷ M acetylcholine-induced relaxation response during constriction evoked by 3 × 10⁻⁶ M phenylephrine.

**Assay of cGMP in Aortic Rings.** Aortic rings were fixed in a 10-ml organ bath filled with Krebs-Henseleit solution as described above. After allowing equilibration for 1 h, IBMX (5 × 10⁻⁵ M) was added to the organ bath. Twenty-five minutes after the addition of IBMX, the rings were stimulated with Ang II (1 × 10⁻⁷ M) for 5 min. In some experiments, l-NAME (1 × 10⁻⁴ M) was added at the same time as the IBMX addition. Thereafter, the rings were frozen in dry ice-acetone and homogenized in ice-cold 6% trichloroacetic acid. cGMP was extracted, acetylated, and quantified by radioimmunoassay using the cyclic GMP ³⁵S-labeled Biotak assay system (Amersham Biosciences Inc., Piscataway, NJ) and is expressed as picomoles per milligram of protein. Protein contents of the rings were measured by the Bio-Rad protein assay kit (Bio-Rad, Hercules, CA) using γ-globulin as a standard.

**Statistical Analysis.** All data are expressed as mean ± S.E. Statistical comparisons of PRC, PRA, blood pressure, AT₂ receptor mRNA levels, and cGMP contents under various treatments were performed with one-way analysis of variance with pairwise comparison by the Bonferroni-Dunn method. Comparison of concentration-response curves of Ang II was carried out by repeated measures analysis of variance followed by the Bonferroni-Dunn method. Differences were considered significant for p < 0.05.

**Results**

**Blood Pressure in the Thoracic Aorta, PRC, and PRA after Aortic Banding.** The increased blood pressure in the thoracic aorta was monitored at the carotid artery after aortic banding. The systolic blood pressure was increased more than 30 mm Hg 4 days after aortic banding, and the levels were maintained for 28 days: systolic blood pressures 4, 7, 14, and 28 days after banding were 127.6 ± 4.7, 128.2 ± 3.3, 129.3 ± 1.1, and 153.4 ± 7.8 mm Hg, respectively, and were significantly higher than 97.2 ± 2.2, 97.5 ± 4.6, 95.9 ± 3.1, and 97.7 ± 5.9 mm Hg in sham-operated rats, respectively, and 96.5 ± 3.1, 97.9 ± 3.7, 98.3 ± 4.5, and 98.1 ± 4.6 mm Hg in nonoperated rats, respectively (n = 5–6; p < 0.01).

PRC and PRA were significantly increased at 2 and 4 days after aortic banding and returned to the levels of sham-operated animals at 7 days; both levels remained low 14 and 28 days after banding (Fig. 1).

**AT₁ and AT₂ Receptor mRNA Levels in the Thoracic Aorta after Aortic Banding.** To determine whether the pressure-overload alters the vascular expression of AT₁ and AT₂ receptor mRNAs, we examined the mRNA levels of these receptors in the thoracic aorta by RT-PCR. As shown in Fig. 2, signals corresponding to AT₁ receptor mRNA could easily be detected in the thoracic aorta of sham-operated animals, whereas only faint signals were detectable for AT₂ receptor mRNA. However, the levels of AT₂ receptor mRNA, but not AT₁ subtype, were increased 4, 7, 14, and 28 days after the banding of the abdominal aorta (Fig. 2). These observations were confirmed by quantitative real-time PCR: the levels of AT₂ receptor mRNA increased more than 300% after 4, 7, and 14 days compared with those of age-matched untreated rats or sham-operated rats (Fig. 2). High levels of AT₂ receptor mRNA were also observed after 28 days.

**Involvement of the AT₁ Receptor in the Up-Regulation of AT₂ Receptor mRNA in the Thoracic Aorta after Aortic Banding.** To examine whether the pressure-overload-induced increase in AT₂ receptor mRNA is mediated by Ang II itself, we studied the effects of the AT₁ receptor antagonists losartan and candesartan on the up-regulation of AT₂ receptor mRNA in the thoracic aorta. As shown in Fig. 3, the up-regulation of AT₂ receptor mRNA 7 days after aortic banding was completely inhibited by successive administration of losartan (1 mg/kg/day i.p.) or candesartan (2 mg/kg p.o.) for 7 days. In contrast, the administration of losartan or candesartan did not affect the blood pressure elevation in the thoracic aorta after aortic banding: 128.3 ± 5.9 mm Hg in untreated rats with aortic banding (n = 5) versus 126.7 ± 5.6 mm Hg in losartan-treated rats with aortic banding (n = 5; p > 0.1) and 127.4 ± 5.2 mm Hg in candesartan-treated rats with banding (n = 5; p > 0.1).

**AT₂ Receptor-Dependent Decrease in the Contractile Response to Ang II in Ring Preparations of Pressure-Overloaded Thoracic Aorta in Vitro.** The contractile response to Ang II was compared between the ring preparations of the thoracic aorta from sham-operated (control rings) and aortic banding rats (pressure-loaded rings). We examined thoracic aortas from rats 7 days after banding or sham operation, because high levels of AT₂ receptor mRNA were detected in thoracic aortas of banding rats during this period. The contractions evoked by Ang II were significantly decreased in the pressure-loaded rings at higher

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**Fig. 1.** PRC and PRA in rats after the banding of the abdominal aorta. Plasma samples were collected from rats 2, 4, 7, 14, and 28 days after sham operation or banding. PRC or PRA in plasma samples was determined by radioimmunoassay of Ang I after the incubation with or without bilaterally nephrectomized rat plasma, respectively. PRC in sham-operated rats (open circles) and in banding rats (closed circles), or PRA in sham-operated (open triangles) and in banding rats (closed triangles) were expressed as nanograms of Ang I liberated by 1-ml plasma sample during 1-h incubation with or without nephrectomized rat plasma. Each data point represents mean ± S.E. (n = 4). **,** **,** p < 0.001, significantly different from sham-operated rats.
concentrations of Ang II, such as $3 \times 10^{-8}$ and $1 \times 10^{-7}$ M, compared with control rings (Fig. 4A). Blockade of the AT$_2$ receptor by PD123319 ($1 \times 10^{-6}$ M) increased the contractile response to Ang II in the pressure-loaded rings (Fig. 4C), but not in control rings (Fig. 4B).

Then we compared the contractile response to Ang II between the ring preparations from rats treated or untreated with losartan for 7 days after aortic banding, because of the observation that the administration of losartan abolished the up-regulation of AT$_2$ receptor mRNA in the thoracic aorta after pressure-overload. Aortic rings were prepared from rats 24 h after the final administration of losartan at day 6. As shown in Fig. 5, losartan administration to sham-operated animals did not alter responses to Ang II in aortic rings compared with those from vehicle-treated sham animals. In contrast, the decrease in the response to Ang II by banding was significantly prevented in aortic rings from losartan-treated rats after banding compared with vehicle-treated banding animals (Fig. 5).

Endothelium-Dependent Decrease in the Contractile Response to Ang II in Ring Preparations of Pressure-Overloaded Thoracic Aorta in Vitro. The contractile response to Ang II was markedly augmented by removal of the endothelium in either control (Fig. 6A) or pressure-loaded rings (Fig. 6B). When the concentration-response curves were compared between control and pressure-loaded rings after removal of endothelium, decreased response to Ang II in pressure-loaded rings was not observed, and rather the response was augmented significantly in the pressure-loaded rings at lower concentrations of Ang II between $1 \times 10^{-10}$ and $1 \times 10^{-9}$ M (Fig. 6C). PD123319 did not influence the response to Ang II in the endothelium-denuded rings of either control or pressure-loaded rings (data not shown).

To determine the involvement of endothelium-derived NO in the decreased response to Ang II in pressure-loaded rings, the ring preparations with intact endothelium were treated with a NO synthase inhibitor L-NAME ($1 \times 10^{-4}$ M) for 30
min before the cumulative addition of Ang II. As shown in Fig. 7, L-NAME pretreatment augmented the contractile response of Ang II in both control and pressure-loaded rings (Fig. 7, A and B), and no significant differences were observed in the response to Ang II between these L-NAME-pretreated rings (Fig. 7C).

Discussion

In the present study, we found up-regulation of the AT2 receptor mRNA, but not of the AT1 subtype, in the thoracic aorta under pressure-overload. Because either the administration of losartan or candesartan blunted the up-regulation of AT2 receptor mRNA without showing changes in the band-
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ing-induced pressure-overload, it seems likely that the mechanical load up-regulates the aortic AT$_2$ receptor through the action of Ang II, probably via activation of AT$_1$ receptor. Although high levels of PRC and PRA were observed in animals 4 days after banding as a response of kidney to decreased blood pressure distal to the aortic banding, the levels returned to ranges of sham-operated animals after 7 days, suggesting that the pressure elevation at carotid artery seems to be related to the mechanical obstruction. In fact, the administration of AT$_1$ receptor antagonists did not decrease blood pressure at carotid artery in rats with aortic banding. These observations may be supported by a finding that enalapril did not reduce the carotid arterial pressure in rats with aortic banding (Baker et al., 1990).

Several lines of evidence have suggested the existence of a link between the effects of transmural pressure and Ang II in blood vessels. Noda et al. (1994) indicated that mechanical stretch and Ang II synergistically stimulated cultured rat aortic smooth muscle cells to induce a marked increase in the expression of parathyroid hormone-related peptide mRNA. Bardy et al. (1996) reported that the increased transmural pressure elevation at carotid artery seems to be related to the mechanical obstruction. In fact, the mechanical load up-regulates the aortic AT$_2$ receptor through the action of Ang II, probably via activation of AT$_1$ receptor.

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Fig. 6. Contractile response to Ang II in endothelium-denuded ring preparations of thoracic aorta. Thoracic aortas were dissected from rats 7 days after sham operation or aortic banding, and then the endothelium was removed before fixation in the organ bath. A, comparison of the cumulative concentration-response curves for Ang II between intact (open circles; $n = 5$) and endothelium-denuded (closed circles; $n = 5$) aortic rings from sham-operated rats. Significantly different from intact rings (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). B, comparison of the cumulative concentration-response curves for Ang II between intact (open circles; $n = 6$) and endothelium-denuded (closed circles; $n = 6$) aortic rings from banding rats. ***, $p < 0.001$, significantly different from intact rings. C, comparison of the cumulative concentration-response curves for Ang II in endothelium-denuded rings between sham-operated (open circles; $n = 5$) and banding (closed circles; $n = 6$) rats. *, $p < 0.05$, significantly different from sham-operated rats. Data represent mean ± S.E.; results are expressed as percentage of contraction evoked by 40 mM KCl.

Fig. 7. Contractile response to Ang II in L-NAME-pretreated ring preparations of thoracic aorta. Aortic rings were prepared from thoracic aortas dissected from sham-operated rats (A; $n = 5$) or rats 7 days after aortic banding (B; $n = 8$), then cumulative concentration-response curves for Ang II were constructed before (open circles) or 30 min after the treatment with L-NAME ($1 \times 10^{-4}$ M) (closed circles). Significantly different from untreated rings (***, $p < 0.001$). Data on L-NAME-treated rings in both A and B are shown together in C, in which open or closed circles represent rings from sham-operated or banding rats, respectively. Data represent mean ± S.E.; results are expressed as percentage of contraction evoked by 40 mM KCl.
pressure in the aorta might have caused the local generation of Ang II, which acted synergistically with the transmural pressure to enhance vascular fibronectin expression via the AT_1 receptor. Recently, Bonnet et al. (2001) demonstrated that the AT_2 receptor mRNA was up-regulated in rat mesenteric arteries after a pressure dose of Ang II infusion for 2 weeks; they suggested the involvement of AT_1-receptor mediation in this Ang II effect, because AT_1 receptor antagonist inhibited the Ang II-induced up-regulation of the AT_2 receptor.

In the aortic banding model, the decreased blood pressure distal to the banding stimulates the kidney to release renin, resulting in increased circulating levels of Ang II. However, as shown in this study and by other investigators (Baker et al., 1990; Doggrell and Brown, 1998), the fact that the elevation of plasma renin is observed only within a few days after aortic banding does not account for the increased levels of the AT_2 receptor mRNA over 3 weeks. Therefore, a rapid increase in AT_2 receptor mRNA levels within 4 days seems to depend on a transient elevation of plasma renin, but the sustained increase in AT_2 receptor mRNA may probably be due to locally generated Ang II in the pressure-overloaded aorta.

In ring preparations of rat thoracic aortas that were dissected 7 days after aortic banding, the contractile response to Ang II was significantly decreased compared with that in control rings. The response to Ang II in the pressure-loaded rings was increased in the presence of the AT_2 receptor antagonist PD123319, but not in the control rings, suggesting a potential role of the AT_2 receptor in the decreased aortic response to Ang II. Indeed, the reduction of Ang II responsiveness in pressure-loaded rings was prevented by the administration of losartan, which was found to inhibit the up-regulation of AT_2 receptor mRNA after aortic banding.

Because Ang II binds to the AT_1 and AT_2 receptor subtypes with similar affinity (Nouet and Nahmias, 2000), the contractile response of the aorta to Ang II seems to be dependent on the relative expression level and/or responsiveness of both receptors. Thus, it seems likely that the decreased response to Ang II in the pressure-overloaded aortas depends on, at least in part, the up-regulation of the AT_2 receptor.

There is evidence that Ang II binding to AT_2 receptors decreases the Gq-coupled phospholipase C activation by the AT_1 receptor (Gyurko et al., 1992), suggesting that the activation of AT_2 receptors on aortic smooth muscle cells directly interacts with the signaling pathway of AT_1 receptors. However, a decreased response to Ang II was not observed in the endothelium-denuded rings of pressure-overloaded aortas, and PD123319 did not alter the response to Ang II in the pressure-loaded rings, as well as in the control rings, after removal of the endothelium. Thus, it seems that the contraction of aortic smooth muscle cells by Ang II via the AT_1 receptor is attenuated by a vasorelaxation factor(s) derived from the endothelium via AT_2 receptor stimulation. A potential factor is NO, because of the observation that, after the inhibition of NO synthase by l-NAME, the response to Ang II became comparable in both control and pressure-loaded rings.

The basal levels of cGMP in pressure-loaded aortic rings were significantly higher than those in control rings. Because the cGMP levels in pressure-loaded rings were significantly reduced after l-NAME treatment, it is likely that the increased levels of basal cGMP in pressure-loaded rings result from the enhanced production of NO by the endothelium of pressure-overloaded aortas. In fact, the protein and mRNA levels of endothelial NO synthase are up-regulated in pressure-overloaded thoracic aortas after banding of the abdominal aorta (Bouloumie et al., 1997; Barton et al., 2001). Thus, the increased levels of basal cGMP in pressure-loaded rings may participate with, at least in part, the attenuation of the AT_1 receptor-mediated contractile response.

Given that the increased expression of AT_2 receptors in pressure-loaded aortas is functionally coupled to the NO-cGMP system, it is logical to expect that the stimulation by Ang II results in the elevation of cGMP levels in pressure-loaded rings, because of observations that the contractile response to Ang II in pressure-loaded rings was significantly increased by PD123319. However, stimulation by Ang II did not affect the cGMP levels in pressure-loaded rings, as in control rings. These results do not support the idea that the decreased response to Ang II observed in pressure-loaded rings simply depends on the NO-cGMP system via the activation of the AT_2 receptor. Thus, it is reasonable to consider mechanisms other than the NO-cGMP system to explain the AT_2 receptor-mediated reduction of Ang II-responsiveness in pressure-loaded aortas. Together, the decreased contractile response to Ang II in thoracic aorta by pressure-overload seems to result from at least two different mechanisms: one is the pressure-overload-induced activation of the NO-cGMP system, and another the AT_2 receptor-dependent vasodilatory mechanisms, such as the activation of phospholipase A_2 and release of arachidonic acid (Zhu et al., 1998).

It has recently been demonstrated that the AT_2 receptor-mediated vasodilator response to Ang II is mediated by kinin in various vessels, such as the rat aorta (Gohlke et al., 1998), canine coronary microvessels (Seyedi et al., 1995), and rat
mesenteric artery (Katada and Majima, 2002). These studies suggest that the AT_2 receptor is coupled to the local generation of kinin in the vascular wall, which stimulates NO production in endothelial cells via the bradykinin B_2 receptor. Bradykinin in the vascular wall, which stimulates NO production in endothelial cells via the bradykinin B_2 receptor. Furthermore, both control and pressure-loaded rings did not exhibit a relaxation response to exogenous bradykinin, even at high concentrations, in contrast to the sensitive relaxation by acetylcholine, indicating that the rat thoracic aorta is essentially insensitive to bradykinin. This observation is supported by a previous study (Wirth et al., 1996) that rat aorta shows vasorelaxation in response to acetylcholine, but not to bradykinin. Thus, it is unlikely that the kinin-NO-cGMP system plays a role in the signaling cascade of the AT_2-receptor in the rat thoracic aorta.

Recent studies on the vascular AT_2 receptor have focused on the pathophysiologic roles under hypertensive conditions in which vascular AT_2 receptors are up-regulated, such as in the mesenteric arteries of young spontaneously hypertensive rats (SHR) (Touyz et al., 1999) and in the thoracic aorta of SHR (Otsuka et al., 1998). Barber et al. (1999) demonstrated that an AT_2 receptor agonist induced a depressor response during simultaneous AT_1 receptor blockade in SHR, suggesting that the AT_2 receptor opposes the action of AT_1 receptor in blood pressure regulation, at least in SHR. Carey et al. (2000) demonstrated a depressor effect of Ang II in the presence of AT_1 receptor blocker via AT_2 receptor stimulation. These studies strongly suggest that the AT_2 receptor acts as a vasodilatory pathway counterregulatory to the vasoconstrictor actions of Ang II through the AT_1 receptor. The present study supports this concept by the finding of the up-regulation of the aortic AT_2 receptor under pressure-overload contributes to the attenuation of the AT_1 receptor-mediated aortic constriction. Thus the up-regulation of the vascular AT_2 receptor under pressure-overload seems to be one of the compensatory responses of vessels counteracting the AT_1 receptor-dependent vasoconstriction to relieve the mechanical overload. However, the beneficial effects of AT_1 receptor antagonists through the activation of the AT_2 receptor (Liu et al., 1997; Gigante et al., 1998; Carey et al., 2001) may be limited under pathological conditions in which the expression of AT_2 receptors is controlled by the activation of the AT_1 receptor, as shown in the present study.

References


Address correspondence to: Dr. Hiroshi Okamoto, Department of Pharmacology, Faculty of Pharmaceutical Sciences, Kobe Gakuin University, Iikawadani-cho, Nishi-ku, Kobe 651-2180, Japan. E-mail: p-okamoto@kobegakuin.ac.jp