Role of AT$_2$ Receptors in Angiotensin II–Stimulated Contraction of Small Mesenteric Arteries in Young SHR

Rhian M. Touyz, Dierk Endemann, Gang He, Jin-S. Li, Ernesto L. Schiffrin

Abstract—This study assesses the receptor subtype (AT$_1$ and AT$_2$) through which angiotensin II (Ang II) mediates contraction in small arteries of young and adult spontaneously hypertensive rats (SHR). Segments of third-order mesenteric arteries (~200 µm in lumen diameter) were mounted in a pressurized system. Systolic blood pressure and media:lumen ratio of small arteries were significantly greater ($P<0.001$) in young SHR and adult SHR than in age-matched Wistar-Kyoto rats (WKY). Ang II–induced contractile effects were significantly increased ($P<0.05$) in young SHR compared with age-matched WKY. AT$_1$ blockade with losartan, and combined AT$_1$ and AT$_2$ blockade with losartan and PD123319, abolished Ang II–stimulated contraction in young and adult rats. AT$_2$ blockade (PD123319) significantly reduced ($P<0.01$) Ang II–elicited contraction in young SHR but had no effect in WKY or adult SHR, indicating that AT$_2$ receptors may contribute to Ang II–induced contraction in young SHR. To determine the Ang receptor status in rat mesenteric vessels, AT$_1$ and AT$_2$ receptor mRNA expression was determined by reverse transcription–polymerase chain reaction. AT$_1$ and AT$_2$ receptor protein expression were detected by Western blot analysis. AT$_1$ receptor mRNA was equally expressed in age-matched rats, but expression was significantly lower in young rats compared with adult rats. AT$_2$ receptor mRNA was weakly expressed in WKY and adult SHR. In vessels from young SHR, AT$_2$ receptor mRNA expression was significantly increased compared with the other groups. AT$_1$ receptor protein was only detectable in young rats, with the magnitude of expression greater in SHR than WKY. In conclusion, Ang II–stimulated contractile responses are augmented in vessels from young SHR. These effects are reduced by selective AT$_1$ blockade and abolished by AT$_1$ blockade, indicating that both Ang receptor subtypes are involved in contraction in young SHR. In WKY and adult SHR, losartan, but not PD123319, inhibited Ang II–induced contraction, indicating the exclusive involvement of AT$_1$ receptors. Thus, in SHR, in the phase of developing hypertension, enhanced Ang II–stimulated vascular contraction may be associated with changes in Ang II receptor status, as evidenced pharmacologically and by increased vascular AT$_2$ receptor mRNA and protein expression. (Hypertension. 1999;33[part II]:366-372.)

Key Words: resistance ■ arteries ■ hypertension ■ receptors, angiotensin ■ vasoconstriction ■ PD123319 ■ losartan

Angiotensin II (Ang II), the final mediator of the renin-angiotensin system, plays a pivotal physiological role in cardiovascular homeostasis. It is a potent vasoconstrictor of the peripheral vasculature and induces hypertrophy, hyperplasia, or both in small resistance arteries, in vascular smooth muscle cells, in endothelial cells, and in cardiomyocytes.1–4 Because of these actions, Ang II may play an important pathophysiological role in the development and maintenance of hypertension.

Cellular responses to Ang II are mediated by specific cell membrane receptors. Two main subtypes of Ang receptors have been pharmacologically defined: AT$_1$ and AT$_2$, which are blocked specifically by losartan and PD123319, respectively.5–7 AT$_1$ has 2 subtypes in rodents, AT$_{1A}$ and AT$_{1B}$, with greater than 95% amino acid sequence homology.6 Most of the known physiological effects of Ang II are mediated by AT$_1$ receptors and, until recently, it was believed that vascular Ang II receptors were exclusively of the AT$_1$ subtype. However, it has recently been demonstrated that the adult rat aorta expresses a small but significant amount of AT$_2$ receptors as well.8 In the aortas of fetal and young rats, the proportion of AT$_2$ receptors is higher, and this predominance of AT$_2$ receptors is reversed during development.8,9 In 2-week-old Sprague-Dawley rats, 81% of Ang receptors in the aorta are of the AT$_2$ subtype, and in 8-week-old rats, this is reduced to 28%, with a predominance of AT$_1$ (71%).8 In 6- to 8-week-old spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY), renal resistance vessels display 20% of Ang II binding sites with affinity for PD123319.10

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From the Medical Research Council Multidisciplinary Research Group on Hypertension, Clinical Research Institute of Montreal and Université de Montréal, Montreal, Quebec, Canada.
Correspondence to Rhian M. Touyz, MD, PhD, Clinical Research Institute of Montreal, 110 Pine Ave W, Montreal (Quebec), Canada H2W 1R7. E-mail touyz@ircm.umontreal.ca
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AT1 receptors may be important in tissue growth and development. Growth-promoting effects of Ang II appear to be mediated primarily via AT1 receptors, and antiproliferative effects have been linked to AT2 receptors. A study by Stoll et al demonstrated an antiproliferative effect of Ang II on coronary endothelial cells, which could be blocked by PD123319 (an AT2 antagonist). Saward and Zahradka reported that PD123319, but not losartan, could block Ang II–induced RNA synthesis in A10 vascular smooth muscle cells. Levy et al recently reported that blood pressure remained high in Wistar rats treated for 3 weeks with Ang II and PD123319 but that fibrosis and vascular hypertrophy were reduced by PD123319 compared with Ang II infusion alone.

AT1 receptors have also been implicated in pathological conditions associated with cardiovascular remodeling. In neointimal formation after vascular injury, AT1 receptor expression is changed to that of the AT2 subtype; in diabetes, postmyocardial infarction, ischemia, and hypertension, AT1 receptor expression may be enhanced. Also, vascular responsiveness to Ang II is altered in hypertension. In SHR with established hypertension, vascular reactivity to Ang II is increased or unchanged, and in portal hypertension, mesenteric artery responses to Ang II are reduced. We and others have shown that Ang II–stimulated portal hypertension, mesenteric artery responses to Ang II are reduced. In young SHR and stroke-prone SHR, renal vascular responses to Ang II are augmented. Underlying mechanisms for altered vascular responses are exaggerated in smooth muscle cells from mesenteric arteries of SHR. In young SHR and stroke-prone SHR, renal vascular responses to Ang II are augmented. Underlying mechanisms for altered Ang II–elicited vascular responses in hypertension are unclear, but changes in receptor status may play a role.

The aims of the present study were (1) to determine the receptor subtype through which Ang II mediates contraction in young and adult SHR, (2) to evaluate the vascular AT1 and AT2 receptor status by determining mRNA expression of the 2 receptor subtypes in rat small mesenteric arteries, and (3) to assess whether AT1 and AT2 receptor mRNA and protein expression is altered in SHR. Contractile effects of Ang II were assessed in mesenteric resistance vessels from 6-week-old SHR in the phase of developing high blood pressure, and in 21-week-old SHR in the phase of established hypertension. Age-matched normotensive WKY were also studied. Arteries were mounted as pressurized preparations, which facilitates assessment of vessels in conditions that resemble those in vivo.

**Methods**

**Materials**

Ang II was obtained from Peninsula Laboratories Inc. PD123319 was from Research Biochemicals International. AT1 (N-10) rabbit polyclonal antibody and AT2 (C-18) goat polyclonal antibody were bought from Santa Cruz Biotechnology Inc. All other chemicals were obtained from Sigma Chemical, Fischer Scientific, and BDH Inc.

**Rats**

Animal experiments were performed according to the recommendations of the Canadian Council for Animal Care and were approved by the Animal Care Committee of the Clinical Research Institute of Montreal. Male SHR and WKY 5 and 20 weeks of age were acquired from Taconic Farms Inc. (Germantown, NY). They were housed under standardized conditions with controlled temperature (22°C) and humidity (60%) and exposed to a 12-hour light/dark cycle. They were fed regular pelleted rat chow and given tap water ad libitum. Indirect systolic blood pressure was measured by the tail-cuff method in conscious prewarmed slightly restrained animals 3 to 4 days before experimentation. Blood pressure was recorded with a model PCPB photoelectric pulse sensor on a Grass model 7 polygraph fitted with a 7P8 preamplifier (Grass Medical Instruments). The average of 3 pressure readings was recorded.

**Preparation and Mounting of Small Arteries**

Rats were euthanized by decapitation. Superior mesenteric arteries were taken from the part of mesenteric bed that feeds the jejunum 8 to 10 cm distal to the pylorus, were dissected out, and were immediately placed in cold physiological salt solution (PSS) of the following composition (mmol/L): NaCl 120, NaHCO3 25, KCl 4.7, KH2PO4 1.18, MgSO4 1.2, CaCl2 2.5, EDTA 0.026, and glucose 5.5. PSS was bubbled with 95% air and 5% CO2 to give a pH of 7.4 and maintained at 37°C. A second or third branch of the mesenteric arterial bed of 130 to 280 μm in lumen diameter and about 2 mm in length was carefully dissected out and cleaned of all adherent connective tissue under a dissecting microscope.

The arterial segments were mounted as pressurized preparations as previously described. Arteries (≈280 μm diameter and 2 to 3 mm in length) were mounted onto 2 glass microcannulae. One cannula was fixed, whereas the other was adjustable and could be positioned as appropriate. Both ends of arterial segments were secured to the cannula with nylon suture. The arterial segments were adjusted by carefully moving the cannula until the vascular walls were parallel without any buckling or stretching. The vessels were pressurized to 45 mm Hg, considered the optimum pressure, because contractile responses reach a maximum at this intraluminal pressure as shown in preliminary studies. After applying intraluminal pressure, the arteries were checked for leaks, which were identified by a reduction in the preset intraluminal pressure. The arterial segments were then allowed to equilibrate for 45 to 60 minutes. A viability test was performed in all arteries, and only those segments in which an extraluminal application of KCl (ie, PSS that contained 125 mmol/L KCl) containing 10 μmol/L norepinephrine, induced vasoconstriction to >50% of their resting lumen diameter were considered viable. The integrity of vascular endothelium was confirmed if arterial segments dilated in response to an extraluminal application of 10 μmol/L acetylcholine in PSS containing 10 μmol/L norepinephrine.

**Experimental Protocol**

After each activation, the arterial segments were perfused with PSS and allowed to regain their resting diameter. Media thickness and lumen diameter were then measured. Measurements were made from the transillumination image with a microcomputer-based videography system at 3 points along a portion of each vessel, and the mean value was calculated. The arteries were perfused extraluminally at a rate of 2 mL/min with PSS containing Ang II (10-10 to 10-6 mol/L) to obtain cumulative concentration-response curves. In the case of antagonists, the arteries were preincubated with drugs 15 minutes prior to starting the experiments. The arteries were stimulated at each concentration until the maximal decrease in lumen diameter was obtained. Each arterial segment was used for only 1 Ang II concentration-response curve.

**Reverse Transcription–Polymerase Chain Reaction Analysis of AT1 and AT2 Receptors**

AT1 and AT2 receptor mRNA expression was measured by reverse transcription–polymerase chain reaction (RT-PCR). Total RNA was extracted from mesenteric arteries using TRIzol (GIBCO Life Technologies). Total RNA samples were treated with RNase-free DNase (GIBCO Life Technologies), and contamination of sample RNAs by genomic DNA was excluded by directly subjecting the sample RNAs to PCR amplification without a RT step. Total RNA

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from adrenal gland was used as a positive control for AT, and AT_2 receptors in RT-PCR. Water was used as a negative control.

Reverse transcription was performed in a reaction volume of 30 µL containing 1 µg RNA, 1.5 µL of 10 mmol/L dNTP, 6 µL of BRL 5 × buffer, 0.6 µL Oligo (dT)_{12-18} primer (0.5 µg/µL), 1.5 µL of 200 U/µL M-MLV RT (Moloney murine leukemia virus reverse transcriptase), 0.9 µL rRNasin (RNAse inhibitor) 40 U/µL, and 3 µL of dithiothreitol 0.1 mol/L at 37°C for 1 hour. The reaction was inactivated at 95°C for 5 minutes. After first-strand synthesis of RNA, 2 µL cDNA was then amplified using specific primers. For amplification of AT, receptor cDNA, the sense primer 5’-GTAGCAAAATCGTCCTGATCATGAT-3’ (extending from base 568 through base 587) and the antisense primer 3’-TATGTCGGGGTTAATGTTACGAC-5’ (extending from base 1006 through base 1030) were used. For amplification of AT_2 receptor cDNA, the sense primer was 5’-ACCTGATGAGTGTGGATAGG-3’ (extending from base 827 through base 848), and the antisense primer was 3’-AAGGTGGCAGATCACCACCCAC-5’ (extending from base 1037 through base 1065). The amplification profile involved denaturation at 95°C for 30 seconds, annealing at 57°C for 30 seconds, and extension at 72°C for 30 seconds for 30 cycles. After amplification, PCR products were electrophoresed on a 1.5% agarose gel for 1 hour at 9V/cm gel. Bands corresponding to RT-PCR products were visualized by UV light after agarose gel electrophoresis, and their intensities were measured by densitometry.

**Western Blot Analysis of Vascular AT_1 and AT_2 Receptors**

Mesenteric arteries, isolated from young and adult WKY and SHR (n=3 per group), were homogenized with VARI-MIX III (Caulk Dentsply Co, Toronto, Ontario, Canada). The homogenate was incubated on ice for 30 minutes in PBS containing 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 10 mg/mL phenylmethylsulfonyl fluoride, and 10 U/mL aprotinin, followed by centrifugation at 15 000 g for 20 minutes at 4°C. The protein concentration was determined with Micro BCA Protein Assay Kit (Pierce). After denaturation at 100°C for 5 minutes, equal amounts of proteins (20 µg) were loaded on a 12% sodium dodecyl sulfate–polyacrylamide gel and transferred to polyvinylidene fluoride membrane for 1 hour at 100 V at 4°C. Membranes were blocked overnight at 4°C in 5% milk washing solution (50 mmol/L Tris-HCl, pH 7.4). Membranes were incubated with rabbit polyclonal antibody against AT_1 receptor (≈50 kDa) or goat polyclonal antibody against AT_2 receptor (≈44 kDa) (Santa Cruz) diluted 1:100 or 1:200, respectively, in washing solution at room temperature for 1 hour. The membranes were then washed, incubated with anti-rabbit or anti-goat horseradish peroxidase–conjugated secondary antibody 1:5000 (or 1:2000) for 1 hour at room temperature, and washed extensively. Membranes were incubated with Chemiluminescence Blotting Substrate (Boehringer Mannheim), according to the manufacturer’s protocol, and exposed to X-ray film that was immediately developed. The film was scanned by ScanJet 6100C/T (Hewlett Packard) and saved to a computer. Band intensity was measured by computer analysis, using the Image Quant program (Molecular Dynamics). Statistical Analysis

Contrastive responses to Ang II were calculated by measurement of percentage decrease in resting diameter relative to the response to 10 µmol/L norepinephrine. The maximal contraction (E_{max}) induced by Ang II was calculated as the maximal percentage decrease in lumen diameter. Media cross-sectional area was calculated by subtraction of luminal cross-sectional area (CSA) from total cross-sectional area: CSA = π(D_0^2 − D_1^2)/4, where D_0 is the external diameter and D_1 is the lumen diameter of blood vessels. Values are presented as mean±SEM. Statistical evaluation of the data was performed by ANOVA when more than 2 mean values were compared or by Student’s t test for comparison of 2 mean values. P<0.05 was considered statistically significant.

**Results**

**Blood Pressure, Body Weight, and Vessel Wall Parameters**

Systolic blood pressure was significantly higher (P<0.001) in young (129±2 mm Hg) and adult (205±2 mm Hg) SHR compared with young (104±2 mm Hg) and adult (114±0.7 mm Hg) WKY.

Lumen diameter was significantly smaller (P<0.001), and media:lumen ratio and media thickness were significantly greater (P<0.001) in SHR than in age-matched WKY (Table 1). Media cross-sectional area was similar in 6-week-old SHR and WKY, but in 21-week-old rats it was greater in SHR than WKY (P<0.05; Table 1). Cross-sectional area was significantly greater (P<0.001) in adult SHR than in young SHR.

**Contractile Effects of Ang II**

Application of Ang II reduced lumen diameter in a concentration-dependent manner in vessels from young and adult rats. In young rats, contractile responses induced by Ang II were significantly greater in SHR than in WKY (Table 2, Figure 1). Ang II–stimulated contraction in 21-week-old SHR was slightly greater than that in vessels of age-matched WKY, but only reached statistical significance (P<0.05) at 10⁻⁹ mol/L Ang II. Sensitivity to Ang II was significantly greater in arteries from adult SHR than in age-matched WKY (Table 2). Vascular contractile responses in SHR were significantly greater in young prehypertensive rats compared with adult rats (Table 2).

**Effects of Losartan and PD123319 on Ang II–Stimulated Contraction**

To determine the receptor subtype through which Ang II mediates vascular contraction, vessels were preexposed to the selective AT_1 antagonist losartan or the selective AT_2 receptor-blocker PD123319. In young rats, PD123319 had no significant effect on Ang II–induced contraction in WKY but significantly attenuated maximum responses in SHR (P<0.05; Figure 1, Table 2). In adult rats, PD123319 had no significant effect on Ang II–elicited maximal contractile responses in SHR or WKY (Figure 2). Losartan inhibited Ang II effects but did not completely block

<table>
<thead>
<tr>
<th>TABLE 1. Structural Properties of Small Mesenteric Arteries From Young (6-Week-Old) and Adult (21-Week-Old) WKY and SHR</th>
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<tr>
<td>Parameter</td>
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<tr>
<td>-----------</td>
</tr>
<tr>
<td>WKY</td>
</tr>
<tr>
<td>Lumen diameter, µm</td>
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<td>Media thickness, µm</td>
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<tr>
<td>Media/lumen ratio, %</td>
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<tr>
<td>Cross-sectional area, 1000 µm²</td>
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Results are mean±SEM. *P<0.05, **P<0.01 vs WKY counterpart.
contractile responses induced by high Ang II concentrations in SHR arteries (Figures 1 and 2). Pretreatment with losartan and PD123319 in combination completely abolished Ang II–stimulated contractions in young and adult WKY and SHR (Figures 1 and 2).

Expression of AT1 and AT2 Angiotensin Receptors

Figures 3 and 4 are representative examples of the PCR products for young and adult WKY and SHR. The amounts of vascular AT1 and AT2 receptor mRNA were measured by scanning and expressed as arbitrary units (Figures 3 and 4). Figure 5 demonstrates the mean±SEM for the amounts of vascular AT1 and AT2 receptor mRNA, expressed as a ratio to GAPDH mRNA, in the different groups (n=3 per group). AT1 receptor mRNA was equally expressed in age-matched WKY and SHR (Figures 3 and 5). However, compared with adult rats, AT1 mRNA expression was significantly less in young rats (Figures 3 and 5). AT2 receptor mRNA was weakly expressed in mesenteric arteries from WKY and adult SHR (Figures 4 and 5). In young SHR, AT2 receptor mRNA expression was greater compared with age-matched WKY and adult SHR (Figures 4 and 5).

Western Blot Analysis of Vascular AT1 and AT2 Receptors

Western blot analysis demonstrated that the AT1 receptor protein was detectable in mesenteric vessels from adult WKY and SHR but not from young rats of either strain (Figure 6). The AT2 receptor protein was weakly expressed in young rats, with the magnitude of expression being higher in vessels from SHR than in WKY (Figure 6). AT2 receptor protein was undetectable in vessels from adult rats of either strain (Figure 6). PC12W cells, which express AT2 receptors exclusively, were used as a positive control for AT2 receptor protein.

Discussion

Results from the present study demonstrate that in the phase of developing hypertension in SHR Ang II–stimulated contraction is mediated via AT1 receptors and receptors that are blocked by PD123319, probably AT2 receptors, whereas in adult SHR with established hypertension Ang II–induced

Table 2. Maximal Contractile Responses (Emax) and Sensitivity to Ang II (pD2) in the Absence and Presence of PD123319 (Selective AT1 Receptor Antagonist) in Mesenteric Vessels of Young and Adult WKY and SHR

<table>
<thead>
<tr>
<th></th>
<th>Young Rats (6-Week-Old)</th>
<th>Adult Rats (21-Week-Old)</th>
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<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
</tr>
<tr>
<td>Ang II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emax</td>
<td>39±4.0 (9)</td>
<td>62±9.4** (10)</td>
</tr>
<tr>
<td>pD2</td>
<td>9.0±0.52</td>
<td>9.2±0.56</td>
</tr>
<tr>
<td>Ang II+PD123319</td>
<td>43±7.2 (10)</td>
<td>34±4.7+ (12)</td>
</tr>
<tr>
<td>Emax</td>
<td>8.9±0.78</td>
<td>9.14±0.8</td>
</tr>
</tbody>
</table>

Emax is expressed as percentage of maximum norepinephrine contraction. Numbers in parentheses are number of rats. Data are mean±SEM.

*P<0.05, **P<0.01 vs WKY counterpart; +P<0.01 vs young SHR Ang II group.

Figure 1. Line graphs demonstrate contractile effects of Ang II in small mesenteric arteries from 6-week-old WKY (left) and SHR (right) in the absence and presence of 10–5 mol/L PD123319 and 10–5 mol/L losartan. Each data point is mean±SEM of 6 to 10 experiments. *P<0.05, **P<0.01 vs Ang II counterpart. NE indicates norepinephrine.

Figure 2. Line graphs demonstrate contractile effects of Ang II in small arteries of 21-week-old WKY (left) and SHR (right) in the absence and presence of 10–8 mol/L PD123319 and 10–8 mol/L losartan. Each data point is the mean±SEM of 6 to 8 experiments. *P<0.05, **P<0.01 vs Ang II. NE indicates norepinephrine.
Vascular Responses to Angiotensin II and AT₂ Receptors

Vasoconstriction is mediated exclusively via AT₁ receptors. The pharmacological findings are supported by molecular data, where AT₂ receptor expression was increased only in arteries from young SHR. These novel data in young SHR provide important information regarding the vasoconstrictor effects of Ang II that may be involved in vascular dysfunction associated with the development of spontaneous hypertension.

Ang II dose-dependently contracted small arteries from WKY and SHR. Using a preparation similar to the one described here, Falloon et al also demonstrated a contractile effect of Ang II on small mesenteric arteries from WKY.²⁹ Responsiveness in vessels from 6-week-old SHR was significantly greater than in WKY and adult SHR. Vascular hyperresponsiveness to Ang II in young SHR and stroke-

Figure 3. Detection of AT₁ mRNAs by RT-PCR in mesenteric vessels from young and adult WKY and SHR. GAPDH mRNA is an internal control. The amount of AT₁ mRNA was measured by scanning and expressed as arbitrary units. (+) indicates the positive control (rat adrenal gland mRNA) and (−) indicates the negative control (water). Measurements represent duplicates made on the same sample. Results are representative of 3 experiments. AT₁R indicates AT₁ receptor.

Figure 4. Detection of AT₂ mRNAs by RT-PCR in mesenteric vessels from young and adult WKY and SHR. GAPDH mRNA is an internal control. The amount of AT₂ mRNA was measured by scanning and expressed as arbitrary units. (+) indicates the positive control (rat adrenal gland mRNA) and (−) indicates the negative control (water). Measurements represent duplicates made on the same sample. Results are representative of 3 experiments. AT₂R indicates AT₂ receptor.

Figure 5. Bar graphs demonstrate amounts of AT₁ and AT₂ receptor mRNA in mesenteric arteries from young and adult WKY and SHR. The amounts of AT₁ and AT₂ mRNA were measured by scanning and expressed as the ratio of optical density of AT receptor mRNA to GAPDH mRNA. Data are expressed as mean±SEM; n=3 rats per group. *P<0.05 vs WKY counterpart. **P<0.01 vs adult counterpart.

Figure 6. Western blot analysis comparing expression of the AT₁ and AT₂ receptor subtypes (20 μg protein per lane) in mesenteric arteries from young and adult WKY and SHR. The AT₁ receptor protein was detected at ~50 kDa, and the AT₂ receptor protein was detected at ~44 kDa. Bar graphs demonstrate arbitrary densitometric units. Data shown are representative of results of 3 separate experiments.
prone SHR has also been demonstrated in renal vessels. Ang II–elicited vascular hyperresponsiveness in the early phase of blood pressure elevation may represent a critical phase in the development of hypertension in this genetic model of hypertension. In support of this are studies that examined long-term cardiovascular effects after a brief period of angiotensin-converting enzyme inhibitor treatment in young SHR. SHR treated with perindopril from 6 to 10 weeks of age was sufficient to prevent the full expression of genetic hypertension and cardiovascular hypertrophy.

Underlying mechanisms for Ang II–related changes in the early phase of blood pressure elevation could be due to alterations in Ang II receptor status. Results from our study demonstrate that the selective AT2 receptor blocker PD123319 had no effect on Ang II–mediated constriction in vessels of WKY or adult SHR. However, Ang II–stimulated constriction was significantly attenuated in young SHR, suggesting that in the early phase of blood pressure elevation, AT1-sensitive receptors, which are probably AT1 receptors, also play a role in Ang II–mediated vasoconstriction. Selective blockade of the AT1 subtype antagonized the constrictor actions of Ang II in all groups. These data indicate that in normotensive rats and in SHR with established hypertension, Ang II–induced vasoconstriction in small mesenteric arteries is mediated exclusively via AT1 receptors, whereas in young SHR Ang II–stimulated contraction is associated with AT1 and PD123319-sensitive receptors, which may be AT2 receptors. Our findings are in agreement with those of others who recently demonstrated that in young rats, Ang II induces vasoconstriction in renal arteries via AT1 and PD123319-sensitive receptors (J.W. Arendshorst, personal communication, 1998). AT2 receptor–mediated vasoconstriction has been implicated in cerebral arteries and in renal vasoconstriction in hydropnephrotic rat kidneys. Other studies have shown that AT2 receptors mediate 20% of Ang II–induced renal vasoconstriction in SHR during the development of hypertension. Furthermore, AT2 receptors are reexpressed in various pathological states of the vasculature, including hypertension. The exact mechanism or mechanisms underlying AT2-associated vasoconstriction are unclear, but the interplay between AT1 and AT2 receptor stimulation in young SHR could be important. Our data demonstrate that Ang II–induced contraction in young SHR is mediated essentially via AT1 receptors (≈90%), and that AT2 receptors contribute ≈20%. Because the magnitude of the losartan-insensitive contractile component is less than that of the PD123319-sensitive component, it may be plausible that AT2-receptor stimulation could modulate AT1 receptor–mediated vasoconstriction. It is also possible that other Ang II receptor subtypes, which have not yet been characterized, may also be playing a role. Hayashi et al reported that PD123319 in addition to binding to AT1 receptors also binds to AT2, particularly AT2a receptor subtypes. This is probably not the case in the present study, because PD123319 did not alter contraction in young WKY and adult rats but had an effect only on vessels from young SHR, which have increased AT2 mRNA and protein levels. Although our data suggest that AT2 receptors may play a role in vasoconstriction in the development of hypertension, most previous studies, which were conducted mainly in normotensive rats, demonstrated that AT2-receptor stimulation mediates signaling pathways associated with vasodilation and inhibition of cell growth. Recent data, however, implicate that vascular remodeling in hypertension is mediated in part via AT2 receptors. The exact role of the vascular AT2 receptor subtype in the development of hypertension is unclear and awaits further clarification.

To investigate in greater detail the significance of Ang II receptor subtypes in mesenteric arteries from SHR, molecular techniques were used to assess AT1 and AT2 receptor mRNA and protein expression. AT1 receptor mRNA expression was not different between age-matched WKY and SHR but was significantly greater in adult rats than in young rats. Our results are in agreement with other studies that demonstrate that AT1 receptors are the major receptor subtype in adult tissue. AT2 receptor mRNA was weakly expressed in vessels from WKY and adult SHR. However, in arteries from young SHR, AT1 receptor mRNA expression was markedly elevated. These data are supported by results obtained from Western blot analysis, which demonstrate that AT2-receptor protein expression is similar between age-matched rats, whereas AT2-receptor protein expression is increased in young SHR compared with young WKY. AT1-receptor protein was undetectable in arteries from adult rats. Previous studies have reported that AT1 receptors are reexpressed or upregulated in experimental cardiac hypertrophy, myocardial infarction, and neointimal lesions after vascular injury, but to the best of our knowledge, data from the present study are the first to demonstrate differential AT1 receptor mRNA and protein expression in mesenteric arteries from SHR in different phases in the development of hypertension.

In conclusion, the present study demonstrates that young SHR have elevated blood pressure, significant vascular hypertrophy, enhanced Ang II–stimulated responsiveness of small mesenteric arteries, increased vascular expression of AT1 receptor mRNA, and involvement of both AT1 and PD123319-sensitive receptors (probably AT2) in Ang II–mediated contraction. In adult SHR with established hypertension, Ang II–elicited contraction is mediated exclusively via AT1 receptors. Thus, augmented Ang II vascular responsiveness in young SHR may be associated with changes in Ang II receptor status, which could contribute to the development of high blood pressure in this model of genetic hypertension.

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