Original Article

Angiotensin II Type 2 Receptor Inhibits Prorenin Processing in Juxtaglomerular Cells

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Long-term treatment with an angiotensin II type 1 receptor blocker (ARB) has been shown to decrease the plasma renin activity (PRA) of hypertensive patients, whereas PRA remains elevated during angiotensin-converting enzyme inhibitor (ACEI) treatment. In the present study, we used rat juxtaglomerular (JG) cells to elucidate the mechanism(s) involved in the differential regulation of PRA between ARB and ACEI treatment. Addition of 100 nmol/l angiotensinogen (Aogen) to JG cells ($n=6$ primary cultures) significantly increased the medium angiotensin (Ang) II levels from 14 ± 2 to 440 ± 9 pg/ml and suppressed the renin secretion rate (RSR) from $39.6 \pm 5.4\%$ to $6.3 \pm 1.8\%$ without affecting active renin content (ARC) or total renin content (TRC). In the Aogen-treated cells, the ACEI, delapril hydrochloride (CV3317, 10 μ mol/l), significantly decreased the medium Ang II levels to 58 ± 14 pg/ml and increased RSR to $39.8 \pm 4.1\%$ without affecting ARC or TRC. The ARB, an active metabolite of candesartan cilexetil (CV11974, 10 μ mol/l), however, significantly increased the medium Ang II levels and RSR to 486 ± 15 pg/ml and $40.9 \pm 9.8\%$, respectively, and decreased ARC from 63.2 ± 6.8 to 21.6 ± 3.6 ng of Ang I $\cdot$ h $^{-1}$ $\cdot$ million cells $^{-1}$ without affecting TRC. The decreases in ARC of the Aogen + CV11974-treated cells ($n=6$ primary cultures) were inhibited by an Ang II type 2 receptor blocker, PD123319 (10 μ mol/l). JG cells ($n=6$ primary cultures) were also treated with an Ang II type 2 receptor agonist, CGP42212A (0.1 μ mol/l). CGP42212A significantly increased RSR from $38.2 \pm 1.6\%$ to $49.7 \pm 4.7\%$ and decreased ARC from 60.8 ± 3.0 to 25.3 ± 2.8 ng of Ang I $\cdot$ h $^{-1}$ $\cdot$ million cells $^{-1}$ without affecting TRC. Addition of CV11974 did not alter the RSR, ARC, or TRC of the CGP42212A-treated cells; however, PD123319 abolished the effects of CGP42212A. These results indicate that, distinct from ACEIs, ARBs inhibit prorenin processing of JG cells through Ang II type 2 receptors. Long-term treatment with an ARB may decrease PRA in part by diminishing the storage of active renin in JG cells. (*Hypertens Res* 2003; 26: 915–921)

Key Words: renin-angiotensin system, angiotensinogen, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, prorenin

Introduction

Angiotensin II type 1 receptor blockers (ARBs) completely inhibit all physiological effects of angiotensin type 1 (AT1)

receptors, including the negative feedback loop of renin secretion. During ARB treatment, therefore, renin secretion from juxtaglomerular (JG) cells should be increased, and the increased plasma renin levels should lead to elevation of plasma angiotensin (Ang) II levels, resulting in predominant

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stimulation of angiotensin type 2 (AT2) receptors. Because AT2 receptor is reported to antagonize the pressor effects and cell-proliferative actions of AT1 receptor and increase plasma bradykinin levels (1, 2), ARBs would seem to have advantages over angiotensin-converting enzyme inhibitors (ACEIs) in terms of conferring protection from hypertensive organ damage. In addition, ACEIs may have some disadvantages, because a chymase can generate Ang II during ACEI treatment. It is therefore reasonable to expect that the overall clinical benefits of ARBs would be superior to those of ACEIs. However, clinical studies have demonstrated that ARBs and ACEIs are equivalent in protecting systemic organs, including the heart and kidneys, from hypertensive damage (3–8).

In a recent clinical report, long-term treatment with an ARB restored or decreased plasma renin activity of hypertensive patients to the baseline level or lower, although plasma renin activity remained elevated during the ACEI treatment (9), and in another study, the increase in plasma renin activity during long-term treatment with an ARB was smaller than that observed during long-term treatment with an ACEI (10). These novel findings suggest that ARB treatment may have some negative influences on plasma renin control, and thus the stimulation of AT2 receptors during ARB treatment may be less than expected.

The present study was designed to delineate the differential effects of ARB and ACEI on renin synthesis and secretion at the level of JG cells. For this purpose, rat JG cells were incubated with the culture medium containing a recombinant angiotensinogen, and the synthesis and secretion of active renin were evaluated in the presence or absence of an ARB and ACEI. In addition, the agonist and antagonist for AT2 receptors were added to JG cells to determine the contribution of AT2 receptors to the ARB effects on renin regulation.

Methods

Primary Culture of Rat JG Cells

In accordance with the guidelines and practices established by the Keio University Animal Care and Use Committee, JG cells were isolated from kidneys of male Sprague-Dawley rats (100–150 g) as previously described (11–14). JG cells were suspended at 10^6 cells/ml in culture medium consisting of RPMI 1640 with 25 mmol/l *N*-2-hydroxyethylpiperazine-*N*-2-ethane sulfonic acid, 0.3 g/l L-glutamine, 100 µg/ml streptomycin, 100 U/ml penicillin, 0.66 U/l insulin, and 10% fetal bovine serum. Cell number was determined using a Coulter counter. The suspended cells were distributed in 1 ml aliquots into individual wells of 8-well chamber slides containing 1 ml of culture medium and incubated at 37°C. JG cells had a 48-h rest period before the beginning of the experiments. Immunofluorescence staining for renin confirmed that $87 \pm 4\%$ of the cells ($n = 5$ primary cultures) were

positive for renin at 60 h after isolation (11–14).

Immunocytochemical Detection of Renin and AT1/AT2 Receptors by Confocal Laser Microscopy

Primary JG cells grown on LabTek chamber slides (154917; Nalgene Nunc, Tokyo, Japan) were fixed with 4% paraformaldehyde for 20 min and prehybridized with Protein Block Serum-Free (Dako, Carpinteria, USA). Cells were then incubated overnight at room temperature with mouse monoclonal anti-renin antibody against rat renin antibody (Swant, Bellinzona, Switzerland) at a dilution of 1:50, followed by 1 h-incubation with FITC-conjugated goat polyclonal anti-mouse IgG (1:500; American Qualex, San Clemente, USA) at room temperature. Cells were then incubated overnight at room temperature with the rabbit anti-AT1 receptor antibody (Santa Cruz Biotech, Santa Cruz, USA) at a dilution of 1:200, followed by 1 h-incubation with AlexaFluor 647 goat anti-rabbit IgG (1:500; Molecular Probes, Eugene, USA) at room temperature. To detect AT2 receptor, goat polyclonal anti-AT2 receptor antibody (Santa Cruz Biotech) was used at a dilution of 1:200 as a primary antibody, and visualized with FITC-conjugated rabbit polyclonal anti-goat IgG (Sigma, St. Louis, USA) at a dilution of 1:500. The cells were mounted in Vectashield (Vector Laboratories, Burlingame, USA) containing PI (Sigma) for nuclear staining. After isolating the secondary antibody by immunoaffinity chromatography, a sample from which the primary antibodies had been omitted was used as a negative control. For confocal imaging, a Bio-Rad Radiance 2000 laser scanning microscope (Bio-Rad, Tokyo, Japan) was used at constant system setting, and digitally captured images were deconvolved with LaserSharp2000 (Bio-Rad) imaging software.

Measurement of Renin Secretion Rate, Active Renin Content, and Total Renin Content in JG Cells

The renin secretion rate (RSR), active renin content (ARC), and total (active + inactive) renin content (TRC) were measured in JG cells as described previously (13, 14). In brief, after the culture medium had been removed, the cells were washed twice with pre-warmed phosphate buffered saline (PBS). Each well was filled with 1 ml of Ca^{2+} -containing PBS. Immediately before (0 h) and at 12 h of culture, the cell-conditioned buffer was removed and centrifuged. The supernatants were stored at -20°C until renin activity was assayed for determination of RSR. After rinsing with PBS, the cells were frozen in liquid nitrogen and stored at -80°C . For assay of ARC and TRC, frozen cells were homogenized in 1 ml of buffer (pH 6.0) containing 2.6 mmol/l ethylenediaminetetraacetate, 1.6 mmol/l dimercaprol, 3.4 mmol/l 8-hydroxyquinoline sulfate, 0.2 mmol/l phenylmethylsulfonyl fluoride, and 5 mmol/l ammonium acetate. The homogenates were centrifuged at $12,000 \times g$ for 30 min, and the supernatant was removed. To measure TRC in JG cells, the sam-

Fig. 1. Immunocytochemical detection of renin and AT₁ receptors by confocal laser microscopy. In the cells containing a nucleus (shown in red) and renin granules (shown in green), AT₁ receptors (shown in purple) were present both at the cell surface and in the cytoplasm (A). AT₂ receptors (shown in blue) were also observed both at the cell surface and in the cytoplasm (B). The negative control without primary antibodies did not have any non-specific signals (C).

ples (900 μ l) were incubated at 0°C for 60 min with 100 μ l of 4 mg/ml trypsin (Sigma) in 500 mmol/l Tris buffer, pH 7.5, containing 5 mmol/l CaCl₂, 0.1% NaCl azide, and 1% bovine serum albumin, as described previously (14). Soybean trypsin inhibitor (Sigma; 8 mg/ml final concentration) was added to stop the reaction. Thus, inactive renin in the samples was converted to active renin, and renin activity was then determined.

Renin activity was determined as previously described (12). Samples were incubated for 1 h at 37°C with plasma from bilaterally nephrectomized male Sprague-Dawley rats as the renin substrate, and renin activity was determined by the generation of Ang I from a plasma angiotensinogen substrate. Ang I levels were measured using a radioimmunoassay coated-bead kit from Dinabott Radioisotope Institute. RSR (%) was calculated as the fractional release of overall active renin (*i. e.*, [buffer renin activity at 12 h - buffer renin activity at 0 h]/[active renin content in JG cells at 12 h + buffer renin activity at 12 h - buffer renin activity at 0 h]). JG cell ARC and TRC were expressed as the renin activity of the sample obtained per million cells.

Experimental Protocols

Primary JG cell cultures were divided into four groups, with

each group including at least 2 wells, and the mean values per primary culture were determined in each group. In the first series of experiments, we assessed the effects of recombinant sheep angiotensinogen (Aogen, 100 nmol/l) (15) on renin synthesis and secretion in JG cells. The cells were conditioned in control buffer or buffer containing CV3317 (delapril hydrochloride, 10 μ mol/l) as an ACEI and CV11974 (an active metabolite of candesartan cilexetil, 10 μ mol/l) as an ARB. The amounts of Ang II generated from Aogen in the culture media with or without Aogen, Aogen + ACEI, or Aogen + ARB were determined by radioimmunoassay. In the second series of experiments, which examined the involvement of AT₁ and AT₂ receptors in the effects of Aogen on renin regulation, the cells were conditioned in buffer containing the AT₁ receptor blocker, CV11974 (10 μ mol/l), the AT₂ receptor blocker, PD123319 (10 μ mol/l; Sigma), or both. The effects of the AT₂ receptor agonist, CGP42212A (0.1 μ mol/l, Sigma) on renin regulation were examined in the third series of experiments. The cells were conditioned in control buffer or buffer containing CV11974 (10 μ mol/l) and PD123319 (10 μ mol/l). Aogen was a gift from Dr. Fumiaki Suzuki (Gifu University, Gifu, Japan). CV3317 and CV11974 were provided by Takeda Chemical Industries (Osaka, Japan).

Statistical Analysis

Data were analyzed by two-way ANOVA followed by multiple comparisons using Scheffé's *F*-test for repeated measures. A value of $p < 0.05$ was considered statistically significant. Data are shown as the means $\pm$ SEM.

Results

AT₁ and AT₂ Receptors of JG Cells

Figure 1 shows the immunocytochemical colocalization of renin and AT receptors. In JG cells containing renin granules, AT₁ receptor signals were observed not only on the cell surface but also in the cytoplasm, and these signals were eradicated by preabsorbing the antibodies with the competing peptide (Santa Cruz Biotech) (data not shown). AT₂ receptor signals were also observed both on the cell surface and in the cytoplasm, but AT₂ receptors were observed separately from renin granules because of their lower expression level. No competing peptide against the AT₂ receptor antibody used in the present study is currently available. In the absence of primary antibodies, however, no signals other than those in the nucleus were observed. These results were consistent with previous studies demonstrating an immunohistochemical localization of AT₁ receptor (16–19) or AT₂ receptor (19) in the JG apparatus and a biochemical localization of AT₂ receptor in afferent arterioles (20) which transform metaplastically into JG cells (21, 22).

Table 1. Angiotensin II Concentrations in Culture Medium

	Treatments			
	Control	Aogen	Aogen + ACEI	Aogen + ARB
Angiotensin II (pg/ml)	14 ± 2	440 ± 9*	58 ± 14*†	486 ± 15*†

Data are the means ± SEM. Aogen, angiotensinogen (100 nmol/l); ACEI, CV3317 (10 μmol/l); ARB, CV11974 (10 μmol/l). The experiment was performed with 6 primary cultures. **p* < 0.05 vs. control. †*p* < 0.05 vs. Aogen.

Fig. 2. Effects of 100 nmol/l recombinant sheep angiotensinogen (Aogen) on renin secretion rate (RSR), active renin content (ARC), and total renin content (TRC) in juxtaglomerular cells incubated with control buffer or buffer containing ACEI (CV3317, 10 μmol/l) and angiotensin II type 1 receptor blocker (ARB; CV11974, 10 μmol/l). The experiment was performed with 6 primary cultures. **p* < 0.05 vs. control. †*p* < 0.05 vs. Aogen alone.

Effects of Angiotensinogen on JG Cells Treated with ACEI and ARB

Table 1 shows Ang II concentrations in the culture medium of untreated JG cells and the cells treated with Aogen alone, Aogen + CV3317, or Aogen + CV11974. In the Aogen-treated JG cells, the medium Ang II levels significantly increased compared to those in untreated JG cells, and the increases were attenuated by addition of an ACEI, CV3317, and enhanced by addition of an ARB, CV11974.

Figure 2 demonstrates the effects of Aogen on the RSR,

ARC, and TRC of the JG cells incubated with control buffer or buffer containing an ACEI, CV3317, and an ARB, CV11974. The RSR, ARC, and TRC of JG cells treated with control buffer averaged 39.6 ± 5.4%, 63.2 ± 6.8 ng of Ang I·h⁻¹·million cells⁻¹, and 98.0 ± 13.5 ng of Ang I·h⁻¹·million cells⁻¹, respectively. Addition of Aogen to the culture medium significantly decreased RSR to 6.3 ± 1.8% but did not alter ARC or TRC. In the presence of CV3317, Aogen did not influence RSR, ARC, or TRC of JG cells. The RSR of JG cells treated with Aogen + ACEI (CV3317) averaged 39.8 ± 4.1% and was similar to that of JG cells treated with control buffer. In the presence of CV11974, however, Aogen significantly reduced ARC to 21.6 ± 3.6 ng of Ang I·h⁻¹·million cells⁻¹ without affecting RSR or TRC. The RSR of JG cells treated with Aogen + ARB (CV11974) averaged 40.9 ± 9.8% and was similar to that of JG cells treated with control buffer.

Functions of AT1 and AT2 Receptors in JG Cells

Figure 3 demonstrates the effects of the AT1 receptor blocker CV11974, the AT2 receptor blocker PD123319, or both on RSR, ARC, and TRC in the Aogen-treated JG cells. The RSR, ARC, and TRC of the Aogen-treated JG cells averaged 9.0 ± 1.2%, 58.5 ± 4.5 ng of Ang I·h⁻¹·million cells⁻¹, and 102.5 ± 6.4 ng of Ang I·h⁻¹·million cells⁻¹, respectively. Addition of CV11974 to the culture medium of the Aogen-treated JG cells significantly increased RSR to 50.5 ± 5.5% and significantly decreased ARC to 20.2 ± 2.7 ng of Ang I·h⁻¹·million cells⁻¹ without affecting TRC. Addition of PD123319 did not significantly influence the RSR, ARC, or TRC of the Aogen-treated JG cells. In the Aogen-treated JG cells, however, treatment with both CV11974 and PD123319 significantly increased RSR to 39.7 ± 2.1% and did not influence ARC or TRC. The RSR of JG cells treated with Aogen + CV11974 + PD123319 was significantly greater than that of the Aogen-treated cells or Aogen + PD123319-treated cells and similar to that of the Aogen + CV11974-treated cells. The ARC of JG cells treated with Aogen + CV11974 + PD123319 was significantly greater than that of the Aogen + CV11974-treated cells and similar to that of the Aogen-treated cells or the Aogen + PD123319-treated cells.

Figure 4 shows the effects of the AT2 receptor agonist CGP42212A on RSR, ARC, and TRC of JG cells incubated with control buffer or buffer containing the AT1 receptor blocker CV11974 and the AT2 receptor blocker PD123319.

Fig. 3. Effects of angiotensin II type 1 receptor blocker (CV11974, 10 μ mol/l), angiotensin II type 2 receptor blocker (PD123319, 10 μ mol/l), and both on renin secretion rate (RSR), active renin content (ARC), and total renin content (TRC) in juxtaglomerular cells incubated with buffer containing 100 nmol/l recombinant sheep angiotensinogen (Aogen). The experiment was performed with 6 primary cultures. * $p < 0.05$ vs. Aogen alone. † $p < 0.05$ for Aogen + CV11974 + PD123319 vs. Aogen + CV11974. § $p < 0.05$ for Aogen + ARB + PD123319 vs. Aogen + PD123319.

Treatment with CGP42212A significantly increased RSR from $38.2 \pm 1.6\%$ to $49.7 \pm 4.7\%$ and significantly decreased ARC from 60.8 ± 3.0 to 25.3 ± 2.8 ng of Ang I·h⁻¹·million cells⁻¹ without affecting TRC (97.7 ± 2.8 and 93.2 ± 3.6 ng of Ang I·h⁻¹·million cells⁻¹ for control and CGP42212A, respectively). In the presence of CV11974, CGP42212A also increased RSR to $55.0 \pm 4.6\%$, decreased ARC to 24.3 ± 1.6 ng of Ang I·h⁻¹·million cells⁻¹, and did not influence TRC in JG cells. These changes were similar to those observed in the JG cells treated with CGP42212A alone. In the presence of PD123319, however, CGP42212A did not influence RSR, ARC, or TRC of JG cells. The RSR of JG cells treated with CGP42212A + PD123319, averaging $36.3 \pm 3.6\%$, was similar to that of JG cells treated with control buffer and significantly lower than the RSR of JG cells treated with CGP42212A alone or CGP42212A + CV11974. The ARC of JG cells treated with CGP42212A + PD123319, averaging 59.0 ± 4.1 ng of Ang I·h⁻¹·million cells⁻¹, was similar to that of the control buffer-treated JG cells and significantly greater than that of JG cells treated with CGP42212A alone or CGP42212A + CV11974.

Fig. 4. Effects of angiotensin II type 2 receptor agonist (CGP42212A, 0.1 μ mol/l) on renin secretion rate (RSR), active renin content (ARC), and total renin content (TRC) in juxtaglomerular cells incubated with control buffer or buffer containing angiotensin II type 1 receptor blocker (CV11974, 10 μ mol/l) and angiotensin II type 2 receptor blocker (PD123319, 10 μ mol/l). The experiment was performed with 6 primary cultures. * $p < 0.05$ vs. control. † $p < 0.05$ for CGP42212A + PD123319 vs. CGP42212A alone.

Discussion

Addition of Aogen to culture medium significantly increased the medium Ang II concentration and significantly decreased RSR, suggesting that the Ang II generated from Aogen in the culture medium can inhibit renin secretion from JG cells. In addition, neither ARC nor TRC increased, despite the great decrease in RSR. It is therefore possible that the Ang II generated from Aogen also suppresses renin synthesis in JG cells. The ACEI, CV3317, significantly attenuated the conversion of Aogen to Ang II and inhibited the Aogen-induced decrease in RSR. The ARB, CV11974, also suppressed the Aogen-induced decrease in RSR, although the medium Ang II levels increased during ARB treatment. These results suggest that Ang II can inhibit renin secretion from JG cells through AT₁ receptors, consistent with previous studies (11, 23).

Although suppression of the overall Ang II effects by ACEI treatment did not influence ARC or TRC of the Aogen-treated JG cells, the selective blockade of AT₁ receptor with CV11974 significantly decreased the ARC of the Aogen-treated JG cells concomitant with a significant increase in the medium Ang II levels, suggesting that stimulation of

AT2 receptors may play an important role in this mechanism. Direct stimulation of AT2 receptors by CGP42212A also decreased the ARC of JG cells. Because selective blockade of AT2 receptors with PD123319 inhibited the decreases in ARC by either Aogen + CV11974 or CGP42212A, AT2 receptors appear to have been responsible for the ARB-induced decrease in ARC of the Aogen-treated JG cells. In addition, none of the treatments used in the present study induced any change in the TRC of JG cells. Based on the assumption that there is no intracellular degradation or inactivation of active renin after it is formed from the conversion of prorenin, a decrease in ARC without a change in TRC suggests an inhibition of prorenin processing—that is, a conversion of inactive renin to active renin. Because intracellular active renin is extremely stable in secretory granules, AT2 receptors can have inhibitory effects on prorenin processing in JG cells. Therefore, in the presence of Aogen (or Ang II), ARB can suppress prorenin processing in JG cells through AT2 receptors. Although short-term treatment with an ARB increases plasma renin activity by stimulating renin secretion from JG cells *in vivo*, long-term treatment with ARB may decrease plasma renin activity by diminishing the stores of active renin in JG cells.

The stimulation of AT2 receptors by Aogen + CV11974 or CGP42212A significantly decreased ARC. Nevertheless, the blockade of AT2 receptors with PD123319 did not influence the ARC of JG cells treated with Aogen. These results suggest that the overall effects of Ang II generated from Aogen are dependent on AT1 receptors. Previous studies have reported that the affinity for Ang II and the number of AT1 receptors are greater than those of AT2 receptors under physiological conditions (18, 20). In the present study, confocal laser microscopy demonstrated abundant AT1 receptors and a low level of AT2 receptors in the JG cells. Therefore, the actions of AT2 receptors may be negligible during a simultaneous stimulation of AT1 receptors, and may be effective only when AT2 receptors are selectively stimulated.

Treatment with CGP42212A increased RSR with a significant decrease in the ARC of JG cells. In the JG cells treated with CGP42212A, however, the absolute value of the renin secreted to the culture medium averaged 25.6 ± 3.9 ng of Ang I·h⁻¹·ml⁻¹ and was significantly lower than that in control JG cells, averaging 38.2 ± 3.6 ng of Ang I·h⁻¹·ml⁻¹. These results suggest that CGP42212A significantly decreased ARC and thereby reduced the content of the renin secreted to the medium. Because of the great decrease in ARC, RSR increased only slightly despite a significant decrease in the absolute value of the renin secreted to the medium.

The culture medium concentrations of Ang II were significantly higher in JG cells treated with Aogen + CV11974 than in the cells treated with Aogen alone. Because Ang II generated from Aogen can be internalized to JG cells through Ang II receptors or degraded to Ang III in the culture medium, several possible mechanisms may be considered. First,

CV11974 may enhance the processing from Aogen to Ang II. The rate-limiting enzyme of this processing is renin, and its concentrations in the culture medium were elevated by the increase in renin secretion induced by CV11974. Thus, treatment with CV11974 may increase the generation of Ang II by elevating the active renin concentration in the culture medium. Secondly, blockade of Ang II binding to AT1 receptors with CV11974 may inhibit the internalization of Ang II into cells, and thereby increase the medium Ang II concentration. Thirdly, CV11974 may inhibit the degradation of Ang II pharmacologically. In any case, the increased concentrations of medium Ang II contributed to the stimulation of AT2 receptors during treatment with Aogen + CV11974.

Treatment with CV3317 significantly decreased the medium Ang II concentration of JG cells treated with Aogen; however, the medium Ang II concentration of JG cells treated with Aogen + CV3317 was still higher than that of JG cells treated with control buffer alone. Nevertheless, CV3317 completely inhibited the Aogen-induced decrease in RSR. There may be a threshold concentration below which Ang II does not further inhibit renin secretion from JG cells. Alternatively, CV3317 itself might have a stimulating effect on renin secretion. However, our preliminary study demonstrated that the RSR of JG cells treated with CV3317 alone was similar to the RSR of untreated JG cells.

In conclusion, an ACEI significantly decreased the medium Ang II levels and increased RSR without affecting ARC or TRC in Aogen-treated cells in the present study. However, ARB significantly increased the medium Ang II levels and RSR and decreased ARC without affecting TRC. The decreases in ARC of the Aogen + ARB-treated cells were inhibited by the blockade of AT2 receptors. In addition, the stimulation of AT2 receptors significantly decreased ARC without affecting TRC. This decrease in ARC was inhibited by the AT2 receptor blocker but not by the AT1 receptor blocker. Therefore, unlike the ACEI, the ARB inhibited prorenin processing of JG cells through AT2 receptors.

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