Inflammation influences vascular remodeling through AT$_2$ receptor expression and signaling

MASAHIRO AKISHITA, MASATSUGU HORIUCHI, HIROYUKI YAMADA, LUNAN ZHANG, GOTARO SHIRAKAMI, KOICHI TAMURA, YASUYOSHI OUCHI, AND VICTOR J. DZAU. Inflammation influences vascular remodeling through AT$_2$ receptor expression and signaling. Physiol. Genomics 2: 13–20, 2000.—The AT$_2$ receptor, which exerts growth inhibitory effects in cell culture, is present scantily in the adult vasculature but is reexpressed after vascular injury. To examine the in vivo role of this receptor in vascular diseases, we developed a mouse model of vascular remodeling and compared the responses in wild-type (Agtr2$^+$) and AT$_2$ receptor knockout (Agtr2$^-$) mice. Polyethylene cuff placement on the femoral artery led to the vascular expression of cytokines, the transcriptional factor interferon regulatory factor-1 (IRF-1), and both the AT$_1$ and AT$_2$ receptors. Although the expressions of IRF-1 and AT$_1$ receptor were induced to comparable levels in both the Agtr2$^+$ and Agtr2$^-$ mice, the neointimal lesion size and the smooth muscle cell proliferation were twice greater in the Agtr2$^+$ than in the Agtr2$^-$ mouse. Correlated with this difference, AT$_2$ receptor expression was induced predominantly in the smooth muscle cells of Agtr2$^+$ mouse. These results demonstrate that the AT$_2$ receptor plays an important role in nonocclusive inflammatory injury by mediating the effects of inflammation on vascular smooth muscle growth inhibition.

angiotensin; smooth muscle; artery; proliferation; apoptosis

The peptide angiotensin II (ANG II) exerts hemodynamic, renal, and electrolyte effects, as well as effects on the structure of the heart and vasculature. Many, if not all, of the actions of ANG II are mediated by the well-characterized type 1 (AT$_1$) receptor, whereas the functions of the recently cloned type 2 (AT$_2$) receptor (19, 25) are still unclear. It has been demonstrated that the levels of the AT$_2$ receptor in the aorta are very low (or undetectable) during early embryonic development but are very high during the later stages of development and in the neonate (34, 41). Following birth, the level of this receptor declines rapidly. In certain pathological conditions such as vascular injury, the AT$_2$ receptor is reexpressed (26).

In vitro studies have shown that the AT$_2$ receptor can mediate growth inhibition in vascular smooth muscle cells (VSMCs) (26), coronary endothelial cells (36), PC12W cells (a rat pheochromocytoma cell line) (24), and cardiomyocytes (2). Also, the AT$_2$ receptor can stimulate differentiation in neuronal cells (22, 24) and/or apoptosis in PC12W cells (13, 44), R3T3 mouse fibroblasts (15), and endothelial cells (3). However, it is critical to point out that the in vivo relevance of these effects has not been proven. Using in vivo gene transfer, we have demonstrated that overexpression of the AT$_2$ receptor transgene inhibited the subsequent development of the neointima, at least in part, by a reduction in DNA synthesis of the medial cells (26). However, the use of gene transfer does not fully address the endogenous pathophysiological function of this receptor. Moreover, we have demonstrated that AT$_2$ receptor expression in vitro can be regulated by interferon regulatory factors (IRF) (14, 15), suggesting that the inflammatory response in vascular disease may regulate AT$_2$ receptor expression. Taken together, these results suggest that upregulation of AT$_2$ receptor in diseased vessel may play an important role in the pathogenesis of vascular remodeling.

In this study, we hypothesize that the inflammatory response to vascular pathological stimuli results in the release of cytokines [such as interferon-γ (IFN-γ)], which induces the expression of IRF-1 in cytokine-responsive cells. IRF-1 subsequently stimulates AT$_2$ receptor expression that modulates VSMC proliferation and vascular remodeling.

We have generated an AT$_2$ receptor knockout mouse strain (11) that provides us with a unique opportunity to address the pathophysiological role of the AT$_2$ receptor in vascular inflammation and remodeling. This mouse strain develops normally but shows an impaired locomotor activity as well as an increased vasopressor response to ANG II (11). These phenotypes were confirmed by another AT$_2$ receptor null strain (17). To define the in vivo role of the endogenous AT$_2$ receptor in vascular remodeling, we developed a model of vascular disease induced by polyethylene cuff placement around the mouse femoral artery. Supporting our hypothesis, the results showed that the neointima lesion was significantly greater in the AT$_2$ receptor knockout (Agtr2$^-$) than the wild-type (Agtr2$^+$) mice.

METHODS

Animals. Adult male Agtr2$^+$ and Agtr2$^-$ mice were used in this study. Since the AT$_2$ receptor gene is located on the X chromosome, heterozygous females were mated with Agtr2$^+$ males to obtain hemizygous and Agtr2$^+$ males. Animal genotyping was performed as previously described (11). In the comparison studies between Agtr2$^+$ and Agtr2$^-$ mice, pairs of Agtr2$^+$ and Agtr2$^-$ littermates were used. These mice, backcrossed for five generations into the FVB/N background, had 97% FVB/N and 3% 129/SV background on average. All mice

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used in this study were 10–12 wk of age and weighed 25–30 g. The mice were housed in a room where lighting was controlled (12 h lights on, 12 h lights off) and room temperature was kept at 22°C. They were given standard diet and water ad libitum. All experimental procedures were approved and carried out in accordance with the guidelines of the Harvard Medical Area Standing Committee on Animals.

Cuff placement. The surgical procedures of cuff placement was performed according to the method described previously (1, 12) with some modification. The mice were anesthetized with ketamine (70 mg/kg) and xylazine (4 mg/kg) by intraperitoneal injection. The left femoral artery of the mouse was isolated from the surrounding tissues. A polyethylene tube (2-mm long PE-90; inner diameter, 0.86 mm; outer diameter, 1.27 mm; Becton-Dickinson) was cut longitudinally to open the tube, loosely placed around the artery and closed with suture. After the experimental period, the mice were killed by an overdose of the anesthesia and perfused with PBS via a catheter placed in the thoracic aorta. Subsequently, the artery was perfusion-fixed at 100 mmHg with 10% neutral buffered Formalin. The cuffed artery was removed and microdissected from the cuff. The arterial tissue was then postfixed in 10% neutral buffered Formalin overnight, dehydrated, and embedded in paraffin. For immunohistochemistry, the artery was perfusion-fixed and immersion-fixed in 4% paraformaldehyde in PBS, cryoprotected in 20% sucrose in PBS, embedded in Tissue-Tek OCT compound (Sakura Finetek, Torrance, CA), and frozen in liquid nitrogen. For RNA and DNA preparation, the tissues were dissected after PBS perfusion, immediately frozen in liquid nitrogen and stored at −80°C.

Some Agtr2−/− mice were treated with the AT2 receptor antagonist, PD-123319 (Research Biochemicals International, Natick, MA). Alzet micro-osmotic pumps (model 1002; Table 1. Primer sequences for RT-PCR

<table>
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<tr>
<th>cDNA and Primer</th>
<th>Sequence of Primer</th>
<th>Product Size</th>
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<td>32</td>
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<tr>
<td>Reverse</td>
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AT1 and AT2, angiotensin receptors; IRF-1, interferon regulatory factor-1; IFN-γ, interferon-γ; IL-1β and IL-6, interleukins; TNF-α, tumor necrosis factor-α; and GAPDH, glyceraldehyde phosphohydrogenase.

Fig. 1. RT-PCR analysis for interferon-γ (IFN-γ), interleukins IL-1β and IL-6, and tumor necrosis factor-α (TNF-α) mRNA (A) and for AT1 receptor, AT2 receptor, and interferon regulatory factor-1 (IRF-1) mRNA (B) in the cuffed mouse femoral artery. Polyethylene cuff was placed around the left femoral artery of the wild-type (Agtr2+/+) mouse. Cuffed arteries at the indicated days and control intact arteries were harvested and frozen. Pooled samples (n = 5–7 for each time point) were used for RNA preparation. RT-PCR products were electrophoresed on agarose gel and visualized by ethidium bromide staining. Glyceraldehyde phosphohydrogenase (GAPDH) served as the internal control. One representative result of four independent RT-PCR (each two from different pools of tissue samples) is shown.
Alza, Palo Alto, CA) were implanted intraperitoneally at the time of cuff placement surgery. The pumps delivered vehicle (PBS) or PD-123319 (20 mg/kg per day) (26) continuously for 14 days at a rate of 0.25 µl/h.

Hemodynamics measurement. In some Agtr2+ and Agtr2− mice, hemodynamic changes after cuff placement were monitored. A polyethylene catheter (PE-10; Becton-Dickinson) was inserted into the right femoral artery, tunneled under the skin, and exteriorized at the back of the neck. The mice were allowed to recover overnight, then arterial pressure and heart rate were recorded over 60 min in a conscious and unrestrained condition through the catheter connected to a Statham pressure transducer.

Morphometric analysis. The middle segment of the artery was cut into three subserial cross sections with 5 µm thickness at an interval of 0.3 mm. The sections were stained by Elastica van Gieson staining and photographed together with an image analyzing software (NIH image) by an observer blinded as to the animal genotype or the treatment. Neointima was defined as the area between the vessel lumen and the internal elastic lamina. Media was defined as the area between the internal and external elastic lamina. Adventitia was defined as the area outside the external elastic lamina. The average of three sections was taken as the value for each animal.

Measurement of DNA synthesis. Bromodeoxyuridine (BrdU; Sigma) was injected 100 mg/kg sc and 30 mg/kg ip at 18 h prior to death and then 30 mg/kg ip at 12 h prior to death (26). Section preparation was done in the same manner as morphometric analysis. Immunohistochemistry using anti-BrdU antibody was performed according to the manufacturer’s protocol (BrdU Staining Kit; Zymed Laboratories, South San Francisco, CA). The sections were counterstained with hematoxylin, and the BrdU index, the ratio of BrdU-positive nuclei vs. total nuclei, was calculated. The numbers of the nuclei were counted by an observer blinded as to the animal genotype. The average index of three sections was taken as the value for each animal.

Immunohistochemistry. Frozen sections (5 µm thick) were immunohistochemically stained by streptavidin-biotin-peroxidase method using a kit (Histostain-SP Kit; Zymed Laboratories). Briefly, endogenous peroxidase and the nonspecific binding of the antibody were blocked with 3% hydrogen peroxide in methanol and 10% FBS in PBS, respectively. Primary antibody diluted in 10% FBS in PBS was applied to the sections and incubated for 16–24 h at 4°C. Subsequently, biotinylated secondary antibody and then streptavidin-peroxidase conjugate were applied. Positive staining was visualized using diaminobenzidine. VSMCs and leukocytes were stained using anti-α-smooth muscle actin antibody (clone 1A4; Sigma; diluted 1:400) and anti-CD45/common leukocyte antigen antibody (clone 30F11.1, diluted 1:100; PharMingen, San Diego, CA), respectively. The AT2 receptor was stained using the polyclonal antibody raised against a synthetic amino-terminal peptide (diluted 1:250; gift from Dr. Robert M. Carey, University of Virginia Health Sciences Center). The characteristics of this antibody have been reported previously (28).

RT-PCR. RNA was prepared from the pooled samples (n = 5–7 for each group) using TRIzol reagent (GIBCO-BRL). First-strand cDNA was synthesized using reverse transcriptase with random hexamers from 1 µg total RNA in 20 µl reaction volume according to the manufacturer’s protocol (GeneAmp RNA PCR Kit; Perkin-Elmer), then one-tenth of the resulting reverse transcription (RT) product was applied to each 25 µl PCR. The PCR reactions contained 1.5 mM Mg2+, 0.2 mM primers, 0.2 mM dNTPs, and 2.5 U/100 µl Taq DNA polymerase (AmpliTaq DNA polymerase; Perkin-Elmer). PCR primers used for IFN-γ, interleukin-1β (IL-1β), IL-6, tumor necrosis factor-α (TNF-α), IRF-1, AT1 receptor, AT2 receptor, and IRF-1 mRNA (A) and immunohistochemistry for the localization of AT2 receptor (B–D). Cuffed arteries at 7 days (7d) in wild-type mice were harvested and micro-separated into two layers, media/intima layers (lane 3) and adventitial layers (lane 4). These tissues as well as the intact arteries (lane 1) and the whole cuffed arteries at 7 days (lane 2) were pooled (n = 7) and used for RT-PCR. Numbers in the cartoon (A, right) correspond to lanes 1–4. One representative result of two independent RT-PCR is shown. Frozen sections prepared from cuffed arteries at 7 days in wild-type mice were stained with antibody to AT2 receptor (B), anti-α-smooth muscle (SM) actin antibody (C), or anti-CD45/common leukocyte antigen antibody (D). Sections were counterstained with hematoxylin. Magnification ×400; bar = 20 µm.

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glyceraldehyde phosphodehydrogenase (GAPDH) are listed in Table 1. PCRs except for GAPDH were carried out with 30 cycles of 1 min of denaturation at 94°C, 1 min of annealing at 55°C, and 2 min of extension at 72°C, followed by 10 min of final extension step. PCRs for GAPDH were carried out with 25 cycles. PCR products were separated by 1.5% agarose gel electrophoresis and were detected as a single band of the expected size in each PCR shown in the Figs. 1–3. The results shown in Figs. 1A, 1B, and 3 are representative of two independent RT-PCR reactions using the same RNA pool and two RT-PCR reactions using a second pool of RNA. The results shown in Fig. 2 are representative of two independent RT-PCR reactions using the same RNA pool.

Data analysis. The values in the text and Figs. 4 and 5 are expressed as means ± SE. The data were analyzed using one-factor ANOVA. If a statistically significant effect was found, then the Newman–Keuls test was performed to isolate the difference between the groups. \( P < 0.05 \) was considered to be statistically significant.

RESULTS

We first examined the mRNA expression of inflammatory cytokines, IFN-γ, IL-1β, IL-6, and TNF-α in the cuffed artery of the Agtr2\(^+\) mouse by RT-PCR. As shown in Fig. 1A, the expression of these cytokines was scarce or undetectable in the control intact artery, whereas the expression was induced markedly by cuff placement. The peak response was observed at 5–7 days after cuff placement. Next, we examined AT\(_1\) and AT\(_2\) receptor mRNA expression in the injured artery of the Agtr2\(^+\) mouse. As shown in Fig. 1B, AT\(_1\) receptor mRNA was upregulated in response to cuff placement. Also, AT\(_2\) receptor mRNA was stimulated markedly from the baseline undetectable level. The peak effect was observed at 5 days after cuff placement, then the expression declined gradually. Since we demonstrated that the cytokine-induced transcription factor IRF-1 transactivated AT\(_2\) receptor in B3T3 cells (14, 15), we examined IRF-1 expression in the blood vessel. Upregulation of IRF-1 coincided with AT\(_2\) receptor expression, suggesting the involvement of IRF-1 in AT\(_2\) receptor induction in this vascular disease model.

To localize the expression of AT\(_1\) receptor, AT\(_2\) receptor, and IRF-1, cuffed arteries at 7 days in the Agtr2\(^+\) mice were harvested and micro-dissected into two regions: the media/intima layers and the adventitial layer as shown in Fig. 2A. RT-PCR showed that AT\(_1\) and AT\(_2\) receptors were predominantly expressed in the media/intima layers, whereas IRF-1 was expressed in both regions. Immunohistochemistry using the antibody to AT\(_2\) receptor (Fig. 2, B–D) also demonstrated that AT\(_2\) receptor expression was mainly observed in the media and neointima and associated with α-smooth muscle actin but not with CD45, indicating that AT\(_2\) receptor was localized to VSMC, although possible endothelial expression of AT\(_2\) receptor could not be denied. No significant staining for AT\(_2\) receptor was observed in the intact artery (data not shown).

Based on these data, we hypothesized that the absence of AT\(_2\) receptor expression in response to inflammation in the Agtr2\(^-\) mouse augmented neointima formation. We confirmed that AT\(_2\) receptor mRNA was not expressed in the Agtr2\(^-\) mice, whereas AT\(_1\) receptor mRNA was comparably expressed in the Agtr2\(^+\) and Agtr2\(^-\) mice at 7 and 14 days after cuff placement (Fig. 3). Figure 4A shows the representative cross sections of the cuffed femoral artery in the Agtr2\(^+\) and Agtr2\(^-\) mice at 7, 14, and 28 days after cuff placement. Concentric neointima was formed and developed up to 28 days. Both the media and the neointima were uniformly stained with anti-α-smooth muscle actin antibody, whereas the adventitia was not (Fig. 2C), indicating that the media and the neointima consisted primarily of VSMCs. We detected numerous CD45-positive cells in the adventitia at 1–7 days (Fig. 2D), documenting leukocyte infiltration. Neointima formation was exaggerated in the Agtr2\(^-\) mouse compared with that in the Agtr2\(^+\) mouse, whereas the medial area did not change throughout the experimental period (Fig. 4B). Accordingly, there was no significant difference in the medial area between the Agtr2\(^+\) and Agtr2\(^-\) mice. On the other hand, the neointimal area was 170–200% greater in the Agtr2\(^-\) mouse than in the Agtr2\(^+\) mouse at 7, 14, and 28 days after cuff placement. The adventitial area did not differ between the Agtr2\(^+\) and Agtr2\(^-\) mice (data not shown).

![Fig. 3. RT-PCR analysis for AT\(_1\) receptor and AT\(_2\) receptor mRNA in the cuffed mouse femoral artery of the wild-type (Agtr2\(^+\), WT) and AT\(_2\) receptor null (Agtr2\(^-\), KO) mice. Cuffed arteries at the indicated days and control intact arteries were harvested and frozen. Pooled samples (\( n = 5–7 \) for each time point) were used for RNA preparation. RT-PCR products were electrophoresed on agarose gel and visualized by ethidium bromide staining. GAPDH served as the internal control. One representative result of four independent RT-PCR (each two from different pools of tissue samples) is shown.](http://physiolgenomics.physiology.org/Downloaded_from/http://physiolgenomics.physiology.org)
To evaluate the effect of AT$_2$ receptor expression on vascular proliferation, we counted BrdU-positive cells and hematoxylin-positive cells. As shown in Fig. 4C, the BrdU index in the media was higher in the Agtr2$^{-}$ mouse than in the Agtr2$^{+}$ mouse at 7 days, whereas no difference was observed at 14 days or 28 days. The BrdU index in the intima was also higher in the Agtr2$^{-}$ mouse than in the Agtr2$^{+}$ mouse at 7 days and at 14 days, but no difference existed at 28 days after cuff placement.

We measured blood pressure and heart rate in the Agtr2$^{+}$ and Agtr2$^{-}$ mice before and after cuff placement, since hemodynamic changes may influence neointima formation and the vasopressor response to ANG II is reported to be exaggerated in the Agtr2$^{-}$ mouse (11, 17). The intact mice showed comparable mean
arterial pressure ($Agtr2^+$, 107 ± 3 mmHg; $Agtr2^{-}$, 106 ± 3 mmHg; $n = 8$) and heart rate ($Agtr2^+$, 593 ± 22 bpm; $Agtr2^{-}$, 607 ± 21 bpm). Moreover, cuff placement for 14 days did not affect these hemodynamic parameters. Thus there was no significant difference in mean arterial pressure ($Agtr2^+$, 107 ± 4 mmHg; $Agtr2^{-}$, 108 ± 2 mmHg; $n = 7$) or heart rate ($Agtr2^+$, 606 ± 20 bpm; $Agtr2^{-}$, 605 ± 25 bpm) between the two strains after cuff placement.

To further confirm that the AT$_2$ receptor mediates the reduction in neointima formation, we infused the AT$_2$ receptor blocker, PD-123319, into $Agtr2^+$ mice for 14 days after cuff placement and examined vascular histomorphometry. As shown in Fig. 5, the neointimal area was increased by PD-123319 treatment, whereas the medial area or the adventitial area (data not shown) was not affected.

DISCUSSION

This study demonstrates that placement of a nonconstricting cuff around the mouse femoral artery induces the expression of inflammatory cytokines and consequently IRF-1, which stimulates AT$_2$ receptor expression in the VSMC. Furthermore, confirming the in vitro data demonstrating growth inhibitory action of the AT$_2$ receptor, VSMC proliferation and neointimal lesion area were two-fold greater in the $Agtr2^-$ mouse than those in the $Agtr2^+$ mouse.

In vitro cell culture studies suggest that AT$_2$ receptor can exert growth inhibitory effects in AT$_2$ receptor cDNA-transfected VSMCs (26), cultured rat endothelial cells (36), and neonatal cardiomyocytes (2). In addition, we have demonstrated that AT$_2$ receptor exerts proapoptotic effects in PC12W cells (13, 44), R3T3 mouse fibroblasts (15), and AT$_2$ receptor-transfected VSMCs (43), whereas the AT$_1$ receptor stimulation inhibits apoptosis in VSMCs (29, 43). Using in vivo gene transfer into the balloon-injured rat carotid artery, we have demonstrated previously that overexpression of the AT$_2$ receptor transgene inhibited the subsequent development of neointima (26). However, the role of the endogenous AT$_2$ receptor in vascular remodeling remains to be defined. Previous studies using AT$_2$ receptor blockers have yielded conflicting results (16, 18). The gene-targeted mouse provides a unique model in which AT$_2$ receptor is knocked out and therefore is particularly useful in studying the role of the endogenous AT$_2$ receptor in vascular remodeling. We chose the nonconstricting cuff model because it is very reproducible, whereas vascular injury induced by intraluminal balloon or wire in the mouse is quite variable. Furthermore, the cuff injury model stimulates an inflammatory reaction that appears to play an important role in mediating the formation of neointimal hyperplasia (12). Indeed, recent data have documented the pivotal role of inflammation in atherosclerosis and vascular diseases (23, 30, 31). In addition, an angiotensin-converting enzyme inhibitor, perindopril, inhibited cuff-induced neointima formation in mice by ~60% (our unpublished results), suggesting the role of renin-angiotensin system in this cuff injury model. Thus cuff injury in the $Agtr2^-$ mouse would also provide us with the model to study the role of pathological inflammation in the regulation of AT$_2$ receptor gene expression and signaling in vivo.

We have shown that IFN-1, which can be induced by inflammatory cytokines, transactivates AT$_2$ receptor expression (14, 15). We have identified IRF binding motif (between −282 and −253) in the mouse AT$_2$ receptor genomic DNA and demonstrated that the expression of AT$_2$ receptor in R3T3 cells is transcriptionally regulated by the competitive binding of two related IRFs (IRF-1 and IRF-2) (14). These IRFs have been previously shown to recognize the same DNA sequence elements: the AAGTGA motif or G(A)AAAG/CT/CGAAAG/CT/C (8, 39). Competitive binding of IRF-1 and IRF-2 was reported to play critical roles in determining growth, transformation, and apoptosis of cells (9, 21, 38, 42). By antisense treatment, we have demonstrated that IRF-1 inhibition abolished the upregulation of AT$_2$ receptor and attenuated ANG II-mediated apoptosis (15). We have examined the role of cytokine IFN-γ on AT$_2$ receptor expression in these cells and demonstrated that IFN-γ induced IRF-1 expression and increased IRF binding to the AT$_2$ receptor promoter region and consequently AT$_2$ receptor upregulation (unpublished results). Taken together, these in vitro results suggest that cytokine-induced IRF-1 is very important for AT$_2$ receptor expression.

To study the potential role of IRF-1 in vascular AT$_2$ receptor in vivo, we examined the mRNA expressions of IRF-1 and AT$_2$ receptor as well as the cytokines, IFN-γ, IL-1β, IL-6, and TNF-α. We demonstrated that at 5–7 days after cuff placement, the expressions of cytokines and IRF-1 were activated. AT$_2$ receptor mRNA was upregulated concomitantly in the $Agtr2^+$ but not in the $Agtr2^-$ mouse. Using regional dissection of the vessel and immunohistochemistry, we demonstrated
that the cytokines and IRF-1 were detected throughout the vessel wall, whereas AT₂ receptor was specifically expressed in the medial and neointimal cells that were stained with α-smooth muscle actin. These observations have led us to hypothesize that the vascular inflammatory reaction in response to pathological stimuli results in the release of cytokines such as IFN-γ that stimulates paracrine IRF-1 expression. IRF-1 subsequently induces AT₂ receptor upregulation in VSMC. This sequence of events enhances AT₂ receptor-mediated antiproliferation of VSMC, leading to the modulation of neointima formation. Furthermore, the AT₂ receptor expression may also mediate, at least in part, the apoptosis after vascular injury. However, our preliminary data failed to detect a difference between Agtr²⁺ and Agtr²⁻ vessels at 7 days after cuff placement (data not shown).

To further confirm the physiological relevance of increased AT₂ receptor in cuff-induced vascular disease model, we examined the effect of AT₂ receptor antagonist administration and demonstrated that this treatment enhanced neointima formation in the Agtr²⁻⁻ mouse. This result further supports the notion that the AT₂ receptor plays a critical role in vascular remodeling in vivo.

The relevance of our findings in humans remains to be defined. Since inflammation appears to play pathological roles in human atherosclerosis and restenosis, it would be intriguing to study the status of AT₂ receptor expression and its role in human vascular diseases.

We are indebted to Dr. Robert M. Carey, University of Virginia Health Sciences Center, for the generous gift of the antibody to AT₂ receptor. This work was supported by National Heart, Lung, and Blood Institute Grants HL-58516 and HL-61661 and by a grant from Longwood Foundation for Translational Research.

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