Stimulation of Different Subtypes of Angiotensin II Receptors, AT\textsubscript{1} and AT\textsubscript{2} Receptors, Regulates STAT Activation by Negative Crosstalk

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Abstract—Angiotensin II type 2 (AT\textsubscript{2}) receptor exerts an inhibitory action on cell growth. In the present study, we report that the stimulation of AT\textsubscript{2} receptor in AT\textsubscript{2} receptor cDNA–transfected rat adult vascular smooth muscle cells (VSMCs) inhibited angiotensin II type 1 (AT\textsubscript{1}) receptor–mediated tyrosine phosphorylation of STAT (signal transducers and activators of transcription) 1α/β, STAT2, and STAT3 without influence on Janus kinase. AT\textsubscript{2} receptor activation also inhibited the tyrosine phosphorylation of STAT1α/β induced by interferon-γ, epidermal growth factor, and platelet-derived growth factor. Similar effects of AT\textsubscript{2} receptor were observed in R3T3 fibroblast and mouse fetal VSMCs, which express endogenous AT\textsubscript{2} receptor. Moreover, AT\textsubscript{2} receptor inhibited serine phosphorylation of STAT1\(a/b\) and STAT3 via the binding of extracellular signal–regulated kinase (ERK) activation. Stimulation of AT\textsubscript{2} receptor inhibited the binding of STATs with cis-inducing element in c-fos promoter, resulting in decreased c-fos expression. Taken together, our results suggest that AT\textsubscript{2} receptor can crosstalk negatively with multiple families of growth receptors by inhibiting ERK and STAT activation. (Circ Res. 1999;84:876-882.)

Key Words: angiotensin receptor STAT vascular smooth muscle cell

The peptide angiotensin (Ang) II exerts hemodynamic, renal, and electrolyte as well as cardiovascular structural effects. Two distinct types of Ang II receptors have been cloned and are designated as type 1 (AT\textsubscript{1})\footnote{Circ Res. 1999;84:876-882.} and type 2 (AT\textsubscript{2}) receptors. Most of the known effects of Ang II in adult tissues are attributable to the AT\textsubscript{1} receptor. In contrast, the AT\textsubscript{2} receptor is abundantly and widely expressed in fetal tissues and reexpressed in certain pathological conditions such as ovarian atresia, myocardial infarction, and vascular injury,\footnote{Circ Res. 1999;84:876-882.} suggesting that this receptor plays a pivotal role in differentiation and growth. Although both AT\textsubscript{1} and AT\textsubscript{2} receptors belong to the 7-transmembrane, G protein–coupled receptor family, recent evidences reveal that the functions of AT\textsubscript{1} and AT\textsubscript{2} receptors are antagonistic. AT\textsubscript{1} receptor promotes cell growth whereas AT\textsubscript{2} receptor mediates growth inhibition.\footnote{Circ Res. 1999;84:876-882.} The stimulation of AT\textsubscript{2} receptor in AT\textsubscript{2} receptor cDNA–transfected rat adult vascular smooth muscle cells (VSMCs), cardiomyocytes, endothelial cells, neuronal cells (PC12W cells), and fibroblasts (R3T3 cells). Growth inhibitory effects of AT\textsubscript{2} receptor are unique in that this is a 7-transmembrane, G protein–coupled receptor that counteracts the growth action of other 7-transmembrane, G protein–coupled receptors as well as other classes of growth factor receptors. It has been reported that these effects of AT\textsubscript{2} receptor are, at least partly, mediated by the activation of protein tyrosine phosphatase (PTPase), which results in the inactivation AT\textsubscript{1} receptor– and/or growth factor–activated mitogen-activated protein (MAP) kinase (p42 and p44 MAP kinases are known as extracellular signal–regulated kinases [ERKs]).\footnote{Circ Res. 1999;84:876-882.}

Given the breadth of its interaction with multiple growth factor receptors, we hypothesize that the AT\textsubscript{2} receptor signaling must interact with other cellular signal transduction pathways in which tyrosine phosphorylation is involved. STATs (signal transducers and activators of transcription) are initially identified as the primary mediators of interferon (IFN)–dependent signaling and now are known to be activated by a number of cytokines and growth factors, as well as AT\textsubscript{1} receptor,\footnote{Circ Res. 1999;84:876-882.} mediating their effects on cell growth.\footnote{Circ Res. 1999;84:876-882.}

The binding of ligands to their receptors triggers tyrosine phosphorylation of STATs via activation of receptor-associated Jak (Janus kinase) family of tyrosine kinases (Jak1, Jak2, Jak3, and Tyk2).\footnote{Circ Res. 1999;84:876-882.} The function of STAT is also influenced by serine phosphorylation. Wen et al\footnote{Circ Res. 1999;84:876-882.} reported that the phosphorylation of serine residues, potential ERK phosphorylation sites in STAT1 and STAT3, is induced by IFN-γ and platelet-derived growth factor (PDGF) and that maximal activation of transcription by STAT1 and STAT3 requires both tyrosine and serine phosphorylation of these proteins. Interestingly, recent studies have revealed that AT\textsubscript{1} receptor stimulation activates STAT transcription factor via binding of STATs with cis-inducing element in c-fos promoter, resulting in decreased c-fos expression. Taken together, our results suggest that AT\textsubscript{2} receptor can crosstalk negatively with multiple families of growth receptors by inhibiting ERK and STAT activation.
tyrosine and serine phosphorylation through the activation of Jak\textsuperscript{2,6,31,35} and ERK,\textsuperscript{26} respectively. Accordingly, we postulated in the present study that AT\textsubscript{2} receptor stimulation inactivates STAT transcription factors through tyrosine dephosphorylation as well as through inhibiting serine phosphorylation by the inhibition of ERK activation.

Materials and Methods

Cell Culture

Adult rat aortic VSMCs were isolated from Sprague-Dawley rat thoracic aorta, cultured, and used at passage 4 to 8 as previously reported.\textsuperscript{7,11} The AT\textsubscript{2} receptor cDNA was constructed into a plasmid pUC-CAGGS that has the cytomegalovirus enhancer and the chicken \( \beta \)-actin promoter.\textsuperscript{3,7,11} AT\textsubscript{2} receptor expression vector or control vector (parental pUC-CAGGS) was transfected into the subconfluent VSMCs using cationic liposome-mediated gene transfer as previously reported.\textsuperscript{11} After the transfection, the cells were fed with the medium containing 10% calf serum for 24 hours and then maintained in the defined serum-free medium containing 0.5 \( \mu \)mol/L insulin, 5 \( \mu \)mol/L transferrin, and 0.2 \( \mu \)mol/L ascorbate for an additional 2 days. The quiescent cells thus obtained were used for the subsequent experiments. Thoracic aortas were removed from mouse fetuses (FVB/N strain) (embryonic day 20) and microdissected free from the surrounding adventitial tissues. The aortas from individual animals were placed into separate individual test tubes and then digested in collagenase (1 mg/mL; type II; Sigma Chemical Co) in DMEM (GIBCO/BRL) for 30 minutes at 37°C. After trituration and centrifugation twice, the cells were seeded in 10-cm culture dishes and cultivated in DMEM supplemented with 10% FBS. Culture medium was changed every other day. At 10 to 14 days after primary culture, the cells were trypsinized and seeded for the following experiments. All experimental procedures were approved and carried out in accordance with the guideline of the Harvard Medical Area Standing Committee on Animals. R3T3 fibroblast cells were cultured as described.\textsuperscript{37}

Immunoprecipitation and Western Blot Analysis

The cells were stimulated with Ang II (Sigma), DuP753 (Merck & Co), PD123319 (Research Biochemicals International), IFN-\( \gamma \), epidermal growth factor (EGF), or PDGF (GIBCO/BRL). In some experiments, the VSMCs were treated with PD98059 (New England Biolabs) 60 minutes before Ang II treatment. At the end of the stimulation, the cells were quickly washed twice with HEPES-buffered saline and frozen in liquid nitrogen. The cells were lysed. All the lysis buffer (20 mmol/L Tris-HCl [pH 7.4], 150 mmol/L NaCl, 2.5 mmol/L EDTA, 1% [vol/vol] Triton X-100, 0.1% [wt/vol] sodium deoxycholate, 0.1% [wt/vol] SDS, 50 mmol/L NaF, 10 mmol/L NaF, 10% glycerol, 1 mmol/L sodium orthovanadate, 10 \( \mu \)mol/L aprotinin, and 1 mmol/L phenylmethylsulfonyl fluoride) and the supernatant fraction was obtained as cell lysate by centrifugation at 12,000 rpm for 25 minutes at 4°C. The cell lysate was incubated with 10 \( \mu \)g of antiphosphotyrosine monoclonal antibody (Upstate Biotechnology) or antiphosphoserine antibody (ZYMED) at 4°C for 12 hours and precipitated by addition of 25 \( \mu \)L of protein A/G-agarose (Santa Cruz Biotechnology). The immunoprecipitate was resuspended in 2\( \times \) Laemli sample buffer and run on the 8% SDS/PAGE. The proteins were then transferred to nitrocellulose membrane (Amersham), blotted with anti-STAT1, STAT2, STAT3, Jak2, or Tyk2 antibody (Santa Cruz Biotechnology), and detected by the enhanced chemiluminescence method (Amersham). Densitometric analysis was performed using an image scanner (Aarcus II, Agfa) and NIH image software. All values are expressed as mean±SEM. Statistical significance was assessed by ANOVA followed by Scheffé test. \( P<0.05 \) was considered significant.

Preparation and Analyses of Nuclear Extract

Nuclear extract was prepared from VSMCs as previously reported.\textsuperscript{37} For immunoblotting for STAT in the nuclear extract, nuclear protein (100 \( \mu \)g) was immunoprecipitated with 10 \( \mu \)g of antiphosphotyrosine or antiphosphoserine antibody and immunoblotted with anti-STAT antibody. For electrophoretic gel mobility shift assay, the oligonucleotide probes for sis-inducing element (SIE) and mutant SIE were synthesized as follows:26,27 SIE: 5'-CAGTTCCCGCTAAC-3'; mutant SIE, 5'-CAGCAGCGTCTAATC-3'. In addition, the AP-1 consensus oligonucleotide, 5'-CGCTTGATGAGTCAGCCGGAA-3', was also used for a competition assay. Complementary oligonucleotides were annealed for 2 hours, while the temperature descended from 80°C to 25°C. The double-stranded SIE and mutant SIE probes were labeled with \( ^{32} \)P]-yATP (3,000 Ci/mmol) (Amersham) and T4 polynucleotide kinase.\textsuperscript{32} P-labeled probe (30,000 cpm) was incubated for 30 minutes at room temperature with 10 \( \mu \)g of nuclear proteins and 1 \( \mu \)g of poly(dI:dC)/dT(dC) and electrophoresed on 5% PAGE as previously described.\textsuperscript{37} The gel was dried and exposed for autoradiography.

Northern Blot Analysis

RNA was extracted from the harvested cells by RNAzol B reagent (Tel-Test). Total RNA (20 \( \mu \)g) was electrophoresed on 1% formaldehyde-agarose gel and transferred to a nylon membrane (Amersham). The membrane was then blotted with \( ^{32} \)P-labeled 1.0-kb PstI fragment of murine c-fos probe and 0.78-kb PstI-XhoI fragment of a human GAPDH.

Results

Tyrosine Dephosphorylation of STAT by AT\textsubscript{2} Receptor Stimulation

We transfected the rat AT\textsubscript{2} receptor cDNA expression vector into cultured VSMCs, because VSMCs prepared from adult rat aorta showed high levels of AT\textsubscript{2} receptor binding but minimally detectable levels of AT\textsubscript{2} receptor.\textsuperscript{7,11} Three days after transfection, the density of AT\textsubscript{2} receptor increased in these cells (AT\textsubscript{2} receptor, 8.89±0.44 fmol/10\textsuperscript{6} cells; AT\textsubscript{2} receptor, 5.84±0.32 fmol/10\textsuperscript{6} cells; \( n=6, \) mean±SE). Lysates were prepared from Ang II (10\textsuperscript{-7} mol/L)–treated VSMCs, immunoprecipitated with antiphosphotyrosine antibody, and immunoblotted with STAT antibodies. In control vector–transfected VSMCs, Ang II stimulated tyrosine phosphorylation of STAT1\( \alpha/\beta \), STAT2, and STAT3 (Figure 1A) as previously reported.\textsuperscript{26,27} However, in AT\textsubscript{2} receptor–transfected cells, the effect of Ang II on the tyrosine phosphorylation of these STATs was attenuated (Figure 1A and Figure 1B), and the addition of DuP753 (an AT\textsubscript{2} receptor–specific antagonist) restored phosphorylation of STATs, whereas the addition of DuP753 (an AT\textsubscript{2} receptor–specific antagonist) attenuated the effect of Ang II (Figure 1A, middle and bottom panels). These results suggest that AT\textsubscript{2} receptor stimulation inhibits the tyrosine phosphorylation of STATs and antagonizes the effect of AT\textsubscript{2} receptor.

To further confirm that AT\textsubscript{2} receptor stimulation dephosphorylates STAT, we next examined the possibility that AT\textsubscript{2} receptor stimulation also inhibits the tyrosine phosphorylation of STAT induced by other families of receptors. As shown in Figure 1B and 1C, IFN-\( \gamma \) or PDGF phosphorylated STAT1\( \alpha/\beta \) in AT\textsubscript{2} receptor–transfected VSMCs. Selective stimulation of AT\textsubscript{2} receptor (Ang II plus DuP753) inhibited the phosphorylation of STAT1\( \alpha/\beta \).

The AT\textsubscript{2} receptor is abundantly and widely expressed in fetal vasculature but is present only at low levels in adult tissues, leading to the hypothesis that this receptor is involved in growth, development, and/or differentiation. To examine the role of endogenous AT\textsubscript{2} receptor on STATs, we used VSMCs prepared from mouse fetal aorta. VSMCs were cultured from the aorta of...
fetal mouse at embryonic day 20, which expresses both AT_1 and AT_2 receptor (AT_1 receptor, 4.72 ± 0.81 fmol/10^6 cells; AT_2 receptor, 2.35 ± 0.81 fmol/10^6 cells; n=4, mean ±SE). Stimulation with Ang II (10^{-7} mol/L) in the presence of the AT_2 receptor–specific antagonist (PD123319, 10^{-5} mol/L) enhanced further the tyrosine phosphorylation of STAT1a/b and STAT3 compared with stimulation with Ang II alone (Figure 3A). The effect of Ang II on tyrosine phosphorylation of STATs was attenuated with the addition of the AT_1 receptor–specific antagonist DuP753 (10^{-5} mol/L) (Figure 3A). To confirm further the observation in other cells, we studied mouse fibroblast R3T3 cells, which express abundant AT_2 receptor but not AT_1 receptor. As shown in Figure 3B, Ang II inhibited the tyrosine phosphorylation of STAT1a/b by IFN-γ, EGF, and PDGF, supporting our notion that AT_2 receptor stimulation dephosphorylates STAT.

It has been reported that the AT_1 receptor stimulation activates STAT via tyrosine phosphorylation through the activation of Jak. To explore the possibility that AT_2 receptor inhibits Jak activation and results in STAT inactivation, we studied the effect of AT_2 receptor on the tyrosine phosphorylation of Jak2 and Tyk2 (Figure 1D). There was no difference in Ang II–stimulated tyrosine phosphorylation of Jak2 and Tyk2 between control vector– and AT_2 receptor–transfected VSMCs, suggesting that AT_2 receptor stimulation does not affect the activation of Jak2 and Tyk2.

Serine Dephosphorylation of STAT by AT_2 Receptor Stimulation

Recently, it has been reported that the function of STAT is also enhanced by serine phosphorylation. We examined the effect of AT_2 receptor stimulation on serine phosphorylation of STAT. AT_2 receptor–transfected VSMCs were treated with Ang II (10^{-7} mol/L) for 15 minutes, and the cell lysates were immunoprecipitated with antiphosphoserine antibody and immunoblotted with STAT antibodies. As shown in Figure 4A and 4C, Ang II stimulation increased the serine phosphorylation of STAT1a and STAT3. Ang II plus PD123319 treatment further

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Figure 1. Ang II–mediated tyrosine phosphorylation and dephosphorylation of STATs and Jak. A, Control vector–transfected VSMCs (CV) or AT_2 receptor cDNA–transfected VSMCs (AT_2R) were treated with Ang II (10^{-7} mol/L) (top panel). AT_2 receptor–transfected VSMCs were also treated with Ang II (10^{-7} mol/L), DuP753 (10^{-7} to 10^{-5} mol/L), and/or PD123319 (10^{-5} mol/L) for 15 minutes. Cell lysates were immunoprecipitated with antiphosphotyrosine antibody and immunoblotted with STAT antibodies. B and C, AT_2 receptor–transfected VSMCs were treated with IFN-γ (1 μg/mL) or PDGF (10 ng/mL) with or without Ang II (10^{-7} mol/L) plus DuP753 (10^{-7} mol/L). Cell lysates were immunoprecipitated with antiphosphotyrosine antibody and immunoblotted with STAT1 antibody. D, VSMCs were treated with Ang II (10^{-7} mol/L), and cell lysates were immunoprecipitated with antiphosphotyrosine antibody and Western blotted with Jak2 or Tyk2 antibody. Data are representative of 6 separate experiments.

Figure 2. Densitometric measurements of tyrosine phosphorylation of STAT1 (A), STAT2 (B), and STAT3 (C) 15 minutes after Ang II (10^{-7} mol/L) stimulation in control vector–transfected VSMCs (CV) or AT_2 receptor cDNA–transfected VSMCs (AT_2R). Values expressed are mean ±SEM of 6 different experiments.
enhanced serine phosphorylation of STAT1α and STAT3, whereas the addition of DuP753 to Ang II attenuated the effect of Ang II on serine phosphorylation (Figure 4A and 4C).

STAT1β and STAT2 lack putative serine phosphorylation sites for ERK, and we did not observe the serine phosphorylation of STAT1β and STAT2 by Ang II (data not shown).

To examine the effect of ERK on serine phosphorylation on STAT1α and STAT3 via AT1 receptor, we treated control VSMCs with a specific inhibitor to MAP kinase kinase 1 (also known as MEK-1 inhibitor), PD98059 (50 μmol/L), 60 minutes before the addition of Ang II (10⁻⁷ mol/L). As shown in Figure 4B, pretreatment with PD98059 inhibited the stimulatory effect of the AT1 receptor on serine phosphorylation of STAT1α and STAT3.

We next examined the protein levels of STATs, Jak2, and Tyk2 3 days after AT2 receptor cDNA transfection into the VSMCs, and we did not observe any detectable changes in these protein levels (Figure 5).

Effect of AT2 Receptor on Nuclear Translocation of STAT

We next examined the possibility that AT2 receptor-mediated dephosphorylation of STATs influences the nu-
cells within 30 minutes. In contrast, the Ang II–induced formation of the SIF complex in the control vector–transfected promoter region. As shown in Figure 7A, Ang II induced the fos assay using the radiolabeled probe for SIE in the c-STAT3 are components of the SIF complex. Next, we studied was not detected (Figure 7D), suggesting that both STAT1 and from nuclear extract by immunoprecipitation, the SIF complex serum response factor. When STAT1 or STAT3 was removed by nuclear extract (100 μg) was immunoprecipitated with antiphosphotyrosine (A) or antiphosphoserine antibody (B) and Western blotted with STAT1 or STAT3 antibody. Data are representative of 3 separate experiments.

Figure 6. Nuclear translocation of STAT1 and STAT3. Nuclear extracts were prepared from control vector (CV) or AT2 receptor cDNA–transfected VSMCs, which were treated with Ang II (10^{-7} mol/L) for 30 minutes. Nuclear extract (100 μg) was immunoprecipitated with antiphosphotyrosine (A) or antiphosphoserine antibody (B) and Western blotted with STAT1 or STAT3 antibody. Data are representative of 3 separate experiments.

clear translocation of STATs. Tyrosine- and serine-phosphorylated STAT1 and STAT3 were shown to increase in the control VSMCs nuclei after Ang II stimulation (Figure 6). In the AT2 receptor–transfected cells, however, accumulation of nuclear phosphorylated STAT1 and STAT3 was attenuated.

Induction and DNA binding of the sis-inducing factor (SIF) complex were assessed by electrophoretic gel mobility shift assay using the radiolabeled probe for SIE in the c-fos gene promoter region. As shown in Figure 7A, Ang II induced the formation of the SIF complex in the control vector–transfected cells within 30 minutes. In contrast, the Ang II–induced formation of the SIF complex was attenuated in the AT2 receptor cDNA–transfected cells. The addition of 100-fold excess of unlabeled SIE as a competitor abolished the SIF complex (lanes 2 and 5, Figure 7B), whereas the unlabeled mutant SIE had no effect (lanes 3 and 6, Figure 7B). In addition, the 32P-labeled mutant SIE probe did not show any specific binding (lanes 7 and 8, Figure 7B). The addition of AP-1 oligonucleotide competitor did not affect the formation of SIF (Figure 7C), indicating that the SIF is distinct from classical AP-1 components such as the serum response factor. When STAT1 or STAT3 was removed from nuclear extract by immunoprecipitation, the SIF complex was not detected (Figure 7D), suggesting that both STAT1 and STAT3 are components of the SIF complex. Next, we studied the expression of c-fos mRNA by Northern blot and observed that Ang II induces c-fos mRNA expression after 30 minutes of stimulation in the control- and the AT2 receptor expression vector–transfected VSMCs (Figure 7E). However, its expression was attenuated markedly in the AT2 receptor–transfected cells.

Discussion

STATs are now known to be activated by many different extracellular signaling proteins including cytokines, growth factors such as EGF and PDGF, and Ang II via the AT1 receptor, and the mechanism of STAT activation is well defined. In contrast, the inactivation mechanism(s) of the Jak/STAT pathway is not well known. Recent evidences suggest that phosphatases may play important roles in regulating Jak/STAT function. Indeed, the use of phosphatase inhibitors can lead to constitutive activation of some STAT pathways.\[500\],\[510\] and SHPTP1 has been implicated in the down-regulation of IFN signaling through STAT.\[520\] We have demonstrated that AT2 receptor activates PTPase, antagonizes the effects of growth factor and AT1 receptors, and exerts antigrowth effects in several types of cells such as VSMCs,\[700\],\[1100\],\[1400\],\[1600\] PTPase activation by the AT2 receptor has also been demonstrated in rat adrenal glomerulosa and PC12W cells,\[18\] neuronal NG 108-15 cells,\[19\] and R3T3 cells.\[20\] These results have led us to examine the possibility that AT2 receptor stimulation dephosphorylates the tyrosine residues of Jak and/or STATs.

The data obtained from the present study have demonstrated that AT2 receptor stimulation inhibits the AT1 receptor–mediated tyrosine phosphorylation of STAT1α/β, STAT2, and STAT3, whereas AT1 receptor does not affect the tyrosine phosphorylation of Jak2 and Tyk2. The fact that STAT, but not Jak, is dephosphorylated via AT2 receptor is interesting. These results also suggest that AT2 receptor–activated PTPases may directly dephosphorylate the tyrosine residues of STAT1α/β, STAT2, and STAT3. Identification of specific phosphatase(s), which is activated by AT2 receptor stimulation and inhibits STAT phosphorylation, is intriguing and may provide new insights into the regulatory mechanism of the function of STATs as well as the signaling mechanism of the AT2 receptor. Recently, the activation of SHPTP1 via AT2 receptor was reported,\[23\] suggesting that SHPTP1 is one of the phosphatases that mediates dephosphorylation of STATs by AT2 receptor stimulation. Moreover, evaluation of the mechanism of dephosphorylation of Jak may reveal further the mechanism of the distinct dephosphorylation of STAT and Jak via AT2 receptor.

Recent evidences have revealed that serine phosphorylation and tyrosine phosphorylation are required for the maximal activation of STATs. It has been reported that phosphorylation of serine residues is induced by IFN-γ and PDGF and that direct association of ERK2 with IFN receptor and STAT1 occurs after IFN stimulation.\[30\] AT1 receptor stimulation has been also reported to phosphorylate serine residue of STAT3 in rat neonatal cardiac fibroblasts and CHO-K1 cells by ERK activation.\[36\] We have also confirmed that AT1 receptor stimulation phosphorylates the serine residues of STAT1α and STAT3 in VSMCs via ERK activation. As expected, on the basis of the previous observation that AT2 receptor stimulation inhibits STAT1α and STAT3 in VSMCs via ERK activation. As expected, on the basis of the previous observation that AT2 receptor stimulation inhibits STAT1α and STAT3 in VSMCs via ERK activation, we have demonstrated that in response to AT1 receptor stimulation, tyrosine- and serine-phosphorylated STAT1 and STAT3 accumulate in the nuclei of VSMCs and become a component of the nuclear SIF complex. In contrast, in AT2 receptor–transfected VSMCs, AT2 receptor–mediated SIF complex binding with SIE in the c-fos gene promoter was attenuated. This decrease was associated with the reduction of the levels
of phosphorylated STAT1 and STAT3. Consistent with these findings, the AT1 receptor–induced c-fos gene expression was decreased significantly by the concomitant stimulation of the AT2 receptor in these cells. The c-fos gene expression is regulated by the net interaction with different transcriptional factors. Partial inhibition of c-fos could be mediated by other mechanisms. The inactivation of ERK by AT2 receptor may also result in a decreased production of serum response factor, and this may act in concert with the inactivation of STAT via the inhibition of serine phosphorylation, thereby resulting in the decrease of c-fos transcription.

The AT2 receptor is abundantly expressed in the fetal vasculature with rapid decline after birth and reexpression in vascular injury, suggesting an important role of the AT2 receptor in vascular development and vascular remodeling. In the present study, we examined the interaction of AT1 and AT2 receptors in fetal VSMCs, which express both receptors. As expected, we demonstrated that AT2 receptor stimulation inhibited AT1 receptor–mediated STAT phosphorylation, suggesting that the negative regulation of STAT activation may also be involved in the AT2 receptor–mediated vasculogenesis in the fetus.

The present study suggests that AT1 receptor activates (1) STAT1α/β, STAT2, and STAT3 by tyrosine phosphorylation via Jak2 and Tyk2 activation and (2) STAT1α and STAT3 by serine phosphorylation via ERK activation. In contrast, the AT2 receptor stimulation inactivates STATs by tyrosine dephosphorylation without affecting Jak as well as by serine dephosphorylation via the inhibition of ERK, thereby antagonizing the AT1 receptor–mediated upregulation of the c-fos gene. Moreover, AT2 receptor stimulation also dephosphorylates STAT, which is activated by IFN-γ, PDGF, and EGF, in VSMCs as well as in R3T3 cells. These results suggest that the AT2 receptor is capable of exerting broad inhibitory effects on STAT activation induced by multiple families of receptors (growth factor, cytokine, and 7-transmembrane receptors).

In summary, our data provide evidence for negative crosstalk of the 7-transmembrane-spanning AT2 receptor with multiple families of receptors via the inactivation of ERK and STAT pathways.

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References


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