Angiotensin AT₁ and AT₂ Receptors Differentially Regulate Angiopoietin-2 and Vascular Endothelial Growth Factor Expression and Angiogenesis by Modulating Heparin Binding–Epidermal Growth Factor (EGF)–Mediated EGF Receptor Transactivation

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Abstract—Angiotensin II (Ang II)–mediated signals are transmitted via heparin binding epidermal growth factor (EGF)–like growth factor (HB-EGF) release followed by transactivation of EGF receptor (EGFR). Although Ang II and HB-EGF induce angiogenesis, their link to the angiopoietin (Ang)–Tie2 system remains undefined. We tested the effects of Ang II on Ang1, Ang2, or Tie2 expression in cardiac microvascular endothelial cells expressing the Ang II receptors AT₁ and AT₂. Ang II significantly induced Ang2 mRNA accumulations without affecting Ang1 or Tie2 expression, which was inhibited by protein kinase C inhibitors and by intracellular Ca²⁺ chelating agents. Ang II transactivated EGFR via AT₁, and inhibition of EGFR abolished the induction of Ang2. Ang II caused processing of pro–HB-EGF in a metalloproteinase–dependent manner to stimulate maturation and release of HB-EGF. Neutralizing anti–HB-EGF antibody blocked EGFR phosphorylation by Ang II. Ang II also upregulated vascular endothelial growth factor (VEGF) expression in an HB-EGF/EGFR–dependent manner. AT₂ inhibited AT₁–mediated Ang2 expression and phosphorylation of EGFR. In an in vivo corneal assay, AT₁ induced angiogenesis in an HB-EGF–dependent manner and enhanced the angiogenic activity of VEGF. Although neither Ang2 nor Ang1 alone induced angiogenesis, soluble Tie2-Fc that binds to angiopoietins attenuated AT₁–mediated angiogenesis. These findings suggested that (1) Ang II induces Ang2 and VEGF expression without affecting Ang1 or Tie2 and (2) AT₁ stimulates processing of pro–HB-EGF by metalloproteinases, and the released HB-EGF transactivates EGFR to induce angiogenesis via the combined effect of Ang2 and VEGF, whereas AT₂ attenuates them by blocking EGFR phosphorylation. Thus, Ang II is involved in the VEGF-Ang-Tie2 system via HB-EGF–mediated EGFR transactivation, and this link should be considerable in pathological conditions in which collateral blood flow is required. (Circ Res. 2001;88:22-29.)

Key Words: angiotensin II • angiopoietin • angiogenesis • vascular endothelial growth factor • endothelial cell

Remodeling and establishment of blood vessels is regulated by paracrine signals from the receptor tyrosine kinases (RTKs).¹ Vascular endothelial growth factor receptor (VEGF-R)–1 and VEGF-R2 are such RTKs with their cognate ligand VEGF, which have been shown to be required for vessel development during embryogenesis²,³ and to augment postnatal angiogenesis.⁴–⁶ The other endothelial cell (EC)–specific RTK is the Tie receptor family, consisting of Tie1 and Tie2, and the latter has been shown to be widely expressed in cardiovascular tissue.⁷,⁸ The studies of Tie1-null⁹¹⁰ and Tie2-null¹⁰,¹¹ mice or of a mutation in the Tie2 gene in humans indicate that the Tie2 system regulates the EC recruitment of stromal cells required to encase and thereby stabilize primitive endothelial tubes.¹²

Ligands for the Tie2 receptor have been identified as angiopoietin (Ang) 1 and Ang2.¹³,¹⁴ Ang1 is the major physiological ligand for Tie2.¹⁴ Ang2 disrupts blood vessel formation in the developing embryo by antagonizing the effects of Ang1.¹³ Ang2-overexpressing transgenic mice die with vascular defects similar to those of Tie2- or Ang1-null mice.¹⁰,¹⁵ A study using a corneal assay showed that Ang1 promotes vascular network maturation, whereas Ang2 works...
Northern Blotting, Stable Transfection of DNA, and Measurement of Alkaline Phosphatase (ALP) Activity

Northern blotting for Ang1, Ang2, and Tie2 was performed using cRNA probes. CMECs expressed 2 different sizes of spliced Ang2 transcripts (2.8 and 2.3 kb) and a single size of Ang1 (4.8 kb) and Tie2 (4.1 kb). Because both Ang2 transcripts responded to Ang II to a similar extent and with an equal time course, we quantitatively measured only the 2.8-kb Ang2 transcript. Signals were measured by densitometry and arbitrarily normalized relative to the GAPDH mRNA level.

We constructed pRC expression vector containing rat ALP cDNA between HB-EGF signal sequence and HB-EGF cDNA (pAlp-HB-EGF). This pAlp-HB-EGF (30 μg) was transfected into CMECs using Lipofectamine Plus (GIBCO BRL) and selected with G418. Three stable cell lines having high ALP activity in the incubation medium were selected and analyzed for the experiment.

Measurement of ERK, EGFR, and Protein Kinase C (PKC) Activation, Metabolic Labeling, and Nuclear Run-Off Assay

Tyrosine phosphorylation of ERK was determined in CMECs exposed to Ang II for 5 minutes using phospho-ERK antibody as described previously. Tyrosine phosphorylation of EGFR was analyzed in CMECs exposed to Ang II for 1.5 minutes; cell lysates were immunoprecipitated with anti-EGFR antibody and then blotted with anti-phosphotyrosine antibody as described. PKC activity was measured in CMECs exposed to Ang II for 1 minute using the Promega TECT-PCK assay system. Immunoprecipitation and Western blotting, metabolic labeling of cells with [35S]methionine and immunoprecipitation with anti-human–specific EGFR antibody (nonreactive to rat EGFR) followed by autoradiography in SDS-PAGE were performed as described previously.

Angiogenesis Assay Using Rabbit Cornea and Tissue Concentration of Ang II

An aliquot of 1 μL of a solution containing Ang II (50 ng) with or without PD123319 (1 μg), CS866 (1 μg), or VEGF (100 ng) was mixed with 10 μL of a solution of ethylene vinylaceta copolymer (Mitsui Dupon) in dichloromethane and frozen. The pellet was inserted surgically into pockets created in the rabbit corneal stroma and set 3 mm from the corneoscleral limbus. Seven days after implantation of the pellet, capillary formation was examined. For measurement of Ang II level, the tissue sample was weighed and homogenized in 0.1 mol/L HCl. The supernatant, obtained by centrifugation of the homogenate at 20,000g for 30 minutes, was applied to a minicolumn (Amprep C8, Amersham) to extract Ang II. Ang II was determined by high-performance liquid chromatography coupled with radioimmunoassay as described.

Statistical Analysis

The results are expressed as mean±SE. Statistical analyses were performed by 1-way ANOVA followed by pairwise comparisons (control versus conditions) using the Dunnett multiple-comparisons test.
This article was retracted in May 2013.

**Results**

**Ang2 mRNA Accumulation Is Increased by AT1, Whereas AT2 Inhibits Its Accumulation**

Ang II (100 nmol/L) significantly stimulated Ang2 mRNA accumulation after 4 hours of incubation, reaching a maximal increase (2.4-fold) after 6 hours and thereafter returning to the control level at 16 hours. Neither Ang1 nor Tie2 mRNA levels were responsive to Ang II (Figure 1A). Ang II–induced Ang2 mRNA levels increased dose dependently with an EC50 of ~30 nmol/L and a maximal peak at 100 nmol/L (data not shown). Subsequent experiments were performed with 100 nmol/L Ang II for 6 hours.

Ang II receptor densities were quantified in CMECs, and the effects of AT1 antagonist CS866 or AT2 antagonist PD123319 were examined. CMECs (n = 5) expressed AT1 and AT2 (Ki = 0.2 ± 0.01 and 0.20 ± 0.01 nmol/L, and Bmax = 108 ± 4 and 73 ± 2 fmol/mg protein, respectively). Induction of Ang2 mRNA by Ang II was abolished by CS866, whereas PD123319 markedly (76%, P<0.001 vs control) enhanced Ang II–mediated Ang2 mRNA accumulation (Figure 1B).

**PKC and Ca2+ Are Involved in AT1-Mediated Ang2 mRNA Accumulation**

AT1 causes generation of diacylglycerol and inositol triphosphate, which induce PKC activation and release of Ca2+, respectively. We tested the role of the PKC inhibitors GF109203X and calphostin C on AT1 (Ang II + PD123319)–mediated Ang2 mRNA levels. Pretreatment with these inhibitors markedly but not completely (67–64%) inhibited Ang2 mRNA accumulation, and depletion of phorbol ester–sensitive PKC showed a similar blocking effect (Figure 2A). AT1–mediated Ang2 mRNA accumulation was moderately inhibited by BAPTA-AM (47%) and TMB8 (44%), which are commonly used as intracellular Ca2+ chelators, whereas combined treatment with GF109203X and BAPTA-AM completely abolished it (Figure 2A). BAPTA-AM and TMB8 reduced Ang II–mediated PKC activities by 33% and 36%, respectively (Figure 2B), suggesting that the actual inhibition ratio by Ca2+ chelators is likely lower than the estimated value and that Ang II induces Ang2 expression mainly via a PKC-dependent mechanism.

**Ang2 mRNA Accumulation by AT1 Is Induced via Downstream Signals of EGFR**

AT1–mediated signals and gene expression were induced via downstream signals of transactivated EGFR. The EGFR antagonist AG1478 abolished AT1–mediated Ang2 mRNA accumulation, and inhibition of ERK activity by the mitogen-activated protein kinase/ERK (MEK) inhibitor PD98059 (10 μmol/L) also blocked it (Figure 3A). To further confirm the involvement of EGFR, we established CMECs stably overexpressing EGFR dominant-negative mutant (EGFR-533del) lacking its kinase domain as previously reported. The inhibilatory action of Ang2 mRNA by Ang II + PD123319 or EGF was abolished by EGFR-533del.

Very recently, Prenzel et al reported that EGF-R transactivation by G protein–coupled receptors is caused by release of HB-EGF due to proteolytic processing by metalloproteinase. Figure 3A shows that addition of the neutralizing anti–HB-EGF antibody heparin (which competes with cell surface-associated heparin sulfate proteoglycans as coreceptors for HB-EGF binding to EGFR) and metalloproteinase inhibitor batimastat completely inhibited AT1–mediated Ang2 mRNA accumulation.

Figure 1. Time-dependent effects of Ang II on Ang2, Ang1, and Tie2 mRNA accumulation in CMECs. A, CMECs were stimulated with Ang II (100 nmol/L) for the indicated periods. Total RNA (30 μg) was analyzed by hybridization with Ang2, Ang1, and Tie2 cRNA probes or GAPDH cDNA probe. Exposure time was 48 hours for Ang2, Ang1, and Tie2 mRNA and 12 hours for GAPDH mRNA. B, To test the effects of PD123319 (1 μmol/L) or CS866 (1 μmol/L), cells were pretreated with these inhibitors for 30 minutes and then exposed to Ang II (100 nmol/L) for 6 hours. mRNA signals were measured by densitometry and normalized relative to those of GAPDH mRNA. Results are arbitrarily indicated as values relative to Ang2 mRNA levels in the unstimulated control cells. Results shown are mean ± SE of 4 separate experiments, and representative data are shown. *P<0.001 vs control.
We established 3 CMEC lines stably transfected with the expression vector containing ALP gene between the HB-EGF signal sequence and cDNA and measured ALP activity in the incubation medium (Figure 3C). ALP activity was rapidly increased after AT1 stimulation (1.9-fold at 1 minute) and reached a 4.3-fold increase at 3 minutes. Pretreatment with batimastat or CS866 completely abolished the AT1-mediated increase in ALP activity. GF109203X and BAPTA-AM greatly inhibited the increase of ALP activity. These inhibitors alone did not affect the ALP activity. Similar results were obtained in 3 different cell lines, indicating that Ang II rapidly stimulates the proteolytic processing of pro–HB-EGF by metalloproteinases to cause the maturation and release of HB-EGF. We also tested HB-EGF–mediated effects on the action of AT2 by vanadate. Phosphatase (PTP)

AT2 Inhibits AT1-Mediated Ang2 mRNA Accumulation via Activation of Protein Tyrosine Phosphatase (PTP)

As shown in Figure 1B, AT2 inhibited AT1-mediated Ang2 expression. AT2 was reported to activate vanadate-sensitive PTP or okadaic acid–sensitive serine/threonine phosphatase to inhibit ERK activities.42 Pretreatment with vanadate but not okadaic acid enhanced Ang II–induced accumulation of Ang2 mRNA (≈83%), whereas CS866 abolished the enhancement by Ang II + vanadate, suggesting the inhibition of the action of AT2 by vanadate.

ERK phosphorylation was maximally activated 5 minutes after addition of Ang II (2.8-fold). PD123319 further increased Ang II–mediated ERK activation (4.7-fold), which was abolished by CS866 (Figure 4B), suggesting that AT2 mediated inhibition of ERK activation. This action of AT2 was completely blocked by vanadate (Figure 4B).

We next examined whether the AT2 effect on ERK is exerted upstream or downstream of EGFR. Ang II–mediated phosphorylation of EGFR was further stimulated (66%) by PD123319. CS866 and vanadate produced inhibition similar to that observed in ERK activation (Figure 4C), suggesting that AT2 effects mainly occur by affecting EGFR level as a result of activation of vanadate-sensitive PTP.

Ang II Induces Ang2 Protein Expression via AT1 but Inhibits It by AT2

CMEC was metabolically labeled, and Ang2 protein was immunoprecipitated. The molecular size of Ang2 protein was reported to range from 55 to 70 kDa as a result of glycosylation.14 The detected size of Ang2 protein was ≈58 kDa, and the expression level was significantly increased (1.8±0.11-fold, n=4) by Ang II treatment (Figure 5A). PD123319 or vanadate enhanced Ang II–induced Ang2 protein expression (3.2±0.14-fold, n=4). Because the antibody used here was an anti-goat polyclonal antibody, we tested the effects of control goat IgG; no specific bands were detected (Figure 5A).

Figure 3. Effects of HB-EGF released by AT1 on Ang2 expression and EGFR phosphorylation. A, Cells were pretreated for 30 minutes with EGFR antagonist AG1478 (250 nmol/L), MEK inhibitor PD98059 (10 μmol/L), neutralizing anti–HB-EGF antibody (×100), heparin (10 μg/mL), and metalloproteinase inhibitor batimastat (5 μmol/L) and then stimulated with PD123319 (1 μmol/L) + Ang II (100 nmol/L) and HB-EGF (20 ng/mL) for 6 hours. Ang2 mRNA levels were determined as described in Figure 1. B, CMECs stably overexpressing pcDNA alone (mock transfection) or containing human EGFR-533del were established.33,34 Expressions of EGFR-533del was confirmed by labeling cells with [35S]methionine and immunoprecipitation with anti-human–specific EGFR antibody (nonreactive to rat EGFR) followed by autoradiography in SDS-PAGE.33,34 Cells were starved for serum for 12 hours and then stimulated with Ang II (100 nmol/L) or EGF (2 and 50 ng/mL) for 6 hours. C, CMECs stably transfected with pALP-HB-EGF were stimulated for 3 minutes by PD123319 (1 μmol/L) + Ang II (100 nmol/L) with or without batimastat (5 μmol/L), CS866 (1 μmol/L), GF109203X (1 μmol/L), or BAPTA-AM (10 μmol/L), and ALP activity in the incubation medium was determined as described in Materials and Methods. The ALP activities (n=4 in each experiment) were shown relative to the values of 0 time control arbitrarily normalized in one. D, CMECs were pretreated as described in panel A and then stimulated for 1.5 minutes. Cellular extracts were immunoprecipitated (IP) with anti-rat EGFR antibody and then blotted with anti-phosphotyrosine (PY) or anti-rat EGFR antibody. Results shown are mean±SE of at least 4 separate experiments, and representative data are shown. *P<0.001, **P<0.005 vs the control.
This article was retracted in May 2013.

Ang II Increases the Transcriptional Rate of Ang2 Gene Without Affecting Its mRNA Stability

We tested the effects of Ang II on Ang2 mRNA stability by inhibiting gene transcription with actinomycin D. In control cells, the half-life of Ang2 mRNA was 6.3±0.2 hours (n=5). When cells were stimulated with Ang II, the half-life was 6.6±0.2 hours (n=5), which was not significantly different from that in control cells (Figure 5B). The runoff assay indicated that the transcriptional rate of the Ang2 gene relative to that of GAPDH was increased 2.7±0.1-fold (n=4) in response to Ang II (Figure 5C).

Ang II Uregulates VEGF Expression via HB-EGF–ERK Pathway

VEGF mRNA levels were significantly increased 2 hours after addition of Ang II and reached a maximal level at 4 hours (2.9-fold). This VEGF induction by Ang II was completely blocked by addition of neutralizing anti–HB-EGF antibody or pretreatment of AG1478 or PD98059 (Figure 5D), suggesting that Ang II also upregulates VEGF expression by stimulating the processing and release of HB-EGF followed by activation of the EGFR-ERK pathway.

Ang II Induces Angiogenesis via HB-EGF

In the cornea assay, pellets containing Ang II (500 ng) induced apparent corneal angiogenesis extending from the limbus across the cornea (Figure 6B) as compared with that by the control buffer pellet (Figure 6A). Pellets containing PD123319+Ang II (selective stimulation of AT1) enhanced Ang II–induced neovascularity (Figure 6C), whereas CS866+Ang II (selective stimulation of AT2) blocked the corneal angiogenesis (Figure 6D). Inhibition of HB-EGF processing and release by addition of batimastat abolished corneal angiogenesis by PD123319+Ang II (Figure 6E). Because Asahara et al.16 reported that neither Ang2 nor Ang1 caused corneal angiogenesis and we also confirmed it, we next tested the effect of Tie2-Fc that binds to angiopoietins to abolish their effects.13,16 Interestingly, excess amounts of Tie2-Fc (10 μg) markedly attenuated AT1 (Ang II+PD123319)–mediated angiogenesis (Figure 6F). VEGF induced enriched neovascularity (Figure 6G), and a combination of VEGF+Ang II+PD123319 induced a further increase in the corneal and circumferential neovascularity (Figure 6H). Addition of Tie2-Fc (10 μg) did not affect VEGF-induced corneal angiogenesis (n=5, data not shown).

We next determined the tissue concentration of Ang II in the cornea 7 days after implantation of the pellet containing Ang II (500 ng). Although Ang II–like activity was not detectable in the normal cornea, it was increased up to 887±17 pg/g tissue in the corneal area surrounding the pellet (n=6). We also determined the tissue concentration of Ang II in the normal rabbit heart and found that it contained 125±7 pg/g tissue of Ang II (n=6). Thus, the pellet containing 500 ng of Ang II did not produce the pharmacologically high level of local Ang II, and this level might be detectable in the pathophysiological conditions such as ischemic myocardium in which local renin-angiotensin system is markedly activated.43

Discussion

The major new findings of this study were that (1) CMECs express Ang1, Ang2, and its receptor Tie2; (2) Ang II upregulates the expression of Ang2 and VEGF, but not Ang1.
This article was retracted in May 2013.

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Figure 6. Macroscopic photographs of rabbit cornea obtained by slit-lamp biomicroscopy 6 days after pellet implantation. Pellets containing Ang II (500 ng) induced corneal angiogenesis (B) compared with that by the control buffer pellet (A). Pellets containing PD123319 (1 μg) + Ang II (500 ng) (selective stimulation of AT₁) enhanced Ang II–induced neovascularity (C), whereas CS866 (1 μg) + Ang II (500 ng) (selective stimulation of AT₂) blocked the corneal angiogenesis (D). Inhibition of HB-EGF release by batimastat (100 ng) or addition of soluble Tie2-Fc (10 μg) abolished corneal angiogenesis by PD123319 (1 μg) + Ang II (500 ng) (selective stimulation of AT₁) and Tie2-Fc containing ectodomain of Tie2 attenuated the resultant greater production of VEGF inhibiting AT₁-mediated apoptotic change. Because the AT₁ numbers are much more in rat CMECs (≈60-fold) than those in human CMECs, the difference in the receptor numbers may account for the lack of AT₁ action in human CMECs.

There is evidence that AT₁ antagonizes AT₂-mediated ERK activation by activating PTP or serine/threonine phosphatases in a cell type–dependent manner. In this study, we found for the first time that AT₁ abolished AT₂-mediated EGFR phosphorylation in a PTP-dependent manner and attenuated the

that this Tie2-Fc binds to angiopoietins to abolish their effects. Neither Ang2 nor Ang1 induces angiogenesis in the corneal assay, whereas VEGF caused enriched capillary formation. Considering the present observation that Ang2 and VEGF expression is selectively induced by Ang II, these findings may suggest that Ang2 itself cannot induce angiogenesis but is indirectly involved in Ang II–mediated angiogenesis by enhancing the angiogenic activity of VEGF. Consistent with our observation, Asahara et al already reported using the corneal assay that Ang2 or Ang1 alone lacked the angiogenic activity, but Ang2 enhanced the sprouting of capillary vessels in the presence of VEGF by antagonizing the stabilization effect of Ang1 on vessel formation. Ang2 was also shown to collaborate at the front of invading vascular sprouts in the presence of VEGF, serving as an initial angiogenic signal. Thus, it is likely that VEGF is a major factor responsible for Ang II–induced angiogenesis and Ang2 complements the angiogenic activity of VEGF by enhancing vascular sprouts.

The present findings demonstrated that ERK activation plays a key role in Ang II–induced Ang2 expression. We showed that ERK is activated downstream of EGFR transactivated by Ang II in cardiac fibroblasts. The involvement of ERK in VEGF-induced Ang2 expression was reported in retinal MECs. This study extended the previous observation and clearly showed that both Ca²⁺ and PKC signaling transactivate the EGFR/ERK cascade to stimulate Ang2 expression. Interestingly, we found that processing of HB-EGF or PKC activation by Ang II was rapidly induced (1 minute) before maximal activation of EGFR (1.5 minutes) and that the transactivation mechanism of EGFR was mostly mediated by HB-EGF processed and released via metalloproteinas.

HB-EGF is a heparin-binding member of the EGF family and is synthesized as a transmembrane precursor that is then proteolytically processed into the mature, soluble growth factor. The proteolytic processing was reported to be regulated by PKC and Ca²⁺ signaling, consistent with the present findings. Thus, AT₂–mediated HB-EGF release followed by EGFR transactivation is a key molecule to induce both Ang2 and VEGF expression in CMECs. Li et al reported that AT₁ caused apoptosis in human CMECs, whereas AT₂ had no effect. Because suppression of apoptosis is important for angiogenesis, our present observation appears to contrast with the report by Li et al. Considering that VEGF, which is known as a strong antiapoptotic ligand, was induced by Ang II in rat CMECs (Figure 5) and that HB-EGF caused angiogenesis via induction of VEGF, it is plausible that the extent of Ang II–mediated HB-EGF release is greater in rat CMECs than in human CMECs and the resultant greater production of VEGF inhibits AT₁-mediated apoptotic change. Because the AT₁ numbers are much more in rat CMECs (≈60-fold) than those in human CMECs, the difference in the receptor numbers may account for the lack of AT₁ action in human CMECs.

There is evidence that AT₁ antagonizes AT₂–mediated ERK activation by activating PTP or serine/threonine phosphatases in a cell type–dependent manner. In this study, we found for the first time that AT₁ abolished AT₂–mediated EGFR phosphorylation in a PTP-dependent manner and attenuated the

and Tie2; (3) induction of Ang2 and VEGF expression is mediated mainly by binding to EGFR of HB-EGF processed and released via AT₁ signaling; (4) AT₂ attenuates EGFR phosphorylation by activating PTP; and (5) AT₁ induces corneal capillary formation via combined effects of Ang2 and VEGF, in which Ang2 enhances angiogenic activity of VEGF, whereas AT₂ attenuates angiogenesis by inhibiting EGFR phosphorylation.

Ang II was shown to augment angiogenesis and cause upregulation of HB-EGF expression. In this study, we found that neutralizing anti–HB-EGF antibody abolished Ang II–induced Ang2 and VEGF expression and that inhibition of HB-EGF processing abolished Ang II–mediated angiogenesis, indicating that HB-EGF is a key molecule mediating Ang II signals toward angiogenesis. Interestingly, we also found that the Tie2-Fc containing ectodomain of Tie2 attenuated Ang II–induced angiogenesis. Previous studies have shown
angiogenic activity by AT$_1$. In the pathological condition in which the renin-angiotensin system is activated in capillary vessels, the treatment with AT$_1$ antagonists may potentially block Ang II–induced angiogenesis by enhancement of anti-angiogenic action via AT$_2$ as well as inhibition of AT$_1$-mediated angiogenic activity. Thus, these findings suggest that AT$_1$ antagonists are useful for the inhibition of retinal angiogenesis in diabetic or hypertensive patients; however, they may attenuate collateral vessel formation in ischemic myocardium. Although further analyses on the clinical level would be required to prove this hypothesis, the novel link between Ang II receptors and the Ang–Tie2 system should be more considerable in the clinical setting in which angiogenesis occurs.

Acknowledgments

This study was supported in part by research grants from the Ministry of Education, Science and Culture of Japan; Study Group of Molecular Cardiology; Japan Medical Association; Japan Smoking Foundation; and Japan Heart Foundation.

References


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Circ Res. 2001;88:22-29
doi: 10.1161/01.RES.88.1.22

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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The American Heart Association (AHA) published an expression of concern\textsuperscript{1-3} simultaneously in \textit{Circulation}, \textit{Circulation Research}, and \textit{Hypertension}, and we have now been notified by Kyoto Prefectural University of Medicine that certain data and figures in these 5 articles were falsified. Kyoto Prefectural University of Medicine sends their deepest apologies to the academic community at large.

The AHA is hereby retracting these articles:


References


This Notice of Retraction is copublished in \textit{Circulation}, \textit{Circulation Research}, and \textit{Hypertension}. \textit{(Circ Res.} 2013;112:e180.)

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\textit{Circulation Research} is available at http://circres.ahajournals.org

DOI: 10.1161/RES.0b013e31829b5cca