Role of Epidermal Growth Factor Receptor and Endoplasmic Reticulum Stress in Vascular Remodeling Induced by Angiotensin II

Takehiko Takayanagi, Tatsuo Kawai, Steven J. Forrester, Takashi Obama, Toshiyuki Tsuji, Yamato Fukuda, Katherine J. Elliott, Douglas G. Tilley, Robin L. Davisson, Joon-Young Park, Satoru Eguchi

Abstract—The mechanisms by which angiotensin II (AngII) elevates blood pressure and enhances end-organ damage seem to be distinct. However, the signal transduction cascade by which AngII specifically mediates vascular remodeling such as medial hypertrophy and perivascular fibrosis remains incomplete. We have previously shown that AngII-induced epidermal growth factor receptor (EGFR) transactivation is mediated by disintegrin and metalloproteinase domain 17 (ADAM17), and that this signaling is required for vascular smooth muscle cell hypertrophy but not for contractile signaling in response to AngII. Recent studies have implicated endoplasmic reticulum (ER) stress in hypertension. Interestingly, EGFR is capable of inducing ER stress. The aim of this study was to test the hypothesis that activation of EGFR and ER stress are critical components required for vascular remodeling but not hypertension induced by AngII. Mice were infused with AngII for 2 weeks with or without treatment of EGFR inhibitor, erlotinib, or ER chaperone, 4-phenylbutyrate. AngII infusion induced vascular medial hypertrophy in the heart, kidney and aorta, and perivascular fibrosis in heart and kidney, cardiac hypertrophy, and hypertension. Treatment with erlotinib as well as 4-phenylbutyrate attenuated vascular remodeling and cardiac hypertrophy but not hypertension. In addition, AngII infusion enhanced ADAM17 expression, EGFR activation, and ER oxidative stress in the vasculature, which were diminished in both erlotinib-treated and 4-phenylbutyrate–treated mice. ADAM17 induction and EGFR activation by AngII in vascular cells were also prevented by inhibition of EGFR or ER stress. In conclusion, AngII induces vascular remodeling by EGFR activation and ER stress via a signaling mechanism involving ADAM17 induction independent of hypertension. (Hypertension. 2015;65:1349-1355. DOI: 10.1161/HYPERTENSIONAHA.115.05344.) • Online Data Supplement

Key Words: angiotensin II • fibrosis • hypertension • hypertrophy • muscle, smooth, vascular • signal transduction

The renin angiotensin system has been strongly implicated in hypertension and its complications. Importantly, it has been suggested that the mechanisms by which angiotensin II (AngII) elevates blood pressure and enhances end-organ damage may be distinct.1 Vascular remodeling associated with hypertension has been strongly implicated in end-organ damage and associated with poor cardiovascular outcomes.2,3 The remodeling predisposes to end-organ damage and pharmacological intervention in vascular remodeling should have special clinical efficacy for prevention of hypertensive complications.2,3 However, the exact signal transduction cascade by which AngII mediates vascular remodeling such as medial hypertrophy and perivascular fibrosis remains insufficiently understood. Therefore, the rationale of the present study is to explore the signal transduction mechanism of AngII required for the initiation of vascular remodeling but not hypertension and to test its functional relevance to seek a novel treatment for hypertensive complications.

AngII mediates vascular smooth muscle cell (VSMC) contraction via Gq-mediated intracellular Ca2+ elevation and G12/13-mediated Rho kinase activation.4 We have shown in vitro that Gq- and disintegrin and metalloproteinase domain 17 (ADAM17)–mediated epidermal growth factor receptor (EGFR) trans-activation via heparin-binding EGF-like growth factor shedding is required for extracellular signal–regulated kinase activation and VSMC hypertrophy but not for intracellular Ca2+ elevation or Rho kinase activation.5-7 Also, EGFR activity and ADAM17 expression are enhanced in the
Figure 1. Effects of epidermal growth factor receptor (EGFR) inhibitor, erlotinib, on cardiovascular remodeling induced by angiotensin II (AngII). C57Bl/6 mice were infused with saline (n=8) for 2 weeks, or AngII (1 μg/kg per minute) for 2 weeks with (n=8) or without (n=8) treatment of erlotinib (10 mg/kg per day IP injection). Hearts and kidneys were stained with Sirius red and aortas were stained with Masson trichrome (mean±SEM). A, Representative staining (×200) is presented. B, Quantification of medial area to internal arterial area of the coronary and renal arteries and quantification of perivascular fibrosis area to vascular area of these arteries. C, Quantification of medial thickness of the thoracic aorta. D, Heart weight (HW) body weight (BW) ratio. E, Mean arterial pressure (MAP) was evaluated by telemetry. F, Heart sections were immunostained with antibodies as indicated (n=4). Antibodies against Lys-Asp-Glu-Leu (KDEL) and CCAAT/enhancer-binding protein homologous protein (CHOP) were used to assess endoplasmic reticulum stress. Antibody against nitrotyrosine (nTyr) was used to assess oxidative stress. *P<0.05 compared with control saline infusion. †P<0.05 compared with AngII infusion.
neointima after angioplasty, and dominant-negative ADAM17 gene transfer prevents the EGFR activation and neointimal hyperplasia. Others have shown that the EGFR activation mediates AngII-induced reactive oxygen species generation in VSMCs, and EGFR antisense or ADAM17 interfering RNA can suppress AngII-induced cardiac hypertrophy. Data from mice having mutant EGFR further support the role of EGFR in AngII-associated cardiac remodeling. However, whether an EGFR inhibitor such as erlotinib used for human cancer treatments has therapeutic potential against hypertensive vascular remodeling remains unclear.

Literature increasingly suggests that prolonged ER stress and the subsequent unfolded protein response likely contribute to the development and progression of cardiovascular diseases such as heart failure and atherosclerosis. Although the downstream consequences of prolonged ER stress generally involve unfolded protein response–specific gene programs, ER stress seems critical for enhancement of reactive oxygen species in many organ and cell systems including VSMCs. AngII has been shown to enhance ER stress in vitro and in vivo, potentially mediating enhancement of oxidative stress and subsequent target organ damage. Supplementation of ER chaperone 4-phenylbutyrate (PBA) prevented AngII-induced vascular hypertrophy, perivascular fibrosis, and cardiac hypertrophy markedly (but not fully except in renal arterial fibrosis) but not hypertension (Figure 3; Tables S1 and S2). In addition, AngII-induced vascular ADAM17 induction, EGFR activation, and enhancement of ER/oxidative stress were prevented by PBA treatment (Figure S3). PBA inhibited AngII-induced ADAM17 protein expression and promoter activity in cultured VSMCs (Figure 4A; Figure S2B). Gene-transfer of ER chaperone, 78-kDa glucose-regulated protein (78-kDa GRP), into the subfornical organ of mice having mutant EGFR further support the role of AngII in AngII-associated cardiac remodeling. However, whether an EGFR inhibitor such as erlotinib used for human cancer treatments has therapeutic potential against hypertensive vascular remodeling remains unclear.

To test for the role of ER stress in AngII-induced vascular remodeling, AngII-infused mice were treated with a chemical ER chaperone, 4-phenylbutyrate (PBA). PBA prevented AngII-induced vascular hypertrophy, perivascular fibrosis, and cardiac hypertrophy markedly (but not fully except in renal arterial fibrosis) but not hypertension (Figure 3; Tables S1 and S2). In addition, AngII-induced vascular ADAM17 induction, EGFR activation, and enhancement of ER/oxidative stress were prevented by PBA treatment (Figure S3). PBA inhibited AngII-induced ADAM17 protein expression and promoter activity in cultured VSMCs (Figure 4A; Figure S2B). Gene-transfer of ER chaperone, 78-kDa glucose-regulated protein, inhibited AngII-induced ADAM17 protein expression and promoter activity in cultured VSMCs (Figure 4A; Figure S2B). Gene-transfer of ER chaperone, 78-kDa glucose-regulated protein, inhibited AngII-induced ADAM17 protein expression and promoter activity in cultured VSMCs (Figure 4A; Figure S2B). Gene-transfer of ER chaperone, 78-kDa glucose-regulated protein, inhibited AngII-induced ADAM17 protein expression and promoter activity in cultured VSMCs (Figure 4A; Figure S2B).

Methods

Extended Methods are provided in the online-only Data Supplement.

Results

To test for the role of EGFR activation in AngII-induced vascular remodeling, AngII-infused mice were treated with or without an EGFR kinase inhibitor, erlotinib. Two weeks of AngII infusion in control mice caused vascular medial hypertrophy in coronary arteries, renal arteries, and aortas that was markedly (but not completely) prevented in mice treated with erlotinib. Perivascular fibrosis in coronary and renal arteries induced by AngII infusion was partially or completely prevented in erlotinib-treated mice, respectively (Figure 1A–1C). AngII-induced cardiac hypertrophy as assessed by heart weight:body weight ratio (Figure 1D) and echocardiogram (Table S1 in the online-only Data Supplement) was partially or completely attenuated in erlotinib-treated mice, respectively. In contrast, hypertension was induced in both nontreated and erlotinib-treated mouse groups infused with AngII (Figure 1E; Table S2). In addition, body weight and heart rate remained the same among the 3 animal groups (Table S2).

The AngII-induced vascular remodeling in control mice was associated with vascular EGFR activation, ER stress, and oxidative stress assessed by immunohistochemistry. These AngII responses were markedly attenuated in mice treated with erlotinib (Figure 1F; Figure S1). We were unable to assess active EGFR in the myocyte area of the heart because of lack of any significant staining in response to AngII infusion. ADAM17 expression was barely detectable in heart tissue but was significantly induced on AngII infusion in the coronary arteries. No such induction was seen in mice treated with erlotinib. Erlotinib also inhibited EGFR activation, 78-kDa glucose-regulated protein induction, ADAM17 protein induction, and promoter activation in VSMCs stimulated with AngII (Figure 2; Figure S2A).

To test for the role of ER stress in AngII-induced vascular remodeling, AngII-infused mice were treated with a chemical ER chaperone, 4-phenylbutyrate (PBA). PBA prevented AngII-induced vascular hypertrophy, perivascular fibrosis, and cardiac hypertrophy markedly (but not fully except in renal arterial fibrosis) but not hypertension (Figure 3; Tables S1 and S2). In addition, AngII-induced vascular ADAM17 induction, EGFR activation, and enhancement of ER/oxidative stress were prevented by PBA treatment (Figure S3). PBA inhibited AngII-induced ADAM17 protein expression and promoter activity in cultured VSMCs (Figure 4A; Figure S2B). Gene-transfer of ER chaperone, 78-kDa glucose-regulated protein, inhibited AngII-induced ADAM17 protein expression and promoter activity in cultured VSMCs (Figure 4A; Figure S2B). Gene-transfer of ER chaperone, 78-kDa glucose-regulated protein, inhibited AngII-induced ADAM17 protein expression and promoter activity in cultured VSMCs (Figure 4A; Figure S2B). Gene-transfer of ER chaperone, 78-kDa glucose-regulated protein, inhibited AngII-induced ADAM17 protein expression and promoter activity in cultured VSMCs (Figure 4A; Figure S2B).
expression (Figure 4B). PBA also attenuated AngII-induced EGFR activation in VSMCs (Figure S2C).

**Discussion**

The major finding of the present study is that AngII-induced vascular hypertrophy and perivascular fibrosis were attenuated in mice treated with inhibitors of EGFR and ER stress, and that these protective effects were independent of hypertension. The suppression of AngII-induced vascular hypertrophy in erlotinib-treated mice is in line with our past in vitro observations that genetic ADAM17 silencing or inhibition of EGFR transactivation prevented the hypertrophic responses in cultured VSMCs. Moreover, mice with sm22α-promoter–dependent EGFR silencing have less baseline arterial wall to lumen ratio while blood pressure increases to the same extent as wild type on acute AngII infusion, thus supporting the role of EGFR in vascular hypertrophy. It is intriguing that pharmacological EGFR inhibition also prevented perivascular fibrosis induced by AngII, as low expression of ADAM17 under normal conditions and enhanced expression in areas of interstitial fibrosis damaged human kidneys have been reported. In addition, AngII-induced renal interstitial fibrosis can be inhibited in proximal tubule–specific EGFR null mice or with erlotinib treatment, and cardiac-specific heparin-binding EGF-like growth factor transgenic mice develop cardiac fibrosis. In our control model of 2-week AngII infusion, interstitial fibrosis within the heart was too marginal to be quantitatively evaluated. However, it is likely that the paracrine production of heparin-binding EGF-like growth factor and activation of EGFR via activation of ADAM17 in VSMCs, as well as other cell types, may be critical for the development of overall tissue fibrosis associated with hypertension.

The present study demonstrating predominantly vascular ADAM17 induction and EGFR activation suggests a vascular contribution to cardiac hypertrophy via EGFR transactivation induced by AngII. It should be noted that vascular smooth muscle (but not cardiac myocyte)–targeted Gq inhibition attenuates cardiac hypertrophy in AngII-infused mice. However, cardiac myocyte–targeted expression of dominant-negative EGFR inhibited cardiac hypertrophy induced by...
inhibitors, albeit using distinct inhibitors and administrative routes. Therefore, further research is needed to clarify the roles of cell type–specific EGFR transactivation induced by distinct G protein–coupled receptors in cardiac pathophysiology such as those using both cardiomyocyte and VSMC EGFR-deficient mice.

Although ER stress has been implicated in cardiovascular diseases, limited information has been available about its role in hypertension and associated complications. It has been recently reported that ER stress inhibitors attenuate AngII-induced aortic apoptotic and fibrotic responses in rats. Our data further suggest a potential prevention of hypertensive cardiovascular remodeling (but not hypertension) by reducing ER stress through inhibition of the ADAM17/EGFR axis of AngII signal transduction. However, PBA treatment was reported to attenuate hypertension in AngII-infused mice. Brain-selective treatment of ER stress inhibitor, tauroursodeoxycholic acid, also attenuates AngII-induced hypertension in mice. It is possible that the antihypertensive effect of PBA might be overridden in the present study because a higher concentration of AngII was infused. In addition, mechanical stretch of VSMCs, which is enhanced during hypertension, may lead to EGFR activation and ER stress in these cells. However, this may not be the case in our study because hypertension was unaltered with either treatment. It should also be noted that most of the remodeling assessments were not completely inhibited by erlotinib or PBA. As EGFR activation or ER stress was completely suppressed by the corresponding inhibitor, respectively, it is likely that there is a minor but still important signaling mechanism causing cardiovascular remodeling independently of the EGFR/ER stress cascade.

Inhibition of ADAM17 induction with EGFR inhibition or ER stress inhibition suggests the presence of a feed-forward ADAM17 signal amplification, which seems to involve transcriptional upregulation of ADAM17. Indeed, the ADAM17 promoter has functional ER stress responsible elements. We have recently demonstrated that ADAM17 mRNA induction in aorta in response to AngII infusion was inhibited by erlotinib treatment. Hypoxia-inducible factor-1α seems to mediate ADAM17 promoter activation by AngII, and AngII is reported to activate hypoxia-inducible factor-1α through EGFR transactivation in VSMCs. ER stress may also be involved in hypoxia-inducible factor-1α activation as reported in the vascular endothelial growth factor promoter. Thus, AngII induction of ADAM17 via EGFR activation may involve downstream signal crosstalk between hypoxia-inducible factor-1α and ER stress.

It has been reported that activation of vascular EGFR or ER stress causes endothelial cell dysfunction, which could be important to enhance vascular remodeling in response to AngII. Aldosterone antagonism also prevents AngII-dependent vascular and cardiac fibrosis. Although the role of EGFR in aldosterone-induced cardiovascular remodeling remains controversial, it will be interesting to test the causal role of ER stress in aldosterone-induced vascular remodeling in the future.

**Perspectives**

EGFR signal transduction seems to be essential for cardiovascular remodeling associated with ER/oxidative stress but...
not for hypertension in mice with AngII infusion. The signal seems to include a feed-forward mechanism involving vascular ADAM17 induction via ER stress acting on its gene promoter, which enhances EGFR ligand production and subsequent EGFR activation and vascular remodeling (Figure S4). ER stress also causes reactive oxygen species generation, enhancing EGFR activation. Additional research in this cascade is warranted to seek for alternative or additive treatments against hypertension and its complications.

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Disclosures

None.

References

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EGF Receptor Mediates Vascular Remodeling


**Novelty and Significance**

**What Is New?**

- Analyses of blood pressure and vascular pathology in the heart, kidney, and aorta with intervention established a role for epidermal growth factor receptor (EGFR) and endoplasmic reticulum stress in angiotensin II–induced pathological vascular remodeling independent of hypertension in mice.
- The concept of the feed-forward induction of vascular disintegrin and metalloproteinase domain 17 (ADAM17) to amplify the EGFR pathway and subsequent vascular remodeling was presented.

**What Is Relevant?**

- Results indicating prevention of vascular remodeling but not hypertension by erlotinib or 4-phenylbutyrate provide a foundation to seek a potential add-on therapy to current pressure-lowering treatments for hypertension.
- The vascular restricted EGFR signal transduction highlights the importance of vascular pathology for subsequent tissue dysfunction in hypertension.

**Summary**

In angiotensin II–infused mice, vascular hypertrophy and perivascular fibrosis were prevented by pharmacological inhibition of EGFR activity and endoplasmic reticulum stress. Angiotensin II infusion showed vascular ADAM17 induction, EGFR activation, and endoplasmic reticulum stress, which were attenuated by respective inhibitors. Cultured vascular cells were used to confirm the potential feed-forward mechanism of ADAM17 induction through transcriptional activation.
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Online Supplemental Methods and Figures

Role of Epidermal Growth Factor Receptor and Endoplasmic Reticulum Stress in Vascular Remodeling Induced By Angiotensin II

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Methods

Animal Studies and the Tissue Analysis

Animal procedures were performed in accordance with National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and Temple University IACUC guidelines. 8-10 week old male C57Bl/6 mice (Jackson) were infused with AngII (Bachem, 1 µg/kg/min) for 2 weeks via osmotic mini-pump with or without treatment of erlotinib (OSI Pharmaceuticals: provided by Genentech) which was solubilized in 20% captisol (Ligand Technology), 10 mg/kg/day intraperitoneal injection, or 4-phenylbutyrate (PBA) provided by Catherine P. Peklak, Scandinavian Formulas, 1 g/kg/day in drinking water. Blood pressure and heart rate were evaluated in the conscious state at day 14 by telemetry (DSI equipped with ADInstrument 6 software) via carotid catheter (PA-C10 transmitter). Cardiac function was measured using VisualSonics Velvo 2100 (M-mode). Extracted hearts, kidneys and aortas were fixed and used for histological studies as described previously \(^1\). To evaluate vascular hypertrophy and perivascular fibrosis in hearts and kidneys, serial cross-sections (5 µm thick) were stained in Sirius Red (EMS, Hatfield PA). Briefly, after de-paraffinization and re-hydration, sections were stained in equal parts Weigert’s Iron Hematoxylin A and B (EMS, Hatfield PA) for 10 min at room temperature. Sections were then washed twice in distilled water for 3 min per wash. Sirius Red was added for 1 h at room temperature. Slides were washed twice in 0.01N HCl for 3 min per wash. Sections were then dehydrated and penetrated using ethanol and xylene, respectively. Images were visualized on an Olympus IX81 inverted microscope using a Photometrics CoolSNAP HQ CCD camera and were acquired with SPOT 4.7 Basic software. Analysis was conducted using ImageJ 1.49g software (http://rsb.info.nih.gov/ij).

To calculate vascular hypertrophy, the value of medial area was divided by the true area of the vessel. True area was calculated by vessel outer perimeter\(^2\) divided by \(4\pi\). The value generated was the area of the vessel in true circular form. To calculate perivascular fibrosis, the value of fibrosis area was subtracted from vessel area and divided by the true area of the vessel. In total, 6-8 randomly selected samples per group were used for analysis. 3 representative vascular images were analyzed per
sample. To evaluate medial hypertrophy of aortas, Masson trichrome staining was performed to distinguish medial area from adventitia in the thoracic aortas. Briefly, after de-paraffinization and re-hydration, sections were incubated with Bouin’s fluid for 1 h at 56°C. Sections were washed three times in distilled water for 3 min per wash and then incubated with Working HE solution for 7.5 min followed by washing in distilled water for 30 sec. Sections were then incubated with Biebrich Scarlet-Acid Fuchsin solution for 1 h at 56°C. After incubation with phosphotungstic-phosphomolybdic acid solution for 5 min, sections were stained with Aniline Blue stain solution for 5 min. Sections were washed in 1% acetic acid for 30 sec and distilled water for 30 sec. Sections were then dehydrated and penetrated using ethanol and xylene, respectively. Medial hypertrophy was quantified by measurements of medial thickness in 4 randomly-selected locations per slide. 3 representative vascular images were analyzed per sample. Adventitia of the aorta was not quantified as the area was occasionally damaged or removed during the dissection. For immunohistochemistry (IHC), serial cross-sections were deparaffinized and blocked in 5% goat serum and 1% BSA for 1h at room temperature, incubated with primary antibody in PBS containing 1% BSA and 0.1% Tween 20 for 18 h at 4 °C, followed by biotinylated secondary antibody for 90 min at room temperature. Slides were incubated with avidin–biotin peroxidase complex for 30 min at room temperature and staining was visualized with the substrate diaminobenzidine (Vector) producing a brown color and counterstained with haematoxylin. An equal concentration of control IgG was used side-by-side with each antibody to ensure staining specificity. Quantification of the antibody staining was performed as reported previously with subtraction of the IgG background staining. All images were visualized on a Photometrics CoolSNAP HQ digital camera and were acquired with SPOT 4.7 Basic software using the same exposure time. Images were loaded into the ImageJ program for analysis. A vascular region of interest was drawn around the coronary arteries with the freehand selection tool. Adventitia was excluded from the quantification, since the adventitial areas were quite limited in the arteries, except those with AngII infusion alone. All images were set to the same hue, saturation and brightness. The area and intensity (optical density) in the region of interest were then measured and analyzed. Data were obtained from 4 mice in each group with 3 to 4 non-overlapping high power
fields for each antibody.

**Cell Culture and Experiments**
VSMCs were prepared from thoracic aorta of male Sprague-Dawley rats by the explant method as described previously. VSMCs were subcultured in DMEM containing 10% fetal bovine serum, penicillin and streptomycin. Cells from passage 3 to 10 at 80~90% confluence were made quiescent by incubation with serum-free medium for 2-3 days. To avoid any potential phenotypic alteration, VSMCs were renewed every 2-3 months and VSMCs from frozen stock were never used. The results were confirmed in at least 2 distinct cell preparations. Immunoblotting (IB) was performed as previously described. Quiescent VSMCs grown on 6-well plates were stimulated with AngII (Sigma) for specified durations. The reaction was terminated by the replacement of medium with 100 µL of 1xSDS lysis buffer. 40 µL of the cell lysates were subjected to SDS-PAGE gel electrophoresis and electrophoretically transferred to a nitrocellulose membrane. The membranes were then exposed to primary antibodies overnight at 4 °C. After incubation with the peroxidase linked secondary antibody for 1 h at room temperature, immunoreactive proteins were visualized using a chemiluminescence reaction kit. *ADAM17* promoter assay was performed as we have reported previously with a modification to replace the luciferase expression vector with an adenovirus encoding the luciferase cassette for VSMC transduction. Recombinant adenoviral vector encoding mouse GRP78 cDNA was obtained from Iowa Gene Vector Core. VSMCs were infected with adenovirus as reported previously.

**Antibodies**
Antibody against Tyr-phosphorylated EGFR for IHC (2234) was purchased from Cell Signaling. Antibody against Tyr-phosphorylated EGFR for IB (44788G) was purchased from Invitrogen. Antibody against KDEL for detection of an ER stress marker GRP78 (ADI-SPA-827) was purchased from Enzo Life Sciences. Antibodies against ADAM17 for IB (sc-13973), EGFR (sc-03) and an ER stress marker, CHOP-10/GADD-153 (sc-575) were purchased from Santa Cruz Biotechnology. Antibodies against ADAM17 for IHC (ab39163) were purchased from Abcam. Antibodies against
GAPDH (MAB374) and an oxidative stress marker, nitro-tyrosine (06-284) was purchased from Millipore.

**Statistical Analysis**
Data were presented as mean±SEM. Differences between the multiple groups were analyzed by 1-way or 2-way ANOVA, followed by the Tukey’s post hoc test. Statistical significance was taken at p<0.05.

**Reference**


Table S1. M-mode echocardiography at 2 weeks after AngII infusion

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<tr>
<th>Measure</th>
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<th>AngII+Erlo</th>
<th>AngII+PBA</th>
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<td>IVSd (mm)</td>
<td>0.668±0.052</td>
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<td>0.675±0.045†</td>
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<td>LVIDd (mm)</td>
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<td>3.49±0.24</td>
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<td>LVPWd (mm)</td>
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<td>FS (%)</td>
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Mean±SEM (n=8), p<0.001 compared with saline* or AngII† infusion.  IVSd: interventricular septum thickness in diastole; LVIDd: LV internal diameter in diastole; LVPWd: LV posterior wall thickness in diastole; LVIDs: LV internal diameter in systole; FS: fractional shortening.

Table S2. Effects of Erlotinib and PBA on characteristics of mice infused with AngII

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<th>AngII+PBA</th>
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<td>BW (g)</td>
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<td>SBP/DBP (mmHg)</td>
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<td>172±9*/120±12*</td>
<td>169±16*/118±29*</td>
<td>163±3*/123±10*</td>
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<tr>
<td>HR (beats/min)</td>
<td>618±84</td>
<td>648±95</td>
<td>583±90</td>
<td>585±58</td>
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</table>

Mean±SEM (n=8), *p<0.001 compared with saline infusion.  BW: body weight; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate.
Figure S1. EGFR inhibitor Erlotinib attenuated ADAM17 induction and ER/oxidative stress induced by AngII in coronary arteries. Specific staining intensity at medial area in Figure 1F was quantified with subtraction of control IgG staining intensity of the corresponding area (n=4, means ± SEM), *p<0.05 compared with control saline infusion. †p<0.05 compared with AngII infusion.
**Figure S2.** Effects of EGFR inhibitor and ER stress inhibitor on ADAM17 promoter activation and EGFR activation induced by AngII in VSMCs. **A.** Rat VSMC infected with adenovirus encoding ADAM17 promoter-Luc construct (100 moi) were pretreated with or without Erlotinib (1 µmol/L for 30 min) and stimulated with AngII (100 nmol/L) for 24 h followed by measurement of cellular luciferase activity (means ± SEM, n=4). **B.** Rat VSMC infected with adenovirus encoding ADAM17 promoter-Luc construct (100 moi) were pretreated with or without PBA (10 mmol/L for 30 min) and stimulated with AngII (100 nmol/L) for 24 h followed by measurement of cellular luciferase activity (means ± SEM, n=4). **C.** Rat VSMCs were pretreated with or without PBA (10 mmol/L for 30 min) and were stimulated with AngII (100 nmol/L) for 18 h. The cell lysates were analyzed by immunoblotting as indicated (means ± SEM, n=4 in each group). *p<0.05 compared with basal. †p<0.05 compared with AngII stimulation.
Figure S3. ER stress inhibitor PBA attenuated ADAM17 induction, EGFR activation and ER/oxidative stress induced by AngII in coronary arteries. 8 week old C57Bl/6 mice were infused with AngII or saline for 2 weeks with or without treatment of PBA. Heart sections were immuno-stained with antibodies as indicated (n=4). Specific staining intensity at medial area in Supplemental Figure IIA was quantified with subtraction of control IgG staining intensity of the corresponding area (n=4, means ± SEM), *p<0.05 compared with control saline infusion. †p<0.05 compared with AngII infusion.
Figure S4. Potential feed-forward signaling mechanism by which AngII induces hypertensive vascular remodeling. Induction of ADAM17 expression by AngII via ER stress acting upon its gene promoter enhances EGFR ligand production and subsequent EGFR activation and vascular remodeling. ER stress also causes ROS generation, enhancing EGFR activation.