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

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Abstract

The mechanisms by which angiotensin II (AngII) elevates blood pressure and enhances end-organ damage appear 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 metalloprotease 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 was also prevented by inhibition of EGFR or ER stress. In conclusion, AngII induces vascular

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Disclosures

None.

remodeling by EGFR activation and ER stress via a signaling mechanism involving ADAM17 induction independent of hypertension.

Keywords

Hypertension; Angiotensin II; Vascular Smooth Muscle; Signal Transduction; Hypertrophy; Fibrosis

Introduction

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 in order to seek a novel treatment for hypertensive complications.

AngII mediates vascular smooth muscle cell (VSMC) contraction via G_q-mediated intracellular Ca^{2+} elevation and $G_{12/13}$-mediated Rho kinase activation[4]. We have shown *in vitro* that G_q- and metalloprotease ADAM17-mediated epidermal growth factor receptor (EGFR) “trans”-activation via heparin-binding EGF-like growth factor (HB-EGF) shedding is required for extracellular signal-regulated kinase activation and VSMC hypertrophy but not for intracellular Ca^{2+} elevation or Rho kinase activation[5–7]. Also, EGFR activity and ADAM17 expression are enhanced in the neointima after angioplasty, and dominant-negative ADAM17 gene-transfer prevents the EGFR activation and neointimal hyperplasia[8]. Others have shown that the EGFR activation mediates AngII-induced reactive oxygen species (ROS) generation in VSMCs[9], and EGFR antisense[10] or ADAM17 interfering RNA[11] can suppress AngII-induced cardiac hypertrophy. Data from mice having mutant EGFR further support the role of EGFR in AngII associated cardiac remodeling[12]. However, whether an EGFR inhibitor such as erlotinib utilized for human cancer treatments[13] has therapeutic potential against hypertensive vascular remodeling remains unclear.

Literature increasingly suggests that prolonged ER stress and the subsequent unfolded protein response (UPR) likely contribute to the development and progression of cardiovascular diseases such as heart failure and atherosclerosis[14, 15]. While the downstream consequences of prolonged ER stress generally involve UPR specific gene programs[16], ER stress appears critical for enhancement of ROS in many organ and cell systems including VSMCs[14, 17]. AngII has been shown to enhance ER stress *in vitro* and *in vivo*[18, 19], potentially mediating enhancement of oxidative stress and subsequent target organ damage.

Supplementation of ER chaperone 78-kDa glucose-regulated protein (GRP78) into the subfornical organ of the brain has been demonstrated to be effective in attenuating AngII-induced ER stress and hypertension[20]. In addition, recent evidence suggests a link between EGFR and ER stress[21].

Taking the above information together, we have tested our hypothesis that activation of EGFR and ER stress are critical components required for vascular remodeling but not hypertension induced by AngII utilizing mice treated with an EGFR inhibitor or ER stress inhibitor. Our findings support this hypothesis and further demonstrate a novel concept of ADAM17 induction by EGFR via ER stress, potentially amplifying hypertensive end-organ damage mediated by the renin angiotensin system.

Methods

Extended methods are provided in the online 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. 2 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 assessed by heart weight to body weight ratio (Figure 1D) and echocardiogram (Supplemental Table S1) was partially or completely attenuated in erlotinib-treated mice, respectively. In contrast, hypertension was induced in both non-treated and erlotinib-treated mouse groups infused with AngII (Figure 1E and Supplemental Table S2). In addition, body weight and heart rate remained the same among the three animal groups (Supplemental 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 and Supplemental Figure S1). We were unable to assess active EGFR in the myocyte area of the heart due to lack of any significant staining in response to AngII infusion. ADAM17 expression was barely detectable in heart tissue but was significantly induced upon AngII infusion in the coronary arteries. No such induction was seen in mice treated with erlotinib. Erlotinib also inhibited EGFR activation, GRP78 protein induction, ADAM17 protein induction, and promoter activation in VSMCs stimulated with AngII (Figure 2 and Supplemental 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 and Supplemental Table S1 and S2). In addition, AngII-induced vascular ADAM17 induction, EGFR

activation, and enhancement of ER/oxidative stress were prevented by PBA treatment (Supplemental Figure S3). PBA inhibited AngII-induced ADAM17 protein expression and promoter activity in cultured VSMCs (Figure 4A and and Supplemental Figure S2B). Gene-transfer of ER chaperone, GRP78, inhibited AngII-induced ADAM17 protein expression (Figure 4B). PBA also attenuated AngII-induced EGFR activation in VSMCs (Supplemental 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[5, 7]. Moreover, mice with sm22α-promoter dependent EGFR silencing have less base-line arterial wall to lumen ratio while blood pressure increases to the same extent as wild type upon acute AngII infusion[22], 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[23]. Additionally, AngII-induced renal interstitial fibrosis can be inhibited in proximal tubule specific EGFR null mice or with erlotinib treatment[24], and cardiac specific HB-EGF transgenic mice develop cardiac fibrosis[25]. 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 HB-EGF and activation of EGFR via activation of ADAM17 in VSMCs, as well as other cell types, may be critical for 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 G_q inhibition attenuates cardiac hypertrophy in AngII-infused mice[26]. However, cardiac myocyte-targeted expression of dnEGFR inhibited cardiac hypertrophy induced by AngII[12]. Cardiac specific deletion of an ER stress sensor, double stranded RNA-activated protein kinase R-like ER kinase exacerbates pressure overload-induced cardiac hypertrophy[27]. Thus, interdependence for vascular and cardiac hypertrophy is most likely. Although vascular EGFR-silenced mice develop cardiac hypertrophy and hypotension with aging[22], this phenotype could be due to cardiac EGFR silencing since the sm22α-promoter also drives significant deletion of EGFR in cardiomyocytes[22]. Indeed recent studies have reported a cardioprotective role for EGFR[28, 29]. However, partially contrasting findings were observed in mice regarding cardiac hypertrophy, contractility and apoptosis under the conditions of chronic isoproterenol infusion with co-treatment of EGFR inhibitors[28, 30], 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 utilizing both cardiomyocyte and VSMC EGFR-deficient mice.

Although ER stress has been implicated in cardiovascular diseases[14], 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[31]. 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[19, 32]. Brain-selective treatment of ER stress inhibitor, tauroursodeoxycholic acid, also attenuates AngII-induced hypertension in mice[20]. It is possible that the anti-hypertensive effect of PBA might be overridden in the present study since a higher concentration of AngII was infused. In addition, mechanical stretch of VSMCs, which is enhanced during hypertension, may lead to EGFR activation[33] and ER stress[34] in these cells. However, this may not be the case in our study since 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 from 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 up-regulation of ADAM17. Indeed, the ADAM17 promoter has functional ER stress responsible elements[35]. We have recently demonstrated that ADAM17 mRNA induction in aorta in response to AngII infusion was inhibited by erlotinib treatment[36]. Hypoxia inducible factor (HIF)-1α appears to mediate ADAM17 promoter activation by AngII[37], and AngII is reported to activate HIF-1α through EGFR transactivation in VSMCs[38]. ER stress may also be involved in HIF-1α activation as reported in the vascular endothelial growth factor promoter[39]. Thus, AngII induction of ADAM17 via EGFR activation may involve downstream signal crosstalk between HIF-1α and ER stress.

It has been reported that activation of vascular EGFR or ER stress causes endothelial cell dysfunction[19, 21], which could be very important to enhance vascular remodeling in response to AngII. Aldosterone antagonism also prevents AngII-dependent vascular and cardiac fibrosis[40]. While the role of EGFR in aldosterone-induced cardiovascular remodeling remains controversial[41], it will be interesting to test the causal role of ER stress in aldosterone-induced vascular remodeling in the future.

Perspectives

EGFR signal transduction appears 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 upon its gene promoter, which enhances EGFR ligand production and subsequent EGFR activation and vascular remodeling (Supplemental Figure S4). ER stress

also causes ROS generation, enhancing EGFR activation. Additional research in this cascade is warranted in order to seek for alternative or additive treatments against hypertension and its complications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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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 EGFR and ER stress in AngII-induced pathological vascular remodeling independent of hypertension in mice.
- The concept of the feed-forward induction of vascular 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 PBA 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 AngII-infused mice, vascular hypertrophy and perivascular fibrosis were prevented by pharmacological inhibition of EGFR activity and ER stress. AngII infusion showed vascular ADAM17 induction, EGFR activation and ER stress, which were attenuated by respective inhibitors. Cultured vascular cells were utilized to confirm the potential feed-forward mechanism of ADAM17 induction through transcriptional activation.

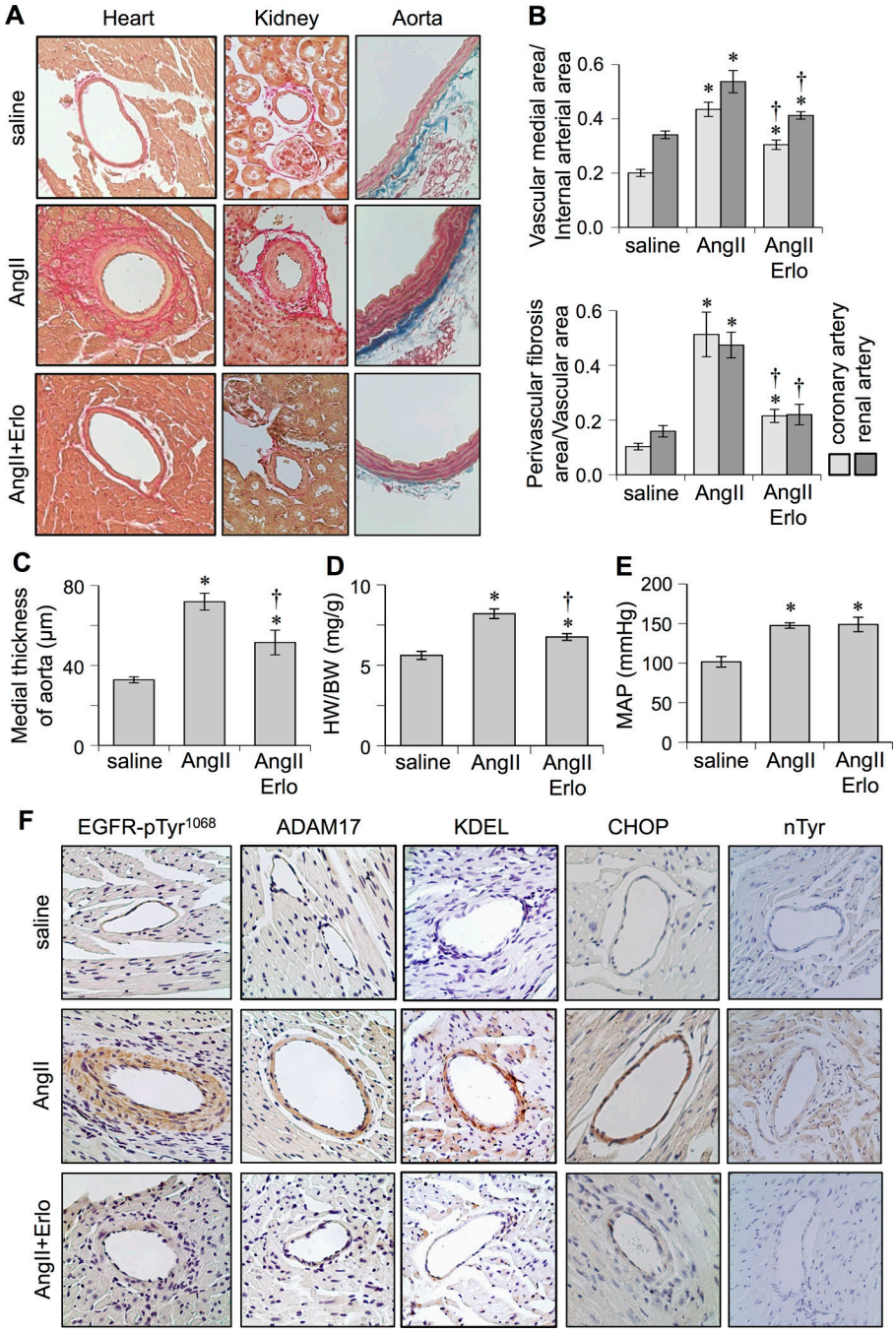

Figure 1.

Effects of EGFR inhibitor, erlotinib, on cardiovascular remodeling induced by AngII. C57Bl/6 mice were infused with saline (n=8) for 2 weeks, or AngII (1 μg/kg/min) for 2 weeks with (n=8) or without (n=8) treatment of erlotinib (10 mg/kg/day intraperitoneal injection). Hearts and kidneys were stained with Sirius red and aortas were stained with Masson trichrome (Mean±SEM). **A**: Representative staining (200x) 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 immuno-stained with antibodies as indicated (n=4). Antibodies against KDEL and CHOP were used to assess ER stress. Antibody against nitro-tyrosine (nTyr) was used to assess oxidative stress. *p<0.05 compared with control saline infusion. †p<0.05 compared with AngII infusion.

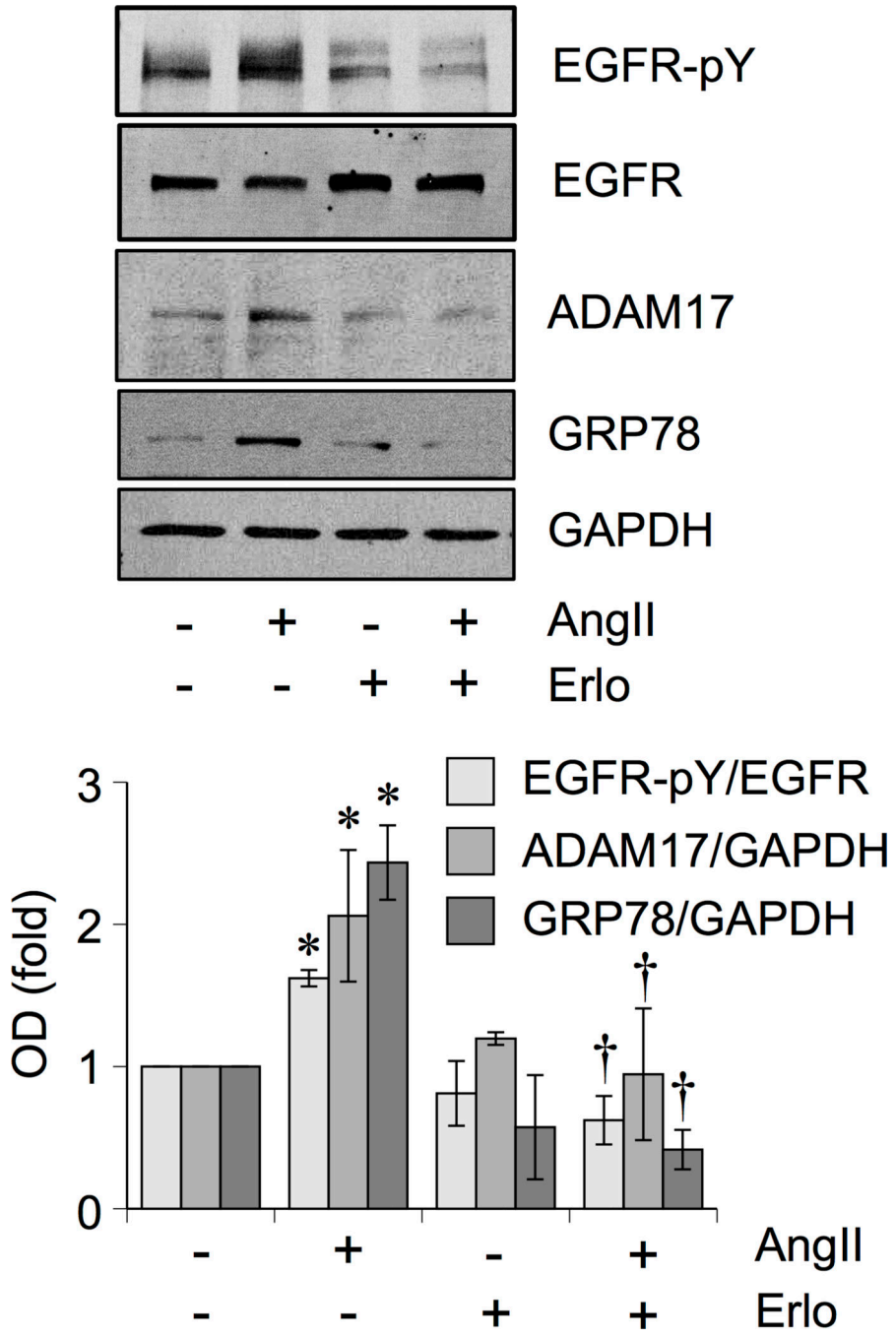

Figure 2.

EGFR inhibitor attenuated induction of ADAM17 and GRP78 in response to AngII in VSMCs. Rat VSMCs were pretreated with or without Erlotinib (1 μmol/L for 30 min) and stimulated with AngII (100 nmol/L) for 18 hours. 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.

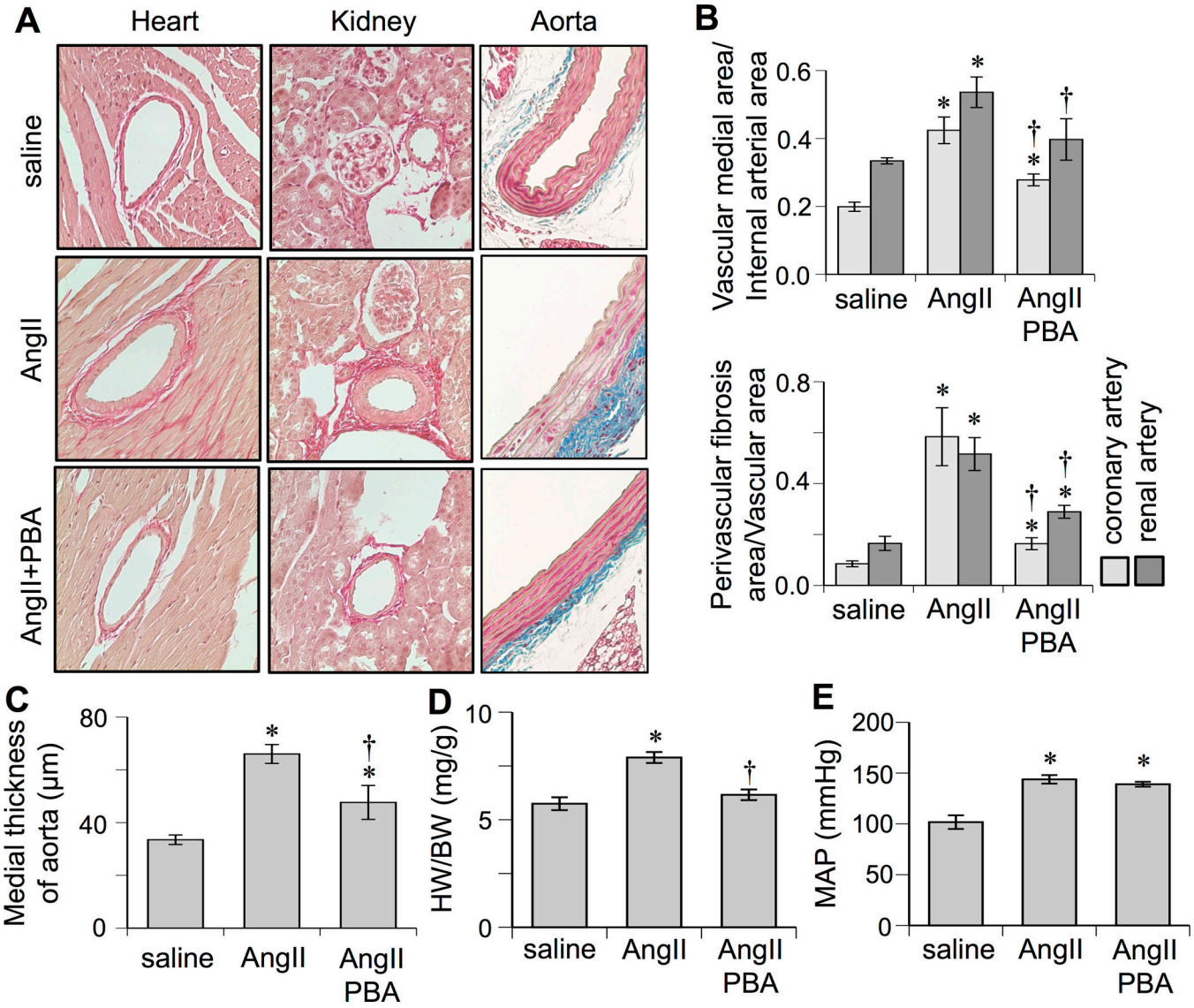

Figure 3.

Effects of ER stress inhibitor, PBA, on cardiovascular remodeling induced by AngII. C57Bl/6 mice were infused with saline (n=8) for 2 weeks, or AngII for 2 weeks with (n=6) or without (n=8) PBA treatment (1g/kg/day in drinking water). 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. *p<0.05 compared with control saline infusion. †p<0.05 compared with AngII infusion.

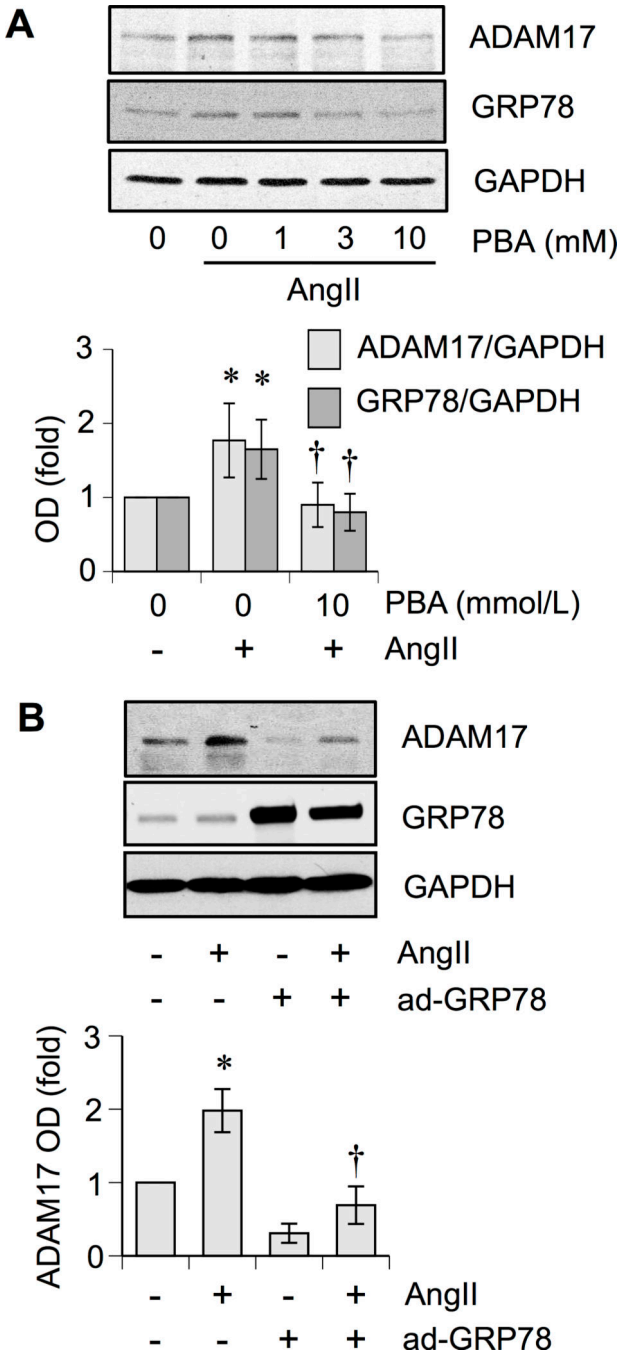

Figure 4.

Inhibition of ER stress attenuated AngII-induced ADAM17 induction in VSMCs. **A.** Rat VSMCs were pretreated with or without PBA (1 – 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). **B.** Rat VSMC infected with adenovirus encoding GRP78 or control LacZ (100 moi) were stimulated with AngII (100 nmol/L) for 24 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.