Vascular ADAM17 as a Novel Therapeutic Target in Mediating Cardiovascular Hypertrophy and Perivascular Fibrosis Induced by Angiotensin II

Takehiko Takayanagi,* Steven J. Forrester,* Tatsuo Kawai, Takashi Obama, Toshiyuki Tsuji, Katherine J. Elliott, Elisa Nuti, Armando Rossello, Hang Fai Kwok, Rosario Scalia, Victor Rizzo, Satoru Eguchi

See Editorial Commentary, pp 849–850

Abstract—Angiotensin II (AngII) has been strongly implicated in hypertension and its complications. Evidence suggests the mechanisms by which AngII elevates blood pressure and enhances cardiovascular remodeling and damage may be distinct. However, the signal transduction cascade by which AngII specifically initiates cardiovascular remodeling, such as hypertrophy and fibrosis, remains insufficiently understood. In vascular smooth muscle cells, a metalloproteinase ADAM17 mediates epidermal growth factor receptor transactivation, which may be responsible for cardiovascular remodeling but not hypertension induced by AngII. Thus, the objective of this study was to test the hypothesis that activation of vascular ADAM17 is indispensable for vascular remodeling but not for hypertension induced by AngII. Vascular ADAM17–deficient mice and control mice were infused with AngII for 2 weeks. Control mice infused with AngII showed cardiac hypertrophy, vascular medial hypertrophy, and perivascular fibrosis. These phenotypes were prevented in vascular ADAM17–deficient mice independent of blood pressure alteration. AngII infusion enhanced ADAM17 expression, epidermal growth factor receptor activation, and endoplasmic reticulum stress in the vasculature, which were diminished in ADAM17–deficient mice. Treatment with a human cross-reactive ADAM17 inhibitory antibody also prevented cardiovascular remodeling and endoplasmic reticulum stress but not hypertension in C57Bl/6 mice infused with AngII. In vitro data further supported these findings. In conclusion, vascular ADAM17 mediates AngII-induced cardiovascular remodeling via epidermal growth factor receptor activation independent of blood pressure regulation. ADAM17 seems to be a unique therapeutic target for the prevention of hypertensive complications. (Hypertension. 2016;68:949-955. DOI: 10.1161/HYPERTENSIONAHA.116.07620.) • Online Data Supplement

Key Words: end-organ damage ■ fibrosis ■ hypertension ■ renin angiotensin system ■ signal transduction

The prevalence and morbidity of hypertension is growing steadily.1 End-organ damage is the most important clinical consequence of hypertension causing cardiac failure, renal failure, and stroke. Despite great achievements in blood pressure therapy, optimally treated hypertensive patients still have a 50% greater risk than untreated normotensive subjects,2 suggesting the urgent need of add-on therapy to specifically target hypertensive end-organ damage. Vascular remodeling has been strongly implicated in hypertensive end-organ damage and associated with poor cardiovascular outcomes. The remodeling predisposes to end-organ damage, and pharmacological intervention in vascular remodeling should have special clinical efficacy for prevention of hypertensive organ damage.1 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 cardiovascular remodeling and end-organ damage may be distinct.4 Although many downstream signaling cascades and target genes/proteins of AngII have been identified, the proximal key event primarily responsible for vascular remodeling, such as vascular hypertrophy and fibrosis, independent of blood pressure, remains largely unclear.5,6 AngII mediates vascular smooth muscle cell (VSMC) contraction via Gα-mediated intracellular Ca2+ elevation and G12/13-mediated Rho kinase activation.7 ADAM (a disintegrin
ADAM17 gene transfer prevents neointimal hyperplasia.\textsuperscript{12} in neointima after angioplasty, and dominant-negative ADAM17flox/flox sm22 diovascular mortality.\textsuperscript{15} However, whether vascular ADAM17 kinase activation.\textsuperscript{9-11} Also, ADAM17 expression is enhanced in neointima and in the left ventricle on AngII infusion\textsuperscript{14} and that an ADAM17 polymorphism is associated with cardiovascular mortality.\textsuperscript{15} However, whether vascular ADAM17 manipulation has therapeutic potential against hypertensive complications remains completely unknown. Therefore, using mice lacking VSMC ADAM17 or treated with a human cross-reactive ADAM17 antibody as well as with in vitro fibrosis assay, we have tested our hypothesis that vascular ADAM17 is indispensable for cardiovascular remodeling but not for hypertension induced by AngII, thus, highlighting a unique therapeutic target in hypertension.

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. Eight- to 10-week-old male ADAM17\textsuperscript{flox/flox} sm22\textsuperscript{Cre\textsuperscript{-/-}} mice\textsuperscript{16} and control ADAM17\textsuperscript{flox/flox} sm22\textsuperscript{Cre\textsuperscript{+/-}} mice were infused with AngII (Bachem, 1 \( \mu \)g/kg/min) for 2 weeks via osmotic mini-pump.\textsuperscript{17} Eight- to 10-week-old male C57Bl6 mice (Jackson) were infused with AngII (Bachem, 1 \( \mu \)g/kg/min) and treated with human cross-reactive ADAM17 inhibitory antibody A98\textsuperscript{18} or control human IgG2 (Athens Research & Technology) which was solubilized in PBS, 10 mg/kg/d intraperitoneal injection, at day 1 and day 7. Blood pressure and heart rate were evaluated in the conscious state by telemetry (Data Sciences International equipped with ADInstrument 6 software) via carotid catheter (PA-C10 transmitter). Cardiac function was measured using VisualSonics Velvo 2100 (M-mode). Plasma B-type natriuretic peptide and blood urea nitrogen concentrations were determined by the ELA kits (RayBiotech Inc and Stanbio Laboratories, respectively). Extracted hearts, kidneys, and aortas were fixed and used for histological studies as described previously.\textsuperscript{19}

To evaluate vascular hypertrophy and perivascular fibrosis in hearts and kidneys, serial cross-sections (5-\( \mu \)m-thick) were stained in Sirius Red (EMS, Hatfield PA). Briefly, after deparaffinization and rehydration, sections were stained in equal parts Weigert’s Iron Hematoxylin A and B (EMS, Hatfield PA) for 10 minutes at room temperature. Sections were then washed twice in distilled water for 3 minutes per wash. Sirius Red was added for 1 hour at room temperature. Slides were washed twice in 0.01 N HCl for 3 minutes per wash. Sirius Red was added for 1 hour at 56°C. Sections were washed three times in distilled water for 3 minutes per wash and then incubated with Bouin’s fluid for 1 hour at 56°C. Sections were washed three times in distilled water for 3 minutes per wash and then incubated with Working HE solution for 7.5 minutes followed by washing in distilled water for 30 s. Sections were then incubated with Biebrich Scarlet-Acid Fuchsin solution for 1 hour at 56°C. After incubation with phoshphotungstic-phosphomolybdic acid solution for 5 minutes, sections were washed with Aniline Blue stain solution for 5 minutes. Sections were washed in 1% acetic acid for 30 s and in distilled water for 30 s. Sections were then dehydrated and penetrated using ethanol and xylene, respectively. Images were visualized on an Olympus IX81 inverted microscope using an Olympus SC30 high-resolution camera and were acquired with Olympus cellSens Entry 1.11 software. Analysis was conducted using ImageJ 1.50f software (http://rsb.info.nih.gov/ij).

To calculate vascular hypertrophy in the heart and kidney, the value of medial area was divided by the true area of the vessel. True area was calculated by vessel outer perimeter divided by \( \pi \). 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 to 8 randomly selected samples per group were used for analysis. Three representative vascular images were analyzed per sample. Medial hypertrophy of thoracic aorta was quantified by measurements of medial thickness in 4 randomly selected locations per slide. Three representative vascular images were analyzed per sample. Adventitia of the aorta was not quantified because the area was occasionally damaged or removed during the dissection.

For immunohistochemistry, serial cross-sections were deparaffinized and blocked in 5% goat serum and 1% BSA for 1 hour at room temperature, incubated with primary antibody in PBS containing 1% BSA and 0.1% Tween 20 for 18 hours at 4°C, followed by biotinylated secondary antibody for 90 minutes at room temperature. Slides were incubated with avidin–biotin peroxidase complex for 30 minutes at room temperature, and staining was visualized with the substrate diaminobenzidine (Vector) producing a brown color and counterstained with hematoxylin. An equal concentration of control IgG was used side-by-side with each antibody to ensure staining specificity.\textsuperscript{19} Quantification of the antibody staining was performed as reported previously with subtraction of the IgG background staining.\textsuperscript{19} All images were visualized on Olympus SC30 high-resolution camera and were acquired with Olympus cellSens Entry 1.11 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 because the adventitial areas were 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 nonoverlapping high-power fields for each antibody.

To evaluate ADAM17 mRNA expression, thoracic aortas and hearts were homogenized using BioMasher (Takara), and total RNA was extracted using TRIzol reagent (Invitrogen). cDNA was synthesized with RevertAid First Strand cDNA Synthesis Kit (Thermo). Quantitative real-time polymerase chain reaction was performed with SYBR Green qPCR Master Mix (Fermentas) as described previously.\textsuperscript{19} cDNA abundance was calculated by normalization to ribosome 18S. The primers used were ADAM17- forward GGC GCG GGA GGA AGT TT, reverse CGC CGC CTC ATG TTC CCG TC, ribosome 18S: forward AGT TCC AGC ACA TTT TGC GAG, reverse TCA TCC TCT GTG AGT TCT CCA.

Cell Culture and Experiments

VSMCs were prepared from thoracic aortas of male Sprague–Dawley rats by the explant method as described previously.\textsuperscript{21} VSMCs were subcultured in DMEM containing 10% fetal bovine serum, penicillin, and streptomycin. Cells from passage 3 to 10 at 80% to 90% confluence were passed at least 3 times to allow for cell growth. The cells were then detached from the dish and reseeded at the desired density. The cells were maintained in a humidified incubator at 37°C in a mixture of 5% CO\textsubscript{2} and 95% air. The medium was changed every 2 to 3 days. To avoid any potential phenotypic alteration, VSMCs were refreshed every 2 to 3 months, and VSMCs from frozen stock were not used. The results were confirmed in at least 2 distinct cell preparations.

Immunoblotting was performed as previously described.\textsuperscript{21} Quiescent VSMCs grown on 6-well plates were stimulated with 100 nmol/L AngII (Sigma) for specified durations. The reaction was terminated by the replacement of medium with 100 \( \mu \)L of 1xSDS lysis buffer. Forty microliter 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 hour at room temperature, immunoreactive proteins were visualized using a chemiluminescence reaction kit.

To evaluate probrifotic response, serum-starved VSMCs were stimulated with 100 mmol/L AngII for 48 hours, and extracellular cellular collagen content was quantified by Sirius Red collagen quantification kit (Chondrex) according to the manufacturer’s protocol. Recombinant adenoviral vector encoding rat ADAM17 siRNA was created, and specificity and efficiency has been reported. VSMCs were infected with 100 m.o.i. adenovirus before the AngII stimulation as reported previously. ADAM17-selective inhibitor compound no 21 (JG26) was generated as reported. VSMCs were pretreated with 1 μmol/L JG26 or vehicle (0.1% DMSO in final) for 30 minutes before the AngII stimulation. 4-Phenylbutyrate was obtained from Scandinavian Formulas and solubilized in DMEM. VSMCs were pretreated with 10 mmol/L 4-phenylbutyrate for 30 minutes. Erlotinib (OSI Pharmaceuticals) was obtained from Genentech. VSMCs were pretreated with 1 μmol/L erlotinib for 30 minutes.

**Antibodies**

Antibodies against Tyr1068-phosphorylated EGFR for immunohistochemistry (2234) and Ser10 phosphorylated eIF2α were purchased from Cell Signaling. Antibody against Tyr1068-phosphorylated EGFR for immunoblotting (44786G) was purchased from Invitrogen. Antibody against KDEL for detection of an endoplasmic reticulum (ER) stress marker GRP78 (ADI-SPA-827) was purchased from Enzo Life Sciences. Antibodies against ADAM17 for immunoblotting (sc-13973), EGFR (sc-03), and an ER stress marker, CHOP-10/GADD153 (sc-575), were purchased from Santa Cruz Biotechnology. Antibodies against ADAM17 for immunohistochemistry (ab59163) were purchased from Abcam. Antibodies against Cre recombinase (MAB3120) and GAPDH (MAB374) were purchased from Millipore.

**Statistical Analysis**

Data are presented as mean±SEM or SD where appropriate. Differences between the multiple groups were analyzed by 1-way or 2-way analysis of variance, followed by the Tukey’s post hoc test. Statistical significance was set at \( P<0.05 \).

**Results**

**Prevention of AngII-Induced Cardiovascular Remodeling in Mice Lacking VSMC ADAM17**

VSMC ADAM17-deficient ADAM17flox/flox sm22αCre−/− mice were generated, and vascular selective Cre expression was confirmed (Figure S1A in the online-only Data Supplement). VSMC ADAM17-deficient mice and littermate control ADAM17flox/flox sm22αCre−/− mice were infused with 1 μg/kg/min AngII for 2 weeks. In control littermate mice, 2 weeks of AngII infusion caused vascular hypertrophy in aorta and coronary arteries that was markedly prevented in VSMC ADAM17-deficient mice. Perivascular fibrosis induced by AngII infusion was also prevented in VSMC ADAM17–deficient mice. Cardiac hypertrophy in control mice was attenuated in VSMC ADAM17–deficient mice. Cardiac ADAM17 expression was barely detectable in heart or kidney but was significantly induced on AngII infusion in the vasculature. No such induction was observed in VSMC ADAM17–deficient mice (Figure 2; Figure S2). Quantitative polymerase chain reaction analysis of aortic mRNA confirmed ADAM17 induction by AngII as well as vascular ADAM17 silencing. ADAM17 mRNA was also increased in whole heart with AngII infusion in control mice but not in VSMC ADAM17–deficient mice (Figure S3). In addition, although statistically insignificant, cardiac ADAM17 mRNA expression tends to be less in saline-infused VSMC ADAM17–deficient mice compared with saline-infused control mice.

**Human Cross-Reactive ADAM17 Antibody Attenuates Cardiovascular Remodeling Induced by AngII**

To ascertain that ADAM17 represents a novel therapeutic target contributing to target organ remodeling, AngII-infused C57B16 mice were treated with a human cross-reactive ADAM17 inhibitory antibody A9B. C57B16 mice were

**Figure 1.** Prevention of cardiovascular remodeling in vascular smooth muscle cell (VSMC) ADAM17−/−deficient mice. VSMC ADAM17−/−deficient mice (A17f/fsmCre−/−) and control littermate mice (A17f/fsmCre+/−) were infused with angiotension II (AngII) or saline for 2 weeks. A, Tissues (heart and kidney) were stained with Sirius Red. Representative images are shown. Data are mean±SEM (n=5). B, Heart weight/body weight (HW/BW) ratio was calculated. Mean±SEM (n=8). C, Mean arterial blood pressure (MAP) was evaluated by telemetry. Mean±SEM (n=8). \( \ast \)P<0.05 compared with saline control. \( \dagger \)P<0.05 compared with AngII control.
infused with 1 μg/kg/min AngII for 2 weeks with treatment of ADAM17 antibody or control IgG (10 mg/kg IP on day 1 and 7). A9B8 prevented AngII-induced cardiovascular hypertrophy and perivascular fibrosis but not hypertension or its development. These responses were associated with suppression of ER stress (Figure 3; Figure S4 and Table S2A and S2B).

**ADAM17 Antibody, a Small Molecule ADAM17 Inhibitor or Adenovirus Encoding ADAM17 siRNA, Attenuates a Fibrotic Response Induced by AngII In Vitro**

To test for the role of ADAM17 in AngII-induced vascular fibrosis, extracellular collagen content was evaluated in VSMCs stimulated with AngII. Adenovirus encoding ADAM17 siRNA attenuated the AngII-induced enhancement in collagen content (Figure 4A and 4B). Treatment of VSMCs with ADAM17 antibody A9B8, a highly selective ADAM17 inhibitor JG26/compound 21, an EGFR kinase inhibitor erlotinib or a chemical ER chaperone 4-phenylbutyrate also attenuated AngII-induced enhancement in collagen content (Figure 4C; Figure S5A–S5C). As shown with the ADAM17 siRNA, A9B8 as well as JG26 inhibited AngII-induced EGFR transactivation assessed with an auto-phosphorylation site antibody (Figure 4D; Figure S5D).

**Discussion**

The major finding of the present study is that AngII-induced cardiovascular hypertrophy and perivascular fibrosis but not hypertension were attenuated in mice lacking VSMC ADAM17 expression, as well as in mice treated with an ADAM17 antibody. The suppression of AngII-induced...
EGFR activation and vascular hypertrophy in ADAM17-deficient mice is in line with our past in vitro observations that genetic ADAM17 inhibition or silencing prevents EGFR transactivation and subsequent hypertrophic responses in cultured VSMCs.9,11 It is intriguing that VSMC silencing of ADAM17 or pharmacological inhibition of ADAM17 under normal conditions and enhanced expression in areas of interstitial fibrosis in damaged kidneys in humans have been reported.23 Additionally, cardiac-specific heparin-binding EGF transgenic mice develop cardiac fibrosis.24 AngII-induced renal interstitial fibrosis is attenuated in proximal tubule EGFR-deficient mice.25 In our 2-week AngII infusion model, interstitial fibrosis is attenuated in proximal tubule EGFR-deficient mice.25 Interstitial fibrosis in the heart or kidney was too marginal to be assessed. However, it is likely that the paracrine production of heparin-binding EGF and activation of EGFR via induction and activation of ADAM17 in VSMCs may be critical for development of perivascular fibrosis associated with hypertension.17,26

In the present study with AngII-infused mice treated with an ADAM17 inhibitory antibody, hypertension was induced within a few days, with blood pressure values comparable to those of the control mice. The values are also in agreement with reported values in C57B16 background mice with AngII infusion.27 Although these data suggest that ADAM17 inhibition or silencing has no alteration on the development of hypertension in response to AngII, lack of continued blood pressure recording is a limitation of the present study. This study could also include an additionally important ADAM17floxed/lox sm22Cre− control mouse group because endogenous lox-lox sites are known to cause Cre-dependent chromosomal rearrangement in a Cre-transgenic mouse in the absence of loxP sequences.28 Although the protocol is different, control sm22αCre− mice develop hypertension, cardiac hypertrophy, and vascular fibrosis in response to AngII infusion.29

We have confirmed vascular dominant expression of the Cre transgene and intact presser responses to AngII in VSMC ADAM17-deficient mice or ADAM17 antibody-treated mice. However, contribution of VSMC ADAM17 to AngII-induced cardiac hypertrophy may require additional confirmation. Although lesser than smooth muscle, the sm22α promoter driver could show transgene expression in cardiac myocytes.30 There is a tendency of cardiac ADAM17 reduction in ADAM17floxed/lox sm22αCre− mice, which could be because of a combination of VSMC and cardiac myocyte silencing in the heart according to literature.31 Although the present study suggests that vascular-dominant ADAM17 and EGFR activation may mediate cardiac hypertrophy induced by AngII, cardiac myocyte-targeted expression of dominant-negative EGFR inhibits cardiac hypertrophy induced by AngII.32 Therefore, future experiments in cardiac myocyte–specific ADAM17−/− deficient mice should be conducted to test the role of cardiac myocyte ADAM17 in cardiac hypertrophy induced by AngII.

ER stress has been implicated in cardiovascular diseases,33 whereas limited information is available for its role in hypertension.14 Our recent study suggests a potential prevention of hypertensive organ damage but not hypertension by 4-phenylbutyrate, which seems to involve VSMC ADAM17 as well as EGFR.34 Inhibition of the fibrotic response in VSMCs with ER stress inhibition further suggests the presence of ER stress–responsible downstream signal transduction leading to vascular fibrosis, which likely involves transcriptional upregulation of several distinct genes.17,20

Perspectives

The vascular ADAM17/EGFR signal transduction axis seems to be essential for cardiovascular remodeling associated with ER stress but not for hypertension by 4-phenylbutyrate, which seems to involve VSMC ADAM17 as well as EGFR.34 Inhibition of the fibrotic response in VSMCs with ER stress inhibition further suggests the presence of ER stress–responsive downstream signal transduction leading to vascular fibrosis, which likely involves transcriptional upregulation of several distinct genes.17,20

Acknowledgment

We thank Dr Gillian Murphy for A9B8 preparation.

Sources of Funding

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Disclosures

None.

References


### Novelty and Significance

**What Is New?**

- Analysis of blood pressure and vascular pathology in the heart, kidney, and aorta with vascular smooth muscle cell ADAM17 deletion or ADAM17 inhibition by an antibody established a role for this metalloproteinase in Angiotensin II–induced pathological vascular remodeling independent of hypertension in mice.

- The concept of the feed-forward induction of vascular ADAM17 to amplify the epidermal growth factor receptor pathway and subsequent endoplasmic reticulum stress.

**What Is Relevant?**

- Vascular-restricted ADAM17 signal transduction highlights the importance of vascular pathology for subsequent tissue dysfunction in hypertension.

- Results indicating prevention of vascular remodeling but not hypertension by human cross-reactive ADAM17 antibody provide a foundation to seek a potential add-on therapy to current pressure-lowering treatments for hypertension.

**Summary**

In vascular smooth muscle ADAM17-deficient mice or mice treated with ADAM17 inhibitory antibody, vascular hypertrophy and peri-vascular fibrosis but not hypertension were prevented. Angiotensin II infusion showed vascular ADAM17 induction, epidermal growth factor receptor activation, and ER stress, which were attenuated in these mice. Cultured vascular smooth muscle cells were used to confirm the involvement of the ADAM17/epidermal growth factor receptor signaling axis in the induction of vascular fibrosis.
Vascular ADAM17 as a Novel Therapeutic Target in Mediating Cardiovascular Hypertrophy and Perivascular Fibrosis Induced by Angiotensin II
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Vascular ADAM17 as a Novel Therapeutic Target in Mediating Cardiovascular Hypertrophy and Perivascular Fibrosis Induced by Angiotensin II


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* These authors contributed equally to this work.

Short title: Vascular ADAM17 mediates organ damage by AngII

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### Supplementary Tables S1

#### S1A. M-mode echocardiography at 2 weeks after AngII infusion

<table>
<thead>
<tr>
<th>Parameters</th>
<th>A17ffsmCre-/- saline</th>
<th>A17ffsmCre-/- AngII</th>
<th>A17ffsmCre+/- saline</th>
<th>A17ffsmCre+/- AngII</th>
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<tbody>
<tr>
<td>IVSd (mm)</td>
<td>0.620±0.050</td>
<td>0.781±0.097*</td>
<td>0.590±0.049</td>
<td>0.581±0.040†</td>
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<td>LVIDd (mm)</td>
<td>3.72±0.15</td>
<td>3.02±0.12*</td>
<td>3.75±0.07</td>
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<td>LVPWd (mm)</td>
<td>0.750±0.017</td>
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<td>0.638±0.060</td>
<td>0.755±0.070†</td>
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<td>LVIDs (mm)</td>
<td>2.95±0.20</td>
<td>2.09±0.15*</td>
<td>2.88±0.11</td>
<td>2.70±0.21†</td>
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<tr>
<td>FS (%)</td>
<td>29.4±4.1</td>
<td>29.5±1.4</td>
<td>33.7±1.9</td>
<td>34.4±5.9</td>
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</tbody>
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Mean±SD (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.

#### S1B. Effects of VSMC ADAM17 deletion on characteristics of mice infused with AngII

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<tr>
<th>Parameters</th>
<th>A17ffCre-/- saline</th>
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<td>BW (g)</td>
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<td>19.9±3.5</td>
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<td>SBP/DBP (mmHg)</td>
<td>121±5/88±13</td>
<td>185±19*/142±19*</td>
<td>117±11/96±10</td>
<td>182±26*/131±18*</td>
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<tr>
<td>HR (beats/min)</td>
<td>496±159</td>
<td>570±100</td>
<td>463±146</td>
<td>585±58</td>
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Mean±SD (n=8), *p<0.001 compared with saline infusion. BW: body weight; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate. No significance was detected among the parameters between Cre+/- and Cre-/- animals regardless of the treatment.
**Supplementary Table S2**

### S2A. M-mode echocardiography at 2 weeks

<table>
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<th>saline</th>
<th>AngII+IgG2</th>
<th>AngII+A9B8</th>
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</thead>
<tbody>
<tr>
<td>IVSd (mm)</td>
<td>0.679±0.020</td>
<td>0.902±0.029*</td>
<td>0.774±0.025†</td>
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<td>LVIDd (mm)</td>
<td>3.84±0.07</td>
<td>3.58±0.10*</td>
<td>3.65±0.12</td>
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<tr>
<td>LVPWd (mm)</td>
<td>0.707±0.020</td>
<td>0.847±0.020*</td>
<td>0.798±0.036†</td>
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<td>LVIDs (mm)</td>
<td>3.11±0.07</td>
<td>2.634±0.08*</td>
<td>2.67±0.15</td>
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<td>FS (%)</td>
<td>28.7±0.7</td>
<td>30.2±0.7</td>
<td>29.7±1.0</td>
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Mean±SD (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.

### S2B. Effects of A9B8 on characteristics of mice infused with AngII

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<th>AngII+A9B8</th>
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<tbody>
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<td>BW (g)</td>
<td>23.3±2.2</td>
<td>25.0±2.0</td>
<td>24.6±2.1</td>
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<td>SBP/DBP (mmHg)</td>
<td>119±15/84±12</td>
<td>172±9*/137±17*</td>
<td>170±7*/141±6*</td>
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<tr>
<td>HR (beats/min)</td>
<td>634±85</td>
<td>586±87</td>
<td>583±90</td>
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Mean±SD (n=6), *p<0.001 compared with saline infusion. BW: body weight; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate.
Supplementary Figure S1. A. Expression of Cre in VSMC ADAM17 deficient mice. Cre expression was analyzed by immunohistochemistry with anti-Cre antibody. B. VSMC ADAM17 deficient mice and control mice were infused with AngII or saline as in Figure 1. Aortas were stained with Masson’s trichrome. Representative images are shown. Data are mean±SEM (n=6). C. Plasma BNP concentration. Plasma BUN concentration. Mean±SEM (n=6). *p<0.05 compared with saline control. †p<0.05 compared with AngII control.
Supplementary Figure S2. a. Suppression of vascular ER stress in VSMC ADAM17 deficient mice. VSMC ADAM17 deficient mice and control mice were infused with AngII or saline as in Fig 1. Tissues were immuno-stained with the antibodies indicated. Representative images are presented (n=4). Data are mean±SEM (n=4). *p<0.05 compared with saline control. †p<0.05 compared with AngII control.
**Supplementary Figure S3.** The aorta (A) and heart (B) samples were evaluated for ADAM17 mRNA expression by qPCR. Mean±SEM (n=6). *p<0.05 compared with saline control. †p<0.05 compared with AngII control.
Supplementary Figure S4. Effects of ADAM17 inhibitory antibody, A9B8, on hypertension development induced by AngII. A: C57Bl/6 mice were infused with AngII from Day 0 with or without treatment of A9B8 on Day 1. Arterial pressure was evaluated by telemetry on Day 1 and Day 2 (Mean±SEM, n=3). Significant blood pressure elevation in response to AngII infusion was observed at Day 2 compared with Day 1 regardless of the antibody treatment. *p<0.05 compared with corresponding Day1 values.
**Supplementary Figure S5.**

**A:** Rat aortic VSMCs pretreated with ADAM17 inhibitor JG26 (1 μmol/L) or vehicle (DMSO final concentration 0.1%) for 30 min were stimulated with 100 nmol/L AngII for 48 hours and extracellular collagen accumulation was quantified. Mean±SD (n=4).

**B:** VSMCs pretreated with EGFR inhibitor erlotinib (Erlo) or vehicle (DMSO final concentration 0.1%) for 30 min were stimulated with 100 nmol/L AngII for 48 hours and extracellular collagen accumulation was quantified. Mean±SD (n=4).

**C:** VSMCs pretreated with or without PBA (10 mmol/L in DMEM) were stimulated with 100 nmol/L AngII for 48 h and extracellular collagen accumulation was quantified. Mean±SD (n=4).

**D:** VSMCs pretreated with JG26 (1 μmol/L) or vehicle (DMSO final concentration 0.1%) for 30 min were stimulated with 100 nmol/L AngII for 2 min and immunoblottings were performed with antibodies as indicated. Mean±SD (n=4). *p<0.05 compared with vehicle control. †p<0.05 compared with AngII control.