Angiotensin II-mediated oxidative stress promotes myocardial tissue remodeling in the transgenic (mRen2) 27 Ren2 rat

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Whaley-Connell A, Govindarajan G, Habibi J, Hayden MR, Cooper SA, Wei Y, Ma L, Qazi M, Link D, Karuparthi PR, Stump C, Ferrario C, Sowers JR. Angiotensin II-mediated oxidative stress promotes myocardial tissue remodeling in the transgenic (mRen2) 27 Ren2 rat. Am J Physiol Endocrinol Metab 293: E355–E363, 2007. First published April 17, 2007; doi:10.1152/ajpendo.00632.2006.—Angiotensin II (ANG II) contributes to cardiac remodeling, hypertrophy, and left ventricular dysfunction. ANG II stimulation of the ANG type 1 receptor (AT1R) generates reactive oxygen species via NADPH oxidase, which facilitates this hypertrophy and remodeling. This investigation sought to determine whether cardiac oxidative stress and cellular remodeling could be attenuated by in vivo AT1R blockade (AT1B) and/or a superoxide mimetic (tempol) in a rodent model of chronically elevated tissue levels of ANG II, the transgenic (mRen2) 27 rat (Ren2). Ren2 rats overexpress the mouse renin transgene with resultant hypertension, insulin resistance, proteinuria, and cardiovascular damage. Young (6–7 wk old) male Ren2 and age-matched Sprague-Dawley rats were treated with valsartan (30 mg/kg), tempol (1 mmol/l), or placebo for 3 wk. Heart tissue NADPH oxidase (NOX) activity and immunohistochemical analysis of subunits NOX2, Rac1, and p22phox, heart tissue malondialdehyde, and insulin-stimulated protein kinase B (Akt) activation were measured. Structural changes were assessed with cine MRI, transmission electron microscopy, and light microscopy. Increases in sepal wall thickness and altered systolic function (cine MRI) were associated with perivascular fibrosis and increased mitochondria in Ren2 on light and transmission electron microscopy (P < 0.05). AT1B, but not tempol, reduced blood pressure (P < 0.05); significant improvements were seen with both AT1B and tempol on NOX activity, subunit expression, malondialdehyde, and insulin-mediated activation/phosphorylation of Akt (each P < 0.05). Collectively, these data suggest cardiac oxidative stress-induced structural and functional changes are driven, in part, by AT1R-mediated increases in NADPH oxidase activity.

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tional changes in the heart, and abrogation of oxidative stress would then attenuate these abnormalities in the heart.

We utilized immunostaining to evaluate NADPH oxidase subunit expression, assessed NADPH oxidase activity via spectrophotometric analysis, and measured malondialdehyde (MDA) as a surrogate end product of oxidative stress. Structural changes were evaluated by in vivo cine MRI, transmission electron microscopy (TEM), and histological, immunohistochemical, and biochemical techniques. We have further evaluated the impact of chronic ANG II stimulation of NADPH oxidase on insulin signaling via Akt and the consequence of in vivo treatment with a SOD/catalase mimetic (tempol) and AT1B (valsartan).

Materials and Methods

Animals and Treatments

All animal procedures were approved by the University of Missouri animal care and use committees and housed in accordance with National Institutes of Health guidelines. Male transgenic Ren2 rats and male Sprague-Dawley controls were kindly provided by Dr. Carlos M. Ferrario, Wake Forest University School of Medicine (Winston-Salem, NC), through the Transgenic Core Facility (supported in part by National Heart, Lung, and Blood Institute Grant P01 HL-51952). Ren2 rats (n = 6; 6–7 wk of age) and age-matched Sprague-Dawley rats (n = 6 each; Ren2-V and SDV, respectively) or tempol-treated (n = 6 each; Ren2-T and SDT, respectively) groups. Ren2-V and SDV animals received valsartan (30 mg·kg⁻¹·day⁻¹) in their drinking water for 21 days. Ren2-T and SDT animals received tempol (1 mM) in their drinking water for 21 days (6, 50). Animals were injected with 2 U of insulin 5 min before death to evaluate Akt signaling (7).

Systolic Blood Pressure

Systolic blood pressure (SBP) was determined at the end of treatment using the tail-cuff method (student oscillometric recorder; Harvard Systems) three times for 10–15 min after acclimatization and restraint conditioning for 48 h (6, 23, 50).

Measurement of NADPH Oxidase Activity

NADPH oxidase activity was determined in plasma membrane fractions as previously described (6). Aliquots of membrane and cytosolic fractions (12.5–150 μg proteins) were incubated with NADPH (100 μM) at 37°C. NADPH activity was determined by measuring the conversion of radical detector (Cayman Chemical) in the absence and presence of NADPH inhibitor diphenylene iodonium sulfate (DPI; 500 μM) using spectrophotometric (450 nm) techniques.

Lipid Peroxidation Measured in Myocardial Tissue

To measure products of lipid peroxidation, tissue MDA levels were determined (25, 50). Butylated hydroxytoluene (5 mM) was added to heart tissue (100 mg) to prevent new lipid peroxidation. Samples were then homogenized in a buffer solution on ice and centrifuged at 4°C at 15,000 rpm for 10 min. The supernatant was collected for MDA and O-phthalaldehyde (OPA) protein assays. Supernatant (200 μl) was used to measure free MDA with the use of an MDA-586 spectrophotometric assay kit. Total protein was measured by OPA fluorometric assay.

Immunofluorescent Studies

Harvested LVs were immersed and fixed in 3% paraformaldehyde (50). After fixation, tissues were placed in histological cassettes and dehydrated with ethanol, infiltrated with low-melting (50°C) paraplast, and embedded in high-melting (56°C) paraplast. Blocks were sectioned, deparaffinized in CitriSolv, and rehydrated in ethanol and HEPES wash buffer. The first section was washed (3 × 15 min) with HEPES wash buffer and then mounted with Mowiol. The second section was washed and incubated with 1:100 primary antibodies in 10-fold diluted blocking agent, and third and fourth sections were washed and kept in the blocker. Over the course of 48 h, a fifth, sixth, and seventh section were incubated with 1:100 goat gp91phox (NOX2) (Santa Cruz Biotechnology, Santa Cruz, CA), 1:100 mouse Rac1 antibody (Upstate Cell Signaling), and 1:100 goat p22phox antibody (Santa Cruz Biotechnology), respectively, in 10-fold diluted blocker. After 24 h, the slides were washed (3 × 15 min), and a seventh section was mounted with Mowiol. Other sections were incubated with 1:300 Alexa fluor rabbit anti-γ-anti mouse 647 (Molecular Probes, Eugene, OR) in 10-fold diluted blocker except the sixth, which was stained with 1:300 of Alexa fluor goat anti-mouse 647. After 4 h, the slides were washed, mounted with Mowiol, and examined with a laser confocal scanning microscope; images were captured with the use of Laser-sharp software (Bio-Rad), and signal intensities were measured by MetaVue analysis (23, 50).

In Vivo Cine MRI

Scans were performed on Ren2 and Sprague-Dawley rats at 4–5 and 8–9 wk of age using a Varian 7T horizontal bore MRI (Varian, Palo Alto, CA) with ECG and respiratory gating (SA Instruments, Stony Brook, NY). Animals were anesthetized with 2–3.5% isoflurane with the use of a nose-cone nonrebreathing system supplying continuous oxygen. Wall thickness measurements were determined with Image-J (NIH, Bethesda, MD) on a single transverse axial image at 0-ms delay after the R wave and with averaging of five septal width measurements per rat. Ejection fraction was assessed on a single long-axis plane captured at 12 equally spaced time points of the cardiac cycle. LV volume was determined with a modified ellipsoid equation (26). The largest volume was considered end diastolic (EDV) and lowest as end systolic ( ESV), Ejection fraction was calculated as (EDV – ESV/EDV) × 100.

Table 1. Systolic blood pressure and oxidative stress parameters

<table>
<thead>
<tr>
<th></th>
<th>SDC</th>
<th>SDV</th>
<th>SdT</th>
<th>SDC</th>
<th>SDT</th>
<th>Ren2-C</th>
<th>Ren2-V</th>
<th>Ren2-T</th>
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<tr>
<td>Systolic blood pressure, mmHg</td>
<td>115.2 ± 4.7</td>
<td>98.0 ± 1.1</td>
<td>113.5 ± 1.1</td>
<td>197.0 ± 7.8</td>
<td>122.5 ± 7.8</td>
<td>194.0 ± 4.1</td>
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**Oxidative stress**

<table>
<thead>
<tr>
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<th>Ren2-C</th>
<th>Ren2-V</th>
<th>Ren2-T</th>
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<tbody>
<tr>
<td>NADPH oxidase activity, nmol·mg protein⁻¹·min⁻¹</td>
<td>10.8 ± 1.2</td>
<td>12.2 ± 1.7</td>
<td>12.8 ± 0.7</td>
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<tr>
<td>+ diphenylene iodium</td>
<td>11.3 ± 3.0</td>
<td>9.1 ± 1.5</td>
<td>8.7 ± 0.8</td>
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<tr>
<td>Malondialdehyde, μM MDA/mg protein</td>
<td>0.47 ± 0.02</td>
<td>0.49 ± 0.03</td>
<td>0.52 ± 0.01</td>
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Values are means ± SE. SDC, SDV, and SdT, control, valsartan-treated, and tempol-treated Sprague-Dawley rats, respectively; Ren2-C, Ren2-V, and Ren2-T, control, valsartan-treated, and tempol-treated Ren2 rats, respectively. *P < 0.05 compared with SDC; †P < 0.05 vs. Ren2-C; ‡P < 0.05 vs. either initial systolic blood pressure or NADPH oxidase control; §P < 0.05 vs. Ren2-V.
**Light Microscopy**

Fixed paraffin sections of LV were evaluated with Verhoeff-van Gieson stain, which stains elastin (black), nuclei (blue black), collagen (red), and connective tissue (yellow), as previously described (23). Slides were analyzed with a Nikon 50i microscope, and \( \times 4, \times 10, \) and \( \times 40 \) images were captured with a Cool Snap cf camera. Morphometric analysis was performed with MetaVue software. A total of 15 images of coronary artery cross sections were taken from each animal. In each image, the perimeter of the adventitia, media, and lumen were traced, and the percent area was then calculated by subtracting the combined areas for media and lumen from the total area and then dividing by the total area.

**TEM Methods**

LV heart tissue was thinly sliced and placed immediately in primary EM fixative (2% glutaraldehyde, 2% paraformaldehyde in 0.1 M sodium cadodylate buffer, pH 7.35), as previously described (50). A Pelco 3440 laboratory microwave was utilized for secondary fixation, with acetone dehydration and Epon-Spurr’s resin infiltration. Specimens were placed on a rocker overnight at room temperature, embedded the following morning, and polymerized at 60°C for 24 h. A Leica Ultracut UCT microtome with a 45° Diatome diamond knife was used to prepare 85-nm thin sections. The specimens were then stained with 5% uranyl acetate and Sato’s triple lead stain. A JOEL 1200-EX transmission microscope was utilized to view all renal samples.

Three fields were randomly chosen per rat to obtain three \( \times 60,000 \) images/LV-containing cross-sectional views of the mitochondria. Mitochondria were visualized and identified based on the identification of cristae. Then three (450 nm \( \times 450 \) nm) images were cropped out of each \( \times 60,000 \) image obtained. These cropped images were then analyzed with Image J (a public domain Java image processing program made possible by the NIH). By viewing the mitochondria in

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**Fig. 1.** A: immunohistochemical (IHC) staining of NADPH oxidase subunits NOX2, Rac1, and p22phox. B: gray-scale intensity measures of IHC in A. SD-C, SDV, and SDT, control, valsartan-treated, and tempol-treated Sprague-Dawley rats, respectively. Ren2-C, Ren2-V, and Ren2-T, control, valsartan-treated, and tempol-treated Ren2 rats, respectively. *\( P < 0.05 \) compared with Sprague-Dawley control (SDC). **\( P < 0.05 \) compared with Ren2 control (Ren2-C) at 4 wk.
cross section in each cropped image, we calculated, measured, and recorded the number in a blinded fashion (25, 50).

**Citrate Synthase Activity**

Mitochondrial fractions of LV prepared during sucrose differential centrifugation were freeze thawed three times to ensure mitochondrial disruption and were diluted as necessary. Samples were analyzed in an incubation buffer consisting of Tris (pH 8.3), 5,5’-dithiobis(2-nitrobenzoic acid) (DTNB), and oxaloacetate, and the assay was initiated by the addition of acetyl-CoA. Samples were analyzed at 37°C using linear kinetics (405 nm). Amount of DTNB converted per minute was determined with the molar extinction coefficient for DTNB. Rates for each sample were normalized to mitochondrial fraction proteins determined by Bradford assay.

**Western Blot Analysis**

Hearts were homogenized with a glass-on-glass Dounce homogenizer in sucrose homogenization buffer and centrifuged at 1,000 g to remove connective tissue. Protein concentrations were measured by OPA as above. Supernatants were analyzed under denaturing conditions with SDS-PAGE using a mini-protein 2 electrophoresis system. Protein (20 μg) was mixed with 5 μl of loading buffer and incubated at 92°C for 5 min. Samples were then loaded into wells of 4 –15% precast gels (Bio-Rad) and run at 180 V for 35 min. Incubation buffer consisting of Tris (pH 8.3), 5,5’-dithiobis(2-nitrobenzoic acid) (DTNB), and oxaloacetate, and the assay was initiated by the addition of acetyl-CoA. Samples were analyzed at 37°C using linear kinetics (405 nm). Amount of DTNB converted per minute was determined with the molar extinction coefficient for DTNB. Rates for each sample were normalized to mitochondrial fraction proteins determined by Bradford assay.

**Statistical Analysis**

All values are expressed as means ± SE. Statistical analyses were performed with SPSS 13.0 (SPSS, Chicago, IL) using ANOVA with Fisher’s least significant difference test as appropriate and Student’s t-test for paired analysis. Phosphorylated Akt was plotted against NADPH oxidase activity and Rac for linear regression analysis. Significance was accepted as P < 0.05.

**RESULTS**

**Systolic Blood Pressure**

At the end of the treatment period (9–10 wk of age), there were improvements in SBP with valsartan in both the Ren2-V and Ren2-T groups (each P < 0.05) (Table 1), although not with tempol treatment.

**Oxidative Stress in Ren2 Heart Tissue**

**NADPH oxidase activity.** NADPH oxidase activity, as measured by radical detector conversion, was significantly higher in the Ren2-C than in SDC group and attenuated by valsartan (Ren2-V) and tempol (Ren2-T) treatments (P < 0.05). This activity was abrogated by DPI, a specific inhibitor of NADPH oxidase in all groups (P < 0.05), thus validating increased plasma membrane NADPH oxidase activity in the Ren2 heart.

End product of lipid peroxidation as measured by MDA levels. MDA levels were elevated in Ren2-C myocardial tissue compared with that shown in SDC (P < 0.05) (Table 1) and significantly decreased by both treatment paradigms (P < 0.05). Furthermore, MDA levels were lower in Ren2-T than in Ren2-V (P < 0.05).

**NADPH oxidase subunits.** Evaluation of NADPH subunits NOX2, Rac1, and p22phox via immunohistochemistry revealed increased expression of each subunit in Ren2-C, as measured by gray-scale intensities (40.2 ± 7.2, 38.8 ± 6.4, 23.6 ± 2.9, respectively), compared with that shown in SDC (26.7 ± 1.0, 21.7 ± 1.2, 16.5 ± 0.8, respectively) (P < 0.05) (Fig. 1). Reduced expression of these subunits, as observed in both Ren2-V and Ren2-T groups, corroborates reductions seen in NADPH oxidase activity (NOX2: 27.9 ± 3.2 and 19.0 ± 2.3, respectively; Rac1: 22.4 ± 3.0 and 18.6 ± 2.6, respectively; and p22phox: 18.4 ± 1.3 and 15.6 ± 0.2, respectively) (each P < 0.05).

**Myocardial Remodeling in the Ren2 Heart**

**Cine MRI.** In vivo cine MRI was used to measure septal wall thickness and systolic ejection fraction (Fig. 2). There was no difference in wall thickness in the Ren2-C (1.25 ± 0.06 mm) compared with SDC (1.21 ± 0.03 mm; P > 0.05) at 4–5 wk. However, as expected, the systolic ejection fraction in Ren2-C animals was higher than in SDC (74.3 ± 5.1 vs. 52 ± 2.1%; P < 0.05). Septal wall hypertrophy was present in Ren2-C animals (1.65 ± 0.05 mm) compared with that shown in SDC (1.35 ± 0.07 mm; P < 0.05) (Fig. 2A). In contrast to SDC (63.2 ± 5.7%), there was a reduction in systolic ejection fraction in the Ren2-C (65.0 ± 3.4%; P < 0.05), with a change of −9.3 ± −8.4 compared with at 4 wk (Fig. 2B).

Fig. 2. In vivo cine MRI measures of heart. A: septal wall thickness. B: systolic ejection fraction (EF) before initiation and end of treatment in the Ren2. A depicts the septal wall thickness in control animals at 4 wk (pretreatment) and 8 wk (posttreatment), and B depicts systolic ejection fraction before initiation and at end of treatment. *P < 0.05 compared with SDC at 4 wk (Ren2-C and SDC, respectively). **P < 0.05 compared with SDC at 8 wk.
Light microscopy and TEM. Perivascular fibrosis, as represented by percent area fibrosis on Verhoeff-van Gieson staining on light microscopy, was increased in Ren2-C animals (70 ± 3.2%) compared with SDC (57 ± 3.7%; P < 0.05) and improved in Ren2-V (53 ± 4.4%) compared with that shown in Ren2-C animals (P < 0.05) (Fig. 3, A and B). Furthermore, Ren2-T animals displayed a trend toward reduction of perivascular fibrosis (62.7 ± 0.03%).

Ultrastructural analysis with TEM (Fig. 3, C and D) corroborated light microscopy findings (Fig. 3A). Pericapillary fibrosis emanated from the perivascular area to the interstitium of the endomysium between cardiomyocytes (Fig. 3D) compared with that shown in SDC animals (Fig. 3C), similar to that observed on light microscopy (Fig. 3A). Figure 3C displays a normal capillary without pericapillary fibrosis in the SDC at ×10,000 and at ×20,000 (boxed area), and Fig. 3D displays pericapillary fibrosis in the Ren2-C at ×10,000 and at ×40,000 (boxed area); asterisks outline the area of pericapillary fibrosis. Both longitudinal banded collagen and cross-sectional collagen are also shown.

Mitochondrial Content in Ren2 Heart

TEM images taken at ×10,000 (Fig. 4) demonstrated striking changes in the mitochondria (Fig. 4A). There were increased numbers of mitochondria in Ren2-C (83.6 ± 7.6) compared with SDC animals (67.2 ± 5.2; P < 0.05) (Fig. 4, A and B), with trends to improvement in the Ren2-V (68.2 ± 4.8) but not in the Ren2-T (80.1 ± 7.9). Increased mitochondria was confirmed with citrate synthase activity, an enzyme used to quantify mitochondrial content. Similar to TEM analysis, there were increases in Ren2 (536.1 ± 60.7) compared with SDC animals (398.2 ± 26.3; P < 0.05) (Fig. 4C), which demonstrates a trend to improvement in the Ren2-V (444.7 ± 48.6) but not in the Ren2-T (556.3 ± 73.1).

Akt Phosphorylation in Ren2 Heart Tissue

Animals were stimulated with 2 U of insulin before death to evaluate Akt signaling. The ratio of Western blot densities of phosphorylated Akt to total Akt in heart tissue of Ren2-C was less than that of the SDC (P < 0.05) (Fig. 5, A and B).
Phosphorylated Akt was inversely correlated with NADPH oxidase activity ($r^2 = 0.39$) and Rac1 protein expression by immunohistochemistry ($r^2 = 0.37$) (Fig. 5C). Akt phosphorylation was improved in Ren2-V and Ren2-T animals (each $P < 0.05$). Furthermore, Akt phosphorylation was increased in the SDT compared with SDC and SDV animals (each $P < 0.05$).

### DISCUSSION

The data from this investigation indicate that the hypertensive, insulin-resistant transgenic (mRen2)27 (Ren2) rat manifests increased oxidative stress in concert with structural and functional changes in the heart. Alterations visible by light microscopy and TEM include perivascular fibrosis, as previously observed, and increased mitochondrial content (20, 23). AT1B of the Ren2 abrogated these structural abnormalities, suggesting that these changes are mediated by the AT1R. Electron microscopy revealed ultrastructural remodeling in the Ren2 heart compared with Sprague-Dawley rats. These changes included increased mitochondria, consistent with limited previous observations in hypertrophied hearts displaying increases in oxidative stress (45). This may represent an adaptive response to greater energy requirements due to increased afterload. These TEM changes are the first such observations made in a model of chronic ANG II overexpression.

In conjunction with enhanced NADPH oxidase activity, there was an increase in lipid peroxidase products in the Ren2, which was similarly reduced by treatment with either an AT1B or a SOD mimetic. Increased cardiac levels of oxidized products have not been previously observed in the Ren2 rat, but studies have demonstrated that ANG II increases ROS in cultured myocardial fibroblasts and in cardiomyocytes (4, 27, 30). The heart possesses a relatively low endogenous antioxidant capacity as contributed by both enzymatic and nonenzymatic free radical scavengers and antioxidants, thus rendering it susceptible to oxidative stress, with attendant structural and functional abnormalities (20). Indeed, increased oxidative stress in the heart has been linked to ventricular hypertrophy, systolic and diastolic functional abnormalities, and abnormal metabolic signaling (20, 33). The observation that in vivo treatment with AT1B or a SOD mimetic normalized NADPH oxidase and MDA products is the first report that these therapeutic approaches can comparably attenuate consequent pathological effects of chronic AT1R activation.

**Fig. 4.** Mitochondrial content in the Ren2 heart. A: TEM images at $\times 10,000$ represent increased mitochondria content observed in Ren2 heart tissue compared with SDC with improvements in the Ren2-V group, as quantified in B. C: mitochondrial content quantified by citrate synthase activity. *$P < 0.05$ compared with SDC.
Immunohistochemical analysis of cardiac tissue NADPH oxidase subunits NOX2, p22phox, and Rac1 demonstrated an association with increased NADPH oxidase activity. Furthermore, either AT1B or a SOD mimetic significantly reduced the observed increases in all three NADPH oxidase subunits. ANG II stimulation of Rac1 expression has been previously observed in prior studies (11, 28). The small GTP binding protein Rac1 is critical for the assembly and function of the multicomponent NADPH oxidase complex (11, 27). Prior studies have shown that treatment of rat cardiomyocytes with ANG II induces hypertrophy, which is partly dependent on increased Rac1 translocation to cell membranes and consequent activation of NADPH oxidase (11, 14, 28). Recent studies suggest that ANG II-induced ROS production is dependent on Rac1 activation, which cascades into JNK and p38 signaling pathways that promote growth (32). In the present study, there was a relationship between Rac1 expression, increased NADPH oxidase activity, and diminished Akt activation in response to insulin. In hepatocytes, Akt signaling suppresses Rac-regulated ROS production (34), and in endothelial cells Rac1 regulates the production of the potent anti-oxidant manganese SOD (1). These data collectively suggest a negative feedback loop between Rac/ROS formation and Akt and the SOD systems in various tissues.

The changes in NADPH oxidase activity and subunits in this investigation were inversely related to insulin-stimulated Akt phosphorylation, an indicator of Akt2 activation (2, 12, 42). Akt is a highly conserved protein kinase with a pleckstrin homology domain, which is required for phosphorylation and/or activation by phosphoinositol 3-kinase (42). Activation of Akt involves translocation to the plasma membrane where activated phosphoinositide-dependent kinases 1 and 2 phosphorylate Akt on threonine 308 and serine 473, with both phosphorylations required for maximum Akt activation (42). Activated Akt then phosphorylates intracellular proteins that regulate growth, metabolism, survival, and cardiac function (2, 12, 18, 42). Akt activity in the rodent heart is regulated by insulin, nutritional status, pressure overload, and redox status (2, 12, 18, 42). Optimal Akt signaling, although it promotes physiological growth, inhibits pathological hypertrophy (2, 12, 18, 42). Our observations that Akt serine 473 phosphorylation in response to insulin is decreased in a rodent model of ANG II-stimulated oxidative stress, hypertrophy, and reduced myocardial contractility are consistent with these previous obser-
vations (2, 12, 18, 42). Indeed, there was an inverse relationship between Rac1 expression, NADPH oxidase activity, and Akt phosphorylation. These findings, in concert with several prior studies, support a reciprocal relationship between reduced insulin-mediated Akt signaling and oxidative stress in the heart (2, 12, 18, 42). Thus enhanced ANG II generation of ROS in the heart, as well as insulin-sensitive skeletal muscle (6), contributes to an insulin resistance state in these insulin-sensitive tissues. Insulin-stimulated Akt signaling is crucial for glucose transport in the heart and in skeletal muscle (44). Thus this ANG II-induced abnormality may contribute to altered cardiac mechanical-energy coupling in the Ren2 model of chronic ANG II overexpression.

Collectively, these data suggest that, in the Ren2 rat, there is a relative shift to heightened myocardial oxidative stress and remodeling, which are corrected by in vivo treatment with AT1B. In interpreting the results of this, or any study, that investigate blood pressure-mediated effects, measurement of SBP is susceptible to large variability. However, the overall consistent trends in our elevated SBP in the Ren2 that improved with AT1B are consistent with the literature and support our conclusions.

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REFERENCES

21. Gray MO, Long CS, Kalinyak JE, Li HT, Gray MO. Activation of protein kinase C by 10.220.33.5 on April 13, 2017 http://ajpendo.physiology.org/ Downloaded from


