Effects of adrenomedullin on cardiac oxidative stress and collagen accumulation in aldosterone-dependent malignant hypertensive rats


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Running title: Adrenomedullin and aldosterone in the heart

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Abbreviations:
Spontaneously hypertensive rat (SHR); Wister-Kyoto (WKY); systolic blood pressure (SBP); left ventricular (LV); reactive oxygen species (ROS); thiobarbituric acid reactive substances (TBARS); body weight (BW); glyceraldehyde-3-phosphate dehydrogenase
(GAPDH); superoxide anion (O$_2^-$)

A recommended section: Cardiovascular
ABSTRACT

We examined the effects of adrenomedullin on cardiac oxidative stress and collagen accumulation in aldosterone-dependent malignant hypertensive rats. Spontaneously hypertensive rats (SHR) were treated with one of the following combinations for 4 weeks: tap water and vehicle (0.5% ethanol, SC, n=5); 1% NaCl in drinking water and vehicle (n=8); 1% NaCl and aldosterone (0.75 µg/h, SC, n=8); and 1% NaCl, aldosterone and adrenomedullin (1.3 µg/kg/h, SC, n=8). Systolic blood pressure (SBP) and left ventricular (LV) weight were higher in aldosterone-treated SHR than vehicle- or vehicle/1%NaCl-treated SHR. Thiobarbituric acid reactive substances (TBARS) levels and NADPH oxidase activity in LV tissues of aldosterone-treated SHR were also higher than those of vehicle- or vehicle/1%NaCl-treated SHR, and these changes were associated with increases in LV mRNA levels of p22phox, gp91phox, fibronectin, collagen types I and III, as well as collagen content. Treatment with adrenomedullin did not alter SBP or LV weight, but attenuated aldosterone-induced increases in TBARS levels, NADPH oxidase activity and mRNA levels of p22phox, gp91phox, fibronectin, collagen types I and III, as well as collagen content in LV tissues. These data suggest that NADPH oxidase-mediated reactive oxygen species production is involved in the pathogenesis of cardiac collagen accumulation in aldosterone-dependent malignant hypertensive rats, and that the cardioprotective effects of adrenomedullin are mediated through the suppression of this pathway.
INTRODUCTION

Excessive collagen accumulation is a potential cause of abnormal tissue stiffness and dysfunction during the development of cardiac hypertrophy (Kim et al., 1995; Brilla, 2000). A growing body of evidence suggests that aldosterone plays an important role in extracellular matrix and collagen synthesis in myocardium (Young et al., 1994; Brilla 2000; Zannad et al., 2000; Pitt et al., 2003; Bos et al, 2005). In patients with essential hypertension (Duprez et al., 1993; Soylu et al. 2004) and primary aldosteronism (Rossi et al., 1997; Nishimura et al., 1999), pathological patterns of left ventricular (LV) geometry are associated with elevated levels of plasma aldosterone. Further, severer and more frequent LV hypertrophy and remodeling were observed in patients with primary aldosteronism than in essential hypertensive patients (Rossi et al., 1997, 2002). Chronic infusion of aldosterone to rats led to LV hypertrophy with collagen accumulation, independent of its hemodynamic effects (Brilla and Weber, 1992; Brilla et al., 1993; Brilla, 2000). In patients with severe heart failure, administration of a mineralocorticoid receptor antagonist, spironolactone, to patients receiving standard therapy including an angiotensin-converting enzyme inhibitor markedly reduced serum levels of procollagen type III amino-terminal peptide, a specific marker for cardiac collagen synthesis (Zannad et al., 2000). Other studies have shown that monotherapy with a mineralocorticoid receptor antagonist significantly decreases LV mass in patients with essential hypertension (Pitt et al., 2003).

Recent studies indicate the potential participation of reactive oxygen species (ROS) in the pathophysiology of aldosterone-induced cardiovascular tissue injury (Sun et al., 2002; Iglarz et al., 2004; Park et al., 2004). In aldosterone/salt- or
deoxycorticosterone acetate/salt-treated hypertensive rats, cardiovascular NADPH oxidase activity and ROS production were markedly augmented (Beshwick et al., 2001; Iglarz et al., 2004; Park et al., 2004). In these animals, treatment with tempol, a superoxide dismutase mimetic, or apocynin, a NADPH oxidase inhibitor, significantly attenuated cardiovascular fibrosis (Iglarz et al., 2004; Park et al., 2004). More recently, it has also been indicated that adrenomedullin protects cardiovascular injury by reducing NADPH oxidase-mediated ROS production (Shimosawa et al., 2002; Kato et al., 2003; Kawai et al., 2004; Yoshimoto et al., 2004, 2005). Adrenomedullin is a vasodilator peptide originally isolated from human pheochromocytoma cells (Kitamura et al., 1993) and is widely distributed in various tissues and organs, including the heart (Kitamura et al., 2002; Eto et al., 2003; Zhao et al., 2006). However, to the best of our knowledge, there is no evidence that clearly demonstrates any beneficial effects of adrenomedullin on cardiac oxidative stress and remodeling that could occur during the development of aldosterone-dependent hypertension.

In the present study, we aimed to investigate whether treatment with adrenomedullin was cardio-protective by reducing NADPH oxidase-mediated ROS production during the development of aldosterone-dependent malignant hypertension. Accordingly, we examined the effects of chronic treatment with adrenomedullin on the expression and activity of NADPH oxidase and on ROS and collagen levels in LV tissues of aldosterone-infused spontaneously hypertensive rats (SHR); presents a new model of malignant hypertension with secondary hyperaldosteronism.
MATERIALS AND METHODS

Materials

Aldosterone was purchased from Wako Co. (Osaka, Japan). Adrenomedullin (52 human), diphenyleneiodonium, lucigenin and NADPH were obtained from Sigma Chemical Co. (St. Louis, MO).

Animals and Experimental Groups

All experimental procedures were performed according to the guidelines for the care and use of animals as established by Kagawa University Medical School. Experiments were performed on 10-week-old male SHR and age matched Wister-Kyoto (WKY) rats (SLC; Shizuoka, Japan). SHR and WKY rats were housed in separate cages, and maintained at room temperature under a 12 h light/dark cycle. At the beginning of the experiments, SHR were randomly treated for 4 weeks with one of the following combinations: group-1, tap water and vehicle (0.5% ethanol, SC, n=5); group-2, 1% NaCl in the drinking solution and vehicle (n=8); group-3, 1% NaCl and aldosterone (0.75 µg/h, SC, n=8); and group-4, 1% NaCl, aldosterone and adrenomedullin (1.3 µg/kg/h, SC, n=8). WKY rats were treated with tap water and vehicle (n=5) or 1% NaCl in the drinking solution and vehicle (n=8). The doses of aldosterone and adrenomedullin were determined according to the results of previous studies on rats (Mori et al., 2002; Nishikimi et al., 2002; Nishiyama et al., 2004; Park et al., 2004).

Rats were anesthetized with sodium pentobarbital (50 mg/kg, IP), and osmotic minipumps (Alzet; Cupertino, CA) were implanted subcutaneously at the dorsum of the
to infuse vehicle, aldosterone or adrenomedullin. Systolic blood pressure (SBP) was measured weekly by tail-cuff plethysmography (BP-98A, Softron Co; Tokyo, Japan). After 4 weeks treatment, 24-hour urine samples were collected using metabolic cages one day before harvesting. Animals were decapitated and trunk blood collected into chilled tubes containing EDTA. LV tissues were removed, immediately snap-frozen in liquid nitrogen and then stored at -80 °C.

**Real-Time Reverse Transcriptase-PCR**

mRNA expression of p22phox, gp91phox, fibronectin, and collagen types I and III were analyzed by real-time PCR using an ABI Prism 7000 Sequence Detection System (Applied Biosystems; Foster City, CA) (Nishiyama et al., 2004; Nagai et al., 2005a,b). All data were expressed as relative differences in vehicle-, aldosterone/1% NaCl-, aldosterone and adrenomedullin/1% NaCl-treated SHR, and vehicle- or vehicle/1%NaCl-treated WKY rats compared with vehicle/1% NaCl-treated SHR after normalization to the expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The primers for GAPDH, p22phox, gp91phox, fibronectin, collagen types I and III were synthesized as described previously (Nishiyama et al., 2004; Nagai et al., 2005a; Nagai et al., 2005b).

**NADPH Oxidase Activity**

NADPH oxidase-derived superoxide anion (O$_2^-$) generation was measured using lucigenin-enhanced chemiluminescence, as described previously (Zalba et al., 2000; Kim et al., 2002; Nakano et al., 2005). Briefly, LV tissues (80-100 mg) were
placed in chilled phosphate-buffered saline containing protease inhibitor, and homogenized on ice. Protein concentration of homogenates was measured using the Bradford protein assay kit (Bio-Rad Laboratories; Hercules, CA). After centrifuging, the supernatant was transferred into a glass test tube containing lucigenin (final concentration 5 µmol/L in Krebs-Hepes buffer). Chemiluminescence was then recorded every 30 sec for 10 min with a luminescence reader (BLR-301, Aloka; Tokyo, Japan); the readings in the last 5 min were averaged. After measurement of background lucigenin chemiluminescence, NADPH was added to a final concentration of 100 µmol/L. Thereafter, chemiluminescence was recorded another 10 min and the readings in the last 5 min were averaged. To verify if the lucigenin signal reflects O$_2^-$ generation, a NADPH oxidase inhibitor, diphenylene iodonium (Hancock and Jones, 1987), was added at the end of measurements (final concentration 10 µmol/L). In all samples, diphenylene iodonium reduced NADPH-induced increases in chemiluminescence to background levels (data not shown). The differences between the values obtained before and after adding the NADPH were calculated and the activity of NADPH oxidase was expressed as counts per min (cpm) per mg of protein.

**Other Analytical Procedures**

The degree of lipid peroxidation in plasma and LV tissues was determined using biochemical assays of the TBARS, as described previously (Nishiyama et al., 2004; Rahman et al., 2004; Nagai et al., 2005b). Collagen content in the LV tissues was determined on the basis of hydroxyproline concentration (Nishiyama et al., 2004; Nagai et al., 2005b). The value of collagen content was expressed as µg per mg dry tissue
weight. Sodium and potassium concentrations in plasma and urine were measured using flame photometry (Hitachi 750, Hitachi; Tokyo, Japan) (Nishiyama et al., 2006).

**Statistical Analysis**

Values are presented as mean±SE. Statistical comparisons of differences were performed using one-way or two-way analyses of variance combined with Fisher’s post-hoc test. P<0.05 was considered statistically significant.
RESULTS

**Blood Pressure and LV Weight**

The changes in SBP are shown in Figure 1. Baseline SBP was significantly higher in SHR than WKY rats. Treatment with vehicle or vehicle/1% NaCl did not alter SBP in WKY rats (133±1 and 127±2 mmHg, respectively, at week 4). Further, SBP was similar between vehicle- and vehicle/1% NaCl-treated SHR (207±4 and 201±5 mmHg, respectively, at week 4). However, aldosterone/1% NaCl-treatment further increased SBP in SHR (220±8 mm Hg, at week 4). In aldosterone/1% NaCl-treated SHR, SBP was not altered by chronic treatment with adrenomedullin (219±4 mm Hg, at week 4). These data are consistent with those of previous studies (Mori et al., 2002; Nishikimi et al., 2002) showing that chronic subcutaneous infusion of adrenomedullin did not alter SBP in hypertensive rats.

As shown in Table 1, body weight (BW) was similar between vehicle- or vehicle/1% NaCl-treated SHR and WKY rats, respectively. Four weeks of infusion of aldosterone or aldosterone plus adrenomedullin to 1% NaCl-treated SHR prevented the rise in BW that was seen in vehicle- or vehicle/1% NaCl-treated SHR and WKY rats. On the other hand, aldosterone/1% NaCl-treatment significantly increased the LV and LV weights to BW ratio. Concurrent administration of adrenomedullin did not significantly alter the aldosterone-induced increases in LV and LV weights to the BW ratio (Table 1).

**Urine Volume (UV), and Urinary Excretion Rates of Sodium (Unav) and potassium (UkV)**

Four weeks of treatment with 1% NaCl markedly increased UV and Unav in
both SHR and WKY rats (data not shown). Aldosterone/1% NaCl-treated SHR showed higher UV (132±15 mL/day) and lower urinary concentrations of sodium (162±4 mEq/L) and potassium (19±4 mEq/L) than those of 1% NaCl-treated SHR (43±10 mL/day, and 329±20 and 133±17 mEq/L, for each). Aldosterone/1% NaCl-treated SHR showed higher U_NaV (21.1±2.1 mEq/day) than 1% NaCl-treated SHR (13.9±3.6 mEq/day). Concurrent administration of adrenomedullin did not significantly alter UV (122±8 mL/day), urinary concentrations of sodium (189±11 mEq/L) and potassium (23±2 mEq/L), U_NaV (22.8±1.6 mEq/day) in aldosterone/1% NaCl-treated SHR. On the other hand, plasma sodium and potassium levels as well as U_KV were not significantly different among the animals (data not shown).

**TBARS**

As shown on Figure 2A, plasma TBARS levels were higher in vehicle (11.0±0.6 nmol/mL) or vehicle/1% NaCl-treated SHR (10.9±0.4 nmol/mL) compared to vehicle- and vehicle/1% NaCl-treated WKY rats (8.8±0.7 and 9.1±0.6 nmol/mL, respectively). TBARS contents in LV tissues were not different among vehicle- and vehicle/1% NaCl-treated SHR (0.34±0.04 and 0.31±0.02 nmol/mg protein, respectively) and WKY rats (0.29±0.03 and 0.38±0.04 nmol/mg protein, respectively, Figure 2B). As compared with vehicle/1% NaCl-treated SHR, aldosterone/1% NaCl-treated SHR showed significantly higher TBARS levels in both plasma (12.9±0.4 nmol/mL) and LV tissues (0.45±0.05 nmol/mg protein). Concurrent administration of adrenomedullin significantly attenuated aldosterone-induced increases in TBARS levels in both plasma (10.9±0.5 nmol/mL) and LV tissues (0.32±0.03 nmol/mg protein), as shown in Figure 2A.
As shown in Figure 3, NADPH oxidase activity in LV tissues was not different among vehicle- or vehicle/1% NaCl-treated SHR and WKY rats. However, NADPH oxidase activity in LV tissues of aldosterone/1% NaCl-treated SHR was much higher than that in vehicle/1% NaCl-treated SHR (346±35 versus 175±12 cpm/mg protein). Administration of adrenomedullin attenuated aldosterone-induced increases in NADPH oxidase activity (221±15 cpm/mg protein, Figure 3).

**NADPH Oxidase Components**

As shown in Figure 4A and 4B, p22phox and gp91phox mRNA levels in LV tissues were not different among vehicle- or vehicle/1% NaCl-treated SHR and WKY rats. However, aldosterone/1% NaCl-treated SHR showed significantly higher p22phox and gp91phox expression than those of vehicle/1% NaCl-treated SHR by 1.5±0.1 and 2.4±0.3-fold, respectively. In aldosterone/1% NaCl-treated SHR, treatment with adrenomedullin prevented increases in the mRNA levels of p22phox and gp91phox (Figures 4A and 4B).

**Fibronectin and Collagen**

Fibronectin and collagen types I and III mRNA levels in LV tissues were similar among vehicle- or vehicle/1% NaCl-treated SHR and WKY rats (Figures 5A-C). However, aldosterone/1% NaCl-treated SHR showed significantly higher mRNA levels
of fibronectin, and collagen types I and III than those of vehicle/1% NaCl-treated SHR (by 2.2±0.1, 2.2±0.2 and 3.1±0.2-fold, for each). In aldosterone/1% NaCl-treated SHR, treatment with adrenomedullin prevented increases in mRNA levels of fibronectin, and collagen types I and III.

As shown in Figure 5D, collagen contents in LV tissues were similar among vehicle- or vehicle/1% NaCl-treated SHR and WKY rats (17±1, 17±2, 15±1 and 16±1 µg/mg, for each). However, aldosterone/1% NaCl-treated SHR showed significantly higher collagen contents than those of vehicle/1% NaCl-treated SHR (22±2 µg/mg). In aldosterone/1% NaCl-treated SHR, treatment with adrenomedullin markedly attenuated aldosterone-induced increases in collagen contents (18±1 µg/mg).
DISCUSSION

In this study, we examined the effects of adrenomedullin on cardiac oxidative stress and collagen accumulation in aldosterone/salt-treated SHR, a new model of human malignant hypertension with secondary hyperaldosteronism. Our results showed that chronic administration of aldosterone/salt led to the development of LV hypertrophy and increased expression of fibronectin, and collagen types I and III, as well as collagen content in SHR. The present study also provided evidence that aldosterone/salt-induced increases in LV ROS levels are associated with increases in NADPH oxidase activity, and p22phox and gp91phox expression. These data indicate that NADPH oxidase-dependent ROS production is involved in cardiac collagen synthesis in aldosterone-dependent malignant hypertensive rats. We also observed that chronic treatment with adrenomedullin prevented the augmentation of p22phox and gp91phox expression, NADPH oxidase activity, and TBARS levels in LV tissues of aldosterone-induced malignant hypertensive rats. In addition, treatment with adrenomedullin resulted in the marked attenuation of fibronectin and collagen types I and III expression, along with collagen content, without affecting blood pressure. These results suggest that exogenously administered adrenomedullin elicits cardio-protective effects via the inhibition of NADPH oxidase-dependent ROS production and collagen accumulation in aldosterone-dependent malignant hypertension.

In agreement with previous studies (Newaz et al., 1998), plasma TBARS levels of SHR were significantly higher than those of WKY rats. However, we did not find any differences in LV tissue TBARS levels, expression of NADPH oxidase components (p22phox and gp91phox) or NADPH oxidase activity between SHR and WKY rats.
Similarly, salt treatment alone did not alter LV TBARS levels, NADPH oxidase activity, and p22phox and gp91phox expression in these animals, indicating that systemic but not cardiac oxidative stress is enhanced in SHR and salt-treated SHR. On the other hand, aldosterone/salt-treatment significantly increased TBARS levels, NADPH oxidase activity and expression of p22phox and gp91phox in LV tissues of SHR. These data suggest that NADPH oxidase-mediated ROS production in LV tissues is enhanced during the development of aldosterone-dependent malignant hypertension. The present study also showed that augmentation of fibronectin and collagen type I and III gene expression as well as collagen content in LV tissues is associated with increases in ROS levels in aldosterone/salt-treated malignant hypertensive rats, suggesting the potential contribution of ROS to the pathogenesis of aldosterone-dependent cardiac collagen synthesis.

Organ protective effects of antioxidants on hypertension and tissue injury have been demonstrated in a variety of animal models (Newaz and Nawal, 1998; Nakano et al., 2003; Nishiyama et al., 2004; Park at el., 2004). Recent studies have also indicated that adrenomedullin elicits antioxidative effects (Shimosawa et al., 2002; Yoshimoto et al., 2004, 2005). In rat aortic vascular smooth muscle and endothelial cells, adrenomedullin attenuates angiotensin II-stimulated increases in intracellular ROS and NADPH oxidase activity (Yoshimoto et al., 2004, 2005). Animal studies have also demonstrated that adrenomedullin gene delivery reduces cardiac O$_2^-$ levels and NADPH oxidase activity in ischemia reperfusion injury (Kato et al., 2003). Kawai et al. (2004) showed that in adrenomedullin knockout mice, severe femoral arterial intimal thickening induced by cuff placement is associated with the upregulation of NADPH oxidase components and enhanced O$_2^-$ production. Collectively, these observations support the concept that
adrenomedullin reduces NADPH oxidase-dependent ROS production. In the present study, we observed that treatment with adrenomedullin significantly attenuated increases in p22phox and gp91phox expression, NADPH oxidase activity and TBARS levels in LV tissues of aldosterone/salt-treated SHR. These data indicate that adrenomedullin attenuates NADPH oxidase-mediated ROS production in aldosterone-dependent malignant hypertensive rats. In vitro studies have indicated that adrenomedullin inhibits collagen deposition by inhibiting NADPH oxidase-mediated O$_2^-$ generation (Yoshimoto et al., 2004). In the present study, treatment with adrenomedullin attenuated aldosterone-induced increases in LV ROS levels, NADPH oxidase expression and activity, as well as in collagen accumulation, independent of blood pressure changes. These data support the hypothesis that the cardio-protective effects of adrenomedullin are associated with the attenuation of NADPH oxidase-mediated ROS production in aldosterone-dependent malignant hypertension. To support this hypothesis further, it will be necessary to determine whether induction of oxidative stress negates cardioprotective effects of adrenomedullin.

In the present study, we aimed to examine the effects of exogenously administered adrenomedullin. Therefore, we did not clarify the role of endogenous adrenomedullin. Additionally, the present in vivo experiments did not allow us to present any data regarding the precise mechanisms by which adrenomedullin attenuates NADPH oxidase-mediated ROS production. Since plasma sodium and potassium levels or their urinary excretion rates were not changed by adrenomedullin infusion, the cardio-protective effects of adrenomedullin cannot be explained by changes in electrolyte balance. Although blood pressure was not changed by adrenomedullin, it is possible that
aldosterone-induced NADPH oxidase activation is attenuated by adrenomedullin-mediated improvement of tissue microcirculation. Alternatively, adrenomedullin may directly inhibit NADPH oxidase activity by some molecular mechanisms, as suggested by other investigators (Yoshimoto et al., 2004, 2005). Clearly, further studies are needed to address these issues.

In conclusion, the present results suggest that exogenously administered adrenomedullin elicits cardio-protective effects through the attenuation of NADPH oxidase-mediated ROS production and collagen accumulation in aldosterone-induced malignant hypertensive rats. Treatment with adrenomedullin might therefore be a potentially useful therapeutic strategy for preventing cardiac injury in aldosterone-dependent malignant hypertension.
REFERENCES


FOOTNOTES

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LEGENDS FOR FIGURES

Figure 1. Systolic blood pressure (SBP) in spontaneously hypertensive rats (SHR) and Wister-Kyoto (WKY) rats. *P<0.05 vs. vehicle/1% NaCl-treated SHR.

Figure 2. Thiobarbituric acid reactive substances (TBARS) levels in plasma (A) and left ventricular (LV) tissues (B) of SHR and WKY rats at 4 week of treatment. Open, dotted, closed and down diagonal bars represent experimental values in which vehicle, vehicle/1% NaCl, aldosterone/1% NaCl, aldosterone or adrenomedullin/1% NaCl, respectively, was administered. *P<0.05 vs. vehicle/1% NaCl-treated SHR. #P<0.05: aldosterone/1% NaCl-treated SHR vs. aldosterone and adrenomedullin/1% NaCl-treated SHR.

Figure 3. NADPH oxidase-dependent superoxide anion production in homogenates from LV tissues of SHR and WKY rats at 4 week of treatment. Open, dotted, closed and down diagonal bars represent experimental values in which vehicle, vehicle/1% NaCl, aldosterone/1% NaCl, aldosterone or adrenomedullin/1% NaCl, respectively, was administered. *P<0.05 vs. vehicle/1% NaCl-treated SHR. #P<0.05: aldosterone/1% NaCl-treated SHR vs. aldosterone and adrenomedullin/1% NaCl-treated SHR.

Figure 4. p22phox (A) and gp91phox (B) mRNA levels in LV tissues of SHR and WKY rats at 4 week of treatment. Open, dotted, closed and down diagonal bars represent experimental values in which vehicle, vehicle/1% NaCl, aldosterone/1% NaCl,
aldosterone or adrenomedullin/1% NaCl, respectively, was administered. *P<0.05 vs. vehicle/1% NaCl-treated SHR. #P<0.05: aldosterone/1% NaCl-treated SHR vs. aldosterone and adrenomedullin/1% NaCl-treated SHR.

Figure 5. Fibronectin (A), collagen type I (B) and collagen type III (C) mRNA levels in LV tissues of SHR and WKY rats at 4 week of treatment. Open, dotted, closed and down diagonal bars represent experimental values in which vehicle, vehicle/1% NaCl, aldosterone/1% NaCl, aldosterone or adrenomedullin/1% NaCl, respectively, was administered. *P<0.05 vs. vehicle/1% NaCl-treated SHR. #P<0.05: aldosterone/1% NaCl-treated SHR vs. aldosterone and adrenomedullin/1% NaCl-treated SHR.
Table 1. Body weight (BW) and left ventricular weight (LVW) in spontaneously hypertensive rats (SHR) and Wister-Kyoto (WKY) rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>SHR /tap water</th>
<th>SHR /1% NaCl + vehicle</th>
<th>SHR /1% NaCl + aldosterone</th>
<th>SHR /1% NaCl + aldosterone + adrenomedullin</th>
<th>WKY /tap water</th>
<th>WKY /1% NaCl + vehicle</th>
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<tr>
<td>Initial BW (g)</td>
<td>244±6</td>
<td>243±2</td>
<td>246±3</td>
<td>241±4</td>
<td>284±2*</td>
<td>270±5*</td>
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<tr>
<td>Final BW (g)</td>
<td>302±7</td>
<td>310±7</td>
<td>263±6*</td>
<td>244±8*</td>
<td>352±4*</td>
<td>334±6*</td>
</tr>
<tr>
<td>LVW (g)</td>
<td>0.88 ± 0.02*</td>
<td>0.98 ± 0.02</td>
<td>1.17 ± 0.03*</td>
<td>1.15 ± 0.04*</td>
<td>0.71 ± 0.02*</td>
<td>0.68 ± 0.02*</td>
</tr>
<tr>
<td>LVW/ BW (mg/g)</td>
<td>2.93 ± 0.09*</td>
<td>3.25 ± 0.07</td>
<td>4.42 ± 0.17*</td>
<td>4.78 ± 0.20*</td>
<td>2.04 ± 0.06*</td>
<td>2.05 ± 0.02*</td>
</tr>
</tbody>
</table>

Data represent mean±S.E.  *P<0.05 vs. vehicle/1% NaCl-treated SHR.
Figure 2

A

Plasma

(nmol/ml)

SHR

WKY

B

Left ventricle

(nmol/mg protein)

SHR

WKY

*  

#  

0.15  

0.25  

0.35  

0.45  

0.55
Figure 3

NADPH-stimulated O$_2^-$ production (cpm/mg protein)

*

#
Figure 4

A

p22phox/GAPDH (fold)

B

gp91phox/GAPDH (fold)

SHR

WKY

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Figure 5

(A) Fibronectin/GAPDH (fold) in SHR and WKY.

(B) Collagen I/GAPDH (fold) in SHR and WKY.

(C) Collagen III/GAPDH (fold) in SHR and WKY.

(D) Collagen content (µg/mg) in SHR and WKY.