Prevention of renovascular and cardiac pathophysiological changes in hypertension by angiotensin II type 1 receptor antisense gene therapy

JEFFREY R. MARTENS*, PHYLLIS Y. REAVES*, DI LU*, MICHAEL J. KATOVICH‡, KATHLEEN H. BERECEK‡, SANFORD P. BISHOP§, MOHAN K. RAIZADA*¶, AND CRAIG H. GELBAND*

*Department of Physiology, College of Medicine and †Department of Pharmacodynamics, College of Pharmacy, University of Florida, Gainesville, FL 23610; and ‡Department of Physiology and Biophysics and §Department of Pathology, University of Alabama, Birmingham, AL 35294

Communicated by George K. Davis, University of Florida, Gainesville, FL, December 10, 1997 (received for review September 23, 1997)

ABSTRACT Hypertension produces pathophysiological changes that are often responsible for the mortality associated with the disease. However, it is unclear whether normalizing blood pressure (BP) with conventional therapy is effective in reversing the pathophysiological damage. The duration and initiation of treatment, site of administration, and agent used all appear to influence the reversal of the pathophysiological alterations associated with hypertension. We have previously established that retrovirally mediated delivery of angiotensin II type 1 receptor antisense (AT1R-AS) attenuates the development of high BP in the spontaneously hypertensive (SH) rat model of human essential hypertension. Our objective was to determine whether this attenuation of high BP is associated with prevention of other pathophysiological changes induced by the hypertensive state. Intracardiac delivery of AT1R-AS in neonates prevented the development of hypertension in SH rats for at least 120 days. Contractile experiments demonstrated an impaired endothelium-dependent vascular relaxation (acetylcholine) and an enhanced contractile response to vasoactive agents (phenylephrine and KCl) in the SH rat renal vasculature. In addition, the voltage-dependent K+ current density, which is believed to contribute to smooth muscle resting membrane potential and basal tone, was decreased in renal resistance artery cells of the SH rat. AT1R-AS treatment prevented each of these renal vascular alterations. Finally, AT1R-AS delivery prevented the pathological alterations observed in the SH rat myocardium, including left ventricular hypertrophy, multifocal fibrosis, and perivascular fibrosis. These observations demonstrate that viral-mediated delivery of AT1R-AS attenuates the development of hypertension on a long term basis, and this is associated with prevention of pathophysiological changes in SH rats.

The elevation of systemic blood pressure (BP) associated with hypertension is a risk factor for cardiovascular disease and renal failure. Often it is the pathophysiological alterations and impairments associated with hypertension that lessen life expectancy. Pharmacological intervention has been relatively successful in normalizing the elevation in BP. However, the assumption that reduction of BP will totally reverse hypertensive damage remains unclear (1–4).

The duration of treatment, age at which the antihypertensive therapy is initiated, site of administration, and specific agent used all appear to influence the reversal of the pathophysiological alterations associated with the disease (5–7). In some instances, reversal of pathophysiological alterations may even be unfavorable, such as when regression of left ventricular hypertrophy and peripheral resistance occur in a disproportionate manner (8). Similarly, other reports have indicated that traditional antihypertensive agents can contribute to target organ injury by altering metabolic processes (i.e., hypercholesterolemia, glucose intolerance, hyperkalemia).

A role for the renin–angiotensin system (RAS) in the development of hypertension is well established in both humans and animal models such as the spontaneously hypertensive (SH) rat. Interruption of the RAS pathway, either by preventing the formation of angiotensin II (i.e., angiotensin-converting enzyme inhibitor) or by blocking its actions at the level of the peptide receptor (i.e., angiotensin II type 1 (AT1) receptor antagonists), has been shown to reduce BP and protect against target organ injury (9–13). However, the attenuation or delay of nonhemodynamic pathophysiological impairments with these agents does not entirely reduce the risk to hypertensive patients (3, 4). In addition, chronic administration of traditional therapies is necessary for longterm antihypertensive benefits. Required daily dosing and undesirable side effects such as sexual dysfunction, coughing, and lethargy diminish patient compliance.

We have previously established that retrovirally mediated delivery of AT1 receptor antisense (AT1R-AS) attenuates the development of high BP on a long term basis (14, 15). A single injection of AT1R-AS into neonatal SH rats prevented the development of hypertension up to 90 days after injection. Our objective in this study was to determine whether this attenuation of high BP was associated with the prevention of renal and cardiovascular pathophysiological changes induced by the hypertensive state.

MATERIALS AND METHODS

Preparation of Viral Particles Containing AT1R-AS. A retroviral vector, LNSV, was used to deliver AT1R-AS into the rats (16, 17). The AT1R-AS was cloned in the LNSV vector. It was transfected to packaging cell line PA317 (American Type Culture Collection). After selection by G418, the medium containing viral particles that expressed AT1R-AS (LNSV-AT1R-AS) was collected and used for all animal experiments. Viral particles that did not contain AT1R-AS (LNSV) were also prepared by the above protocol and used as a control.

Abbreviations: BP, blood pressure; RAS, renin–angiotensin system; SH, spontaneously hypertensive; AT1, angiotensin II type 1; AT1R-AS, AT1 receptor antisense; WKY, Wistar Kyoto; PSS, physiological saline solution; ACh, acetylcholine; Kv, voltage-dependent K+; PhE, phenylephrine; VSM, vascular smooth muscle; SHR, SH rat(s).

To whom reprint requests should be addressed: Dept. of Physiology, College of Medicine, University of Florida, P.O. Box 100274, Gainesville, FL 32610. e-mail: mraizada@ufl.edu.
Animals and Experimental Protocols. Five-day-old Wistar Kyoto (WKY) and SH rats were used in this study (Harlan Sprague–Dawley). They were divided into three groups: vehicle (control), virus alone (LNSV), or virus containing AT1-R-AS (LNSV-AT1-R-AS). Treatments were injected via the cardiac route under methoxyflurane (metofane, Pitman–Moore, Mundelein, IL) anesthesia. A bolus of 5 × 10^{10} plaque-forming units of viral particles in 10 μl of physiological saline was used per animal. There was an ~95% survival rate 24–48 hr after viral administration.

At the end of 120 days, rats from each group were used for determining the mean BP through an indwelling catheter implanted in the carotid artery essentially as described (14). Rats were anesthetized with a mixture of ketamine (30 mg/kg), xylazine (6 mg/kg), and acepromazine (1 mg/kg) administered intramuscularly (0.7 ml/kg). Direct BP was recorded from the carotid artery catheter (PE-50) in free-moving, nonrestrained animals with a pressure transducer coupled to a Digi-Med BP analyzer (Micromed, Louisville, KY).

Smooth Muscle Cell Isolation. Male rats were killed by decapitation, and the right and left kidneys were removed and placed in cold, oxygenated (95% O₂/5% CO₂) physiological saline solution (PSS). Renal resistance vessels were identified as the fourth to fifth branch distal to the renal artery. These vessels were dissected free of fat and connective tissue under a dissecting microscope. The vascular fragments were then cut into small pieces (~4 mm) and incubated at room temperature in Ca^{2+}-free PSS for 30 min. The pieces were subsequently resuspended in Ca^{2+}-free PSS digestion buffer for 20–30 min at 37°C. The Ca^{2+}-free PSS digestion buffer contained (in mg/15 ml): collagenase 4.5 (151 units/mg, Worthington), BSA 30, trypsin inhibitor 30, ATP (sodium salt) 1.7, and protease 1.5 (type XXIV, Sigma). After the digestion, the pieces were removed from the digestion buffer, rinsed in Ca^{2+}-free PSS, and gently triturated until a large number of elongated smooth muscle cells were observed. The isolated cells were collected and stored at 4°C until used. Cells were used between 2 and 10 hr after isolation.

Current Recording and Analysis. Single cells were voltage-clamped, and membrane currents were measured by using the whole-cell patch-clamp technique (18). Patch pipettes were made from borosilicate glass capillaries, pulled on a vertical puller (PP-83, Narishige, Tokyo), and fire-polished with a microforge (model MF-83, Narishige); the pipettes had resistances of 3–5 MΩ when filled with the pipette solution.

Voltage-clamp commands potentials were applied to the cells, and membrane current was recorded by using an Axopatch-1D patch-clamp amplifier (Axon Instruments, Foster City, CA). Membrane current was digitized on-line (10.0 KHz) with an analog-to-digital interface (Labmaster TL-1 DMA interface, Axon Instruments) and filtered at 2.0 kHz. Data analysis was performed with pCLAMP 6.0 software (Axon Instruments). All experiments were performed at room temperature.

Tension Measurements. A segment of the rat renal artery was dissected free and cleaned of fat and adventitia. Ring segments (3 mm long) were mounted onto two triangular tungsten wires (35 μm in diameter) and hung vertically in an isolated organ chamber (10 ml). The bottom triangle was mounted to a stable hook whereas the top triangle was attached to a Gould strain gauge. The bath was maintained at 37°C in PSS. A resting force of 1.5 g was applied to the vessels. Vessel segments were equilibrated for 1 hr with three exposures to phenylephrine (Phe, 1 μM). KCl, Phe, and acetylcholine (ACh) dose–response curves were performed in a cumulative manner.

Assessment of Ventricular Hypertrophy and Cardiac and Perivascular Fibrosis. Animals were sacrificed, hearts were excised and rinsed in 0.9% physiological saline, and total heart and ventricular weights were recorded. These weights were normalized to body weight (in g), and the ratios were compared among groups. In a separate series of experiments, the heart, liver, kidney, and adrenal gland were removed during deep anesthesia after arresting contraction by left ventricular injection of saturated KCl. These tissues were fixed in 3% freshly prepared paraformaldehyde in 0.15 M phosphate buffer and stored at 4°C for 24–72 hr and then placed in phosphate buffer with 0.25 M sucrose added (approximately 300 mosM solution) at 4°C. Slices of right and left ventricles, liver, kidney, and adrenal were dehydrated through a graded series of ethanol and xylene and embedded in paraffin. Sections were cut at 5 μm thickness, stained with hematoxylin, eosin, and aldehyde fuchsin Gomori trichrome, and examined by light microscopy. Heart tissue was qualitatively evaluated for type (perivascular, focal, or interstitial) and distribution (left or right ventricle, subendocardial, subepicardial) of connective tissue.

Solutions. The PSS used during cell isolation procedures and contraction experiments contained (in mM): NaCl 120, KCl 4.2, KH₂PO₄ 1.2, MgCl₂ 0.5, CaCl₂ 1.8, glucose 5.5, and Heps 30 (pH 7.35 with NaOH). For all whole-cell voltage-clamp experiments examining voltage-dependent K⁺ (Kv) currents, the bath solution contained (in mM): NaCl 125, KCl 4.2, KH₂PO₄ 1.2, MgCl₂ 2.3, D-glucose 5.5, tetraethylammonium 5.0, and Heps 30 (pH 7.4 with NaOH). The pipette solution for the whole-cell experiments contained (in mM): KCl 140, MgCl₂ 0.5, EGTA 1.0, ATP (magnesium salt) 5.0, and Heps 5.0 (pH 7.2 with KOH).

Statistics. Results are expressed as mean ± SE. Statistical significance was evaluated with repeated measures: ANOVA and Student’s t test were used for unpaired data. Differences were considered significant at P < 0.5. Membrane currents were measured from the zero current level and normalized to cell capacitance.

RESULTS

Effect of AT1-R-AS Treatment on BP. A single intracardiac injection of LNSV-AT1-R-AS to neonatal rats prevented the increase in BP exclusively in SH rats at 120 days postinjection (Fig. 1). The mean BP was 28.9% lower in SH rats treated with LNSV-AT1-R-AS than in untreated SH rats (116.3 ± 16.6 vs. 163.7 ± 6.0 mmHg; n = 6, P < 0.05). In addition the mean BP measured in LNSV-AT1-R-AS-treated SH rats was not significantly different from that of the normotensive WKY control rats (116.3 ± 16.6 vs. 115.6 ± 8.3 mmHg; n = 6, P > 0.05). This effect was specific for LNSV-AT1-R-AS treatment because treatment with LNSV alone had no significant effect on mean BP compared with control SH rats (n = 6). Antisense treatment or vector alone did not affect the BP of WKY rats (n = 6).

Effect of LNSV-AT1-R-AS Treatment on Renal Vascular Reactivity. Alterations in vascular contractile response are known to exist in the SH rats (19, 20). LNSV-AT1-R-AS treatment prevented the alterations in renal artery reactivity measured in the SH rats. Enhanced contractile responses to both potassium chloride (KCl, Fig. 2A) and phenylephrine (Phe, Fig. 2B) were observed in the SH rat model. A leftward shift in the KCl and Phe concentration–response relationships was observed in SH rats (Fig. 2A and B) when compared with WKY controls. The medium effective concentrations (EC₅₀) for KCl and Phe were 31.0 and 46.8% lower (n = 6) in the SH (24 mM, KCl and 202 nM, Phe) than in WKY (35 mM, KCl and 380 mM, Phe) rats, respectively. The shifts in the concentration–response relationships for KCl or Phe were not observed in LNSV-AT1-R-AS-treated SH rats (EC₅₀: 34 mM, KCl and 470 mM, Phe), and LNSV alone (EC₅₀: 26 mM, KCl and 132 mM, Phe) did not change the renal artery contractile properties to KCl or Phe. Finally, an impaired endothelial-dependent relaxation of precontracted renal arteries was also observed in

Physiology: Martens et al.


See Retraction Published October 6, 2004
the SH rat (Fig. 2C). When the arteries were precontracted with Phe (500 nM), the ACh dose–response curve was right shifted and the maximal relaxation was decreased 36% ($n = 6$) in the renal artery from the SH rat (Fig. 2C). The EC$_{50}$ value for ACh-induced relaxation in SH rat (143 nM) renal artery was increased 4.7-fold compared with WKY (30 nM). This shift was not seen in the LNSV-AT$_1$R-AS-treated animals (73 nM). However, it was still present in SH rat treated with LNSV alone (196 nM).

Effect of LNSV-AT$_1$R-AS on Kv Current Density. We have previously shown that Kv current density was decreased in vascular smooth muscle (VSM) cells from the interlobar arteries of the SH rat when compared with WKY control (20). Fig. 3 illustrates the characteristics of Kv current in single cells isolated from renal resistance vessels of WKY and SH rats. During voltage step depolarizations in the presence of 5 mM tetraethylammonium (to block the calcium-activated K* current), Kv current displayed two components. The first is a rapidly activating and inactivating component, which we termed the peak component of Kv current. The second is a slow inactivating sustained component.

Under the above conditions, the Kv current was reduced in cells isolated from the SH rat. This decrease was not evident in SH rats treated with LNSV-AT$_1$R-AS but was apparent in those treated with LNSV alone. Fig. 4 shows the mean current voltage relationship for both the peak (Fig. 4A) and sustained (Fig. 4B) component of Kv current. In each experiment the Kv current was reduced in cells from SH rats as compared with those obtained from WKY controls. Again, this decrease was not observed in cells from LNSV-AT$_1$R-AS-treated SH rats. It should be noted that the cell capacitance was not significantly different between all of the groups tested.

Effect of LNSV-AT$_1$R-AS on Heart Weights and Cardiac Morphology. Ventricular weights were recorded for all groups and normalized to body weight. The ventricular weights of SH rats treated with LNSV alone were significantly greater than the weights recorded for the normotensive WKY (Table 1). This increase was not seen in SH rats treated with LNSV-AT$_1$R-AS. There was no difference in the weights of the LNSV-treated and LNSV-AT$_1$R-AS-treated WKY ventricles. Fig. 5 shows the effect of LNSV-AT$_1$R-AS on cardiac and perivascular fibrosis. Fig. 5A shows photomicrographs of representative sections taken from left ventricular subendomyocardium from (top) WKY, SH, and LNSV-AT$_1$R-AS-treated SH rats, respectively. Multiple focal areas of fibrosis were evident in hearts from SH rats (indicated by arrows). This phenomenon was not seen in hearts from WKY or LNSV-AT$_1$R-AS-treated SH rats. Multiple focal areas of fibrosis were evident in hearts from SH rats (indicated by arrows). This phenomenon was not seen in hearts from WKY or LNSV-AT$_1$R-AS-treated SH rats. Multiple focal areas of fibrosis were evident in hearts from SH rats (indicated by arrows). This phenomenon was not seen in hearts from WKY or LNSV-AT$_1$R-AS-treated SH rats.

DISCUSSION

The results of this study demonstrate that delivery of AT$_1$R-AS prevents the development of hemodynamic and pathophysio-
logical alterations associated with hypertension in the SH rat. Neonatal SH rats that were given a single intracardiac injection of AT1R-AS did not develop an elevation in mean BP, altered renal vascular reactivity, decreased smooth muscle Kv channel current density, left ventricular hypertrophy, or cardiac and perivascular fibrosis. These novel data suggest that interruption of AT1 receptor in the developing SH rat, with a virally mediated gene delivery system, may be used to prevent hypertension and its associated renal and cardiovascular risk factors.

The role of the kidney in the control of fluid volume and BP implies its participation in the development of essential hypertension. The results of earlier renal transplant studies in humans and animals suggest that in a proportion of hypertension cases there exists structural or functional alterations in the kidney (21–24). In addition, evidence indicates that regulation of renal blood flow is an important element of the hypertensive process. Multiple studies have demonstrated that in hypertension there exists an enhancement in renal vascular tone (1, 25). This elevation in tone may lead to increased renal vascular resistance, a common observation in hypertension and elevating BP. Possible mechanisms responsible for the increase in renal vascular resistance include: (i) an enhanced contractile sensitivity to vasoactive agonists; (ii) an impaired endothelial-dependent relaxation; (iii) an increased Ca\(^{2+}\) transport across VSM membranes; (iv) an altered ion channel activity in VSM; and (v) smooth muscle hypertrophy or hyperplasia (26). Our results show that both KCl and Phe produced an enhanced contractile response in renal arteries of SH rats. However, this was not observed in SH rats treated with AT1R-AS. The augmented contractile response to vasoactive agents appears to depend on the vascular bed tested and the type of vasoconstrictor agent (27, 28). However, this does not diminish the significance of the result that alterations in both electromechanical coupling, which is membrane potential-dependent (KCl), and pharmacomechanical coupling, which is agonist-dependent (Phe), were prevented with AT1R-AS treatment.

A second mechanism that may contribute to increased renal vascular resistance and/or decreased renal blood flow is endothelial dysfunction. It is well established that endothelial-derived vasoactive agents regulate blood flow through vessels by controlling the contractile state of VSM cells (29). One such substance, nitric oxide (NO), is released by the endothelium to cause relaxation of the VSM cells (30). It has recently been reported that ACh-induced relaxation of coronary arteries is mediated by endothelial-derived NO (8). Our results show that there exists an impaired endothelial-dependent relaxation to ACh in the renal artery of SH rats. This alteration is prevented in SH rats treated with AT1R-AS. Reports of altered endothelial function appear to depend on a number of factors including vascular bed, model of hypertension, and constrictor agent used (31–33). Some reports suggest that the altered endothelial relaxation in hypertension is not because of im-

---

**Fig. 3.** Characteristics of Kv current in single VSM cells isolated from rat renal resistance arteries. The Kv current was reduced in cells from SHR and SHR + LNSV-treated rats. Delivery of AT1R-AS prevented this decrease. The voltage protocol is displayed at the top. The membrane capacitances of all 4 cells were similar (WKY, 28 pF; SHR, 28 pF; SHR + AT1R-AS, 29 pF; SHR + LNSV, 28 pF).

**Fig. 4.** Mean current–voltage relationships for peak (A) and sustained (B) components of Kv current. There was no significant difference in current density in the WKY (●, n = 7) and SHR + AT1R-AS-treated (▲, n = 6) rats. However, the Kv current density for both the peak and sustained components was significantly lower in the SHR (●, n = 12) and SHR + LNSV-treated (▲, n = 6) animals.
paired NO release but rather because of changes in release of vasoconstricting prostaglandins (31, 33). Regardless of the mechanism, our data demonstrate an impaired endothelial relaxation in the SH rats, which was prevented with AT1R-AS treatment.

A third mechanism by which renal vascular resistance may be increased in hypertension is altered ion channel function in VSM cells. It has been demonstrated that Kv current modulates membrane potential (34, 35). Tonic changes in membrane potential regulate Ca\(^{2+}\) influx and hence contractile tone in VSM cells. Blockade or reduction of this current may lead to increases in tone and ultimately to an increase in vascular resistance. There is substantial evidence for electrophysiological changes in hypertension (36–41). We have previously demonstrated that the Kv current is decreased in rat renal resistance vessels from both a genetic and nongenetic model of hypertension (18). Herein we show that this alteration can be prevented in the SH rat by treatment with AT1R-AS. The parallel between the prevention of the Kv current alterations and normalization of BP suggests that, in hypertension, the alterations in VSM cell K\(^+\) channel function represent feedback mechanisms occurring secondary to the change in arterial BP (42) or distal to a RAS-triggered event.

Cardiovascular ultrastructural changes are a major risk factor for morbidity and mortality in hypertension. Ventricular hypertrophy is a compensatory response to the increased pressure load attributable to the elevation in peripheral resistance (3). Whereas traditional pharmacological therapies have been shown to be effective in controlling and at times reversing these pathophysiological changes, there are often times when this approach is not successful (3, 4). It is important to note that regression of left ventricular hypertrophy has been reported in only 50% of patients treated with traditional antihypertensive drugs and that it is difficult to determine which patients will benefit from these therapies (4). Combination therapies may improve benefits because of the additive BP lowering effects of some antihypertensive agents. However, this may also increase the number and intensity of undesirable side effects and hence further decrease patient compliance. We found an increase in ventricular mass in SH rats treated with LNSV alone. However, this ventricular hypertrophy was prevented with AT1R-AS treatment. Other structural alterations (i.e., cardiac and perivascular fibrosis) were also prevented with AT1R-AS treatment.

Finally, evidence suggests that simply normalizing the BP by pharmacological means is not sufficient to completely regress pathophysiological changes associated with the hypertension. AT1R-AS gene therapy may be superior. It results in the prevention of the increase in mean BP and the associated pathophysiological impairments in hypertension. It may also offer an alternative to the compliance problem and complications of vascular and target organ injury. Finally, the AT1R-AS therapy does not produce a significant increase in plasma angiotensin II levels compared with the antihypertensive effects of Losartan, an AT1 receptor antagonist (15). Therefore, AT1R-AS therapy may have prolonged antihyper-

---

**Table 1.** WKY and SHR ventricular weights normalized to body weight

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>VW, g</th>
<th>VW/BW, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>4</td>
<td>1.05 ± 0.11</td>
<td>3.2 ± 0.09</td>
</tr>
<tr>
<td>WKY + LNSV</td>
<td>3</td>
<td>1.07 ± 0.20</td>
<td>3.0 ± 0.32</td>
</tr>
<tr>
<td>SHR</td>
<td>6</td>
<td>1.14 ± 0.08</td>
<td>3.6 ± 0.13</td>
</tr>
<tr>
<td>SHR + LNSV</td>
<td>3</td>
<td>1.54 ± 0.15*</td>
<td>4.8 ± 0.85*</td>
</tr>
</tbody>
</table>

SH rats treated with LNSV alone demonstrated a greater ventricular weight when compared with all other groups. Ventricular weights (VW) have been normalized to the body weight (BW) for all groups. Values are mean ± SE.

*P < 0.05.

---

**FIG. 5.** Effect of AT1R-AS on cardiac and perivascular fibrosis. (A) Photomicrographs of sections taken from left ventricular subendocardium from WKY, SHR, and SHR + AT1R-AS, respectively. Photographs were ×50 magnification at ×4 objective. Calibration bar is 12 mm = 250 μm. Arrows in SHR panel indicate multiple focal areas of fibrosis. This phenomenon was not seen in hearts from WKY or SHR + AT1R-AS. (B) Sections from midmyocardium showing small arteries and arterioles (<100 μm diameter) from WKY, SHR, and SHR + AT1R-AS-treated hearts. Photographs were taken at ×308 magnification at ×40 objective. Calibration bar is 15 mm = 50 μm. Vessels from SHR demonstrated dense perivascular fibrosis. This was not seen in vessels from WKY or AT1R-AS-treated rats.
tensive effects without the possible adverse side effects of elevated plasma angiotensin II.

A role for RAS in the development or maintenance of hypertension is well established (9, 10, 43–45). Our results confirm a fundamental role for AT1 receptors in both the development of high BP and the production of pathological organ damage. The interruption of the RAS pathway at an early age by using AT1R-AS offers the potential to prevent the development of hypertension and its associated pathophysiological alterations with a single antisense treatment. Of course this approach depends on the identification of genetic determinants of hypertension or on the demonstration of reliable prehypertensive risk factors. Studies are now under way to determine whether this gene delivery approach can be used to reverse the hemodynamic and pathophysiological alterations in the adult hypertensive animal.

LSNV was provided by Geoffrey Owens, University of Colorado, Health Science Center. This work was supported by grants from the National Institutes of Health (HL-52189 to C.H.G. and HL-56921 to M.K.R.) and the Council for Tobacco Research (to C.H.G.) and a predoctoral fellowship from the American Heart Association, Florida Affiliate (to J.R.M.).