Angiotensin II Type 1 Receptor Antisense Gene Therapy Prevents Altered Renal Vascular Calcium Homeostasis in Hypertension

Craig H. Gelband, Phyllis Y. Reaves, Jenafer Evans, Hongwei Wang, Michael J. Katovich, Mohan K. Raizada

Abstract—Intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)\(\text{i}\)]) homeostasis regulates vascular smooth muscle tone, and alteration in [Ca\(^{2+}\)\(\text{i}\)] handling is associated with the development and establishment of hypertension. We have previously established in the spontaneously hypertensive rat (SHR) that virally mediated delivery of angiotensin II type 1 receptor antisense (AT\(_1\) R-AS) prevents the development of high blood pressure and some pathophysiology associated with hypertension for 120 days. In light of this, our objectives in this study were to determine whether AT\(_1\) R-AS gene therapy (1) could have a longer duration in the prevention of hypertension and (2) would attenuate the alterations in renal vascular Ca\(^{2+}\) homeostasis and therefore vasoconstriction, characteristics of hypertension. Intracardiac delivery of AT\(_1\) R-AS in neonates prevented the development of hypertension in SHR for at least 210 days. At this time, untreated SHR renal resistance arterioles showed a significantly enhanced contractile response to KCl and angiotensin II (Ang II) when compared with normotensive Wistar-Kyoto rats. In addition, L-type Ca\(^{2+}\) current density and Ang II–dependent increases in [Ca\(^{2+}\)\(\text{i}\)] were significantly increased in cells dissociated from renal resistance arterioles of the untreated SHR. AT\(_1\) R-AS treatment prevented all of the above vascular alterations associated with the hypertensive state in SHR. Finally, Western blot analysis of L-type Ca\(^{2+}\) channel (\(\alpha_{1C}\)) protein levels in renal resistance arterioles of untreated SHR showed no significant difference when compared with control. These results are novel and demonstrate that viral-mediated delivery of AT\(_1\) R-AS not only attenuates the development of hypertension on a long-term basis but prevents changes in renal vascular Ca\(^{2+}\) homeostasis associated with the disease. (Hypertension. 1999;33[part II]:360-365.)

Key Words: angiotensin II ■ arterioles ■ calcium, intracellular ■ Ca\(^{2+}\) current ■ excitation-contraction coupling ■ gene therapy

Essential hypertension is characterized by normal cardiac output and an increase in total peripheral resistance.\(^1\) Hypertension is one of the most important risk factors for stroke, congestive heart failure, myocardial infarction, end-stage renal diseases, and peripheral vascular disease.\(^2\)–\(^4\) Studies from the last 2 decades have established that both circulating and tissue renin-angiotensin systems (RAS) are important, that their coordinated interaction is essential in the regulation of blood pressure, and that they play a key role in the development, establishment, and maintenance of hypertension.\(^2\),\(^5\),\(^6\)

The relevance of the RAS to blood pressure control is further supported by reports that various genes that encode renin, angiotensinogen, angiotensin-converting enzyme (ACE), and the angiotensin II type 1 receptor (AT\(_1\) R) have been associated with hypertension in both human and animal models.\(^7\)–\(^9\) Additionally, interruption in the expression of the RAS attenuates high blood pressure and other pathophysiological aspects of hypertension.\(^4\),\(^10\),\(^11\) In fact, blockade of the RAS has become a well-accepted treatment for Ang-dependent hypertension and congestive heart failure.\(^11\) Because ACE inhibition and AT\(_1\) R blockade are standard means to treat hypertension and because AT\(_1\) R encoding gene polymorphism is coupled with hypertension in both humans and in animal models of hypertension,\(^6\)\(^9\) it would appear only logical that AT\(_1\),R is an important target in the management of high blood pressure. Although major strides have been made in developing drugs that interfere with either angiotensin II (Ang II) formation or its action toward the management of Ang-dependent hypertension, there is neither a long-term preventive measure nor a cure for this disease.

The most widely used animal model for studying human essential hypertension is the spontaneously hypertensive rat (SHR). Pharmacological intervention has been relatively...
successful in normalizing the elevation in blood pressure associated with hypertension. However, the assumption that reduction of blood pressure will totally reverse hypertension-induced pathophysiological changes remains unclear. Several animal studies suggest that gene therapy would be useful in the treatment of hypertension. Olivero et al\(^{12}\) have shown that disruption of AT\(_R\) expression in mice causes blunting of pressor responses of Ang II. Phillips et al\(^{13}\) have used antisense oligonucleotides for the AT\(_R\) in the brain to reduce high blood pressure in SHR on a short-term basis. Recently, we have established that retrovirally mediated delivery of AT\(_R\) antisense (AT\(_R\)-AS) attenuates the development of high blood pressure on a long-term basis (up to 120 days) as well as prevents some of the pathophysiological alterations observed with the disease (eg, endothelial dysfunction, decreased K\(^+\) channel activity, increased heart weight, and increased cardiac and vascular fibrosis and necrosis) while having no inflammatory response.\(^{14}\) However, a more important pathophysiological alteration, the prevention of changes in cellular Ca\(^{2+}\) handling and therefore the level of vascular tone, has yet to be investigated.

Alterations in cellular Ca\(^{2+}\) homeostasis using \(^{45}\)Ca\(^{2+}\) influx, fluorometric, and patch-clamp measurements in large conduit SHR arteries and cells have been described.\(^{15}\) Renal transplant studies have suggested that the genetic defect in a missense had no effect on AT\(_R\) number or the actions of Ang II in Wistar-Kyoto rats (WKY) or SHR.\(^{14,17–19}\)

Methods

Preparation of Viral Particles Containing AT\(_R\)-AS

A retroviral vector, LNSV, was used to deliver AT\(_R\)-R-AS into the rats as previously described.\(^{14}\) Briefly, the AT\(_R\)-R-AS was cloned in the LNSV vector. It was transfected to packaging cell line PA317 (American Type Culture Collection). After selection by neomycin, the LNSV vector. It was transfected to packaging cell line PA317 containing AT\(_R\)-AS, was collected and used for all animal experiments. The LNSV-AT\(_R\)-AS was injected directly into the left ventricle of the heart under methoxyflurane anesthesia (metofane, 20 mg/kg, intraperitoneal). The animals were killed after 10 days by decapitation, and the right and left kidneys were removed and placed in cold oxygenated (95% O\(_2\)-5% CO\(_2\)) physiological saline solution (PSS). Renal resistance arterioles were identified as the fifth to sixth branch distal to the renal artery, cut into small pieces, and subsequently resuspended in Ca\(^{2+}\)-free PSS digestion buffer containing 20 to 30 minutes at 37°C. The Ca\(^{2+}\)-free PSS digestion buffer contained (in mg/15 mL) collagenase (151 U/mg Worthington Biochemical Corp) 4.5, bovine serum albumin (BSA) 30, trypsin inhibitor 30, ATP (sodium salt) 1.7, and protease (type XXIV, Sigma Chemical Co) 1.5. After digestion, the pieces were gently triturated until a large number of elongated smooth muscle cells were observed. The isolated cells were collected and stored at 4°C until use.

Current Recording and Analysis

Single cells were voltage-clamped, and membrane currents were measured using the whole-cell patch-clamp technique.\(^{14,20}\) Data analysis was performed with pCLAMP 6.0 software (Axon Instruments). All experiments were performed at room temperature. For whole-cell voltage-clamp experiments examining Ca\(^{2+}\) currents, the bath solution contained (in mmol/L) CaCl\(_2\) 1.8, d-glucose 5.5, TEA 5.0, and HEPES 10 (pH 7.4 with CsOH). The pipette solution for the whole-cell experiments contained (in mmol/L) CsCl 140, MgCl\(_2\) 0.5, EGTA 10, ATP (magnesium salt) 5.0, and HEPES 5.0 (pH 7.2 with CsOH).

Tension Measurements

Rat renal resistance arteriole rings (1.5 mm long) were mounted onto 2 triangular tungsten wires (35 mesh, 0.1 mm diameter) and hung vertically in an isolated organ chamber (5 mL) as previously described.\(^{14}\) The bath was maintained at 37°C in PSS.

[Ca\(^{2+}\)] Measurement

[Ca\(^{2+}\)] was measured in dissociated renal resistance arteriolar cells using epifluorescence microscopy. Briefly, the cells were loaded with fura 2 by incubation with 5 mmol/L membrane-permeable fura 2 acetoxymethyl ester (fura 2-AM dissolved in 1 mmol/L DMSO stock) for 30 minutes at 37°C. The cells were illuminated alternately with UV light of 340 and 380 nm wavelength using an IonOptix chopped, electronically controlled, dual-excitation imaging fluorescence system. Cell fluorescence (emitted light) was collected through a 510-nm barrier filter before acquisition by a photomultiplier tube. The fluorescence signals, F\(_{340}\) and F\(_{380}\), were background subtracted during the experiment. The mean change F\(_{340}\)min and F\(_{380}\)max ratios are graphed, which gives a relative indication in the changes observed in [Ca\(^{2+}\)]. The R\(_{340}\) and R\(_{380}\) ratios of WKY and SHR cells treated with and without virus is not significantly different.

Western Blot Analysis

Renal resistance arterioles were snap-frozen with liquid nitrogen. To isolate proteins, 0.1 g of tissue was homogenized in 2.0 mL of cold Tris-HCl buffer containing 2.5 mg/mL leupeptin and 4 mg/mL calpain inhibitors, pH 7.4. The suspension was centrifuged at 30,000g for 20 minutes at 4°C. The resultant pellet was resuspended in the above solution and recentrifuged. This pellet was resuspended in 1.2% digitonin, 300 mmol/L KCl, 150 mmol/L NaCl, 10 mmol/L NaPO\(_4\), peptatin A (0.1 \(\mu\)g/mL), leupeptin (0.1 \(\mu\)g/mL), aprotinin (0.1 \(\mu\)g/mL), AESFB (0.02 mmol/L), benzamidine (0.01 mg/mL), and calpain (0.8 \(\mu\)g/mL), pH 7.4. The protein concentration in the supernatant was determined by Bio-Rad assay using BSA for the standard curve.

For immunoblotting, 20 \(\mu\)g protein was denatured with Laemmli’s sample buffer in a boiling water bath for 3 minutes. Each mixture was electrophoresed in 4% to 15% SDS–polyacrylamide gel and

\[\text{[Ca}^{2+}\text{]}\]
transferred onto nitrocellulose. The membrane was blocked with 10% nonfat dried milk in PBST-BSA for 1 hour followed by incubation overnight at 4°C with either anti-\(\beta\)-actin (1:100, Sigma) or anti-\(\alpha_{\text{c}}\) Ca\(^{2+}\) channel protein (1:1000, Alamone Labs). Protein-bound antibody was detected by incubation of the membrane with horseradish peroxidase–labeled secondary antibody (1:50 000, Jackson ImmunoResearch Laboratory), enhanced by chemiluminescent assay reagents, and recognized by exposure to film.

**Statistics**

Results are expressed as mean±SEM. Statistical significance was evaluated using repeated-measures ANOVA and Student’s t test for unpaired data. Differences were considered significant at \(P<0.05\). Membrane currents were measured from the zero current level and normalized to cell capacitance.

**Results**

**Effect of AT\(_{1}\)-R-AS Treatment on Blood Pressure**

A single intracardiac injection of LNSV-AT\(_{1}\)-R-AS to neonatal rats prevented the increase in blood pressure exclusively in SHR for at least 210 days after injection (Figure 1). The mean blood pressure was 36.6% lower in SHR treated with LNSV+AT\(_{1}\)-R-AS than in untreated SHR (128±6 mm Hg, \(n=6\); \(P<0.01\)). In addition, the mean blood pressure measured in LNSV-AT\(_{1}\)-R-AS–treated SHR was not significantly different from that of the normotensive WKY (Figure 1). It has been previously demonstrated that L-type Ca\(^{2+}\) current in small arterioles of the kidney, which play a significant role in regulating peripheral resistance. LNSV-AT\(_{1}\)-R-AS treatment prevented the alterations in voltage- and receptor-dependent renal arteriolar vascular reactivity measured in SHR (Figure 2). Enhanced contractile responses to both KCl (Figure 2A) and Ang II (Figure 2C) were observed in the SHR. A significant leftward shift in the KCl and Ang II concentration-response relationships was observed in SHR when compared with WKY controls (Figure 2A and 2C). The media effective concentrations (EC\(_{50}\)) for KCl and Ang II were 11.1±2.1 mmol/L and 19.7±2.8 mmol/L (\(n=24\) rings from 6 animals) in the untreated SHR and 36.6±3.4 mmol/L and 76.4±3.3 mmol/L (\(n=24\) rings from 6 animals) in the WKY, respectively. LNSV-AT\(_{1}\)-R-AS prevented the shift in the EC\(_{50}\) for both KCl and Ang II (Figure 2B and 2D). The EC\(_{50}\) for KCl and Ang II in the LNSV-AT\(_{1}\)-R-AS–treated SHR was 39.1±4.2 mmol/L and 68.7±4.2 mmol/L (\(n=24\) rings from 6 animals), which was not significantly different from that of the WKY. Finally the EC\(_{50}\) for KCl and Ang II in the LNSV-treated SHR was 21.1±3.8 mmol/L and 17.7±2.1 mmol/L (\(n=24\) rings from 6 animals), which was not significantly different from that of the untreated SHR.

**Effect of LNSV-AT\(_{1}\)-R-AS Treatment on Voltage and Receptor-Dependent Renal Arteriolar Vascular Reactivity**

Enhanced contractile responses to both KCl (Figure 2A) and Ang II (Figure 2C) were observed in the SHR. A significant leftward shift in the KCl and Ang II concentration-response relationships was observed in SHR when compared with WKY controls (Figure 2A and 2C). The media effective concentrations (EC\(_{50}\)) for KCl and Ang II were 11.1±2.1 mmol/L and 19.7±2.8 mmol/L (\(n=24\) rings from 6 animals) in the untreated SHR and 36.6±3.4 mmol/L and 76.4±3.3 mmol/L (\(n=24\) rings from 6 animals) in the WKY, respectively. LNSV-AT\(_{1}\)-R-AS prevented the shift in the EC\(_{50}\) for both KCl and Ang II (Figure 2B and 2D). The EC\(_{50}\) for KCl and Ang II in the LNSV-AT\(_{1}\)-R-AS–treated SHR was 39.1±4.2 mmol/L and 68.7±4.2 mmol/L (\(n=24\) rings from 6 animals), which was not significantly different from that of the WKY. Finally the EC\(_{50}\) for KCl and Ang II in the LNSV-treated SHR was 21.1±3.8 mmol/L and 17.7±2.1 mmol/L (\(n=24\) rings from 6 animals), which was not significantly different from that of the untreated SHR.

**Effect of LNSV-AT\(_{1}\)-R-AS on L-Type Ca\(^{2+}\) Current Density**

It has been previously demonstrated that L-type Ca\(^{2+}\) current is increased in large conduit arteries of the SHR. Figure 3 illustrates the characteristics of L-type Ca\(^{2+}\) current in single cells isolated from renal resistance vessels of WKY and SHR. During voltage step depolarizations in the presence of block-
ers of K⁺ current, an inward Ca²⁺ current was observed. This Ca²⁺ current was L-type because it is completely inhibited by the dihydropyridine nifedipine (100 nmol/L, data not shown). It is quite evident from the current recordings (Figure 3A) and the current-voltage relationships (Figure 3B, n=21 cells from 6 animals) that Ca²⁺ current density in the cells from the SHR was increased when compared with cells from the WKY. This increase in Ca²⁺ current was not evident in SHR treated with LNSV alone (Figure 3A and 3B). A representative Western blot of L-type Ca²⁺ channel protein is shown. No change was evident in the amount of α₁c Ca²⁺ channel protein in any treatment group when normalized to β-actin protein level (n=6).

**AT,R-AS Gene Delivery Does Not Affect Ca²⁺ Current Kinetics**

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<thead>
<tr>
<th>Species and Treatment</th>
<th>Activation</th>
<th>Inactivation</th>
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<tbody>
<tr>
<td></td>
<td>V₁/₂, mV</td>
<td>Slope</td>
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<tr>
<td>WKY</td>
<td>-1.5±0.6</td>
<td>7.8±0.3</td>
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<tr>
<td>SHR</td>
<td>-0.9±1.0</td>
<td>8.1±0.4</td>
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<tr>
<td>SHR+AT,R-AS</td>
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<tr>
<td>SHR+LNSV</td>
<td>-1.4±0.4</td>
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n=21 cells from 6 animals for each group.

Boltzmann function, membrane potential at which one-half activation or inactivation (V₁/₂) or the slope of the Boltzmann function was unaffected in cells from the WKY, SHR, SHR+LNSV, or SHR+AT,R-AS. Finally, we examined whether there was an increase in Ca²⁺ channel protein density in the renal vasculature of the SHR. Western blot analysis (Figure 4) using an antibody that recognized α₁c Ca²⁺ channel proteins illustrated that when normalized to β-actin, there was no increase in the number of L-type Ca²⁺ channels in the SHR renal resistance arterioles when compared with those in the WKY. Administration of LNSV-AT,R-AS did not alter these data.

**Effect of LNSV-AT,R-AS on Voltage- and Receptor-Dependent Changes in [Ca²⁺]**

Because the KCl and Ang II concentration-response relationship was shifted to the left in the SHR and this was prevented in the SHR treated with LNSV-AT1R-AS, we wanted to examine next whether alterations in intracellular Ca²⁺ handling would be prevented using LNSV-AT,R-AS gene therapy. We have previously shown that basal and Ang II–stimulated increases in [Ca²⁺], were greater in renal arteriolar cells of the SHR when compared with WKY. Figure 5 shows the change in [Ca²⁺], when renal resistance artery cells were challenged with KCl (30 mmol/L, Figure [fig5]A) or Ang II (30 mmol/L, Figure [fig5]B). These doses were chosen because they are approximately 50% effective. Both KCl and Ang II significantly increased [Ca²⁺], in renal arteriolar cells of the SHR when compared with WKY cells.

**Figure 3.** Characteristics of L-type Ca²⁺ current in single vascular smooth muscle cells isolated from rat renal resistance arteries. A, The Ca²⁺ current was increased in cells from SHR and SHR+LNSV when compared with WKY. Delivery of AT,R-AS prevented this increase. The membrane capacitances of all 4 cells were similar (WKY, 27 pF; SHR, 29 pF; SHR+AT,R-AS, 28 pF; SHR+LNSV, 28 pF). B, There was no significant difference in current density of the cells from the WKY (n=21 cells from 6 animals) and SHR+AT,R-AS (n=18 cells from 6 animals). However, the Ca²⁺ current density for both the peak and sustained components was significantly greater in the SHR (n=25 cells from 6 animals) and SHR+LNSV (n=20 cells from 6 animals).

**Figure 4.** Western blot analysis of α₁c in cells from renal resistance arterioles. A representative Western blot of L-type Ca²⁺ channel protein is shown. No change was evident in the amount of α₁c Ca²⁺ channel protein in any treatment group when normalized to β-actin protein level (n=6).
The kidneys, which exquisitely control blood pressure and extracellular fluid volume and ultimately renal blood flow, participate 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 exist structural or functional alterations in the kidney. An elevation in renal vascular tone and therefore renal vascular resistance exists in hypertension; this elevation may be due to a number of factors including (1) an enhanced contractile sensitivity to vasoactive agonists, (2) an impaired endothelium-dependent relaxation, (3) an increased Ca\textsuperscript{2+} transport across vascular smooth muscle membranes, (4) an altered ion channel activity in vascular smooth muscle, and (5) smooth muscle hypertrophy or hyperplasia.

One fundamental reason why renal vascular resistance may be increased in hypertension is an altered ion channel function in vascular smooth muscle cells. Tonic changes in membrane potential and thus Ca\textsuperscript{2+} influx regulate contractile tone of resistance arterioles. A number of reports show that Ca\textsuperscript{2+} current density is increased in the SHR model of hypertension. However, these studies have been in conduit arteries, which play a less significant role in the regulation of peripheral resistance. Here we show that not only is Ca\textsuperscript{2+} current density increased in the SHR, but there is no change in the level of Ca\textsuperscript{2+} channel protein in renal resistance vessels. These data suggest that there may be an altered intracellular regulation of L-type Ca\textsuperscript{2+} channels that may underlie the enhanced Ca\textsuperscript{2+} influx in vascular smooth muscle cells and increases in vascular resistance observed in the kidney in hypertension. It is possible that an increase in Ca\textsuperscript{2+} influx could tend to load the sarcoplasmic reticulum with more Ca\textsuperscript{2+}, enabling more to be released on challenge by a physiological stimuli. Here we show that this altered Ca\textsuperscript{2+} handling by the sarcoplasmic reticulum can be prevented using AT\textsubscript{1}R-AS. This prevention would underlie the shift of the concentration-response curve back to normal with AT\textsubscript{1}R-AS treatment. Physiologically, this would tend to decrease the amount of vascular tone in the kidney for a given concentration of circulating Ang II.

Finally, cardiovascular ultrastructural changes are a major risk factor for morbidity and mortality in hypertension, and Ang II has been speculated to play a role in the increased vascular hypertrophy and/or hyperplasia in hypertension. Could gene therapy delivery systems interrupting the RAS be used to control the vascular remodeling/hypertrophy seen in various forms of hypertension? We have previously shown that AT\textsubscript{1},R-AS treatment could prevent the observed increase in perivascular fibrosis and ventricular hypertrophy in the SHR. Liao et al\textsuperscript{14} have demonstrated that receptor-dependent stimulation of a Ca\textsuperscript{2+}-dependent PKC isoform activates mitogen-activated protein kinase (MAPK) and thus regulates vascular smooth muscle cell growth. The above associated complications occur on a long-term basis, and their cellular mechanisms of action appear to be a Ca\textsuperscript{2+}-dependent process. Therefore, interference in the RAS using gene therapy may be an important pharmacological target to block vascular ultrastructural and remodeling changes in hypertension.

**Discussion**

The results of this study are novel and demonstrate for the first time that the delivery of AT\textsubscript{1},R-AS prevents (1) the development of hypertension for up to 210 days and (2) the alterations in [Ca\textsuperscript{2+}], homeostasis associated with hypertension in the SHR. Neonatal SHR that were given a single intracardiac injection of AT\textsubscript{1},R-AS did not develop (1) elevated blood pressure; (2) increased voltage-dependent Ca\textsuperscript{2+} influx, as measured by an increase in Ca\textsuperscript{2+} current density, across renal vascular smooth muscle membranes; (3) an increase in Ang II–mediated Ca\textsuperscript{2+} influx; or (4) increased Ca\textsuperscript{2+} release from sarcoplasmic reticulum. These findings, along with our previous observations,\textsuperscript{14} suggest that interruption of the RAS with virally mediated gene delivery may be used to prevent hypertension and its associated renal pathophysiological complications.

Figure 5. KCl- and Ang II–mediated changes in [Ca\textsuperscript{2+}]. Both KCl (A) and Ang II (B)–mediated increases in [Ca\textsuperscript{2+}] were significantly greater in SHR and SHR-LNSV than in WKY (n=162 cells from 4 animals; *P<0.01). AT\textsubscript{1},R-AS prevented the alteration in both KCl- and Ang II–mediated increases in [Ca\textsuperscript{2+}]. (n=134 cells from 4 animals; **P<0.01).

(n=162 cells from 4 animals). These increases in [Ca\textsuperscript{2+}], were completely prevented when SHR were treated with LNSV-AT\textsubscript{1},R-AS (n=134 cells from 4 animals). There was no significant difference between SHR+LNSV and the untreated SHR (n=122 cells from 4 animals). When experiments were performed in zero external calcium, all of the KCl-dependent contractions were abolished. However, Ang II still produced a greater peak [Ca\textsuperscript{2+}], measurement in renal arteriolar cells from the SHR than from the WKY. These increases in [Ca\textsuperscript{2+}], were completely prevented when SHR were treated with LNSV-AT\textsubscript{1},R-AS (data not shown). These data support the conclusion that LNSV-AT\textsubscript{1},R-AS gene therapy prevents altered Ca\textsuperscript{2+} handling in hypertension.
Is antisense gene therapy targeting the RAS a therapeutic step forward? In short, the answer is yes. It results in the prevention of the increase in mean blood pressure and the associated pathophysiological impairments in hypertension. It also offers an alternative to the compliance problem and complications of vascular and target-organ injury. Finally, AT₁R-AS therapy does not produce a significant increase in plasma Ang II levels compared with losartan, the AT₁R antagonist. Therefore, AT₁R-AS gene delivery and therapy do have prolonged antihypertensive effects without the possible adverse side effects produced by traditional pharmacological therapies. One caveat of the approach taken in this study is that AT₁R-AS gene delivery prevents the pathophysiological complications occurring with the development of hypertension. Moreover, this type of gene delivery/therapy depends on the identification of genetic determinants of hypertension or on the demonstration of reliable prehypertensive risk factors before it can be used in human trials. Another approach currently being investigated in our laboratory is to develop the next generation of viral vectors, which would increase the feasibility of delivering RAS-relevant genes into the adult SHR to reverse pathophysiological complications observed in hypertension. Preliminary studies show that delivery of RAS-relevant antisense into the adult SHR reverses the increase in blood pressure as well as the differences in excitation-contraction coupling observed in hypertension (C.H. Gelband, unpublished observations, 1998). In summary, gene therapy holds promise in single-dose, long-term treatment for hypertension.

Acknowledgments

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.), the Council for Tobacco Research (to C.H.G.), and the American Heart Association, Florida Affiliate (postdoctoral and predoctoral fellowships to P.Y.R. and J.E., respectively). We would like to thank Rebecca Kocerha for technical assistance.

References

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