Abstract—Angiotensin I–converting enzyme (ACE) inhibitors have been proven to be highly effective and are for the most part the drugs of choice in the treatment and control of hypertension, congestive heart failure, and left ventricular dysfunction. Despite this, questions regarding side effects and compliance with this traditional pharmacological strategy remain. In view of these observations, coupled with recent advances in gene-transfer technology, our objective in this study was to determine whether the expression of ACE could be controlled on a permanent basis at a genetic level. We argued that the introduction of ACE antisense to inhibit the enzyme would be a prerequisite in considering the antisense gene therapy for the control of hypertension and other related pathological states. Retroviral vectors (LNSV) containing ACE sense (LNSV-ACE-S) and ACE antisense (LNSV-ACE-AS) sequences were constructed and were used in rat pulmonary artery endothelial cells (RPAECs) to determine the feasibility of this approach. Infection of rat RPAECs with LNSV-ACE-S and LNSV-ACE-AS resulted in a robust expression of transcripts corresponding to ACE-S and ACE-AS, respectively, for the duration of these experiments, ie, 8 consecutive passages. The expression of ACE-AS but not of ACE-S was associated with a permanent decrease of 70% to 75% in ACE expression and a 50% increase in the B_max for the AT_1s. Although angiotensin II caused a concentration-dependent stimulation of intracellular Ca^2+ levels in both ACE-S– and ACE-AS–expressing cells, the stimulation was significantly higher in ACE-AS–expressing RPAECs. In vivo experiments demonstrated a prolonged expression of ACE-AS transcripts in cardiovascularly relevant tissues of rats. This was associated with a long-term reduction in blood pressure by 15 mm Hg, exclusively in the spontaneously hypertensive rat. These observations demonstrate that delivery of ACE-AS by retroviral vector results in a permanent inhibition of ACE and a long-term reduction in high blood pressure in the spontaneously hypertensive rat. (Circ Res. 1999;85:614-622.)

Key Words: virally mediated delivery ▪ angiotensin-converting enzyme inhibitor ▪ endothelial cell ▪ hypertension ▪ gene therapy

The renin-angiotensin system (RAS) plays an important role in the control of blood pressure (BP), body fluid homeostasis, and several other cardiovascular actions.1–3 Hyperactivity of the RAS has been implicated in such pathophysiological states as hypertension, congestive heart failure, left ventricular hypertrophy, and atherosclerosis. The importance of RAS in the pathogenesis of these diseases is further underscored by the fact that pharmacological agents that interrupt either the formation of angiotensin (Ang) II or its actions are a proven therapy for hypertension and many other cardiovascular- and organ-related diseases. Ang I–converting enzyme (ACE) inhibitors, which inhibit the formation of physiologically active Ang II from its inactive precursor, Ang I, are one such class of drugs. These drugs have achieved a widespread usefulness and, for the most part, are the drugs of choice in the treatment and management of hypertension, congestive heart failure, and left ventricular hypertrophy.4,5 The physiological mechanism by which ACE inhibitors produce such diverse benefits is based on the conclusion that ACE is strategically poised to regulate both the RAS and the kallikrein-kinin system. Thus, as a result of ACE inhibition, Ang II formation is blocked, resulting in the reduction of the effects of Ang II on vascular smooth muscles, vascular endothelium, central nervous system, heart, kidney, and other organ systems involved in the regulation of BP and tissue remodeling.4–6 In addition, ACE inhibition promotes bradykinin-mediated stimulation of nitric oxide production, which provides beneficial effects on endothelium, vasodilation, and tissue remodeling.4–6 In summary, ACE inhibitors are a proven pharmacological therapy and appear to exert...
major effects on the vascular endothelium, both to prevent and, in some cases, to reverse the endothelial dysfunction induced by pathological states.

Despite overwhelming evidence in support of effectiveness of ACE inhibitors in the treatment of cardiovascular diseases, these agents are not without major side effects. They are known to cause hypotension (especially in the renin-dependent state of hypertension), hyperkalemia, a reversible decline in renal damage, and cough, to name a few.\(^7,8\) For example, cough is a single side effect, which has been associated with the lack of compliance in patients.\(^8\) The compliance problem is further compounded by the fact that the ACE inhibitors have to be administered on a regular basis for them to provide continuous beneficial effects.

We decided to explore the hypothesis that inhibition of ACE at a genetic level would produce highly specific and long-term antihypertensive effects. This approach, if successful, might resolve the compliance issue and other side effects common with pharmacological therapy. Moreover, it would provide a model to elucidate the cellular and molecular mechanisms associated with the beneficial effects of ACE inhibition in specific Ang II–responsive organs. Antisense cDNA targeted toward ACE for a gene therapy approach offers an additional advantage, given that ACE inhibitors have a proven track record for the treatment of hypertension, and that ACE gene polymorphism has been linked with hypertension.\(^9,10\)

In this study, we present in vitro and in vivo evidence to demonstrate that virus-mediated delivery of ACE-AS causes permanent inhibition of ACE and a long-term reduction in high BP in the spontaneously hypertensive rat (SHR).

**Materials and Methods**

**Materials**

Wistar Kyoto rats (WKY) and SHR bred from our own colony were used for this study. The original source of breeding animal was purchased from Charles River Breeding Laboratories (Wilmington, Mass). Rat pulmonary artery endothelial cells were kindly provided by Gary A. Visner, University of Florida College of Medicine. Amphotropic PA317 packaging cells and NIH3T3 cells were obtained from American Type Culture Collection. DMEM, M199 medium, geneticin (G-418 sulfate), and Superscript II RNase H–reverse transcriptase were from Life Technologies. FBS and cadet bovine serum were from BioCell. Trypsin was from Worthington Biochemical Corp. [\(^{32}\)P]dCTP (3000 Ci/mmol) was from DuPont/NEN. 

**Methods**

**Construction of LNSV-ACE-S and LNSV-ACE-AS Retroviral Recombinants**

Rat ACE cDNA was generated by RT-PCR with the use of rat ACE-specific primers 1 essentially as described previously.\(^11,14\) In brief, the RT reaction was performed with 5 µg of total RNA from rat lung as described previously.\(^11,14\) Two microliters of RT solution was subjected to PCR with the use of 50 pmol of primers for 30 cycles (94°C for 1 minute, 58°C for 1 minute, and 72°C for 1 minute). PCR products were electrophoresed, and a DNA band of 938 bp was excised and purified.\(^15,16\) The identity of the cDNA was confirmed by sequencing.

**Recombination of ACE-S and ACE-AS With LNSV**

The retroviral vector LNSV was digested with HindIII followed by cloning of ACE-S and ACE-AS sequences corresponding to nucleotides 254 to 1181 in the coding region of the rat ACE into the unique HindIII site by blunt ligation. Recombinant DNA was transformed into competent HB101 bacterial cells, and ACE-S and ACE-AS colonies were selected. The colonies that produced ACE-S or ACE-AS were then grown in Luria Bertani medium with ampicillin (100 µg/ml), and recombiant DNAs were purified. LNSV has a neomycin-resistance gene for selection driven by a long-terminal repeat promoter (Figure 1A).

Various restriction enzymes were used to characterize the recombinants. For example, a SacI digestion of LNSV-ACE-S provided bands corresponding to ~1.2, ~2.9, and ~3.1 kb. Bands of ~0.9, 3.0, and ~3.2 kb were obtained for a similar digestion of ACE-AS (Figure 1B). EcoRI digestion provided 3 bands of ~1.6, ~2.3, and ~3.2 kb for ACE-S (Figure 1B), and 3 bands of ~1.6, ~1.69, and ~3.8 kb for ACE-AS. These bands were observed as anticipated on the basis of the localization of various restriction sites within the
plasmid (Figure 1A). Final characterization was performed by sequence analysis of all the fragments. The ACE-S sequence cannot and did not encode for active ACE protein, even though it contained the first active site of ACE. This was a result of a deliberate designing of the primers.

**Preparation of Retroviral Medium Containing LNSV-ACE-S and LNSV-ACE-AS Viral Particles**

Exponentially growing PA317 packaging cells in 60-mm-diameter tissue culture dishes were used for transfection and preparation of viral particles. Cells were seeded at 1×10⁵ and were grown for 1 day in DMEM containing 10% FBS. Cells were transfected with 2 μg of recombinant DNA containing LNSV-ACE-S or LNSV-ACE-AS mixed with 2 μL of lipofectin in 200 μL of serum-free DMEM. Culture media were replaced 6 hours after transfection, and cells were allowed to grow for 24 hours in DMEM containing 10% FBS. This was followed by G418 (800 μg/mL) selection and extension as described previously. Initially, linear relationships between the PCR products and PCR cycles and concentration dependence of the PCR reaction were carried out to establish optimum PCR conditions as described elsewhere.

**RT-PCR Measurement of AT₁, AT₂, and Endogenous ACE mRNAs**

Total RNA from LNSV-ACE-S and LNSV-ACE-AS–infected RPAECs were isolated and subjected to RT and PCR reactions essentially as described above and elsewhere with the use of AT₁, AT₂, and rat ACE primers. Initially, linear relationships between the PCR products and PCR cycles and concentration dependence of the PCR reaction were carried out to establish optimum PCR conditions as described elsewhere. Previously established optimum conditions for AT₁ and AT₂ and for endogenous ACE mRNAs were subsequently used for semiquantification. In brief, 5 μL of PCR samples containing γ-32P–labeled PCR products were mixed with 5 μL of 2× gel loading buffer (4% Ficoll 400, 20 mmol/L EDTA [pH 8.0], 0.2% SDS, 0.05% bromphenol blue, and 0.05% xylene cyanol) and applied to a 5% acrylamide gel (29:1, acrylamide/bis-acrylamide ratio) in 1× TBE buffer (in mmol/L, Tris base 89, boric acid 89, and EDTA [pH 8.0] 2). The gel was run for 45 minutes at 120 V in a minigel system (Bio-Rad Laboratories). After the electrophoresis, the gel was wrapped in a plastic bag and exposed to x-ray film, which was then developed, and bands representing PCR products were quantified with Bio-Rad Molecular Analysis software. β-Actin mRNA levels were measured in parallel samples for normalization.

**Measurement of ACE Activity in LNSV-ACE-S– and LNSV-ACE-AS–Infected RPAECs**

Uninfected and LNSV-ACE-S– and LNSV-ACE-AS–infected RPAECs were grown to confluence. Medium was replaced with serum-free M199 for 24 hours. Cells were collected and used to determine levels of ACE activity essentially as described by Neels et al. Briefly, cells were scraped off the dish and homogenized in PBS (pH 7.4) for 10 seconds, followed by centrifugation at 1500 g for 10 minutes at 4°C. The supernatants (25 μL) were incubated at 37°C with 25 mmol/L Hip-Gly-Gly substrate in HEPE5 buffer (pH 7.4) for 40 minutes, and the reaction was stopped by addition of sodium tungstate and dilute sulfuric acid. After centrifugation, borate buffer was used to adjust the pH to 9.6 to deproteinize, and the Gly-Gly generated was analyzed.

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**Figure 1.** A, Map of LNSV-ACE-S and LNSV-ACE-AS. LTR indicates long-terminal repeat; Neo R, neomycin resistance gene; and SV40, simian virus 40. Various restriction sites with corresponding nucleotides are represented. B, Restriction enzyme analysis of LNSV-ACE-S and LNSV-ACE-AS. Recombinant DNAs for LNSV-ACE-S and LNSV-ACE-AS were purified and subjected to EcoRI and SacI digestion. The bands generated by these 2 enzymes were consistent with the presence of various restriction sites for them and were predicted.
Measurement of \textsuperscript{125}I-[\text{Sar}\textsuperscript{1}-\text{Ile}\textsuperscript{8}]-Ang II Binding to LNSV-ACE-S– and LNSV-ACE-AS–Infected RPAECs

Uninfected and LNSV-ACE-S– and LNSV-ACE-AS–infected RPAECs were established in 35-mm-diameter tissue culture dishes. Cells attached to these dishes were used to measure the binding of \textsuperscript{125}I-[\text{Sar}\textsuperscript{1}-\text{Ile}\textsuperscript{8}]-Ang II to Ang II receptors as described previously.\textsuperscript{11} In brief, confluent cultures of RPAECs infected with LNSV-ACE-S and LNSV-ACE-AS, were rinsed with PBS (pH 7.2) and incubated with 1 mL of PBS (pH 7.2), containing 0.3 nmol/L \textsuperscript{125}I-[\text{Sar}\textsuperscript{1}-\text{Ile}\textsuperscript{8}]-Ang II and 1% BSA, in triplicate for the determination of total binding. In addition, triplicate cultures that also contained increasing concentrations of losartan (1 mmol/L to 10 μmol/L) or PD 123319 (1 nmol/L to 10 μmol/L) were used for competition-inhibition experiments. Binding data were subjected to Scatchard analysis for the determination of $K_D$ and $B_max$ values essentially as described previously.\textsuperscript{11}

Measurement of [Ca\textsuperscript{2+}], in LNSV-ACE-S– and LNSV-ACE-AS–Infected RPAECs

[Ca\textsuperscript{2+}], was measured in nonconfluent RPAECs using epifluorescence microscopy as described previously.\textsuperscript{22} Briefly, cells were loaded with fura-2 by incubation with 5 μmol/L membrane-permeant fura-2/acetoxymethylester dissolved in 1 mmol/L DMSO and then centered in the optical field of a \texttimes100 oil-immersion fluorescence objective of an inverted microscope (Nikon Diaphot). Before an experiment, the cells were superfused with Tyrode’s solution for 10 minutes to remove excess external fura-2/acetoxymethylester. The superfusion rate was \textapprx 2 mL/min. The cells were illuminated alternately with ultraviolet light (10 per second) of 340- and 380-nm wavelength using an IonOptix chopper-based, 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. Fluorescence signals were digitized online using an IBM-compatible computer and IonOptix fluorescence image acquisition and analysis software. Autofluorescence was minimal, and background images were obtained from a region of the chamber away from the cells to be examined. The fluorescence signals, $F_{340}$ and $F_{380}$, were background subtracted during the experiment.

In Vivo Experiments

Administration of Viral Particles Into WKY and SHR

Five-day-old WKY and SHR were divided into 3 groups: vehicle (control), LNSV-ACE-S (viral control), and LNSV-ACE-AS (experimental). The treatments were injected directly into the left ventricle of the heart under methoxyflurane anesthesia (metofane, Mallinckrodt); treatments consisted of 1 bolus of 5 × \texttimes10^5 CFU/mL of LNSV-ACE-S (S lanes 1, 3, and 5) or with LNSV-ACE-AS (AS lanes 2, 4, and 6) as described in Materials and Methods. Cultures were subjected to G418 selection for 14 days followed by subculture. This subculture was termed passage 1. After confluence, cells were collected and RNA was isolated and subjected to RT-PCR for the detection of ACE-S and ACE-AS mRNAs in corresponding cells with the use of specific primers as described in Materials and Methods. β-Actin mRNA was used for determination of equal loading and quantification. Top, Representative ethidium bromide–stained gel. Bottom, Mean ± SE (n = 3).

β-actin mRNA in samples and presented as relative absorbency essentially as described previously.\textsuperscript{17} Because of the uncertainties involved in the calculation of [Ca\textsuperscript{2+}], signals are reported here as a percentage change in the $F_{340}/F_{380}$ ratio. Data are presented as mean ± SE, and statistical significance was determined using the Student t test. Indirect BP measurements were performed on 4 to 8 animals per group and analyzed by repeated-measures ANOVA.

Results

Infection of RPAECs With Viral Particles Containing LNSV-ACE-AS

First, a series of experiments were carried out to establish conditions for the infection of RPAECs with LNSV-ACE-AS viral particles. Viral particles containing LNSV-ACE-S have been used as the control throughout the study. Infection followed by the G418 selection process yielded cultures expressing ACE-S and ACE-AS mRNAs in LNSV-ACE-S– or LNSV-ACE-AS–infected cells, respectively. The expression was robust and was maintained for the duration of the experiment, ie, 8 passages (Figure 2). The expression of ACE-S and ACE-AS appeared to be comparable at each

**Figure 2.** Expression of ACE-S and ACE-AS in LNSV-ACE-S– and LNSV-ACE-AS–infected RPAECs. RPAEC cultures were infected with 5 × \texttimes10^6 CFU/mL of LNSV-ACE-S (S lanes 1, 3, and 5) or with LNSV-ACE-AS (AS lanes 2, 4, and 6) as described in Materials and Methods. Treatment for 14 days followed by subculture. This subculture was termed passage 1. After confluence, cells were collected and RNA was isolated and subjected to RT-PCR for the detection of ACE-S and ACE-AS mRNAs in corresponding cells with the use of specific primers as described in Materials and Methods. β-Actin mRNA was used for determination of equal loading and quantification. Top, Representative ethidium bromide–stained gel. Bottom, Mean ± SE (n = 3).

β-actin mRNA in samples and presented as relative absorbency essentially as described previously.\textsuperscript{17} Because of the uncertainties involved in the calculation of [Ca\textsuperscript{2+}], signals are reported here as a percentage change in the $F_{340}/F_{380}$ ratio. Data are presented as mean ± SE, and statistical significance was determined using the Student t test. Indirect BP measurements were performed on 4 to 8 animals per group and analyzed by repeated-measures ANOVA.
These observations establish that infection with LNSV-ACE-S or LNSV-ACE-AS viral particles resulted in a stable expression of ACE-S and ACE-AS by RPAECs. This appeared to be specific, given that the parent RPAECs that were not infected did not express either ACE-S or ACE-AS (data not shown). Furthermore, other endothelial cells in culture (human retinal endothelial cell) and astroglial cells in primary culture when infected with LNSV-ACE-AS virus specifically expressed the ACE-AS transcript.

Next, we studied the effect of ACE-AS transcript expression on endogenous ACE in RPAECs. Figure 3 depicts characterization of RT-PCR conditions to semiquantify ACE-S and ACE-AS and to determine the effects of ACE-AS expression on endogenous ACE mRNA. Total RNA from RPAECs infected with ACE-S and ACE-AS showed a PCR cycle–dependent linear relationship with the concentrations of endogenous ACE and β-actin mRNAs (Figure 3A and 3B). A linear relationship also was seen between the optical density of the PCR band and the concentration of the mRNAs after RT reactions (Figure 3C). Thus, optimum conditions were used to determine the effect of ACE-S and ACE-AS expression on endogenous ACE mRNA. Expression of ACE-AS caused a 75% decrease in the ACE mRNA when the levels were compared with the parent noninfected endothelial cell cultures (Figure 3D). In contrast, expression of ACE-S showed no significant effect on ACE mRNA. The reduction in ACE-mRNA levels in ACE-AS cells was maintained as the cells were subcultured for 8 passages (Figure 3D). ACE-S–expressing RPAECs showed a specific activity of ACE enzyme of 1.45 mU/mg protein. This was comparable with the level of ACE activity observed in uninfected, parent RPAECs (Figure 4). However, ACE-AS–expressing RPAECs showed a 70% decrease in ACE activity compared with the ACE-S–expressing cells (Figure 4). As for the ACE mRNA, ACE activity was reduced throughout the duration of the experiment (8 passages) in ACE-AS–expressing cells. These observations indicate that infection of RPAECs with LNSV-ACE-AS results in a permanent expression of ACE-AS transcript that is associated with a parallel decrease in transcription of the endogenous ACE gene and ACE activity.

**Effects of ACE-AS Expression on Ang II Receptors and Ang II Actions**

There are conflicting reports in the literature regarding the effects of ACE inhibitors on Ang II receptor level.26,27
Because ACE inhibitors have diverse effects, not only on the plasma and tissue RAS but also on the bradykinin system, it has been difficult to reconcile these differences. Our in vitro gene delivery system appears to be more selective, given that it would only influence ACE at the genetic level and thus is poised to resolve the issues in a specific manner. With this rationale in mind, we studied the effects of ACE-AS expression on Ang II receptors in RPAECs. Binding of $^{125}$I-\[Sar^1\text{-}Ile^8\]-Ang II to ACE-AS-expressing RPAECs was 40% to 50% higher compared with ACE-S–expressing cells. Losartan caused a dose-dependent inhibition of this binding in both cell types, although the binding was significantly higher in the ACE-AS–expressing cells at all concentrations of losartan (Figure 5). In contrast, PD-123319, an AT$_2$ subtype–specific antagonist, failed to compete for $^{125}$I-binding. This indicated that RPAECs predominantly express the AT$_1$ subtype and that the expression of ACE-AS results in an increase in AT$_1$ binding. This conclusion was confirmed by the Scatchard analysis of the binding data. Table 2 summarizes the observations, which indicate that the increase in the binding in ACE-AS cells resulted from a 50% increase in the B$_{max}$ for the AT$_1$, without a significant change in the K$_d$ values.

RT-PCR was carried out with the use of specific AT$_1$ and AT$_2$ primers to determine whether the increase in the AT$_1$s occurs at the transcriptional level. Optimum conditions used for this semiquantitative analysis of AT$_1$ and AT$_2$ mRNAs have been established previously for cultured cells. Figure 6 shows that AT$_1$ mRNA levels were predominant in both ACE-S– and ACE-AS–expressing RPAECs. AT$_2$ mRNA levels, although detectable, were very low. No significant changes in the levels of AT$_1$ mRNA or AT$_2$ mRNA were observed between the ACE-AS– and ACE-S–expressing cells. These data led us to conclude that the increase in the AT$_1$s in ACE-AS cells could be a posttranscriptional event.

Finally, we sought to determine whether the AT$_1$ in RPAECs is functional and if so, whether an increase in its numbers in ACE-AS cells would be associated with a parallel increase in its function. Because many AT$_1$-mediated cellular actions of Ang II are linked to the changes in $[Ca^{2+}]_i$, we studied the effect of ACE-AS expression on Ang II stimulation of $[Ca^{2+}]_i$. Ang II caused a concentration-
dependent increase in $[Ca^{2+}]_{i}$ in both ACE-S– and ACE-AS–expressing cells (Figure 7A). The response was maximal, with 100 nmol/L Ang II in both cell types, although the ACE-AS–expressing cells showed a higher response at each concentration of Ang II compared with ACE-S–expressing RPAECs. Quantification of $[Ca^{2+}]_{i}$ data showed that the levels of $[Ca^{2+}]_{i}$ were significantly higher in Ang-II–treated ACE-AS cells compared with ACE-S cells (Figure 7B). This was consistent with a higher $B_{max}$ for AT$_1$ receptors in these cells. In contrast to its effect on $[Ca^{2+}]_{i}$, Ang II showed no significant effects on the incorporation of [3H]thymidine into macromolecules or on cell numbers in both ACE-S– and ACE-AS–expressing cells.

**Effects of ACE-AS on the Development of High BP in the SHR**

Five-day-old WKY and SHR were injected with LNSV-ACE-S and LNSV-ACE-AS, and indirect BPs were measured. BP in the SHR was significantly higher than in the WKY by 60 days of age (152 ± 3 versus 121 ± 3, n = 4, P < 0.01). BPs of LNSV-ACE-S–treated SHR were comparable with their respective saline-treated controls at 92 days. However, treatment of SHR with LNSV-ACE-AS resulted in a −15 mm Hg lower BP when compared with LNSV-ACE-S treatment (Figure 8A). No such reduction in BP was observed in the LNSV-ACE-AS–treated WKY rats. These observations provide a molecular basis for our physiological data and set the stage to use this technology for studies on the role of blood and tissue ACE in the control of hypertension.

**Discussion**

The most significant observation of this study is that it demonstrates, for the first time, that a retrovirus based vector can be used to deliver ACE-AS. This delivery results in a permanent attenuation of ACE expression and long-term antihypertensive effects exclusively in the SHR. Thus, the gene delivery system that inhibits ACE expression at the genetic level provides a potentially important tool to study the mechanism by which ACE inhibition causes antihypertensive actions and produces beneficial effects on the cardiovascular system.

Infection of RPAECs in culture resulted in the expression of ACE-S and ACE-AS transcripts. The expression was maintained throughout the duration of RPAECs in culture, up to 8 passages. This is equivalent to ≈32 generations if one assumes that the doubling time for RPAECs in culture is ≈24 hours. LNSV-ACE-AS was further established by treatment with captopril. Captopril caused a 15- to 18-mm Hg lowering of BP in the LNSV-ACE-S–treated SHR (Figure 8B). This treatment showed little effect on BP of LNSV-ACE-AS–treated SHR.

Various Ang target tissues from 120-day-old SHR that were treated with LNSV-ACE-S and LNSV-ACE-AS were harvested to determine whether these prolonged antihypertensive effects are associated with a long-term expression of ACE-AS in vivo. RNA was prepared and used for RT-PCR essentially as described previously. Data in Figure 9 show that a robust expression of ACE-AS and ACE-S (Figure 9B) transcripts was seen in lungs, heart, kidney, liver, and spleen. These observations provide a molecular basis for our physiological data and set the stage to use this technology for studies on the role of blood and tissue ACE in the control of hypertension.
that the permanent expression is a result of integration of ACE-S and ACE-AS into the RPAEC genome. A similar integration was observed when an LNSV vector containing AT1 subtype–specific antisense cDNA was incubated with neuronal and astroglial cells. Expression of ACE-AS was accompanied by a decrease in ACE gene expression as evidenced by decreases in the levels of both ACE mRNA and ACE activity. However, there are other reports that indicate a decrease in AT1s after ACE inhibitor treatment. This conflicting effect of ACE inhibitors would be easily resolved with the use of a gene therapy approach such as the one described here, because it can selectively target Ang-sensitive tissue. The increase in the AT1 was posttranscriptionally regulated, because the mRNA levels for the AT1 did not change between the ACE-AS and ACE-S cells. The mechanism of this control remains to be determined; however, on the basis of our previous observations it is tempting to speculate that the increase could be a result of an increased recycling of AT1s in ACE-AS–expressing cells. Thus, an increased mobilization of AT1s to the plasma membrane from the intracellular compartment could be predicted. This conclusion is based on the fact that significant levels of intracellular AT1s are shown to be present in a wide variety of cells.

Why should one attempt to develop the means to inhibit ACE at a genetic level when proven pharmacological agents are available to do so? The question is particularly relevant, because ACE inhibitors are highly successful in the treatment of hypertension, congestive heart failure, and other related diseases. The rationale for a genetic approach includes the following: (1) ACE inhibitors need to be administered on a regular basis to maintain their beneficial effects, whereas the gene therapy approach could potentially be accomplished with one treatment; (2) ACE inhibitors have significant side effects; (3) compliance appears to be an important impediment in any traditional pharmacological strategy, particularly when it is associated with significant side effects; (4) it has been difficult to elucidate the precise mechanism of action of ACE inhibitors because of their diverse effects on the cardiovascular system, especially the kinin system, whereas gene transfer could produce organ-specific targets for ACE inhibition, which could be used to evaluate the mechanism; and (5) genetic intervention holds the potential for both the long-term control and the prevention of hypertension, given that ACE polymorphism is associated with hypertension. Thus, targeting ACE to investigate its potential in the use of gene therapy for the control of hypertension seems to be a rational approach. This is particularly important in view of well-established pharmacological literature indicating that ACE inhibition improves endothelial dysfunction, controls hypertension, and improves vascular remodeling, among many other beneficial effects.

Delivery of ACE-AS into rats caused a long-term expression of ACE-AS transcript in various Ang-target tissues. This was associated with a significant attenuation of high BP, which was exclusive for the SHR. The fact that captopril failed to significantly reduce BP in the ACE-AS–treated SHR clearly indicated that the LNSV-ACE-AS strategy is targeting the expression of endogenous ACE. These observations provide preliminary evidence for the usefulness of this system to study the precise physiological mechanism of antihypertensive effects of ACE.
inhibition. It also sets the stage to determine the involvement of the bradykinin-nitric oxide system in the beneficial effects of ACE inhibitors on cardiovascular system.

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Sustained Inhibition of Angiotensin I–Converting Enzyme (ACE) Expression and Long-Term Antihypertensive Action by Virally Mediated Delivery of ACE Antisense cDNA

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