Viral transduction of renin rapidly establishes persistent hypertension in diverse murine strains

Shannon M. Harlan, Robert A. Ostroski, Tamer Coskun, Loudon D. Yantis, Matthew D. Breyer, and Josef G. Heuer
Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, Indiana

Submitted 17 March 2015; accepted in final form 17 June 2015

Harlan SM, Ostroski RA, Coskun T, Yantis LD, Breyer MD, Heuer JG. Viral transduction of renin rapidly establishes persistent hypertension in diverse murine strains. Am J Physiol Integr Comp Physiol 309: R467–R474, 2015. First published June 24, 2015; doi:10.1152/ajpregu.00106.2015.—Mice provide a unique platform to dissect disease pathogenesis, with the availability of recombinant inbred strains and diverse genetically modified strains. Leveraging these reagents to elucidate the mechanisms of hypertensive tissue injury has been hindered by difficulty establishing persistent hypertension in these inbred lines. ANG II infusion provides relatively short-term activation of the renin-angiotensin system (RAS) with concomitant elevated arterial pressure. Longer-duration studies using renin transgenic mice are powerful models of chronic hypertension, yet are limited by the genetic background on which the transgene exists and the exposure throughout development. The present studies characterized hypertension produced by transduction with a renincoding adeno-associated virus (ReninAAV). ReninAAV mice experienced elevated circulating renin with concurrent elevations in arterial pressure. Following a single injection of ReninAAV, arterial pressure increased on average +56 mmHg, an increase that persisted for at least 12 wk in three distinct and widely used strains of adult mice: 129/S6, C56BL/6, and DBA/2J. This was accomplished without surgical implantation of pumps or complex breeding and backcrossing. In addition, ReninAAV mice developed pathophysiological changes associated with chronic hypertension, including increased heart weight and albuminuria. Thus ReninAAV provides a unique tool to study the onset of and effects of persistent hypertension in diverse murine models. This model should facilitate our understanding of the pathogenesis of hypertensive injury.

The study of end-organ injury from hypertension relies heavily on human patient data and representative animal models. In mice, experimental hypertension is frequently achieved through activation of the renin-angiotensin system (RAS). Common approaches include chronic ANG II infusion via surgically implanted osmotic minipumps. Osmotic minipumps are advantageous in that they provide continuous infusion of ANG II at a predetermined dose for up to 6 wk in any model of choice. This approach, however, has limitations, including uncertain drug stability in the pump, surgical complications, and a relatively short duration of exposure, restricting researchers from examining more chronic (months of duration) effects of hypertension, limiting their use in developing animal models of chronic hypertension. Moreover, some reports have shown a decline in pressor effect after 2 wk of infusion, limiting their use to more acute studies. Lastly, implantation of the pump requires a minor surgical procedure, exposing mice already implanted with devices for recording arterial pressure to added stress. In addition, surgical implantation limits their use in mouse models with compromised immunity or healing. Despite these limitations, ANG II infusion is a widely used approach to experimentally induce hypertension in rodents.

Others have generated transgenic rodents that overexpress renin, providing chronic models of hypertension. For example, Caron et al. (1–3) developed a transgenic mouse in which renin is overexpressed, independent from intrinsic homeostatic feedback mechanisms, by controlling expression from a liver-specific promoter and targeting the transgene into a liver-specific locus. A similar approach has been used in rats, in which the renin transgene is expressed preferentially from the liver to establish high levels of circulating renin. These transgenic models provide effective means to chronically activate the RAS; however, they too have several limitations. The most significant limitation is the inability to rapidly establish these transgenes on a genetic background isogenic with a novel mouse strain, such as the BXD-recombinant inbred strains, NOD skid gamma model, or genes specific to knockout mouse models. This, consequently, limits their utility in genetic mapping studies via recombinant inbred strains or knockout mice to identify susceptibility loci and genes implicated in hypertension and associated diseases.

A second limitation is the inability to modulate transgene expression levels in many of these models. Furthermore, the transgene may be expressed during development stages; thus, animals could be hypertensive in utero and during postnatal development, which rarely is the case in human patients. Lastly, cross-institutional transfer of transgenic mice is typically administratively cumbersome, and establishing colonies is costly.

To circumvent these limitations, we explored the feasibility of generating a virally transduced model of hypertension via adeno-associated virus (AAV) delivery of murine Ren1d gene (ReninAAV). Of the three Renin isoforms in mice (Ren1d, Ren1c, and Ren2), Ren1d and Ren1c are the circulating isoforms, and murine Ren1d was chosen for incorporation into the AAV, as this is the isoform expressed in DBA and 129 strains, which are susceptible to renal disease. In addition, given the similarity of sequence and enzymatic activity between mRen1d and mRen1c, we anticipate mRen1d should be active in all genetic backgrounds.

Delivery of transgenes using an adenoviral approach has proven a safe and effective means to overexpress a transgene of interest in the clinic and as a scientific research tool. The data demonstrate that ReninAAV is a rapid and transferrable approach to induce persistent hypertension in diverse murine models.
Innovative Methodology

METHODS

Animals. All animal studies were conducted in accordance with the American Association for Laboratory Animal Care institutional guidelines. All in vivo procedures were performed using experimental protocols approved by the Eli Lilly and Company Animal Care and Use Committee. Animals were purchased from the following vendors: Taconic: 129/Sv and C57BL/6J (with or without vendor-performed surgical removal of three-quarters of kidney mass at 8 wk of age) and Jackson Labs: DBA/2J. Unless noted, mice were fed Purina 5008 diet, with free access to normal drinking water. In some studies, drinking water was supplemented with lisinopril (100 mg/l; Sigma, St. Louis, MO).

ReninAAV generation and validation. Mouse Ren1d (accession number: NM_031192.3) cDNA was purchased from Openbiosystems (cat. no.: MMM1013-98076717, Clone ID: 30313659, accession no.: BC061053). The mouse Ren1d (F61R)(P65S) mutation was created using PCR-based mutagenesis (current Protocols in Molecular Biology). These mutations were cloned into a furnA SV40 plasmid enabling cleavage of renin from prorenin in nonrenal tissues, and an N-linked glycosylation site for added stability, as previously described (2). The nucleotide sequence encoding full-length mouse Ren1d (F61R)(P65S) was placed downstream of the liver-specific thyroxin-binding globulin (TBG) promoter to render expression independent of normal transcriptional control and inserted into AAV2/8 vector pENN AAV TBG PI or the pCDNA3.1 expression vector. Driving renin expression from the liver has been shown to result in high levels of circulating renin in transgenic (1–3). The AAV2/8 serotype backbone was used as it preferentially integrates transgenes into the liver, which will foster high levels of expression from the TBG promoter (5). Proper insertion and sequence were confirmed by DNA sequencing. After validating the construct in vitro on the pCDNA3.1 vector backbone, the construct pENNAATTBGPl-mRen1d (F61R)(P65S) was sent to ReGenX for custom AAV prep (ReGenX, Washington, DC) with vendor-determined titer of 3.3 × 10^11 genomic copies (GC) total yield.

Human embryonic kidney (HEK) 293T cells were used to confirm the expression of mRen1d (F61R)(P65S)-pCDNA3.1 construct in vitro. Cells were grown to ~85% confluence in six-well plates and transfected with either Ren1d (F61R)(P65S)-pCDNA3.1 (3 μg/well) or mock-transfected using FuGene HD transfection kit (Promega, Madison, WI). Forty-eight hours after transfection, supernatant was collected for renin activity assay, and RNA was harvested using TRIzol (Invitrogen, Grand Island, NY).

Retro-orbital injections of AAV. AAV was diluted to the desired concentration in sterile PBS. Under isoflurane anesthesia, AAV was administered in 200-μl volume through the retro-orbital vein using a 27-gauge needle with the beveled edge down. The needle was inserted at a 35–45° angle into the medial canthus of the eye until the sphenoid region was reached. The needle was slightly retracted, and the solution was injected slowly into the retro-orbital sinus. Any injections that demonstrated leaking or misplacement of needle were excluded. Mice were allowed to recover in their cages and remained quarantined for 72 h.

In vivo assessment of ReninAAV expression. Mice received a single retro-orbital injection of ReninAAV at ~12 wk of age. A dose of 1 × 10^10 GC ReninAAV or 1 × 10^8 GC LacZAAV was used unless otherwise noted. The following parameters were monitored weekly for the first 4 wk postinjection and bimonthly for an additional 8 wk (12 wk total): body weight, urine albumin-to-creatinine ratio (ACR), and systolic blood pressure (SAP; tail cuff); values stated are for the 12-wk postinjection time point provided, unless otherwise noted. Plasma was obtained at 2 wk postinjection with subsequent collections obtained every 3 or 4 wk. Early and more frequent collections were not permissible under our animal care and use guidelines. Mice were killed at 12 wk postinjection (unless early signs of clinical distress were observed requiring euthanasia). At necropsy, heart and right kidney weights were obtained, and the tissue was fixed in 10% neutral buffered formalin for histological analysis. Liver and left kidney were collected for gene expression analysis.

Measurement of blood pressure. Systolic blood pressure was obtained by tail cuff plethysmography (Coda, Kent Scientific, Torrington, CT). Mice were placed in a restraining tube on a warming platform (Kent Scientific) and allowed to acclimate for 5–10 cycles of recording, after which additional 10–15 recordings were taken and used for analysis. All recordings were performed at the same time of day to minimize effects of diurnal arterial pressure variation. Baseline recordings were first determined at ~10–11 wk of age, and repeated measurements were taken until stable recordings were obtained (~10 mmHg). Calculation of baseline blood pressures was obtained by averaging the measurement of 3 or 4 baseline recordings with single measurements thereafter.

Radiotelemetry recording of arterial pressure was performed in conscious freely moving C57BL/6 mice (PA-C10; Data Science Instruments, St. Paul, MN), as described previously (8). At 10 wk of age, mice were anesthetized to a surgical plane using 2.5% isoflurane. Using aseptic surgical conditions, we inserted a telemetry catheter into the left carotid artery and secured it using silk suture. The transmitter was placed subcutaneously in the midabdominal region. The cervical incision was sutured, and the animals were monitored closely for immediate recovery. Mice were pretreated with ketofen (3 mg/kg) before the surgery followed by 3 days with amoxicillin (100 mg/kg) and ketofen (3 mg/kg) after the surgery.

Animals were allowed to recover for 7 to 10 days with units turned off. Telemetry units were then activated, and arterial pressure was recorded continuously in the conscious unrestrained state for 6 days for baseline recording. Under light isoflurane anesthetic, mice were injected with a single retro-orbital injection of ReninAAV (1 × 10^10 GC) and immediately returned to the cage for continuous recording of arterial pressure. Mice were ~12 wk of age at the time of ReninAAV injection to further validate arterial pressure data obtained by tail cuff measurement. Thirteen days after injection of ReninAAV, mice were given lisinopril (100 mg/l; Sigma, St. Louis, MO) ad libitum in the drinking water for 4 days, followed by a return to normal drinking water for recovery. Hemodynamic parameters were recorded for 10 s every 60 s and stored on a personal computer using Data Science Instruments software and analyzed in GraphPad Prisms (La Jolla, CA).

Plasma renin activity. Renin activity was measured in EDTA-collected plasma or cell supernatant using the SensoLyte 520 mouse renin assay kit (Anaspec no. 72161, Freemont, CA) following the manufacturer’s directions with a few modifications. For measurement of plasma renin, 1.5 μl of plasma was diluted in 50 μl of assay buffer with 1× protease inhibitor (Halt protease and phosphatase inhibitor, no. 1861281; Thermo Scientific Waltham, MA) in an optical grade clear 96-well plate. Controls included the kit provided activated renin with and without the provided renin inhibitor, as well as appropriate negative controls for background fluorescence. Diluted substrate solution (50 μl) was added to each well and incubated in the dark for 3 or 4 h at 37°C. The plate was then read at 490/520 nm using a SpectraMax (Molecular Devices) plate reader. Data are presented as relative fluorescence units.

Renin and aldosterone ELISA. Renin ELISA was performed using the mouse Renin 1 ELISA kit (no. DY4277; R&D Systems, Minneapolis, MN), which measures total renin (prorenin and renin). EDTA-collected plasma was diluted 80-fold in reagent diluent (no. DY995, R&D Systems). A seven-point standard curve was created. The protocol followed the manufacturer’s directions and used suggested reagents, with the only exception being the use of 100 μl of stop solution versus the recommended 50 μl. The samples were read at 450 nm with wavelength correction at 540 nm using the SpectraMax (Molecular Devices) plate reader. An aldosterone ELISA was performed on 25 μl of terminal serum and 25 μl of assay buffer following the manufacturer’s directions (no. R CAN-ALD-450R; BioVendor, Asheville, NC). Samples were read at 450 nm with
wavelength correction at 540 nm using the SpectraMax (Molecular Devices) plate reader. Concentrations were calculated by interpolation using the standard curve in both renin and aldosterone assays.

**Gene expression.** Liver and kidney were collected at necropsy and immediately placed in liquid N₂ and stored at −80°C. Approximately 50 mg of tissues was removed and placed in a bead-beat (no. 6913-100; MP Biomedicals, Solon, OH) with 1 ml of TRIzol reagent (no. 15596-026; Invitrogen, Carlsbad, CA) and placed on ice. Tubes were placed in a FastPrep-24 (MP Biomedicals), vortexed at level 6 for 30 s, and centrifuged for 10 min at 14,000 rpm at 4°C.

For in vitro cell-based studies, 1 ml of TRIzol was added to each well of a six-well plate, and cells were disrupted using a cell scraper. After lysis with TRIzol, 200 µl of chloroform was added to each tube followed by a brief vortex for 30 s and 10 min on ice, and then centrifuged for 15 min at 12,000 rpm at 4°C. Supernatant was transferred to a new tube—the concentration and quality were measured using a spectrophotometer (NanoDrop ND-1000)—and stored at −80°C. RNA was reverse-transcribed following the manufacturer’s protocol (SuperScript III, no. 18080-051, Invitrogen, Grand Island, NY). For TaqMan analysis, probes were purchased from Applied Biosystems (renin no. MM02342887; Grand Island, NY), and loading control 18S (no. 4310893E) was used at 1:20 dilution with TaqMan universal PCR master mix (Applied Biosystems). Detection and quantification were performed on the ABI 7900HT sequence detection system (Applied Biosystems), and data were collected and analyzed with the SDS 2.2.2 software from Applied Biosystems. Data were analyzed by calculating the Ct value, ΔCt in reference to 18S, ΔΔCt, and ultimately fold change calculated from the ΔΔCt value (n = 3 for each group with each sample run in triplicate).

For RT analysis of transfected cells, cDNA from mock- and renin-transfected cells was run on a thermocycler using the following primer pair (Integrated DNA Technologies, Coralville, IA): forward: 5’-ATGGACAGAGGAGATGCG-3’ and reverse: 5’-CAACTT-CAGGGAGCTCGTAG-3’. PCR products were resolved on a 2% agarose gel.

**Clinical histology and pathological assessments.** After at least a 24-h 10% formalin fixation, heart, and kidneys were paraffin-embedded, sectioned at 4 µm, and stained with periodic acid-Schiff stain (PAS), hematoxylin and eosin (H&E), or Masson’s trichrome stain (MTS). Slides were read and scored by a certified anatomic pathologist using the following scoring criteria: 0, no lesions; 1, minimal; 2, slight; 3, moderate; 4, marked; and 5, severe. In addition, at necropsy serum was collected via retro-orbital bleed for measurement of serum creatinine. Urine was collected by cage side spot collection. Urine samples were centrifuged for 15 min at 12,000 rpm at 4°C. Supernatant was stored at −80°C.

**Statistical analysis.** For in vivo studies mice were randomized on the basis of baseline body weight, ACR, and SAP using BRAT v1.0 (block randomization allocation tool). Animals with baseline values two times standard error were excluded. Data were analyzed with JMP v. 8.0 software (SAS Institute) for comparison among multiple groups. Log-transformed data were analyzed in JMP using Tukey-Kramer comparison of pairs or one-way ANOVA. Radiotelemetry analysis was performed using repeated-measures ANOVA. Comparisons of two parameters used Student’s t-test. A P value of < 0.05 was considered statistically significant. Time course studies were further analyzed using two-way ANOVA.

**RESULTS**

**In vitro characterization of mRen1d (F61R)(P65S) construct.** A cDNA-encoding mouse Ren1d was modified by adding a synthetic furin cleavage site (F61R) and an N-linked glycosylation site (P65S) for added stability, and was inserted downstream of the liver-specific TBG promoter (Fig. 1). To validate the construct was functional prior to generation of ReninAAV, HEK 293 cells were transfected with Ren1d (F61R)(P65S)-pCDNA3.1, and renin expression and activity were analyzed. HEK 293 cells transfected with Ren1d (F61R)(P65S)-pCDNA3.1 exhibited increased renin mRNA expression compared with mock-transfected cells (Fig. 1B). Supernatants from cells transfected with Ren1d (F61R)(P65S)-pCDNA3.1 exhibited 130-fold greater renin activity compared with mock-transfected cells (624.8 vs. 4.8 relative fluorescent units, P < 0.001) (Fig. 1C).

**Dose-dependent effects of ReninAAV in vivo.** A single injection of various doses of ReninAAV (3 × 10⁹ GC, 1 × 10¹⁰ GC, and 3 × 10¹⁰ GC) into male 129/S6 mice significantly (P < 0.05) elevated systolic arterial pressure (SAP) after 1 wk, and this increased SAP persisted for the duration of the 12-wk study, except for the 3 × 10⁹ GC dose at weeks 2 and 3 postinjection when only a trend was detected (3 × 10⁹ GC, 1 × 10¹⁰ GC 156 ± 10 mmHg, 3 × 10¹⁰ GC 185 ± 10 mmHg, 3 × 10¹⁰ GC 188 ± 8 mmHg) compared with LacZ controls (126 ± 12 mmHg) (Fig. 2A). The lowest dose tested (1 × 10⁹ GC) showed significantly increased SAP (154 ± 11 mmHg, P < 0.01) only at 10- and 12-wk measurements (Fig. 2A). ReninAAV transduction also resulted in albuminuria in a dose-dependent manner. The two high doses (1 × 10¹⁰ GC and 3 × 10¹⁰ GC) increased ACR (albumin-to-creatinine ratio, 2.065 ± 620 and 2.977 ± 855,

![Image of diagram showing renin-AAV construct](http://apregu.physiology.org/)(Roche, Basel Switzerland) using a polyclonal rabbit anti-human albumin antibody and enzymatic creatinine.

![Fig. 1. In vitro validation of ReninAAV construct. A: diagram of modified mRen1d. Point mutations were made at residues 61 and 65 to add a furin cleavage site and N-linked glycosylation site, respectively, and cloned downstream of the liver-specific TBG promoter. B: RT-PCR analysis identified human embryonic kidney (HEK) 293 cells transfected with Ren1d (F61R)(P65S)-pCDNA3.1 had robust renin mRNA expression compared with mock-transfected cells. C: renin activity assay identified HEK 293 cells transfected with Ren1d (F61R)(P65S)-pCDNA3.1 had 130-fold increase in renin activity compared with mock-transfected cells. *P < 0.01; n = 3/group.](http://apregu.physiology.org/)
Fig. 2. In vivo dose-dependent activity of ReninAAV in 129/S6 mice. A: tail cuff measurement of systolic arterial pressure (SAP); ReninAAV elevated SAP (P < 0.05) within 1 wk postinjection and persisted for 12 wk of study in all doses tested except for the 3 × 10^10 genomic copies (GC) dose at weeks 2 and 3, when only a trend was detected, and the 1 × 10^9 GC dose reached statistical significance only at the 10- and 12-wk time points compared with LacZ controls. B: reninAAV increased ACR typically within 1 wk of injection and persisted throughout the study in all doses tested (1 × 10^0 to 3 × 10^10 GC, P < 0.01 weeks 1–12; 3 × 10^9 GC, P < 0.01 weeks 2–12; and 1 × 10^9 GC, P < 0.02 weeks 10 and 12 only). C: dose-dependent effect of ReninAAV on total renin levels in plasma. D: ReninAAV caused a dose-dependent increase in plasma renin activity. E: increased renin mRNA in livers of ReninAAV-injected mice. F: ELISA demonstrated that ReninAAV causes dose-dependent elevation of plasma aldosterone at 12 wk post-AAV injection. (*P < 0.05 compared with LacZ, n = 4 for each dose tested except dose 3 × 10^9 GC had n = 3 after week 1 postinjection due to a mortality).

Renal and cardiovascular effects of ReninAAV on three genetic backgrounds. To validate facile transferability of ReninAAV-associated hypertension, the effects of ReninAAV were evaluated in three distinct and widely used strains of inbred mice: C57BL/6, DBA/2J, and 129/S6. A single injection of ReninAAV (1 × 10^10 GC) elevated SAP (average +56.1 ± 5.5 mmHg, P < 0.01, 12 wk postinjection) in all three strains compared with respective LacZ controls from each strain (Table 1, Fig. 3A). Significant (P < 0.05) elevations in SAP were observed without any mortality.

Table 1. Comparison of physiological effects of ReninAAV on three genetic backgrounds

<table>
<thead>
<tr>
<th>Model</th>
<th>Injection</th>
<th>ACR</th>
<th>SAP, mmHg</th>
<th>Heart Weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>LacZ</td>
<td>19 ± 4</td>
<td>111 ± 11</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Renin</td>
<td>67 ± 16*</td>
<td>170 ± 6*</td>
<td>0.24 ± 0.02*</td>
</tr>
<tr>
<td>C57BL/6 3/4 nx</td>
<td>LacZ</td>
<td>28 ± 4</td>
<td>139 ± 19</td>
<td>0.18 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Renin</td>
<td>800 ± 101*</td>
<td>167 ± 9*</td>
<td>0.22 ± 0.03</td>
</tr>
<tr>
<td>DBA/2J</td>
<td>LacZ</td>
<td>29.4 ± 15</td>
<td>124 ± 11</td>
<td>0.22 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Renin</td>
<td>480 ± 83*</td>
<td>175 ± 12*</td>
<td>0.24 ± 0.01*</td>
</tr>
<tr>
<td>129/S6</td>
<td>LacZ</td>
<td>10 ± 2</td>
<td>127 ± 14</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Renin</td>
<td>2065 ± 620*</td>
<td>186 ± 16*</td>
<td>0.22 ± 0.01*</td>
</tr>
</tbody>
</table>

Numerical averages of albumin-to-creatinine ratio (ACR), systolic arterial pressure (SAP) (mmHg), and heart weight (g) obtained at 12 wk of renin-coding AAV (ReninAAV) or LacZ-coded AAV (LacZAAV) in C57BL/6, DBA/2J, and 129/S6 mice. n = 5 ± per group. Data are expressed as average with SD. *P < 0.01, †P < 0.05.
were detected after 1 wk in C57BL/6 and 129/S6 mice, and after 2 wk in the DBA/2J mice, which persisted for the duration of the studies (Fig. 3A). Consistent with the elevations in arterial pressure, significantly (P < 0.05) increased heart weight was observed in ReninAAV mice from all three genetic backgrounds (Table 1). Radiotelemetry recordings were also performed in C57BL/6 mice to obtain a comprehensive arterial pressure profile. A baseline mean arterial pressure (MAP) of 100.5 ± 2.5 mmHg was obtained with anticipated diurnal variation (Fig. 3B). Administration of ReninAAV promptly elevated MAP (33 mmHg; P < 0.01) by 19 h postinjection (Fig. 3B). After ~2 wk, when a plateau in arterial pressure was observed, mice were treated with the ACE inhibitor lisinopril, which significantly reduced arterial pressure (Fig. 3, B and C). Lisinopril was discontinued after 3 days, resulting in a prompt reestablishment of hypertension (+5 mmHg; P = 0.2 compared with ReninAAV pretreatment period) (Fig. 3, B and C).

The effect of ReninAAV on kidney function was also measured in the context of three distinct genetic backgrounds. ReninAAV (1 × 10^{10} GC) resulted in robust (P < 0.05) elevations in albuminuria (ACR) in DBA/2J (475 ± 131) and 129/S6 (2,065 ± 620) mice, with minimal, yet significant, elevations of ACR in C57BL/6 mice (67 ± 16) compared with respective LacZ controls (C57BL/6 19 ± 4, DBA/2J 29 ± 15, and 129/S6 10 ± 2) from each strain (Table 1, Fig. 3D). Significant elevations in ACR were observed within 1 wk of ReninAAV injection in 129/S6 and C57BL/6 mice and 2 wk postinjection in DBA/2J, which persisted through the duration of the studies. Histopathological assessment revealed that ReninAAV treatment was associated with moderate, but significant (P < 0.05), renal pathological changes in 129/S6 and DBA/2J mice, but not C57BL/6 mice. Changes observed included tubular protein and basophilia, interstitial fibrosis, interstitial inflammation, and glomerulopathy (Table 2). In C57BL/6 mice injected with ReninAAV, pathological changes of the heart included fibrosis and arteriopathy; however, notable pathological changes in hearts of 129/S6 or DBA/2J mice were not observed (Table 2).

ReninAAV in the kidney disease-resistant 3/4th subtotal nephrectomized C57BL/6 model. ReninAAV (1 × 10^{10} GC) was administered to renal disease-resistant 3/4th nephrectomized (nx) C57BL/6 mice to determine whether RAS activation might induce renal disease. ReninAAV led to significant (P < 0.01) elevations in SAP in both intact (+59 mmHg) and 3/4th nephrectomy C57BL/6 (+28 mmHg) mice compared with respective LacZ controls (Fig. 4A). Control LacZ 3/4th nephrectomy C57BL/6 mice did not have albuminuria compared with intact controls (Fig. 4B). Within 1 wk of ReninAAV
treatment, the 3/4th nephrectomy cohort exhibited marked albuminuria, which persisted for the duration of the study (Fig. 4B). Of note, intact C57BL/6 ReninAAV mice exhibited slight, but significant, elevations in ACR compared with LacZ controls (67 ± 16 vs. 19 ± 4, respectively). Statistically significant elevations in ACR were observed in both intact and 3/4th nephrectomy groups given ReninAAV within the first week of injection (P < 0.05 postinjection weeks 1–3) and persisted for the duration of the study (P < 0.01, weeks 4–12). Renal histopathological changes were not observed among intact ReninAAV C57BL/6 mice, LacZ intact, or LacZ 3/4th nephrectomy (Table 2, Fig. 4). In contrast, the ReninAAV 3/4th nephrectomy C57BL/6 mice had significant (P < 0.05), albeit relatively mild, pathological alterations in the kidney, which included glomerulopathy with mesangial matrix expansion, interstitial inflammation, and tubular basophilia and fibrosis (Table 2, Fig. 4C). ReninAAV-mediated pathological changes in the heart were similar between intact and 3/4th nephrectomy C57BL/6 and included significant (P < 0.05) arteriopathy and a trend toward minimal fibrosis (observed in 2/5 mice in both groups) (Table 2, Fig. 4C).

DISCUSSION

The data presented illustrate a novel, yet simple, approach to rapidly induce chronic hypertension on diverse murine genetic backgrounds. Using AAV approach to overexpress mRen1d, we were able to circumvent several limitations of the available rodent models exhibiting RAS hyperactivity. To ensure high levels of circulating renin, expression was driven by a hepatic specific promoter to render expression independent of normal physiological regulation. Hepatic expression of the ReninAAV was further favored by use of the AAV2/8 serotype that preferentially integrates into the liver (5). Previously, in transgenic mice, renin was overexpressed specifically from the liver, and this approach resulted in increased circulating renin and ANG II, elevated arterial pressure, and renal and cardiac pathological changes (1–3), similar to what was observed with ReninAAV.

As compared with previously reported models of RAS-driven hypertension, ANG II infusion, or renin transgenic mice, ReninAAV yielded comparable elevations in arterial pressure (between 30 and 60 mmHg) (2, 6, 23). ReninAAV transduction was not only associated with hypertension, but also with cardiac and renal pathophysiological consequences, consistent with an overactive RAS. The albuminuria resulting from ReninAAV is consistent with the contribution of ANG II and hypertension to chronic kidney disease (9). In addition, increased heart weight due to cardiac hypertrophy was observed, as seen in other forms of RAS-induced hypertension (1). The varying degrees of end-organ damage observed among the three murine genetic backgrounds underscores the advantage of a readily transferable method to induce hypertension in mice of the appropriate genetic background. Only C57BL/6 mice developed cardiac fibrosis after 12 wk of ReninAAV. Whether 129/S6 and DBA/2J mice exposed to ReninAAV for longer durations would lead to cardiac fibrosis, similar to the pathology observed in aged RenTg mice on the 129/S6 background remains to be established (1). Similarly, the degree of albuminuria induced by ReninAAV differed among the three genetic backgrounds. The DBA/2J and 129/S6 mice developed significantly more albuminuria than C57BL/6 mice, which appeared to be protected. This finding is consistent with published data, which characterized C57BL/6 mice as resistant to developing kidney disease (18, 19). A recent study suggested that ANG II infusion promotes the renal injury in C57BL/6 renal ablation model (18). Supporting this published study, the present study identified increased renal injury in ReninAAV-treated C57BL/6 mice with renal ablation. The ability to easily promote kidney disease in C57BL/6 mice with ReninAAV will provide researchers the opportunity to investigate the role of a specific gene in the pathogenesis of kidney disease using genetically modified strains, which are commonly found in the C57BL/6 background.

The data highlight ReninAAV as a facile and transferrable means to induce chronic hypertension. This approach allows for control of disease onset, facilitating development of chronic models of hypertension that mimics human disease progression. A cardinal advantage of the ReninAAV approach is it allows for long-term expression needed for animal model development without the encumbering need for multiple surgeries required with chronic ANG II infusion. In addition, in our hands ReninAAV is a far more cost-effective and time-efficient approach compared with ANG II infusion. One potential limitation of the ReninAAV is that it induces rapid hypertension, with effects observed within 24 h of injection. This could limit evaluation of the slow pressor effect of ANG II (11). It is plausible that a slow pressor effect may be achieved in the lower doses of ReninAAV or on the DBA/2J background. A final limitation is that this model is intrinsically dependent on elevated ANG II as the cause of hypertension and may not be suitable for studies of ANG II-independent hypertension; however, a similar AAV approach could be used to...
activate other protein-dependent pathways implicated in hypertension (e.g., endothelin).

**Perspectives and Significance**

This study demonstrates that using ReninAAV is an effective approach to obtain ANG II-mediated elevations in arterial pressure of 30–50 mmHg for extended periods of time in three strains of mice readily used in the scientific community: the C57BL/6, 129/S6, and DBA/2J. In addition, the data demonstrate strain-dependent susceptibility in developing complications associated with hypertension. This strain-dependent susceptibility to disease development reiterates the need for a readily transferable tool for inducing hypertension on any genetic strain or background. ReninAAV is a powerful tool in that it will allow for the development of mouse models of hypertension that more faithfully resemble human disease progression, to develop progressive models of diseases complicated by hypertension and to allow researchers to reliably test their hypothesis on what role a gene of interest may have in the disease process by testing the effects of ReninAAV in any knockout, knockin, or transgenic strain. In summary, ReninAAV will hopefully provide an important reagent to establish novel animal models of cardiovascular disease and facilitate research not only in the field of hypertension but also in disease areas that are complicated by hypertension, such as chronic kidney disease.

**ACKNOWLEDGMENTS**

The authors thank Hana Baker, Derek Yang, Martin Cramer, Dianna Jaqua, and Charlie Hu for assistance with in vivo studies and necropsy. Yuewei Qian for cloning assistance, Han Wu for statistical analysis, Brenda Hanssen for

---

**Figure 4.** ReninAAV (1 × 10^10 GC) transforms the kidney disease-resistant C57BL/6 mice to develop kidney disease. **A:** ReninAAV elevated SAP in both intact and 3/4th nephrectomized C57BL/6 mice compared with respective LacZ controls. **B:** ReninAAV elevated ACR in both intact and 3/4th nephrectomized (nx) mice with more robust effect observed in the 3/4th nx mice. **C:** representative photomicrograph (20×) of cardiac and renal pathology observed in ReninAAV intact and 3/4th nx mice compared with LacZ controls. ReninAAV-injected intact and 3/4th nx mice exhibited cardiac fibrosis (arrows) compared with LacZ controls. ReninAAV caused renal pathological change in 3/4th nx C57BL/6 mice, but not C57BL6 intact mice, compared with LacZ controls. The 3/4th nx ReninAAV mice had notable glomerulopathy identified by extensive mesangial matrix expansion (pink staining), which was not observed in intact ReninAAV mice or LacZ controls. Arrows indicate glomerulus, and arrowheads indicate mesangial matrix expansion. MTS, Masson’s trichrome stain; PAS, periodic acid-Shiff stain. n = 5/group in A, with all post-ReninAAV data points (P < 0.05) compared with respective LacZAAV controls except the 3/4 nx 6-wk time point in B (*P < 0.05).
Innovative Methodology

R474

REFERENCES


