

Role of metabolism and receptor responsiveness in the attenuated responses to Angiotensin II in mice compared to rats

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Abstract

Chronic infusion of Angiotensin II (AngII) to rats is a well-characterized model for determining AngII physiology. Genetic manipulations have strengthened knowledge of AngII; however, they do not permit an increase in AngII to be initiated at a selected age, duration and dose. Therefore, exogenous AngII administration remains an important technique to define its biological effect. We previously noted that infusion of AngII to mice had minimal effects compared to the same dose given to rats. In this study, we compared the effects of chronic infusion of the same dose of AngII to C57BL/6 mice and Sprague–Dawley rats, two commonly used rodent models. Rats administered AngII exhibited reductions (by 22%) in body weight, which were not evident in mice. AngII increased blood pressure by 54 mm Hg in rats, but had no effect in mice. Vascular histology demonstrated that AngII caused medial hypertrophy in rats, with adventitial expansion in mice. Plasma concentrations of AngII and its catabolic fragments were elevated (twofold) in mice compared to rats. Angiotensin receptor affinity, density and distribution were similar in rats and mice. Infusion of AngII decreased AngII receptor density in the kidney (by 78%) and spleen (by 29%) of mice, but had no effect in rats. AngII produced a sustained contractile response in rat aortic strips, but minimal responses in mouse aorta. These results demonstrate that differences in circulating angiotensin peptides, AngII receptor regulation, and vascular reactivity contribute to diminished responses to AngII infusion in mice compared to rats. Results from this study suggest that considerably higher doses of AngII may be required to elicit physiologic effects of AngII in mice.

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1. Introduction

The renin–angiotensin system plays a pivotal role in maintaining blood pressure and fluid electrolyte balance [1]. AngII also exerts a variety of other effects including cellular hypertrophy and growth, extracellular matrix deposition and pro-inflammatory effects. A common method to define the biological effects of AngII is to chronically infuse the peptide subcutaneously into rats via Alzet osmotic mini-pumps. The infusion of low dose (50–200 ng/kg/min) AngII to rats has been used as a model of hypertension

with slow onset elevations in blood pressure from vascular remodeling and sympathetic activation [2,3]. Infusion of higher doses (>250 ng/kg/min) of AngII results in a model of hypertension with rapid elevations in blood pressure from direct pressor and renal mechanisms [4]. Additional effects of AngII which have been defined by chronic infusion to rats include cardiac and vascular hypertrophy [5,6], fibrosis [7] and loss of body weight [6,8–10].

While studies with rats have provided valuable insight into AngII physiology, there has been an increased focus of the use of mice to define physiological and pathological effects of the renin–angiotensin system. This preference is facilitated by the relative ease of developing mice that either over-express or are deficient in specific proteins of the renin–angiotensin system. Genetic manipulations have led to mice with altered genes involved in the synthesis of AngII (angiotensinogen [11], renin [12], ACE [11]) and in

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responsiveness to AngII (AT1 [13,14] and AT2 receptors [15,16]). While these genetic manipulations have led to new insights into the renin–angiotensin system, they do not permit an increase in circulating AngII concentrations to be initiated at a selected age of mouse, for a controlled duration and dose. Therefore, exogenous AngII administration remains an important technique to define its effect in vivo.

Previously, we have chronically infused AngII to rats at doses ranging from 100 to 400 ng/kg/min to initiate marked changes in arterial blood pressure, cardiac hypertrophy, and body weight [6,9,10]. However, during initial studies in which we infused AngII at similar doses into mice, we are unable to demonstrate any of these effects. Subsequent studies demonstrated that higher doses (i.e., 1000 ng/kg/min) of AngII were required to initiate even modest changes in these parameters in mice [17–19]. Given the importance of AngII and the evolving use of mice, we defined whether the diminished responsiveness of mice to chronic subcutaneous AngII infusion compared to rats was due to differences in circulating AngII and related peptides, vascular reactivity to AngII, or AngII receptor expression and regulation. We conclude that mice exhibit a diminished blood pressure response to infusions of AngII compared to rats due to differences in AngII receptor responsiveness and regulation.

2. Methods

2.1. Chronic infusion model

Alzet osmotic minipumps (Model 2002; ALZA Scientific Products, Mountain View, CA, USA) were implanted into male, C57BL/6J mice (2 months of age; $n=8$ /group; National Cancer Institute, MD) or male, Sprague–Dawley rats (4 months of age; $n=5$ /group; Harlan, IN). Pumps were filled either with saline vehicle or Ang II (500 ng/kg/min; Sigma Chemical, St. Louis, MO, USA) for subcutaneous infusion for 14 days. Pumps were placed into the subcutaneous space of ketamine/xylazine anesthetized mice and rats through a small incision in the back of the neck that was closed with surgical glue (for mice) or wound staples (for rats). All incision sites healed rapidly without the need for any medication. All animal procedures were in accordance with institutional guidelines at the University of Kentucky.

2.2. Systolic blood pressure measurement

Systolic blood pressures were obtained from the tail of conscious mice and rats using a cuff attached to a computerized system (BP-2000 Visitech Systems, Apex, NC). A separate platform with different size restraining holders was used for measurements in rats versus mice. Mice and rats were acclimated to the instrument for at least 1 week prior to the implantation of osmotic pumps. Measurements were

recorded at the same time of day throughout the study. Individual animals received 10 initial pressure readings to acclimate them to the procedure, then 10 additional cycles were measured to obtain the daily mean systolic pressure. Criteria for acceptance of measurements was at least five recorded pressures per run that had a standard deviation of <30 mm Hg, per animal. The maximum pressure detection of the system was set at 200 mm Hg.

2.3. Angiotensin receptor autoradiography

Tissues (spleen, kidney, heart) were removed from rats and mice and immediately snap frozen in isopentane. Frozen tissues were mounted to a cryostat and sectioned at 16 μ m thickness. Three sets of adjacent sections were cut from each tissue. Analysis of angiotensin receptor density and localization was performed by incubating duplicate tissue sections in a phosphate buffer (20 mM Na_2HPO_4 , 150 mM NaCl, 10 mM MgCl_2 , 5 mM EDTA, bacitracin 50 mg/l, bovine serum albumin 0.2%; pH 7.4) containing 400 pM of [125 I]Sar¹Ile⁸AngII (2200 Ci/mmol; Peptide Radioiodination Center, Washington State University) for 2 h at 22 °C. The sets of adjacent sections were used to determine total binding, nonspecific binding (incubation in the presence of 10 μ M unlabelled AngII) and AT1 receptors (binding displaced by 10 μ M of losartan). At the end of the incubation, tissue sections were washed extensively, air-dried and stored overnight in a desiccator at room temperature. Slides were exposed to film based on the relative abundance of AngII receptors (i.e., 1 day for kidney/spleen sections, 3 weeks for heart sections). A set of radioactive standards was included in each film cassette during the exposure to determine the autoradiographic film response to varying concentrations of radioactivity. Binding results were quantified by video densitometry using the public domain NIH Image 5.2 software. A standard curve was generated for each set of films from the standards by plotting optical densities versus nCi of standards. The amount of ligand bound was determined by interpolating radioactive intensity from the standard curve, which was fit to a third degree polynomial. Binding data is presented as arbitrary densitometry units.

In the spleen, saturation isotherms for [125 I]Sar¹Ile⁸ AngII binding (0.1–5 nM) were performed. Sections were incubated in duplicate with increasing concentrations of [125 I]Sar¹Ile⁸ AngII in the absence or presence of unlabelled AngII (10 μ M) for 2 h at 22 °C. Specific bound in pixel optical density was plotted against radioligand concentration and K_d and B_{max} determined by nonlinear regression with GraphPad Prism (GraphPad Software, San Diego, CA).

2.4. Measurement of circulating angiotensin peptides

Blood was collected by aortic puncture from rats and mice into ice-cold tubes containing protease inhibitors (100 μ l/tube; pepstatin A, 146 mM; 1,10 phenanthroline, 20 mM;

neomycin sulfate, 2 mg/ml; EDTA, 125 mM, ethanol, 2%; DMSO, 2%), and plasma was separated by centrifugation at 4 °C. Preliminary studies demonstrated that the addition of this inhibitor cocktail to blood immediately upon removal from rats and mice markedly reduced detectable levels of catabolic angiotensin peptides (data not shown). Plasma angiotensin peptide concentration was measured in rats (1 ml plasma) and mice (250 μ l plasma) using C18 column pre-purification followed by HPLC [9]. The HPLC system consisted of a Beckman Model 125 Binary Gradient Pump, a Beckman Model 166 UV detector set at 214 nm, a Beckman C18 Ultrasphere ODS column (4.6 mm \times 25 cm) and a Gilson FC204 Fraction Collector. The mobile phase (1.5 ml/min) at time 0 was 80% buffer A (0.1 M Na_2HPO_4), 20% buffer B (0.1 M Na_2HPO_4 containing 33% acetonitrile). Gradient elution of angiotensin peptides was achieved by increasing the concentration of buffer B from 20 to 90% over 33 min, followed by 100% buffer B for 10

min to clean the column. At the end of each day a set of angiotensin standards (AngII, AngIII (2–8), AngIV (3–8), Ang4–8, Ang5–8; 5 μ g/ml of each; Sigma) was injected onto the system to define angiotensin peptide retention times. A buffer blank was injected and collected for assay intermittently to check for carryover of peptides across individual runs, and exhibited minimal angiotensin immunoreactivity (<3 pg). Fractions (1 min) from the HPLC were collected from minute 15 to 30, when individual peptides eluted. HPLC fractions were evaporated in a vacuum concentrator overnight, and reconstituted in 250 μ l of AngII RIA buffer (0.1M K_2HPO_4 , 3 mM EDTA, 0.15 mM 8-hydroxyquinoline, 0.2% bovine serum albumin; pH 7.4). Angiotensin content was measured by RIA using a polyclonal AngII antibody exhibiting minimal cross reactivity to Angiotensin I (2%) and Ang5–8 (4%), but 100% cross reactivity to AngIII, AngIV and Ang4–8. The sensitivity of the RIA was 2.5 pg/100 μ l.

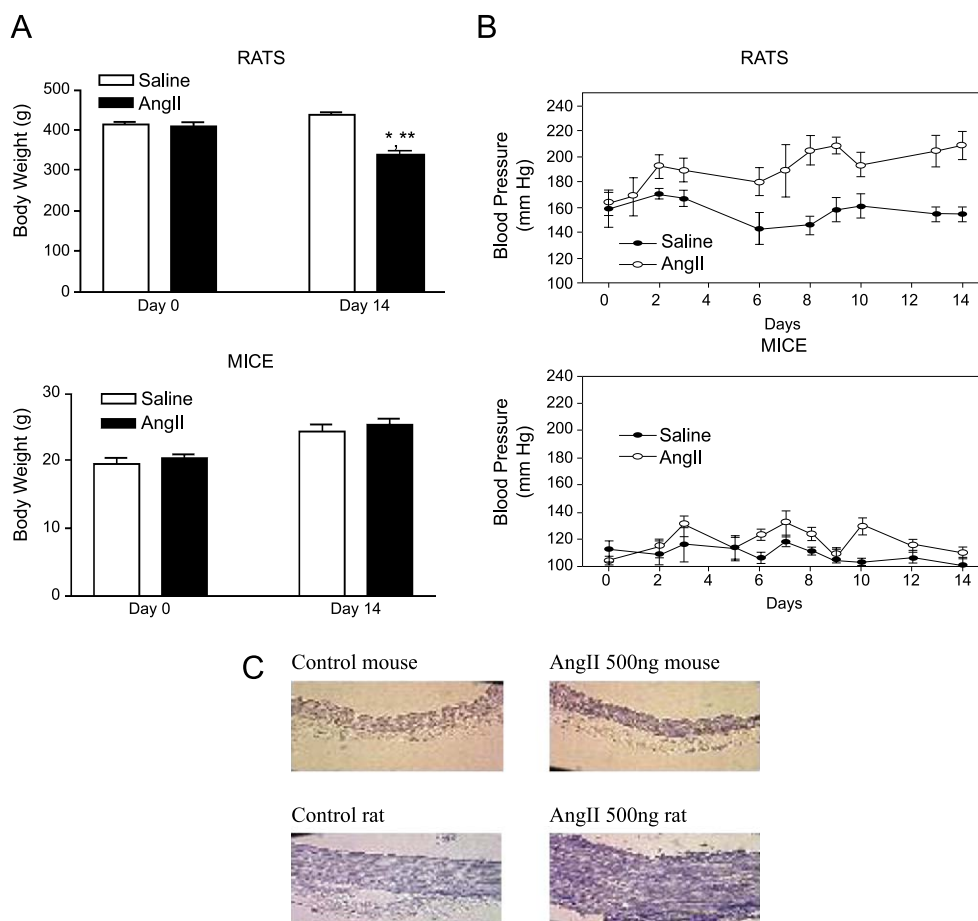


Fig. 1. The effect of chronic infusion of AngII (500 ng/kg/min) to rats and mice on (A) body weight, (B) blood pressure and (C) vascular remodeling. Male Sprague–Dawley rats ($n=5$ /group, saline and AngII) and C57BL/6 mice ($n=8$ /group, saline and AngII) were infused with saline or AngII for 14 days. (A) Body weight at baseline (day 0) and on the final day of infusion. Rats exhibited a reduction in body weight from AngII infusion compared to baseline and compared to saline. Infusion of AngII to mice had no effect on body weight. (B) Systolic blood pressure was measured by tail cuff on conscious rats and mice. Infusion of AngII to rats increased blood pressure by day 2, which was maintained. In mice, there was no effect of AngII infusion on blood pressure. (C) Sections of thoracic aorta from mice and rats were stained with hematoxylin. Mice exhibited adventitial expansion in response to AngII infusion, while rats exhibited pronounced medial hypertrophy. * denotes significantly different from baseline body weight; ** denotes significantly different from saline ($P<0.05$).

2.5. Vascular histology

Aortas were removed and fixed by immersion in paraformaldehyde (4% wt/wt in PBS). Aortas (between the second and third intercostal artery) were sectioned (8 μm thick) using a cryostat and stained with hematoxylin as described previously [20]. Representative sections of aorta from mice and rats were photographed (40 \times magnification).

2.6. Isometric tension measurement

The contractile response to AngII of vascular smooth muscle was evaluated in spiral aortic strips isolated from male, Sprague–Dawley rats (250 g; $n=5$) and C57BL/6 mice (3 months of age; $n=4$). After removing connective tissue, small spiral strips (3 mm long, 150–200 μm wide and 50–75 μm thick) were cut under a stereomicroscope. Two strips of thoracic aorta were obtained from each animal. The endothelium was removed from the strips with razor blades and the successful denudation of endothelium was verified by the loss of acetylcholine-induced relaxation. The normal

external solution was a HEPES-buffered modified Krebs solution containing (mM): Na^+ , 137.4; K^+ , 5.9; Ca^{2+} , 1.2; Mg^{2+} , 1.2; Cl^- , 148.1; glucose, 11.5; and HEPES, 11.6 (pH 7.3 with NaOH at 20 $^\circ\text{C}$). Isometric tension was measured with a force transducer (AE801; AME, Horten, Norway) in a well on a ‘bubble’ plate at 24 $^\circ\text{C}$ as described previously [21]. The muscle strips were stretched to about 1.2 times their resting length and then they were stimulated several times by incubation with a depolarization solution containing 143 mM KCl (replacement of sodium with potassium) until a stable contraction was obtained. Finally, 100 nM AngII was added. The amplitude of contractions was expressed as mean \pm standard error.

2.7. Statistics

For each parameter, the mean and standard error of the mean (S.E.M.) were calculated. Blood pressure data was analyzed by two-way repeated measures ANOVA followed by Tukey post hoc test (SPSS statistical software). Between-group differences in the plasma angiotensin peptide con-

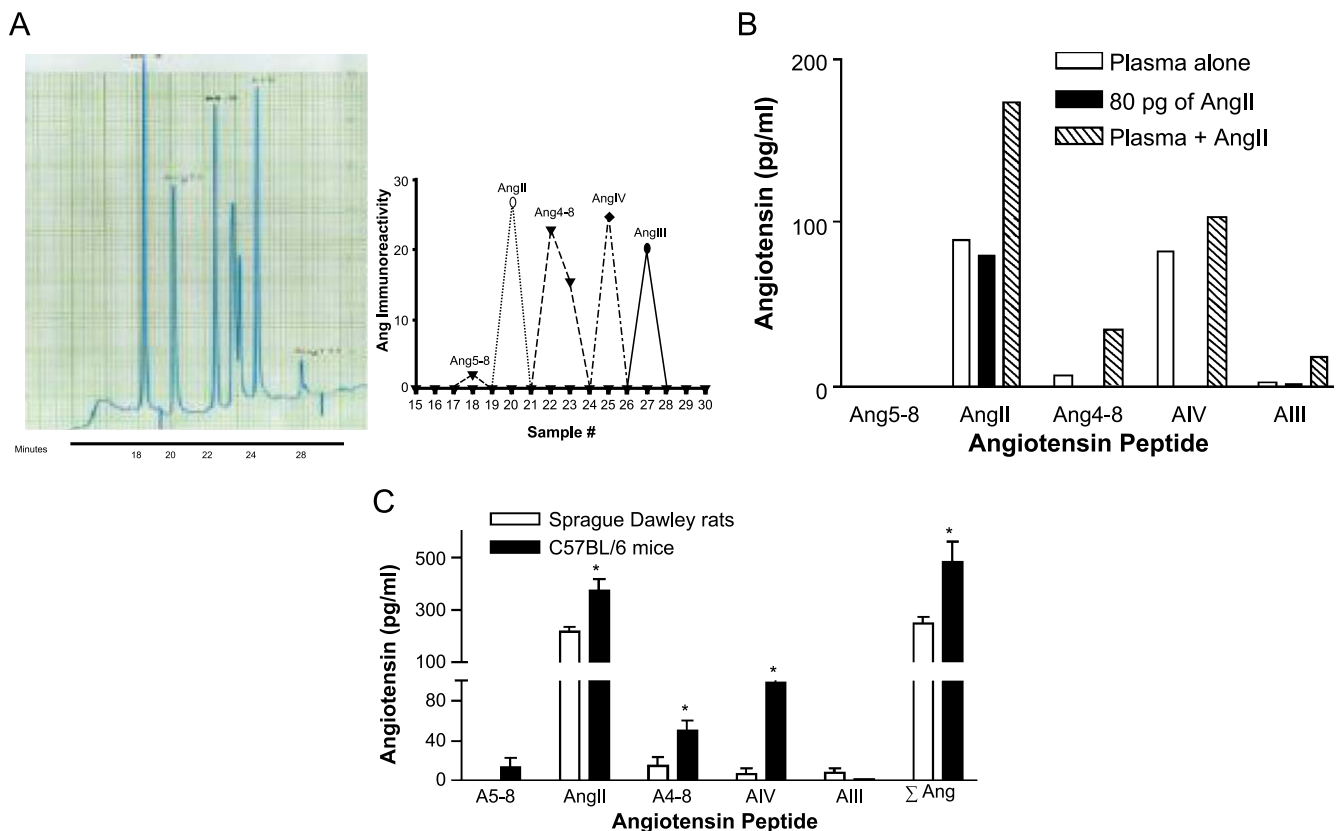


Fig. 2. Measurement of angiotensin peptides in plasma from rats and mice. (A) Left, a representative HPLC chromatogram illustrating the resolution profile of five different angiotensin peptides. Right, HPLC fractions were collected from angiotensin peptide standards (30 pg/standard) and angiotensin peptides quantified by radioimmunoassay. The sample number refers to the time in minutes after injection, and matches the HPLC retention times of standards. The recovery of peptides through the system was 80%. (B) Plasma was processed alone, or after the addition of 80 pg of AngII. The AngII fraction increased by approximately 80 pg in plasma spiked with AngII, with minimal elevations in other angiotensin peptide fragments. (C) The area under the curve was used to determine the concentration of angiotensin peptide fragments in plasma from saline-infused rats and mice on the final day of the study. The concentrations of AngII, A4–8 and AIV in plasma from mice were significantly ($P<0.05$) greater than in rats, resulting in a twofold increase in the sum (Σ) concentration of circulating angiotensins. * denotes significantly ($P<0.05$) different from rat.

centration were determined using a two-way ANOVA followed by post hoc analysis using Tukey test (SPSS). Other parameters (body weight, receptor density) were analyzed for significance by independent *t*-test ($P < 0.05$).

3. Results

We infused the same dose of AngII (500 ng/kg/min) or saline for 14 days to C57BL/6 mice ($n = 8$ /group) and Sprague–Dawley rats ($n = 5$ /group). This strain of mouse

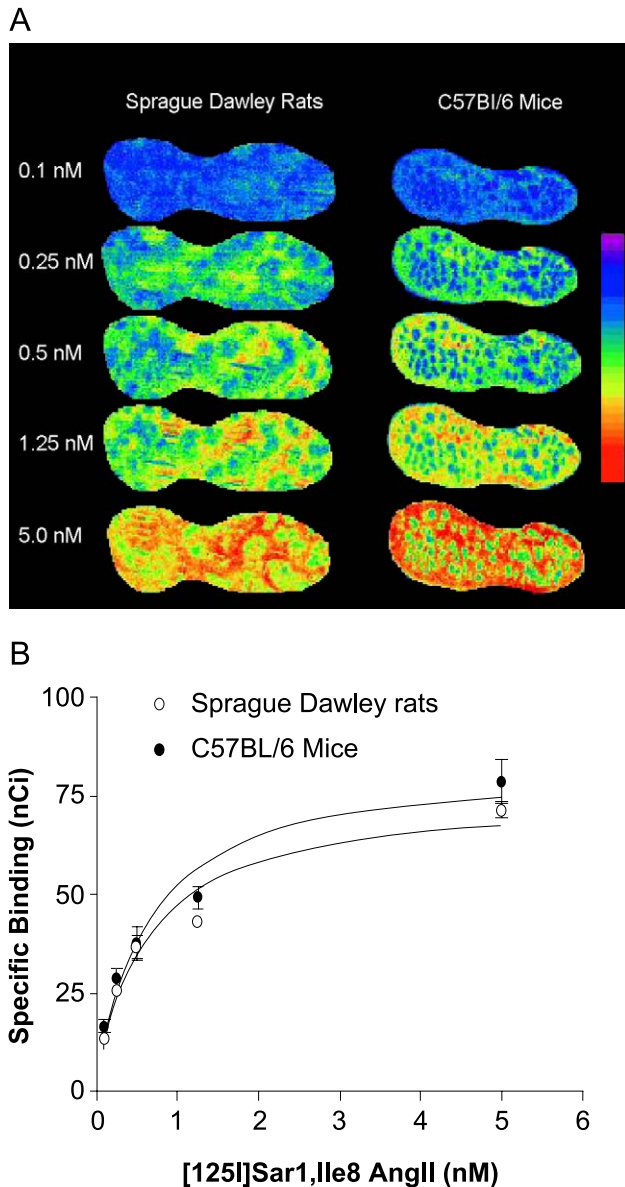


Fig. 3. Angiotensin receptor autoradiography in spleen from rats and mice. Tissue sections from the spleen of non-treated rats and mice were incubated with increasing concentrations of [125 I]Sar 1 ,Ile 8 AngII. (A) Representative total binding in spleen sections from rats and mice with increasing concentrations of [125 I]Sar 1 ,Ile 8 AngII. (B) Saturation binding isotherm for specific [125 I]Sar 1 ,Ile 8 AngII binding in spleen sections from rats and mice. The affinity and density of binding sites were similar between rats and mice.

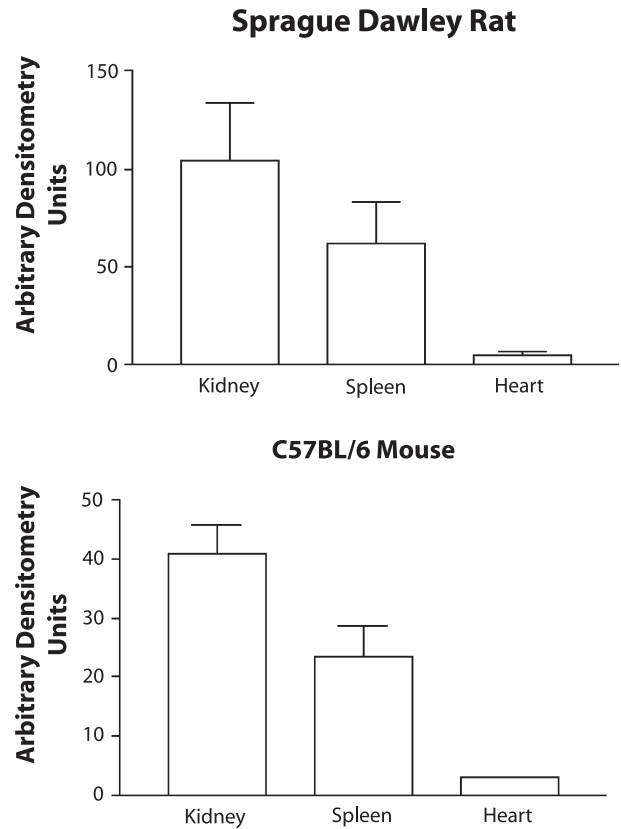


Fig. 4. Relative [125 I]Sar 1 ,Ile 8 AngII binding in tissues from rats and mice. Tissue sections from kidney, spleen and heart were incubated with [125 I]Sar 1 ,Ile 8 AngII (400 pM) for 2 h. Arbitrary densitometry units for [125 I]Sar 1 ,Ile 8 AngII binding were used to determine the rank order (kidney>spleen>heart) for binding in tissues within a species, which was similar in rats and mice.

and rat were chosen for these studies based on their common experimental use. In rats infused for 14 days with 500 ng/kg/min of AngII, body weight was significantly decreased (by 22%) compared to baseline (day 0) and compared to saline controls (Fig. 1A). In contrast, mice infused with this dose of AngII gained weight to the same extent as saline-infused controls. Systolic blood pressure increased within 2 days in rats infused with AngII, with a maximal increase of 54 mm Hg compared to saline on day 14 (Fig. 1B). In contrast, infusion of AngII to mice did not significantly influence systolic blood pressure throughout infusion. Aortic sections obtained from rats and mice infused with saline or AngII were stained with hematoxylin (Fig. 1C). In aorta from rats infused with AngII, pronounced medial hypertrophy was evident. In contrast, adventitial thickening was present in aorta from mice infused with AngII, but there was no evidence of medial hypertrophy.

We used HPLC resolution coupled with radioimmunoassay to determine the concentration of 5 different angiotensin peptides [AngII, AngIII(2–8), AngIV(3–8), Ang4–8, Ang5–8] in the plasma of rats ($n = 5$ /group, saline and AngII-infused) and mice ($n = 8$ /group, saline and AngII-infused). A representative HPLC chromatogram illustrates

the retention times of angiotensin peptide standards (Fig. 2A). Angiotensin immunoreactivity in the HPLC fractions matched the retention times of individual angiotensin peptide standards (30 pg/standard), with 80% recovery through the entire procedure (Fig. 2A). Moreover, plasma spiked with a known amount (80 pg) of AngII immediately upon removal exhibited an appropriate increase in concentration in the AngII fraction with minimal (<5%) breakdown to angiotensin peptide fragments during the extraction and quantification procedure (Fig. 2B). Using this methodology, we detected a single immunoreactive angiotensin peptide in plasma from rats which eluted with a retention time (20.3 min) consistent with AngII (Fig. 2C). In contrast, mouse plasma exhibited three different immunoreactive angiotensins with retention times of AngII, Ang4–8 (22.4 min) and AngIV (25.4 min). For each immunoreactive peak, the area

under the curve was used to quantify the plasma angiotensin concentration (Fig. 2C). The concentrations of AngII, AngIV and Ang4–8 were higher in mice compared to rats (Fig. 2C). Elevations in these angiotensin peptides in mouse plasma resulted in a twofold increase in the sum concentration of circulating angiotensins in mice compared to rats (Fig. 2C). In rats infused with AngII for 14 days, the sum concentration of circulating angiotensins was significantly increased compared to saline (Σ angiotensins: saline, 250 ± 26 ; AngII, 382 ± 40 pg/ml, $P < 0.05$); however, mice infused with AngII did not exhibit elevations in the concentration of circulating angiotensins (saline, 482 ± 81 ; AngII: 505 ± 51 pg/ml).

Receptor autoradiography for [125 I]Sar¹Ile⁸AngII binding was performed on tissue sections from spleen, kidney and heart from rats and mice. Complete saturation binding

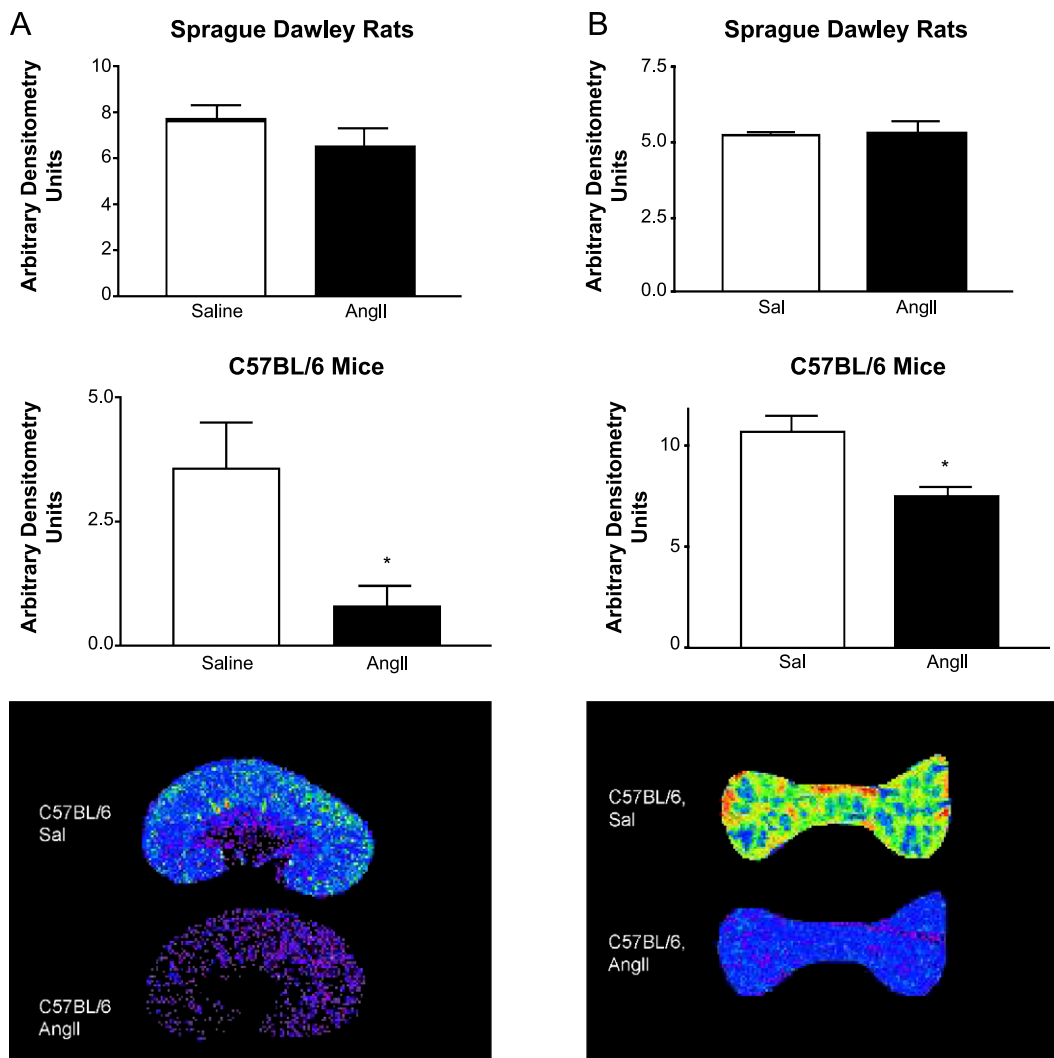


Fig. 5. Regulation of [125 I]Sar¹Ile⁸AngII binding in the kidney (A) and spleen (B) by chronic AngII infusion to rats and mice. Tissue sections from the kidney and spleen of saline- and AngII-infused rats (top histogram) and mice (bottom histogram) were incubated with [125 I]Sar¹Ile⁸AngII (400 pM) for 2 h. Bottom, representative total [125 I]Sar¹Ile⁸AngII binding in the kidney (A) and spleen (B) from saline- and AngII-infused mice. In rats, infusion of AngII for 2 weeks had no effect on [125 I]Sar¹Ile⁸AngII binding in kidney or spleen. In contrast, [125 I]Sar¹Ile⁸AngII binding was reduced by 78% in the kidney and 29% in the spleen of AngII-infused mice compared to saline. * denotes significantly ($P < 0.05$) different from saline.

isotherms for [125 I]Sar¹Ile⁸AngII were performed in spleen tissue sections from rats and mice and demonstrated that binding was to a single site of similar high affinity (K_d , C57BL/6 mice: 0.60 ± 0.08 nM; Sprague–Dawley rats: 0.58 ± 0.06 nM). Moreover, the maximal number of binding sites in spleen was similar in rats and mice (B_{max} , C57BL/6 mice: 84 ± 6 ; Sprague–Dawley rats: 75 ± 3 arbitrary densitometry units) (Fig. 3). We examined the relative density of AngII receptor sites in three different organs from non-treated rats and mice ($n = 5$ /species). In tissues from the rat, [125 I]Sar¹Ile⁸ AngII binding was greatest in the kidney, followed by the spleen, with minimal AngII receptor sites in the heart (Fig. 4). In mice tissues, the same rank order for [125 I]Sar¹Ile⁸ AngII binding was observed. Angiotensin receptor autoradiography was also performed on spleen and kidney tissue sections from rats and mice infused for 14 days with 500 ng/kg/min of AngII. Alternate sections were incubated with losartan to determine the relative AT1/AT2 receptor distribution. In the spleen from saline-infused animals, losartan displaced 63% of the binding in mice and 58% in rats. In the kidney of both rats and mice, losartan displaced 100% of the binding in both saline- and AngII-

infused animals. In rats, infusion of AngII did not alter [125 I]Sar¹Ile⁸ AngII binding density in the kidney or spleen (Fig. 5). However, losartan displacement of binding in the spleen decreased from 63% to 40% in AngII-infused rats; no changes in losartan displacement of AngII binding in the spleen were evident in mice infused with AngII. In mice infused with AngII, [125 I]Sar¹Ile⁸ AngII binding density was decreased by 78% in the kidney and by 29% in the spleen (Fig. 5).

We examined vascular reactivity to AngII in isolated thoracic aortic strips from rats and mice. Tracings illustrating the contractile response in representative strips from rats and mice are depicted in Fig. 6A. Mouse aortic strips exhibited robust and sustained contractile responses to KCl and serotonin. In contrast, the contractile response to AngII (100 nM) in mouse aortic strips was smaller in magnitude and was not maintained. In rat aortic strips, the same concentration of AngII resulted in a significant contractile response, which was maintained for the duration of exposure. For comparisons between rats and mice, the maximal force generated in aortic strips in response to AngII was expressed as a percentage of the KCl response.

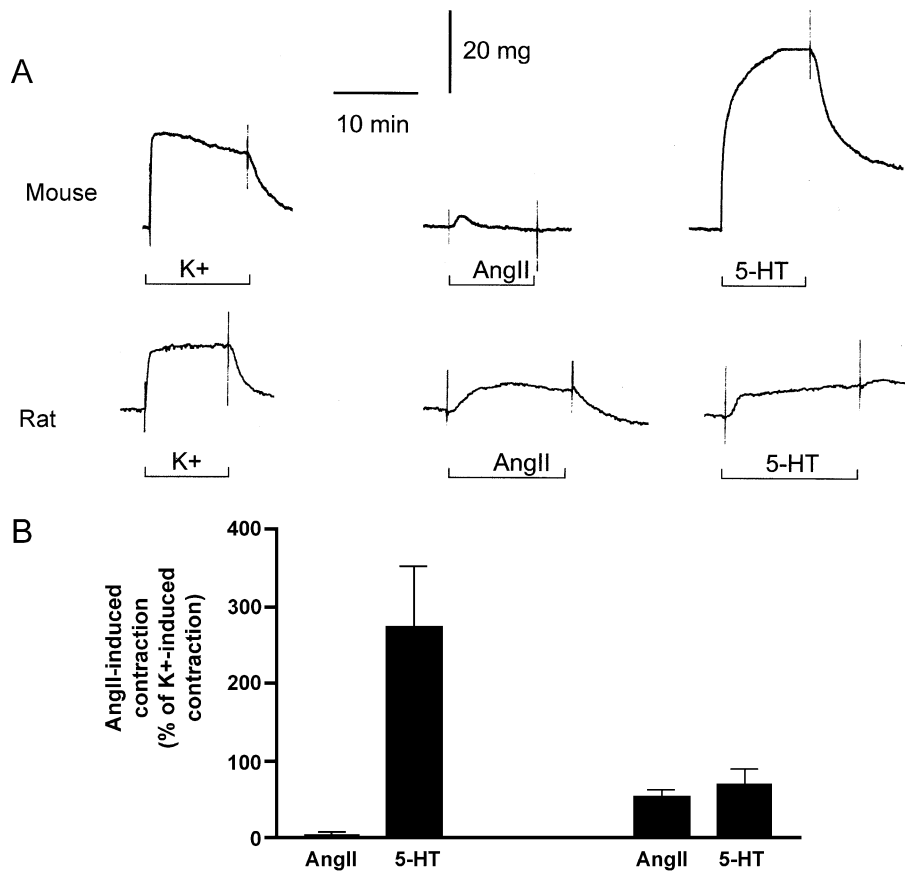


Fig. 6. Reactivity of thoracic aorta strip segments from rats and mice. Thoracic aorta from non-treated rats and mice were cut into spiral strip segments and mounted for determination of contractile force in response to KCl (K⁺; 143 nM), AngII (100 nM), or serotonin (5-HT; 1 μ M). Top, representative tracings for contractile responses to different agonist stimulation in thoracic aorta from rats and mice. Mouse aorta exhibited a diminished, non-sustained contractile response to AngII. Bottom, AngII contractile responses expressed as a percentage of the response to K⁺. Mice exhibited minimal contractile response to AngII, but gave robust responses to 5-HT. In contrast, rats exhibited similar contractile responses to AngII and 5-HT.

Aortic strips from mice generated far less contractile force to AngII compared to rats, but were responsive to serotonin (Fig. 6B). In contrast, aortic strips from rats exhibited contractile responses to AngII which were similar in magnitude to those generated by serotonin.

4. Discussion

This study demonstrated that mice exhibit a diminished blood pressure response to chronic subcutaneous infusion of AngII compared to rats. In addition, reductions in body weight from chronic AngII infusion to rats were not observed in mice. Moreover, vascular remodeling differed between mice and rats infused with AngII, with rat aorta demonstrating medial hypertrophy and mouse aorta exhibiting adventitial thickening. Potential mechanisms contributing to diminished responses to AngII in mice include higher basal circulating angiotensin concentrations, diminished vascular reactivity to AngII, and pronounced receptor down-regulation in response to chronic AngII infusion. The significance of these results relates to the common use of chronic subcutaneous infusion of AngII as a mechanism to define the pharmacology and physiology of AngII. Results from this study suggest that considerably higher doses of AngII may be required to elicit effects from chronic subcutaneous infusion of the peptide to mice.

4.1. Differences in the blood pressure response to AngII between rats and mice

Results from this study demonstrate that chronic administration of an equivalent dose of AngII to rats versus mice resulted in markedly different blood pressure responses. Using chronic subcutaneous infusion, Bendall et al. [22] demonstrated that 200 ng/kg/min of AngII did not elevate blood pressure in C57BL/6 mice. In this study, chronic subcutaneous infusion of as much as 500 ng/kg/min of AngII to mice did not appreciably alter systolic pressure in conscious mice, but robustly increased blood pressure in rats. Due to the magnitude of blood pressure elevation and other effects in rats infused with 500 ng/kg/min of AngII, we did not perform comparative studies in rats and mice at larger AngII doses. However, previous results from our laboratory demonstrate that chronic subcutaneous infusion of 1000 ng/kg/min of AngII to hyperlipidemic mice results in an approximate 25 mm Hg increase in systolic pressure [23]. Thus, higher subcutaneous infusion doses of AngII are capable of elevating systolic pressure in mice.

4.2. Differences in circulating angiotensin peptides between rats and mice

To our knowledge, this is the first report of measurement of five different angiotensin peptides in the plasma of mice. Results from this study demonstrate a higher level of

circulating AngII in mice compared to rats. In mice, previously reported plasma AngII measurements have ranged from 14 to 172 pg/ml [24–27]. Thus, the concentration of angiotensin peptides in plasma from mice in this study is slightly higher than previously reported values. Potential variables in methods for measurement of AngII that may contribute to differences in reported values across studies include the affinity and specificity of the AngII antibody, and the potential for breakdown or formation of angiotensins during the extraction procedure. To assure minimal AngII production or degradation during the extraction procedure used in this study, a variety of protease inhibitors were added immediately to blood. Moreover, recovery of a known amount of AngII added to mouse and rat plasma samples before extraction was high (>80%). Elevations in plasma AngII concentration in mice compared to rats would be anticipated to influence the responsiveness to exogenous AngII infusion. For example, infusion of the same dose of AngII to mice exhibiting a higher baseline plasma AngII concentration compared to rats may have contributed to diminished overall responses in mice.

In addition to differences in plasma AngII concentration between mice and rats, catabolic fragments of AngII were present in plasma from mice, but not rats. Angiotensin II is catabolized by aminopeptidase A (EC3.4.11.7), which removes the N-terminal aspartate to produce AngIII [28,29]. Angiotensin IV is formed from aminopeptidase B (ED3.4.11.6) removal of arginine from AngIII [30]. A higher basal metabolic rate with increased activity of these enzymes in blood from mice compared to rats may have contributed to elevations in these catabolic fragments of AngII in mice. Of these angiotensins, AngIV, which binds with low affinity to AT1/AT2 receptors [31] but high affinity to insulin regulated aminopeptidase [32], exhibits distinct biologic activities from AngII [33]. Cardiovascular effects of AngIV include a reduction in vascular resistance in the renal and cerebral vascular bed [34] and stimulation of endothelial nitric oxide in pulmonary artery ring segments [35]. These effects of AngIV would suggest that elevated concentrations of circulating AngIV in mice compared to rats would cause lower blood pressure in mice compared to rats. Indeed, baseline systolic pressure was lower in mice than rats in this study. Interestingly, circulating angiotensin peptides were elevated in rats infused with AngII, but not in mice, suggesting differences in the handling of AngII with chronic subcutaneous infusion. The inability to detect an increase in circulating angiotensin peptides in mice infused chronically with AngII may have resulted from increased clearance of angiotensins, or sequestration of the peptide in tissue.

4.3. Differences in vascular remodeling between rats and mice

An additional difference between C57BL/6 mice and Sprague–Dawley rats included medial hypertrophy in rat aorta, but adventitial thickening in aorta from mice infused

with AngII. We previously observed marked expansion of the adventitia in aorta from hyperlipidemic LDL receptor $-/-$ or Apolipoprotein E $-/-$ mice infused with AngII [17,18]. Differences in vascular remodeling between rats versus mice infused with AngII could arise from differences in AngII receptor sites in cells of the vascular wall. Alternatively, sustained elevations in blood pressure in AngII-infused rats may have contributed to medial hypertrophy of the aorta. In contrast, elevations in blood pressure were not sustained in mice infused with AngII.

4.4. Differences in angiotensin receptor regulation between rats and mice

In agreement with previous reports, angiotensin receptor binding in the spleen of mice and rats was of high density and affinity [36,37]. In addition, relative AngII receptor density across three different organs was similar in rats and mice, demonstrating similar regional localization of AngII receptors. Moreover, losartan displacement was similar in organs from rats and mice, demonstrating that AT1/AT2 receptor subtype distribution did not markedly differ within an organ from rats compared to mice.

The regulation of AngII receptor density in response to chronic agonist exposure was markedly different between rats and mice. The literature for regulation of AngII receptors following chronic AngII infusion differs according to the AngII dose, route of administration, and duration of infusion. Infusion of AngII to rats has been reported to result in no change in AngII receptor density in liver or kidney [38], an increase in the vasculature [39], but a decrease in the kidney [40]. Previous results demonstrating downregulation of AngII receptor density in the kidney were obtained following intravenous infusion of AngII [41], rather than subcutaneous infusion used in the present study. To our knowledge, there are no previous reports on regulation of AngII receptor density following chronic infusion of AngII to mice. Results from this study demonstrate differential regulation of AngII receptor density in response to chronic AngII infusion between rats and mice. Downregulation of AngII receptor density in mouse tissues following chronic AngII infusion is suggested to contribute to diminished blood pressures responsiveness in mice.

4.5. Differences in vascular reactivity to AngII between rats and mice

Vascular reactivity to AngII in thoracic aortic strips, expressed as a percentage of KCl responsiveness, was markedly decreased in C57BL/6 mice compared to rats. Tanaka et al. [42] reported rapid desensitization to AngII in aortic strips from C57BL/6 mice. Moreover, these investigators demonstrated relatively low AngII contractile responses in mouse aorta compared to contractions elicited by KCl. In addition, Russell et al. [43] reported weak efficacy of AngII to contract thoracic aorta strips from

C57BL/6 mice. These authors demonstrated that lack of a significant contractile response to AngII in mouse aorta does not arise from rapid destruction of AngII, functional antagonism through the AT2 receptor, or age of the mice. Rather, rapid desensitization of mouse aorta in response to AngII was suggested as a potential mechanism for the weak contractile response to AngII. In this study, given that circulating AngII was increased in mice compared to rats, desensitization and/or downregulation of AngII receptors in mouse vasculature may have contributed to diminished blood pressure responsiveness of mice to exogenous AngII.

In conclusion, Sprague–Dawley rats and C57BL/6 mice exhibit differences in the renin–angiotensin system related to circulating angiotensin peptides, AngII receptor regulation, vascular remodeling and responsiveness. Collectively, these differences favor enhanced responsiveness to chronic subcutaneous infusion of AngII to rats compared to mice. Differences in responsiveness between Sprague–Dawley rats and C57BL/6 mice infused with AngII warrant further investigation in relation to vascular pathologies elicited by AngII.

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