

# Activity and responsiveness of the renin-angiotensin system in the aging rat

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**Thompson, Michele M., Terry T. Oyama, Francis J. Kelly, Thomas M. Kennefick, and Sharon Anderson.** Activity and responsiveness of the renin-angiotensin system in the aging rat. *Am J Physiol Regulatory Integrative Comp Physiol* 279: R1787–R1794, 2000.—The systemic renin-angiotensin system (RAS) is suppressed in normal aging, but the activity of the tissue RAS is not well defined. We examined the systemic and intrarenal RAS status of aging normal rats and responses to suppression and stimulation of the production of endogenous ANG II. Studies were performed in young (3 mo) and early aging (15 mo) male Sprague-Dawley rats. Angiotensin-converting enzyme inhibitors modestly decreased mean arterial pressure (MAP) in young (3 mo) and early aging (15 mo) rats and limited proteinuria in the older rats. There were no significant age-related effects on renal function or on endogenous RAS activity. Intravenous infusion of the precursor ANG I led to comparable increases in MAP in younger and older rats. In contrast, the renal effects (reduction in glomerular filtration and plasma flow rates) were exaggerated in the older animals. Intrarenal arterial ANG I did not affect MAP in any group. In young rats, there were no significant hemodynamic effects in either the ipsilateral (infused) or the contralateral (noninfused) kidney. In the older rats, both kidneys had a significant fall in renal plasma flow rate (RPF) with left renal arterial infusion of ANG I. Accordingly, these studies early in the course of aging found only subtle changes in the activity, responsiveness, and metabolism of the RAS. Thus early aging is associated with a modest but important increase in sensitivity to RAS stimulation.

glomerular filtration rate; proteinuria; kidney

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NORMAL AGING IS CHARACTERIZED by changes in the activity or responsiveness of a number of hormonal systems. Among these is the renin-angiotensin system (RAS), which has classically been considered to be suppressed in aging. Changes in activity of the end products of this cascade, ANG II and aldosterone, are believed to contribute to the increased incidence of fluid and electrolyte disorders in the elderly. However, despite the fact that plasma renin concentration (PRC) falls with age (2, 22), interventional studies have invoked a role for the RAS in mediating age-related renal disease. In

aging animal models, angiotensin-converting enzyme (ACE) inhibitors (ACEI), whether started early or late, retard the progression of age-related nephropathy (2, 11, 12, 17, 19, 21, 24). This benefit might not have been predicted given the age-related fall in PRC. However, it is now recognized that, in addition to the circulating (plasma) RAS, there are a number of independent, locally regulated tissue RASs, whose activity does not always parallel that of the systemic circulation (9). These studies were designed to examine several aspects of RAS activity and responsiveness in the course of normal aging in the rat. Our hypothesis was that the reduction in systemic RAS activity might not be reflected in the kidney and that upregulation of the ANG II receptors might manifest functionally as impaired responsiveness to RAS blockade but enhanced responsiveness to RAS stimulation. First, we sought to determine whether the sensitivity of blood pressure, renal function, proteinuria, and/or RAS parameters to chronic ACE inhibition are altered with aging. Second, we sought to determine whether responsiveness to exogenous ANG II is altered as well as the effectiveness of conversion of ANG I to ANG II in the systemic circulation. Third, we sought to determine the efficiency of intrarenal conversion of ANG I to ANG II and the renal consequences of the same in the course of aging.

## METHODS

*The aging model.* Studies were conducted in adult male Sprague-Dawley rats (Harlan, Indianapolis, IN) at two time points: young (3 mo) and older (15 mo). The 15-mo age was selected because these animals exhibit modest age-related renal injury (20) but do not yet have injury sufficient to seriously compromise renal function. All rats were fed standard rat chow (Rodent Laboratory Chow 5001, Purina Mills, Richmond, IN) ad libitum and had free access to water. These studies were approved by the Portland Veterans Affairs Institutional Animal Care and Use Subcommittee.

*Protocol 1: studies of chronic ACEI.* Baseline studies assessed awake systolic blood pressure (SBP), by the tail cuff method and metabolic cage studies for 24-h measurements of urinary protein excretion ( $U_{\text{prot}}$ ). Thereafter, rats were subdivided to receive 4 wk of therapy with the ACEI enalapril

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Table 1. *Chronic ACEI: baseline and posttreatment measurements*

Group	n	SBP-1, mmHg	SBP-2, mmHg	$\Delta$ SBP, mmHg	$U_{\text{prot}}V$ -1, mg/day	$U_{\text{prot}}V$ -2, mg/day	$\Delta U_{\text{prot}}V$ , mg/day	BW, g	LKW, g	LKW/100 g BW
3 Mo	26	131 $\pm$ 3	128 $\pm$ 2	-3 $\pm$ 3	7 $\pm$ 1	10 $\pm$ 2	3 $\pm$ 1	409 $\pm$ 5	1.31 $\pm$ 0.02	0.33 $\pm$ 0.01
3 Mo + low-dose ACEI	24	121 $\pm$ 3	110 $\pm$ 2*	-12 $\pm$ 4	8 $\pm$ 1	9 $\pm$ 1	1 $\pm$ 1	388 $\pm$ 6	1.25 $\pm$ 0.02	0.33 $\pm$ 0.01
3 Mo + high-dose ACEI	12	129 $\pm$ 5	106 $\pm$ 3*	-23 $\pm$ 6	8 $\pm$ 1	9 $\pm$ 1	1 $\pm$ 1	381 $\pm$ 11	1.26 $\pm$ 0.04	0.33 $\pm$ 0.01
15 Mo	24	126 $\pm$ 3	127 $\pm$ 2	1 $\pm$ 4	61 $\pm$ 8	78 $\pm$ 9*	17 $\pm$ 6	566 $\pm$ 9	1.80 $\pm$ 0.04	0.32 $\pm$ 0.01
15 Mo + low-dose ACEI	19	125 $\pm$ 3	118 $\pm$ 3*	-8 $\pm$ 4	60 $\pm$ 7	55 $\pm$ 7	-5 $\pm$ 6	524 $\pm$ 13	1.81 $\pm$ 0.08	0.35 $\pm$ 0.01
15 Mo + high-dose ACEI	11	130 $\pm$ 4	111 $\pm$ 5*	-19 $\pm$ 4	72 $\pm$ 15	60 $\pm$ 16	-11 $\pm$ 14	541 $\pm$ 14	1.91 $\pm$ 0.07	0.35 $\pm$ 0.01
<i>P</i> value, ANOVA										
Age		NS	NS	NS	<0.001	<0.001	NS	<0.001	<0.001	NS
Treatment		NS	<0.0001	<0.0001	NS	NS	<0.02	NS	NS	<0.02
Age + treatment		NS	NS	NS	NS	NS	<0.05	NS	NS	NS

Values are mean  $\pm$  SE; *n*, no. of rats. ACEI, angiotensin-converting enzyme inhibitor; 1, before treatment; 2, posttreatment; SBP, systolic blood pressure;  $U_{\text{prot}}V$ , 24-h urinary protein excretion; BW, body weight; LKW, left kidney weight; NS, not significant ( $P > 0.05$ );  $\Delta$ , change. \* $P < 0.05$  vs. baseline in same group, by paired *t*-test.

(Sigma, St. Louis, MO), at high or low doses, in the drinking water. Untreated young and older groups served as controls. The low-dose rats received 100 mg/l, a dose that caused SBP to fall  $\sim$ 10 mmHg in both age groups. The high doses of 150 mg/l for the young and 200 mg/l for the older were chosen after pilot studies established that these doses led to SBP reductions of  $\sim$ 20 mmHg. After 4 wk of treatment, SBP measurements and metabolic cage studies were repeated. Thereafter, *protocol 1A* involved renal functional studies with measurement of mean arterial pressure (MAP), glomerular filtration rate (GFR), renal plasma flow (RPF), filtration fraction (FF), and renal vascular resistance (RVR). *Protocol 1B* assessed the renal and blood ANG II levels and PRC.

*Protocol 2: studies with intravenous ANG I.* These studies were designed to assess the functional conversion of ANG I to ANG II in the circulation and resultant systemic and renal hemodynamic and biochemical responses in young and older rats. After surgical preparation and baseline measurements of renal hemodynamic function as described below, rats received an intravenous infusion of normal saline vehicle (Veh) at 0.1 ml/h and baseline hemodynamic status was assessed. During the second (experimental) period, rats received either continuous ANG I (0.166  $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ; Sigma) or saline Veh at the same infusion rate. After 20 min of infusion, hemodynamic studies were repeated. At the end of the experiment, blood and kidneys were taken for RAS measurements.

*Protocol 3: studies with intra-arterial ANG I.* These studies were designed to assess the intrarenal conversion of ANG I to

ANG II so as to minimize the effects of changes in the circulatory RAS. During the course of surgical preparation, all rats were instrumented with an indwelling pipette in the left renal artery, as described in *Renal function studies*. Left (infused) kidney and right (noninfused) kidney function were measured, first during a saline Veh period and then during a continuous left renal arterial infusion of ANG I (0.166  $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) or continued saline Veh. At the end of the experiment, blood and kidneys were taken for RAS measurements.

*Renal function studies.* Rats were anesthetized with Inactin (100 mg/kg ip) and placed on a thermoregulated table. The left femoral artery was cannulated, and a baseline sample of blood was collected for determination of hematocrit (Hct) and inulin and para-aminohippurate (PAH) blanks. This arterial catheter was used for subsequent blood sampling and for estimation of MAP via an electronic transducer connected to a direct-writing recorder. After tracheostomy, bilateral internal jugular catheters were inserted for infusions of rat serum and 10% inulin (Cypros, Carlsbad, CA) with 0.8% PAH (Merck, West Point, PA) in 0.9% NaCl (1.2 ml/h). The left ureter was catheterized for urine collections. To maintain euvolesmia, rat serum was infused at 0.1 ml/min for a total equal to 1% of the body weight, followed by a reduction in infusion rate to 0.007 ml/min for young rats and 0.012 ml/min for older rats, to maintain a constant Hct. In *protocol 3*, additional preparation included placement of a catheter in the right ureter. In addition, the left renal artery was carefully dissected and punctured with a glass capillary

Table 2. *Chronic ACEI: systemic and renal hemodynamic studies*

Group	n	MAP, mmHg	GFR, ml/min	RPF, ml/min	Filtration Fraction	RVR, mmHg $\cdot$ ml $\cdot$ min $^{-1}$
3 Mo	13	126 $\pm$ 3	2.27 $\pm$ 0.11	7.69 $\pm$ 0.51	0.30 $\pm$ 0.01	9.7 $\pm$ 0.7
3 Mo + low-dose ACEI	12	113 $\pm$ 3	2.21 $\pm$ 0.13	7.23 $\pm$ 0.52	0.31 $\pm$ 0.01	9.1 $\pm$ 0.7
15 Mo	10	132 $\pm$ 3	2.06 $\pm$ 0.14	7.46 $\pm$ 0.82	0.29 $\pm$ 0.02	11.5 $\pm$ 1.3
15 Mo + low-dose ACEI	7	130 $\pm$ 2	2.14 $\pm$ 0.25	9.49 $\pm$ 1.25	0.24 $\pm$ 0.03	8.6 $\pm$ 1.2
<i>P</i> value, ANOVA						
Age		<0.005	NS	NS	<0.05	NS
Treatment		<0.001	NS	NS	NS	NS
Age + treatment		NS	NS	NS	NS	NS

Values are means  $\pm$  SE. MAP, mean arterial pressure; GFR, glomerular filtration rate; RPF, renal plasma flow rate; RVR, renal vascular resistance.  $P > 0.05$ .

Table 3. *Chronic ACEI: renin-angiotensin system measurements*

Group	n	PRC, ng ANG I· ml <sup>-1</sup> ·h <sup>-1</sup>	Blood ANG II, fmol/ml	LK ANG II, fmol/g
3 Mo	13	10 ± 1	9 ± 2	63 ± 6
3 Mo + low-dose ACEI	12	312 ± 100	15 ± 6	37 ± 6
3 Mo + high-dose ACEI	12	261 ± 47	5 ± 2	28 ± 2
15 Mo	12	9 ± 1	7 ± 1	71 ± 6
15 Mo + low-dose ACEI	11	154 ± 33	5 ± 1	32 ± 5
15 Mo + high-dose ACEI	11	362 ± 87	5 ± 1	27 ± 3
P value, ANOVA				
Age		NS	NS	NS
Treatment		P < 0.0001	P < 0.05	P < 0.0001
Age + treatment		NS	NS	NS

Values are means ± SE. PRC, plasma renin concentration; LK, left kidney. P > 0.05.

pipette (30 μm) (15). After cannulation, saline was infused (2 μl/min) during the equilibration period, until the beginning of the experimental period.

After equilibration, duplicate or triplicate 20-min urine collections with midpoint blood collections were made for measurement of inulin and PAH clearances. Blood was obtained simultaneously for measurement of Hct, inulin, and PAH. GFR (from inulin clearance), RPF (from PAH clearance), FF, and RVR were determined with standard formulas. For the intra-arterial experiments, any experiment with a discordance in GFR between the left and right kidneys of 20% or greater during the baseline period was considered technically unacceptable and was discarded.

**RAS measurements.** To minimize RAS stimulation, rats in protocol 1B (chronic ACEI studies) were anesthetized for <10 min before taking kidney tissue and blood samples. These rats were anesthetized with Inactin (100 mg/kg ip) and placed on a thermoregulated table before sample collection. In protocols 2 and 3, RAS measurements were of necessity performed after longer anesthesia and surgery. The left kidney was quickly excised and homogenized in cold methanol for ANG II measurement. Blood was rapidly obtained by cardiac puncture into prechilled syringes and separated into tubes of either cold methanol (for ANG II) or EDTA (for determination of PRC).

**Analytical methods.** For the calculation of GFR, inulin concentrations in plasma and urine were determined by the macroanthrone method. RPF was determined by PAH clearance, using colorimetric methodology. Urinary total protein content was measured by precipitation with 3% sulfosalicylic acid precipitation (Sigma). PRC was measured by radioimmunoassay using commercially available reagents (New En-

gland Nuclear, Boston, MA). ANG II was measured with the method of Fox et al. (8). ANG II was quantified in a competitive single-antibody radioimmunoassay using rabbit anti-ANG II antibody (Peninsula, Belmont, CA) and monoiodinated I<sup>125</sup>-labeled ANG II (Amersham, Arlington Heights, IL) and ANG II standards (Sigma).

**Statistics.** Values are reported as means ± SE. Statistical analysis was performed by one- or two-way ANOVA, as appropriate. Normality was assessed with the Shapiro-Wilk statistic, and homogeneity of variance was tested with Hartley's  $F_{max}$  test. In some cases, the paired *t*-test was also used. Values that were not normally distributed or homogeneous in variance were analyzed using nonparametric methods. The statistical programs used were SPSS and SAS. Statistical significance was defined as P < 0.05.

## RESULTS

**Protocol 1: studies of chronic ACEI.** Pooled measurements of systemic and urinary parameters in young and older rats, before and after ACEI treatment, are summarized in Table 1. All groups were normotensive at baseline. Enalapril treatment caused a dose-related, parallel decline in SBP both in young and older rats (P < 0.0001). Baseline values for  $U_{prot}V$  were higher in older rats than in young rats (P < 0.0001) and continued to be so throughout treatment, indicating the presence of moderate age-related glomerular injury. Although  $U_{prot}V$  declined numerically with enalapril treatment in the older rats, a significant treatment effect was not found in this relatively brief period of treatment. However, given that  $U_{prot}V$  tended to increase slightly in the untreated rats and to decline in the treated rats, there may have been a modest effect to delay the progression of proteinuria. Older rats were larger at baseline and throughout treatment (P < 0.0001), and enalapril did not alter body weight. As body mass increased with age, so did kidney weight (P < 0.0001), so that the kidney-to-body weight ratio did not change. Treatment had a slight, significant influence on the kidney-to-body weight ratio.

Results of functional measurements in the chronic ACEI studies are depicted in Table 2. During the experiments, effective blockade of ACE was confirmed by infusing a test dose of the precursor ANG I. Enalapril-treated animals showed only a slight (11 ± 2 mmHg) rise in MAP with ANG I, whereas untreated animals showed a 20 ± 4 mmHg rise with ANG I (P < 0.05). Enalapril lowered MAP in young (P < 0.005) but not older rats; thus age altered the blood pressure response to ACEI in the setting of anesthesia (P < 0.05).

Table 4. *Intravenous ANG I studies: baseline parameters*

Group	BW, g	LKW, g	LKW/100 g BW	MAP, mmHg	GFR, ml/min	RPF, ml/min	FF	RVR, mmHg·ml·min <sup>-1</sup>
3 Mo (n = 18)	408 ± 11	1.37 ± 0.45	0.34 ± 0.01	127 ± 2	1.61 ± 0.09	6.12 ± 0.29	0.27 ± 0.01	12.1 ± 0.5
15 Mo (n = 15)	578 ± 11	1.90 ± 0.07	0.33 ± 0.02	129 ± 4	2.03 ± 0.11	7.51 ± 0.45	0.28 ± 0.01	10.6 ± 0.7
P value	P < 0.001	P < 0.001	NS	NS	P < 0.05	P < 0.02	NS	NS

Values are means ± SE. FF, filtration fraction. P > 0.05.

Table 5. Intravenous ANG I: systemic and renal hemodynamic studies

Group	Period	MAP, mmHg	GFR, ml/min	RPF, ml/min	FF	RVR, mmHg·ml·min <sup>-1</sup>
3 Mo + Veh (n = 9)	1	125 ± 4	1.60 ± 0.13	6.00 ± 0.34	0.27 ± 0.02	11.8 ± 0.5
	2	119 ± 4*	1.67 ± 0.17	6.24 ± 0.50	0.27 ± 0.02	11.3 ± 0.7
	Δ	-6 ± 2	+0.07 ± 0.09	+0.24 ± 0.42	0.00 ± 0.01	-0.5 ± 0.8
3 Mo + ANG I (n = 9)	1	130 ± 2	1.62 ± 0.13	6.25 ± 0.38	0.26 ± 0.01	12.4 ± 0.8
	2	146 ± 2*†	1.52 ± 0.09	3.99 ± 0.17*	0.38 ± 0.02*†	21.4 ± 0.8*†
	Δ	+16 ± 3†	-0.10 ± 0.05	-2.26 ± 0.27†	+0.12 ± 0.01†	+9.0 ± 0.9†
15 Mo + Veh (n = 7)	1	123 ± 4	2.19 ± 0.19†	7.91 ± 0.85†	0.29 ± 0.02	9.8 ± 1.0
	2	121 ± 4	2.32 ± 0.20	7.93 ± 0.81†	0.30 ± 0.02	9.5 ± 0.8
	Δ	-2 ± 1	+0.13 ± 0.09	+0.01 ± 0.24	+0.01 ± 0.01	-0.2 ± 0.4
15 Mo + ANG I (n = 8)	1	133 ± 5	1.89 ± 0.11	7.16 ± 0.42	0.27 ± 0.01	11.3 ± 0.9
	2	154 ± 6*†	1.61 ± 0.11*†	3.30 ± 0.25*†	0.49 ± 0.01*†	28.8 ± 2.6*†
	Δ	+20 ± 3†	-0.27 ± 0.05†	-3.86 ± 0.26†‡	+0.23 ± 0.01†‡	+17.5 ± 1.8†‡

Values are means ± SE. *Period 1*, baseline; *period 2*, experimental; Δ, difference between *periods 1* and *2*; Veh, normal saline vehicle; \**P* < 0.05 vs. baseline (*period 1*); †*P* < 0.05 vs. Veh group or change in Veh group; ‡*P* < 0.05 vs. change in corresponding 3-mo group.

GFR was not altered by either age or enalapril, although it declined with age when adjusted for body weight (data not shown; *P* < 0.0001). Similarly, both age and treatment failed to influence renal plasma flow, although when adjusted for body mass, RPF declined with age (data not shown; *P* < 0.05). Enalapril did not affect RPF. Of note, there was no fall in RPF in the older rats treated with enalapril; indeed, their values for RPF were numerically the highest of all groups. Although not detectable by the two-way ANOVA analysis, enalapril may have attenuated the age-related fall in RPF. Age was associated with a significant decrease in filtration fraction (*P* < 0.05) that likewise was not adjusted by enalapril treatment. Values for RVR did not differ among the groups. RAS measurements taken at the end of the study are summarized in Table 3. Enalapril treatment influenced biochemical measurements in the expected fashion: increased PRC (*P* < 0.0001), decreased whole blood ANG II concentrations (*P* < 0.05), and decreased renal ANG II concentrations (*P* < 0.0001). Aging exerted no significant influence on any of these parameters in either treated or untreated groups (Table 3).

*Protocol 2: studies with intravenous ANG I infusion.* Baseline parameters in the groups subjected to intravenous infusion studies are summarized in Table 4 and functional studies in Table 5. As in the preceding protocol, at baseline, the older rats exhibited higher values for body and kidney weights and equivalent values for MAP. In this protocol, values for GFR and RPF were slightly, but significantly, higher in the older

rats (Table 4), although not when factored for body weight (data not shown). Values for FF and RVR were similar.

Results of the ANG I infusion studies are depicted in Table 5. Saline Veh had no significant effect in any group. In the younger rats, ANG I induced a significant increase in MAP. At this dose, GFR was not affected, but RPF fell accompanied by significant increases in FF and RVR. In the older animals, an equivalent increase in MAP was associated with a significant decrease in GFR and more prominent changes in RPF, FF, and RVR than were seen in the younger rats. Thus, whereas the systemic (blood pressure) responses to ANG I were similar, the renal responses were accentuated in the older rats. RAS measurements in the rats subjected to intravenous infusions are summarized in Table 6. All values were higher than those in Table 3 because of the longer duration of anesthesia and the preceding surgery. PRC and renal ANG II levels did not differ between young and older groups that received the saline Veh, but plasma ANG II levels were significantly lower in the 15-mo-old rats. Both groups exhibited the expected responses to ANG I: suppression of PRC and increases both in plasma and renal ANG II levels. There were no significant differences between the younger and older groups receiving ANG I. Thus, whereas the hemodynamic responses were accentuated in the older animals, these could not be correlated with measurable changes in systemic or renal RAS parameters.

*Protocol 3: Studies with intra-arterial ANG I infusion.* Pooled baseline values for systemic and renal parameters in rats subjected to intra-arterial infusions are described in Table 7. Body weights (530 ± 10 vs. 366 ± 8 g, *P* < 0.001) and kidney weights (2.04 ± 0.08 vs. 1.33 ± 0.04 g, *P* < 0.001) were again higher in the older rats, and blood pressures were equivalent. Baseline values for GFR did not differ between young and old rats, whereas values for RPF were higher, and values for FF were lower in the older rats. Baseline values for renal function in the left kidney (containing the pipet) and the right (noninfused) kidney did not

Table 6. Intravenous ANG I studies: renin-angiotensin system studies

Group	PRC, ng ANG I·ml <sup>-1</sup> ·h <sup>-1</sup>	Plasma ANG II, fmol/ml	Renal ANG II, fmol/g
3 Mo + Veh	25 ± 11	89 ± 31	529 ± 217
3 Mo + ANG I	8 ± 1†	1,053 ± 256†	2,790 ± 462†
15 Mo + Veh	22 ± 5	27 ± 9*	293 ± 50
15 Mo + ANG I	6 ± 3†	1,460 ± 475†	885 ± 308†

Values are means ± SE. \**P* < 0.05 vs. corresponding 3-mo group; †*P* < 0.05 vs. corresponding Veh group. There were no significant differences between the 3-mo + ANG I and 15-mo + ANG I groups.

Table 7. Intra-arterial ANG I studies: baseline parameters

Group	MAP mmHg	LK GFR ml/min	LK RPF ml/min	LK FF	RK GFR ml/min	RK RPF ml/min	RK FF
3 Mo (n = 17)	129 ± 2	1.52 ± 0.08	5.31 ± 0.23	0.29 ± 0.01	1.47 ± 0.06	5.37 ± 0.20	0.28 ± 0.01
15 Mo (n = 13)	124 ± 4	1.49 ± 0.11	7.88 ± 0.76	0.20 ± 0.02	1.47 ± 0.11	7.91 ± 0.73	0.20 ± 0.02
P value	NS	NS	P < 0.02	P < 0.02	NS	P < 0.005	P < 0.001

Values are means ± SE.  $P > 0.05$ . There were no significant differences between baseline LK and RK values in the same animals.

differ, and thus function was not demonstrably altered by the presence of the infusion pipet.

There were no significant changes in blood pressure in any group after infusion of ANG I or Veh (data not shown). Changes in GFR and RPF in the four groups are summarized in Fig. 1. Neither ANG I nor saline Veh affected GFR or RPF in the young rats. GFR was similarly unaffected in the older rats. However, RPF was more sensitive in the older rats. ANG I induced a significant decrease in both left and right kidneys of older rats, whereas saline Veh was without effect.

Values for RAS parameters in the rats receiving intra-arterial infusions are summarized in Table 8. Compared with younger rats receiving saline, the Veh-

treated older rats had lower values for PRC but no other significant changes in RAS parameters. Both in young and older rats receiving saline Veh, values for left and right kidney ANG II levels were comparable, confirming that the infusion pipet per se did not affect the RAS. Infusion of ANG I generally had the expected effects in both age groups, with minor differences. PRC was significantly suppressed in the younger rats but only numerically in the older rats. Plasma ANG II levels did not change in the younger rats, whereas they were significantly increased in the older rats receiving ANG I. Both in younger and older groups, the left (infused kidney) showed a significantly increased ANG II level, whereas the ANG II levels did not increase in

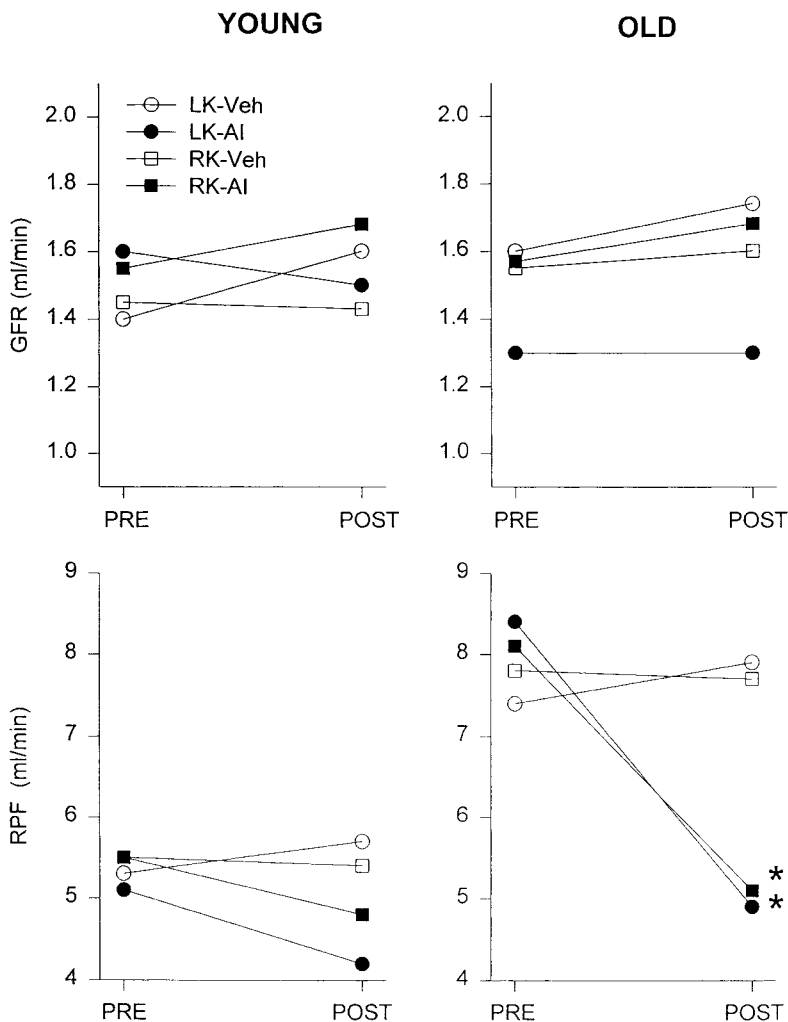


Fig. 1. Left kidney (LK) and right kidney (RK) responses to intra-arterial infusion of ANG I (AI) or normal saline vehicle (Veh) in young and older rats. \* $P < 0.05$  vs. baseline and vs. change in young. PRE, before infusion; POST, after infusion.

Table 8. *Intra-arterial ANG I studies: renin-angiotensin system studies*

	PRC, ng ANG I·ml <sup>-1</sup> ·h <sup>-1</sup>	Plasma ANG II, fmol/ml	LK ANG II, fmol/g	RK ANG II, fmol/g	LK/RK
3 Mo + Veh	39 ± 9	56 ± 15	442 ± 63	464 ± 62	1.0 ± 0.04
3 Mo + ANG I	10 ± 3 <sup>†</sup>	46 ± 19	1,106 ± 180 <sup>†</sup>	703 ± 128	1.7 ± 0.1 <sup>†</sup>
15 Mo + Veh	14 ± 4*	36 ± 20	267 ± 91	313 ± 99	0.9 ± 0.1
15 Mo + ANG I	5 ± 1	176 ± 69 <sup>†</sup>	1,361 ± 246 <sup>†</sup>	761 ± 214	1.8 ± 0.1 <sup>†</sup>

Values are means ± SE. \**P* < 0.05 vs. corresponding 3-mo group; <sup>†</sup>*P* < 0.05 vs. corresponding Veh group. LK/RK, ANG II ratio of LK to RK.

the contralateral, noninfused kidney. Accordingly, the left-to-right kidney ANG II ratios were significantly increased in both groups receiving ANG I but not in the saline-infused groups.

## DISCUSSION

Until recent years, relatively little attention had focused on the potential role of the RAS in the pathophysiology of renal aging. However, several developments have prompted investigation into this pathophysiological process. First, recent improvements in the ability to measure the components of the RAS and, particularly, its expression in the tissue have led to a reevaluation of its role in the course of disease. Second, studies in a variety of other renal diseases have confirmed that RAS inhibition protects against the development of glomerular and tubulointerstitial injury, even in the absence of overt stimulation of the systemic RAS. Third, growing recognition of the alterations in vascular reactivity in the course of normal human aging have stimulated studies of the mechanisms that underlie these changes.

Recent studies have helped to clarify the changes that occur in the RAS in the course of normal aging. PRC falls with aging in normal humans (22) and animals (6, 13, 14, 18), although recent studies in awake, unstressed animals indicate that this may be less apparent in the absence of anesthesia (4). Plasma ANG II levels have not been well studied. Baylis and colleagues (4) noted absence of any significant changes in plasma ANG II in aging, unstressed animals, whereas Corman et al. (5) found that plasma ANG II falls with age when samples are collected under anesthesia. Regarding the intrarenal RAS, most studies have found downregulation of renal renin mRNA (6, 14) and single-nephron renin content (10), although this finding is not universal (5). Renal ANG II levels have not often been reported, although a preliminary study in our laboratory found elevation of whole kidney ANG II levels with advancing age (1). Taken together, the available studies indicate that the plasma RAS is normal or more commonly downregulated with aging. In contrast, the intrarenal system may not be so unequivocally suppressed.

Whether regulation of the RAS by ACEI is altered in normal aging is also incompletely defined. Baylis et al. (4) noted impaired increases in plasma renin activity (PRA) in unstressed aging animals subjected to acute ACEI. Michel and co-workers (18) also found stimula-

tion of PRA with ACEI in aging WAG/Rij male rats, and the present studies confirm preservation of this effect.

The first protocol further examined these aspects of the normal aging process. We intentionally chose 15-mo-old rats as the "older" model, recognizing that this is more analogous to middle age than to true senior citizen status in the rat population. In comparing aging studies, attention must be paid to the variations in the rat models as well as the age. Female rats exhibit less age-related renal disease, and there is wide variation in disease severity among and within rat strains. Among rat strains, Sprague-Dawley rats (as used here) exhibit higher levels of injury than do Munich-Wistar rats, and the WAG/Rij model exhibits a very low level of age-related nephropathy.

We found similar blood pressure responsiveness to ACEI in the older rats compared with the younger. Our proteinuria studies were not particularly conclusive; the very low values in young animals precluded detecting a change with enalapril, whereas the studies in the older animals tended to confirm previous studies (2, 11, 16, 24) that ACEI exert an antiproteinuric effect in the aging kidney. However, the relatively short treatment period may have limited our ability to detect significant antiproteinuric activity, which may have been more evident had the animals been studied for a longer period of time.

Our studies did not find significant differences in the effects of ACEI on renal function at this relatively early point in the course of aging. Effects of ACEI tend to vary, depending on the age and specific animal model. In male (11) and female (6) Wistar rats, ACEI have generally improved renal function, at least until very advanced age. In senescent male Munich-Wistar rats, we previously noted no effect on whole kidney GFR (2), whereas acute inhibition of the RAS with losartan will increase GFR in 15-mo-old Sprague-Dawley rats (20). Compared with responses in young rats, Baylis (3) found enhanced reduction in RVR with ACEI in conscious aging male Sprague-Dawley rats. One may tentatively conclude that there is no consistent effect of ACEI on whole kidney renal function in normal aging, a situation analogous to that in most other experimental models of renal disease, and to humans in the absence of serious renal insufficiency. More importantly, there is no apparent deleterious effect of ACEI on GFR in the absence of advanced nephropathy.

We also found only modest changes in the RAS at this early aging timepoint. Under brief anesthesia, studies in *protocol 1* did not find any significant differences in values for PRC or blood or renal ANG II levels in untreated rats. ACEI induced the expected increase in PRC and reduction in renal ANG II levels. Significant changes in blood ANG II levels were not detected, although the baseline values were all quite low and approaching the lower limits of detection of the assay. Thus it appears that biochemical responsiveness to inhibition of the RAS is preserved at this time point in the aging process.

Whereas inhibition of the RAS does not invoke consistent responses in the aging animal, most studies have found enhanced vasoconstriction in response to ANG II in the aging kidney. This effect has been noted in aging humans (7), in rats with advanced age (23), and in rats studied earlier in the aging process at ages similar to those in the present study (20). Having previously shown increased renal responsiveness to ANG II (20), we next chose to indirectly assess systemic conversion of ANG I to ANG II (*protocol 2*). In the present studies, the fact that ANG I led to similar pressor responses but enhanced renal vasoconstriction in the older rats confirms that renal vascular reactivity to ANG II is enhanced during the aging process. However, an alternate explanation may come from recent observations in much older rats, in which Baylis et al. (4) noted an increase in the metabolic clearance rate of ANG II. In that study, the finding of an enhanced metabolic clearance rate, in the absence of changes in plasma levels, was interpreted to suggest either increased synthesis or impaired degradation of ANG II. Whereas we did find slightly lower plasma ANG II levels in the Veh-treated older rats, we were unable to detect any differences in the plasma ANG II levels in the groups receiving intravenous ANG I. Subtle changes in ANG II degradation rates may, however, have been masked by the preponderant effects of anesthesia and prior surgical experimentation. We would also note that whereas the dosing of ANG I was performed by the conventional method (e.g., according to body wt), the larger size of the older animals resulted in significantly higher doses being infused. This difference in absolute doses may have had an impact. However, we would also note that the blood pressure responses were equivalent. This could reflect diminished blood pressure responsiveness in the older animals given the larger absolute doses, in which case the discordance between blood pressure and renal functional responses is even more striking. We would also note that the differences in basal values in fact contributed to differences when expressed as percent change from values seen in Veh-treated animals. By that analysis, ANG I induced a greater increment in plasma ANG II levels in older rats and a smaller increment in renal ANG II levels. However, the absolute values achieved did not differ between young and old rats infused with ANG II.

The intra-arterial studies lend further confirmation to the possibility of changes in the metabolism of ANG I in the aging kidney. In *protocol 3*, we are confident that there were no technical differences between the groups. In the younger animals, PRC was slightly reduced with ANG I, but there were no detectable differences in plasma ANG II levels, and the increase in renal concentration of ANG II was confined to the left (infused) kidney. In contrast, in the older animals, the plasma ANG II levels were increased as were the levels in the left and (only numerically) in the right kidneys. Because there was no increase in blood pressure in the older animals, any spillover into the systemic circulation must have been minimal, and yet plasma ANG II levels increased. We encountered a similar pattern in a previous study of diabetic rats receiving intra-arterial infusion of ANG I (15) and concluded that in that model, differences in metabolism of ANG II were likely to explain the difference from findings in nondiabetic controls. In the present study, the findings of elevated levels (and enhanced vascular reactivity) in the older animals are consistent with the metabolic studies by Baylis et al. (4). The equivalent pressor responses in our animals seem to weigh against enhanced systemic conversion of ANG I to ANG II but may well suggest impaired degradation of ANG II once formed. Again, the limitation of studying these compounds under conditions of anesthesia and surgery preclude specific evaluation of this hypothesis under these experimental conditions. Furthermore, these functional studies do not provide information as to the site(s) of intrarenal conversion of ANG I to ANG II nor to potential differences in localization of ANG II within the kidney.

In summary, changes in the RAS in early aging are modest. Responsiveness to ACEI is preserved, as previously noted with a specific ANG II (AT<sub>1</sub>)-receptor antagonist (20). However, whereas systemic (blood pressure) responsiveness to RAS stimulation is similar to that in younger animals, renal hemodynamic responsiveness is enhanced; modest changes in angiotensin metabolism may accentuate this effect. Age-related nephropathy appears similar to many other clinical renal diseases: sensitive to the protective actions of ACE inhibition but also to the detrimental effects of stimulation of the RAS. Accordingly, the dangers of volume depletion and other RAS stimuli may be amplified in the aging kidney and should be respected.

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