Furosemide Treatment, Angiotensin II, and Renal Growth and Development in the Rat

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ABSTRACT

Diuretics are commonly used to treat a variety of conditions, including progressive nephropathies, cardiovascular, and pulmonary diseases. Such treatment stimulates the production of angiotensin II, an important mediator of renal growth which may accelerate progressive glomerulosclerosis. To further study the effects of diuretic treatment on normal renal growth and development, weanling male Munich-Wistar rats received no drug, enalapril, furosemide, or both drugs for 6 wk; morphometric studies were then performed using standard light and electron microscopic techniques. Plasma renin activity was elevated by furosemide treatment. Cortical tubular growth was stimulated in rats receiving furosemide or both drugs; enalapril did not affect cortical tubular growth when compared with untreated animals. Glomerular volume was increased in furosemide-treated animals, primarily due to an increase in the proportion of mesangial cells, whereas enalapril decreased glomerular volume. Furosemide also increased the filtration surface area per glomerulus whereas enalapril decreased it. Concurrent enalapril treatment blocked the furosemide-induced changes in filtration surface area as well as attenuating overall glomerular and mesangial growth. Glomerular changes correlated with plasma renin activity. Furosemide stimulated glomerular growth, especially of mesangial cells, probably via stimulation of AngII production. Given the relationship of mesangial cell growth and progressive renal disease, diuretic therapy could thus accelerate glomerulosclerosis. Cortical tubular growth also increased with furosemide; however, enalapril had no effect, so factors besides angiotensin II appeared to modulate tubulointerstitial growth in these animals. (*Pediatr Res* **37:** 747–754, 1995)

Abbreviations
AngII, angiotensin II
ACEI, angiotensin converting enzyme inhibitor
PRA, plasma renin activity
GBM, glomerular basement membrane
FSGS, focal segmental glomerulosclerosis
$Vv_{x/y}$, fractional volume of x per volume of y ($Vv_{cells/glomerulus}$ = fractional volume of mesangial cells per volume of glomerulus)
$\bar{V}_G$, mean glomerular volume
Sv_{PGBM}, surface density of peripheral glomerular basement membrane per volume of glomerulus
$PGBM_{glomerulus}$, mean surface area of peripheral glomerular basement membrane per glomerulus
$PGBM_{cortex}$, total surface area of peripheral glomerular basement membrane per renal cortex

The link between renal hypertrophy and progressive glomerulosclerosis has made it imperative that factors mediating renal growth, particularly mesangial cell proliferation and matrix synthesis, be identified (1, 2). AngII is a renal growth factor, especially in the developing kidney (3–5). All of the components of the renin-angiotensin system are active in the fetus and neonate (6–8). Studies *in vitro* demonstrate that AngII stimulates proliferation and hypertrophy in vascular smooth muscle, mesangial, and proximal tubular cells (9–17). Proliferation of mesangial cells is especially pronounced in fetal cells (16). Suppression of AngII with ACEI or the AngII receptor blocker losartan in the growing rat results in attenuation of glomerular growth independent of hemodynamic effects (18, 19). ACEI in adult rats does not change glomerular size, probably because there is a low rate of cellular turnover in the normal adult rat (18). ACEI or AngII receptor blockade can attenuate renal and glomerular hypertrophy associated with models of chronic renal diseases in adult animals in which cellular proliferation is increased (20–24).

The effect of increased AngII on normal renal growth *in vivo* is unknown. Johnson *et al.* (25) showed that 2 wk of AngII infusion in the adult rat increased glomerular cell proliferation and induced rare areas of FSGS. Quantitative morphometry was not done, but glomeruli were otherwise described as normal (25). Miller *et al.* (26) found no increase in whole kidney or glomerular volume in adult rats receiving AngII infusion for 8 or 12 wk although AngII did induce hypertension

and FSGS. Similar studies have not been performed in growing rats.

Diuretics are commonly used to treat cardiovascular and renal syndromes which are often associated with proteinuria and progressive renal failure (27). These drugs stimulate renin secretion and AngII production, as does volume depletion of any cause (28). Furosemide may also directly stimulate renin release from the macula densa, but this effect is short-lived, lasting less than 4 h, and would appear to play little role in the hyperreninemia produced by chronic diuretic administration (29). If AngII is an important mediator of normal and pathologic glomerular growth and sclerosis, then diuretic therapy may be capable of inducing or accelerating progressive renal disease. Furosemide treatment has been shown to accelerate cystogenesis in the diphenylthiazole model of polycystic kidney disease in the rat (24). Because sodium depletion also accelerated this model and ACEI attenuated it, activation of the renin-angiotensin system was presumed to be the mechanism for these findings (24). Clinical findings also suggest that diuretics may induce or accelerate renal disease. Walker et al found that patients with diabetic kidney disease who received a diuretic as part of their antihypertensive regimen had higher AngII levels and a faster decline in renal function over time when compared with patients who received other types of drugs (30). Diuretic treatment has also been associated with increased cardiovascular mortality in diabetic patients (31). Long-term furosemide treatment has been associated with renal insufficiency in patients with idiopathic edema; biopsy findings in these patients include glomerulosclerosis in some patients and tubular atrophy with interstitial expansion in most patients (32).

The present study was designed to examine the effect of furosemide treatment on normal renal growth and development in the rat, especially the potential role of AngII in this situation. Furosemide treatment for 6 wk resulted in elevated PRA without systemic hypertension. Renal structure in these animals was compared with that of untreated controls, animals treated with enalapril, an ACEI, and animals receiving furosemide and enalapril to delineate the effects of furosemide treatment on normal renal growth and development.

METHODS

Animals

In a series of experiments, 50 weanling male Munich-Wistar rats, 82 ± 4 g (mean $\pm$ SD), were studied. Final body weight, kidney weight, and morphometric parameters were similar for animals within each treatment group between experiments. All animals were fed a set amount of a paste made of 15 g of standard meal rat chow to 25 mL of tap water using a feeding schedule developed in pilot studies in which animals received this paste *ad libitum* and intake quantitated. This feeding schedule resulted in growth similar to that seen in male Munich-Wistar rats fed *ad libitum* chow for a similar period (18). If all animals consumed all offered food, the amount offered was then increased; if any animal failed to consume all offered food, then the amount offered was decreased for every animal until all animals did consume all offered food. Through-

out these experiments, all food was consumed by all animals, so protein, calorie, and electrolyte intake were similar for all animals. The animals had free access to tap water throughout the study. Drugs were thoroughly mixed into the food paste. Ten animals received furosemide (group F), 40 mg/d, as described by Warshaw (33). Thirteen animals received enalapril (group E), 2.5 mg/d, an amount equivalent to that ingested by animals receiving 50 mg/L in the drinking water (Lane PH, unpublished observations). Ten animals received both furosemide, 40 mg, and enalapril, 2.5 mg, per day (group B). The remaining 17 animals received no drug and served as controls (group C). During the final week of study tail-cuff blood pressure was determined for 19 concurrently treated animals (5 F, 5 E, 5 B, and 4 C), and 24-h urine collections were obtained for 17 animals (3 F, 4 E, 5 B, and 5 C). After 6 wk the animals underwent euthanasia in a CO_2 chamber and blood was obtained. The kidneys were excised, weighed, and immersed in Karnovsky's fixative. These studies were approved by the Animal Care Committee of St. Louis University in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Biochemical Studies

PRA was determined for 5 F and 5 C animals in the laboratory of Dr. Steven Katz, Hennepin County Medical Center, Minneapolis, MN, using a modified radioimmunoassay as previously reported (34). Urine protein excretion was measured using a modification of the Lowry method (35).

Morphometric Studies

After at least 24 h of fixation, one kidney from all 50 animals was cut into transverse slices of 4-mm thickness. The cut surface area of each slice was estimated by point counting with a 2-mm lattice grid. Kidney volume (V_{kidney}) could then be estimated with the Cavalieri principle (36). This states that the volume of an object is equal to the area of its cut surfaces multiplied by the mean section thickness. Therefore,

$$V_{kidney} = (\sum P_{kidney} \cdot 4 \text{ mm}^2) \cdot 4 \text{ mm}$$

where P_{kidney} are the points falling on the kidney surface, 4 mm^2 is the area per point for the grid used, and 4 mm is the mean section thickness.

All light and electron microscopic morphometric studies were performed by a single observer who was masked to the identity of the tissue. All 50 animals had all light and electron microscopic morphometric studies performed as described below.

Light microscopy. A single transverse section from the middle of the kidney was embedded in glycol methacrylate and sectioned at 2-μm thickness through 150–200 μm of tissue for light microscopy studies. Sections were stained with Jones silver stain. Final magnification was determined by a stage micrometer. A single randomly selected section was examined at a mean magnification of 58, and a 2-cm lattice grid superimposed for determination by point counting of the volume fractions of medulla and cortex ($Vv_{medulla}$ and Vv_{cortex}),

the proportions of the kidney composed of medulla and cortex (μm^3 of medulla or cortex per μm^3 of kidney). The volumes of cortex and medulla in each kidney ($cortex_{kidney}$ and $medulla_{kidney}$) could then be determined in the following manner:

$$Cortex_{kidney}(mm^3) = Vv_{cortex/kidney} \cdot V_{kidney}(mm^3)$$

The same section was examined with the same grid at a mean magnification of 145 for determination of the volume fractions of glomeruli, tubules, and interstitium ($Vv_{glomeruli/cortex}$, $Vv_{tubules/cortex}$, and $Vv_{interstitium/cortex}$), the proportions of the renal cortex composed of these structures (μm^3 of glomeruli, tubules, or interstitium per μm^3 of renal cortex). Interstitium was defined as that portion of the renal cortex not composed of glomeruli, tubules, arteries, arterioles, or veins at least the size of a tubule in diameter (37). Subsequent fields of cortex were examined and the points falling on glomeruli, tubules, interstitium, and total cortex were recorded until the points on cortex reached 500, approximately 28 fields at the magnification described. Previous studies of the interstitium have shown that a minimum of 12 fields are required to give reproducible results. Five hundred cortical points resulted in a similar number of points on interstitium and glomeruli, which gave reproducible results on repeated measurements. Total volumes of glomeruli ($glomeruli_{cortex}$), cortical tubules ($tubules_{cortex}$), and cortical interstitium ($interstitium_{cortex}$) could then be determined in the following manner:

$$Glomeruli_{cortex}(mm^3) = Vv_{glomeruli/cortex} \cdot cortex_{kidney}(mm^3)$$

In addition, at least 50 glomerular profiles from each kidney were examined for sclerosis on this section and the smallest diameter of 15 randomly selected proximal tubular profiles were measured to estimate mean proximal tubular diameter (38). Mean glomerular volume ($\bar{V}_G$) was determined by measuring the volume of at least 15 individual glomeruli per animal using the Cavalieri principle as previously described (39).

Electron Microscopy. Several 1-mm cubes of cortex from each animal were processed for electron microscopy. The centermost glomeruli from one or more blocks were selected for thin sectioning. At least two glomeruli were studied from each animal. Sections were mounted on Formvar (Monsanto Co., St. Louis, MO)-coated slot grids, stained with uranyl acetate and lead citrate, and examined and photographed with a JEOL 100S microscope as described below. A calibration standard was photographed at each microscopy session to determine final magnification.

Micrographs of an entire glomerular profile were taken and printed at a mean final magnification of 5646. These micrographs were taped together to form a montage of the entire glomerular profile. The minimal convex polygon surrounding the tuft was drawn, and a 3-cm lattice grid was superimposed on the montage to determine volume fraction of mesangium ($Vv_{mesangium/glomerulus}$), the proportion of the glomerular capillary tuft composed of mesangial cells and matrix (μm^3 of mesangium per μm^3 of glomerulus) (40). At least 100 points

on mesangium from two or three glomeruli were counted for each animal.

A grid with points 6 cm apart and two parallel 3 cm (30,000 μm) line segments associated with each point was superimposed on the glomerular montage to determine surface density of the PGBM filtration surface (Sv_{PGBM}), the length of peripheral glomerular filtration surface per volume of glomerulus (μm^2 of surface per μm^3 of glomerulus) (40). At least 100 intercepts from two or three glomeruli were counted for each animal. The amount of PGBM surface per glomerulus ($PGBM_{glomerulus}$) could then be calculated as follows:

$$PGBM_{glomerulus}(mm^2) = Sv_{PGBM}(\mu m^2/\mu m^3) \cdot \bar{V}_G(\times 10^6 \ \mu m^3)$$

Sv_{PGBM} was also used to determine the total PGBM surface area per kidney ($PGBM_{cortex}$):

$$PGBM_{cortex}(\times 10^3 \ mm^2)$$
$$= Sv_{PGBM}(\mu m^2/\mu m^3) \cdot glomeruli_{cortex}(mm^3)$$

Multiple micrographs were taken from each glomerulus in an unbiased systematic manner and printed at a mean final magnification of 18,654. A grid with intersecting line segments 2 cm apart was placed over each micrograph. GBM width was determined by the orthogonal intercept method (41). At least 100 GBM intercepts were classified from two or three glomeruli per animal. From these same micrographs, the volume fractions of mesangial cells and mesangial matrix ($Vv_{cells/mesangium}$ and $Vv_{matrix/mesangium}$) were determined from at least 100 points falling on mesangial areas. These are the proportions of the mesangium composed of mesangial cells and matrix (μm^3 of cells *or* matrix per μm^3 of cells *and* matrix) (42). $Vv_{cells/glomerulus}$ and $Vv_{matrix/glomerulus}$, the proportions of the glomerulus composed of mesangial cells and matrix (μm^3 of mesangial cells or matrix per μm^3 of glomerulus), were then calculated as follows:

$$Vv_{cells/glomerulus} = Vv_{cells/mesangium} \cdot Vv_{menangium/glomerulus}$$

Statistics

All values are reported as mean $\pm$ SD. Values for the four groups were compared with ANOVA and Scheffé F tests. $p < 0.05$ was considered significant. Linear regression analysis was used to examine correlations between parameters. Because of the number of regression analyses performed between structural parameters and PRA, $p < 0.01$ was considered significant. All computations were performed with Statview 4.01 software (Abacus Concepts, Inc., Berkeley, CA).

RESULTS

Because of small but statistically significant differences in body weight among the four groups at the onset of these experiments, the percent of initial body weight at the end of study was used to assess the effect of treatment regimens on somatic growth (Table 1). Administration of enalapril was associated with decreased weight gain over the course of the

Table 1. *Body weight and the relationship of selected parameters to body weight*

	Control (C) (n = 17)	Enalapril (E) (n = 13)	Both drugs (B) (n = 10)	Furosemide (F) (n = 10)
Initial body weight, gm*	82 ± 4	85 ± 2	79 ± 6	80 ± 4
Final body weight, gm†	216 ± 20	197 ± 17	181 ± 19	204 ± 18
Final weight, % of initial body weight‡	266 ± 30	232 ± 18	229 ± 20	254 ± 16
Kidney weight, gm§	2.22 ± 0.33	2.02 ± 0.22	2.33 ± 0.18	2.67 ± 0.30
Kidney weight per body weight, m/gm¶	0.010 ± 0.001	0.010 ± 0.001	0.013 ± 0.001	0.013 ± 0.001
Cortical tubular volume per body weight, mm³/gm¶	2.07 ± 0.31	2.14 ± 0.35	3.51 ± 0.58	3.22 ± 0.51
Glomerular volume per body weight, 10^3 μm³/gm‖	1.52 ± 0.36	1.29 ± 0.25	1.18 ± 0.31	2.12 ± 0.33

All values mean ± standard deviation. ANOVA $p<0.001$ for each parameter shown.
* $p<0.05$ for E vs B or F.
† $p<0.01$ for C vs B.
‡ $p<0.01$ for C vs E and C vs B.
§ $p<0.01$ for C vs F and E vs F.
¶ $p<0.0001$ for C vs F or B and E vs F or B.
‖ $p<0.001$ for F vs C, E, or B.

study as the E and B groups weighed a smaller proportion of their baseline weight than the C animals (Table 1). Systolic blood pressure was not significantly altered by any of the treatment regimens, although it did tend to be lower in all of the treatment groups (C 129 ± 28; E 103 ± 8; B 102 ± 11; and F 104 ± 20 mm Hg). PRA was significantly elevated in F compared with C animals (26.0 ± 9.4 *versus* 7.8 ± 2.3 ng/mL/h; $p < 0.01$). Urine output did not differ among the four groups (C 26 ± 2; E 31 ± 4; B 38 ± 6; and F 36 ± 11 mL/animal/24 h), nor did urine protein excretion (C 27 ± 2; E 32 ± 4; B 38 ± 6; and F 36 ± 10 mg/animal/24 h). Furosemide treatment did increase 24-h urine output when animals receiving it (F + B; 37 ± 8 mL/animal/24 h) were compared with those which did not (C + E; 28 ± 4 mL/animal/24 h; $p < 0.01$). Using these same combined groups, furosemide increased 24 h protein excretion (F + B 37 ± 7 and C + E 29 ± 4 mg/animal/24 h; $p < 0.01$). Enalapril had no effect when groups were combined for this drug (urine output: E + B 35 ± 7 and C + F 30 ± 8 mL/animal/24 h; protein excretion: E + B 35 ± 6 and C + F 30 ± 8 mg/animal/24 h).

Kidney weight and kidney weight per body weight were greater in F and B animals than in those in the C and E groups (Table 1). Kidney weight and final body weight correlated on regression analysis ($r = 0.46$, $p < 0.001$). V_{kidney} was greater in the groups receiving furosemide than in the groups which did not receive this drug (C 756 ± 132; E 678 ± 115; B 1152 ± 324; and F 1019 ± 139 mm³; $p < 0.01$ for B *versus* C, B *versus* E, F *versus* C, and F *versus* E by Scheffé test). Renal cortical volume was affected by furosemide administration, primarily via an increase in cortical tubular volume (Fig. 1). These differences were present even when differences in final body weight were taken into consideration (Table 1), and there were no correlations between final body weight and renal cortical volume ($r = 0.01$) or cortical tubular volume ($r = 0.06$). The volumes of glomeruli and interstitium per renal cortex did not differ significantly among the groups (Fig. 1). Proximal tubular diameter was unaffected by the treatment regimens (C 43 ± 4, E 43 ± 4, F 42 ± 5, and B 41 ± 2 μm). Renal medullary volume did not differ among the groups (C 227 ± 56; E 182 ± 57; B 186 ± 17; and F 252 ± 107 mm³).

$\bar{V}_G$ was significantly increased in F rats when compared with C, E, or B animals (Fig. 2). Enalapril treatment reduced $\bar{V}_G$
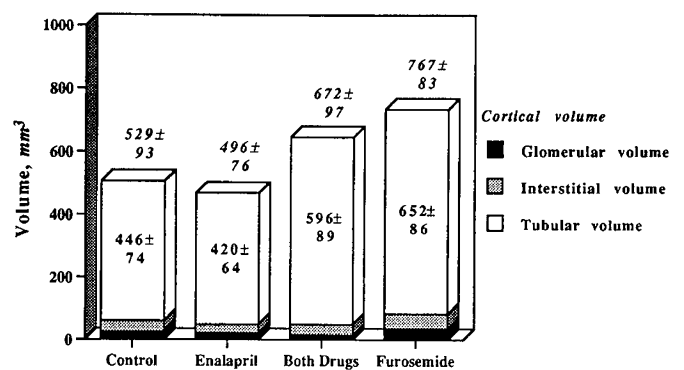

Figure 1. Total glomerular volume (*solid black portion*: control 25 ± 11; enalapril 22 ± 7; both drugs 15 ± 5; and furosemide 36 ± 23 mm³), cortical interstitial volume (*shaded portion*: control 35 ± 18; enalapril 26 ± 8; both drugs 34 ± 16; and furosemide 46 ± 21 mm³), cortical tubular volume (*white portion*, values in plain type), and total cortical volume (*whole bar*, values in italics) in animals receiving no drug, enalapril, both drugs, or furosemide for 6 wk beginning at weaning. Values shown are mean ± SD. ANOVA $p < 0.001$ for cortical tubular volume and total cortical volume; p = NS for total glomerular volume and cortical interstitial volume. By Scheffé F test furosemide or both drugs are greater than control or enalapril for cortical tubular volume and total cortical volume ($p < 0.01$ for each comparison).

because both E and B animals were smaller than C animals (Fig. 2). Retarded overall growth may have played a role in these changes, because differences between control animals and those receiving enalapril alone or with furosemide were eliminated when differences in final body weight were taken into account (Table 1), although $\bar{V}_G$ did not correlate with final body weight ($r = 0.36$) or with the percent weight gain ($r = 0.32$). No glomerulosclerosis was noted in any animal in this study.

Glomerular ultrastructural studies revealed no differences in GBM width (C 99 ± 11; E 98 ± 9; B 105 ± 8; and F 101 ± 15 nm). $Vv_{mesangium/glomerulus}$ was significantly increased by furosemide treatment, and this increase was primarily due to an increase in $Vv_{cells/glomerulus}$ (Fig. 3). These parameters were not different in B than in C animals (Fig. 3). $PGBM_{glomerulus}$ appeared to be increased by furosemide and decreased by enalapril (C 0.075 ± 0.020; E 0.058 ± 0.006; B 0.049 ± 0.018; and F 0.086 ± 0.019 mm²; $p < 0.01$ by Scheffé test for E *versus* F and B *versus* F; $p < 0.05$ for C *versus* B; other
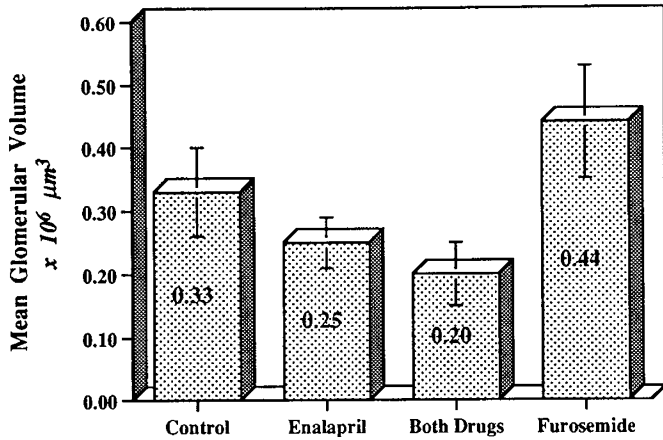
Figure 2. Mean glomerular volume in animals receiving no drug, enalapril, both drugs, or furosemide for 6 wk beginning at weaning. Values shown are mean ± SD × 10^6 μm^3. ANOVA $p = 0.0001$. By Scheffé F test furosemide is greater than control ($p < 0.01$), enalapril ($p < 0.001$), or both drugs ($p < 0.001$). Control is greater than enalapril ($p < 0.05$) or both drugs ($p < 0.01$).
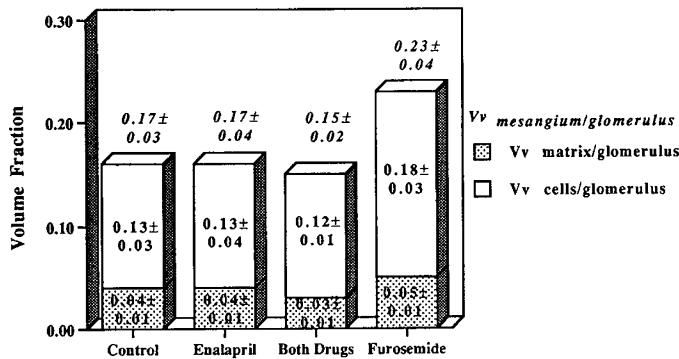
Figure 3. Volume fractions of the glomerulus composed of mesangial matrix ($Vv_{matrix/glomerulus}$; *shaded portion*), mesangial cells ($Vv_{cells/glomerulus}$; *white portion*), and total mesangium ($Vv_{mesangium/glomerulus}$; *whole bar*) in animals receiving no drug, enalapril, both drugs, or furosemide for 6 wk beginning at weaning. Values shown are mean ± SD. ANOVA $p < 0.001$ for $Vv_{cells/glomerulus}$ and $Vv_{mesangium/glomerulus}$; $p = NS$ for $Vv_{matrix/glomerulus}$. By Scheffé F test furosemide is greater than control, enalapril, or both drugs for $Vv_{mesangium/glomerulus}$ and $Vv_{cells/glomerulus}$ ($p < 0.01$ for each comparison).

comparisons $p = NS$). No significant effect on $PGBM_{cortex}$ could be demonstrated (C 5.96 ± 3.14; E 4.97 ± 1.40; B 3.70 ± 1.56; and F 7.13 ± 4.56 × 10^3 mm^2).

Regression analysis revealed many structural parameters that correlated with PRA in the five furosemide-treated animals and the five control animals in which PRA was assessed (Fig. 4). These included $Cortex_{kidney}$ ($r = 0.83, p < 0.01$), Tubule-S_{cortex} ($r = 0.82, p < 0.01$), ($r = 0.81, p < 0.01$; Fig. 4A), $Vv_{mesangium/glomerulus}$ ($r = 0.88, p < 0.001$; Fig. 4B), and $Vv_{cells/glomerulus}$ ($r = 0.83, p < 0.01$; Fig. 4C). $PGBM_{cortex}$ showed a trend toward correlation ($r = 0.74, p = 0.01$). These parameters were not additive on stepwise multiple regression analysis. The following parameters did not correlate with PRA: V_{kidney} ($r = 0.71$), $Vv_{cortex/kidney}$ ($r = 0.45$), $Vv_{medulla/kidney}$ ($r = 0.45$), $Vv_{glomeruli/cortex}$ ($r = 0.30$), $Vv_{tubules/cortex}$ ($r = 0.18$), $Vv_{interstitium/cortex}$ ($r = 0.30$), $medulla_{kidney}$ ($r = 0.25$), $glomeruli_{cortex}$ ($r = 0.64$), $interstitium_{cortex}$ ($r = 0.43$), GBM ($r = 0.33$), Sv_{PGBM} ($r = 0.02$), $Vv_{matrix/glomerulus}$ ($r = 0.31$; Fig. 4D), and $PGBM_{glomerulus}$ ($r = 0.68$).
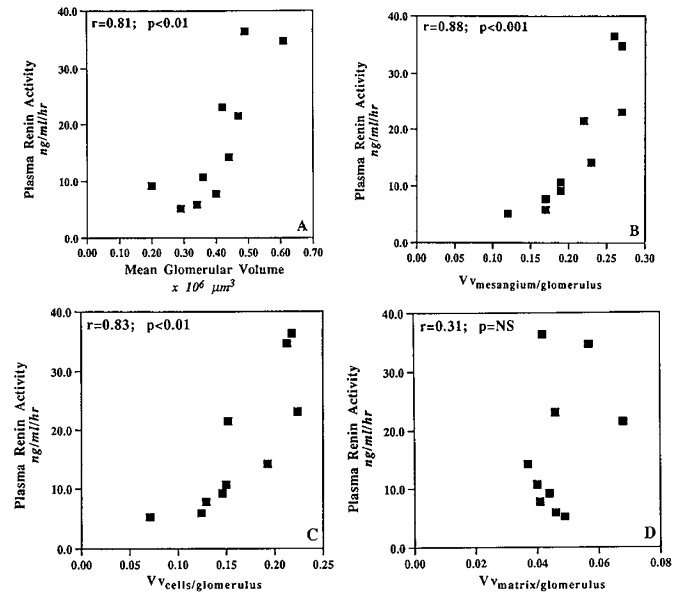
Figure 4. Correlation of glomerular structure with plasma renin activity in rats treated with furosemide or no drug for 6 wk beginning at weaning. *A*, Mean glomerular volume; *B*, mesangial volume fraction ($Vv_{mesangium/glomerulus}$); *C*, mesangial cellular volume fraction ($Vv_{cells/glomerulus}$); and *D*, mesangial matrix volume fraction ($Vv_{matrix/glomerulus}$).

DISCUSSION

Six weeks of furosemide treatment during a period of growth and development resulted in renal hypertrophy in the rats described here. This hypertrophy primarily involved the renal cortex, especially the glomeruli and the cortical tubules. Glomerular hypertrophy was associated with an enlargement of the glomerular mesangium, primarily the mesangial cells. Whether this increase in mesangial cell volume represented hypertrophy, hyperplasia, or both processes cannot be ascertained from the quantitative studies performed. Glomerular changes induced by furosemide correlated with PRA and were attenuated by concurrent treatment with enalapril, suggesting that they were mediated by AngII. Cortical tubular hypertrophy induced by furosemide was not blocked by ACEI treatment, suggesting that the diuretic induced this growth via some other mechanism. Proximal tubular diameter was unaffected by any treatment regimen, suggesting that changes in the cortical tubules were in more distal portions of the nephron. As in Fogo *et al.*'s (18) work, ACEI attenuated normal glomerular growth. This decrease in glomerular size affected all portions of the glomerulus as there were no striking ultrastructural differences between ACEI treated and untreated animals. ACEI did not affect normal cortical tubular growth, suggesting that AngII has little effect in cortical tubular growth and development, despite the presence of AngII receptors on proximal tubule and *in vitro* modulation of proximal tubule cell growth by AngII (4).

AngII is an important renal growth factor in normal and pathologic renal growth (3–5). Fogo *et al.* first demonstrated the importance of AngII in normal glomerular growth by treating weanling rats with the ACEI enalapril for 6 wk (18). This treatment did not result in significant lowering of systemic or glomerular blood pressures, but glomerular growth was

attenuated in those animals who received the drug. This effect was only seen in growing animals; adult rats showed no change in glomerular volume with similar treatment (18). Tufro-McReddie et al. (19) have confirmed the role of AngII in this process by treating weanling rats with the AngII receptor blocker losartan for 3 wk. This treatment resulted in lower body weight, kidney weight, and kidney weight to body weight ratios. Renal growth retardation appeared to be due to decreased cell proliferation because renal DNA content was reduced by losartan, but protein-to-DNA and RNA-to-DNA ratios were similar in treated and untreated animals (19).

ACEI or AngII receptor blockade can prevent hypertrophy associated with pathologic states in adult rats including reduced renal mass, streptozocin-induced diabetes mellitus, and diphenylthiazole-induced cystic disease (20–24). In vitro studies also provide evidence that AngII modulates glomerular cell growth. In cultured vascular smooth muscle cells AngII induces the expression of early proto-oncogenes, increases activity of growth factors, and stimulates cell hypertrophy (9–14, 17). Cultured adult human mesangial cells, which share many characteristics with vascular smooth muscle cells, show only a weak mitogenic response to AngII; however, human fetal mesangial cells show a striking proliferative response in vitro (16, 43). Mesangial cells may also increase collagenous and noncollagenous protein production in the presence of AngII, suggesting that, in some conditions, AngII may stimulate mesangial matrix production as well (44).

AngII may also promote proximal tubular cell growth (4, 5, 15). AngII receptors have been identified on the brush border and basolateral membranes of proximal tubular cells, and these cells express the mRNA and protein products for all components of the renin-angiotensin system (15, 45–48). In a murine proximal tubular cell line AngII stimulates cell hypertrophy but not proliferation (15). AngII may enhance the proliferative effects of other growth factors on tubular cells in vitro (15). The present study suggests that AngII plays little role in normal or furosemide-induced cortical tubular growth in vivo. However, it may still play an important role in tubular growth and interstitial fibrosis during pathologic renal growth.

Few studies have examined the effect of increased AngII on renal morphology in vivo. Johnson et al. (25) infused AngII into adult rats for 14 d at 200 ng/min, a dose that resulted in moderate hypertension. Other than rare areas of FSGS, the glomeruli of these rats were normal by light microscopy; however, quantitative morphometry was not performed. Glomerular cells showed evidence of mesangial proliferation. Small arteries demonstrated focal fibrinoid necrosis and medial hyperplasia, and mononuclear infiltrates and fibrosis were seen in the interstitium. It is possible that at least part of these changes are mediated directly by AngII; however, any or all may be due to the hypertension resulting from AngII infusion (25). Miller et al. (26) infused AngII in adult rats for 8–12 wk. Renal weight and mean glomerular volume were not increased by doses of 100 ng/min of AngII, even though systemic and glomerular pressures were significantly increased and glomerulosclerosis occurred (26). AngII thus appears to be required to induce hypertrophy in the adult animal with renal disease because ACEI treatment or AngII receptor blockade can pre-

vent such growth, but AngII alone is not sufficient to stimulate renal or glomerular hypertrophy in the normal adult animal (20–24, 26). The response of the glomerulus to AngII may also be due to the proliferative state of the mesangial cell. Normal adult animals have relatively slow mesangial cell turnover which is increased in pathologic states (49–52).

Both studies of AngII infusion in adult animals demonstrated glomerulosclerosis whereas none was noted in the present study (25, 26). These differences may be due to age-related differences, because these earlier studies dealt with adult animals whose mesangial cells probably respond differently to AngII than those of the juvenile rats in the present study. Furosemide treatment stimulates PRA without increasing systemic blood pressure; this may be why the juvenile rats did not develop glomerulosclerosis over the time period of the present study. Longer treatment might result in glomerulosclerosis in animals without hypertension. No studies of AngII infusion in juvenile rats have been performed.

Elevated AngII may not be the sole reason that furosemide treatment induces renal hypertrophy in the growing rat. The primary therapeutic use of furosemide is as a diuretic (27). This effect is achieved through competitive blockade of the Na^+-K^+-$2Cl^-$ carrier in the thick ascending limb of the loop of Henle. This results in decreased reabsorption of NaCl and limits the concentration gradient achieved in the medulla, further increasing water excretion (27). Furosemide also stimulates kaliuresis primarily by increased distal nephron delivery of NaCl with increased distal Na reabsorption and K secretion (27). Calcium and magnesium reabsorption are also inhibited, probably because their reabsorption is driven by transepithelial voltage which is eliminated by furosemide therapy (27).

Changes in distal tubules may result from changes in volume status independent of the renin-angiotensin system (53, 54). Rabbits fed high NaCl diets develop hypertrophy of distal convoluted tubule cells (53). Ellison et al. (54) studied the effects of different levels of dietary NaCl and furosemide infusion on renal structure and function in the adult rat. High or low sodium diets did not alter renal tubular structure or distal tubular sodium delivery, even though sodium excretion patterns changed accordingly. Chronic intraperitoneal furosemide infusion greatly increased distal tubular NaCl delivery and reabsorption and produced distal tubular cell hypertrophy without changes in more proximal nephron structures, presumably due to these changes in NaCl delivery (54). Results of the present study would support a mechanism such as this because concurrent ACEI did not block furosemide-induced tubular hypertrophy, and the proximal tubule diameter did not change, suggesting that changes in cortical tubules were restricted to more distal portions of the nephron.

Hypokalemia causes renal hypertrophy and may result from furosemide treatment (55–57). Elger et al. (57) found that in adult rats this hypertrophy primarily involves the inner stripe of the outer medulla, and glomerular tuft volume is not increased. In the study of Warshaw et al. (33), using the same diuretic regimen described here, potassium excretion was increased after the first day of treatment by furosemide, although no statistically significant differences in serum potassium levels were noted throughout the 26 d of their study. It thus seems

unlikely that potassium depletion is responsible for the renal hypertrophy demonstrated in the present study, because furosemide-treated rats developed cortical hypertrophy with increased and did not develop medullary enlargement, although it is possible that this is the pattern of hypokalemic hypertrophy in juvenile animals. In the present study blood was obtained at the time of euthanasia by CO_2. Although this method does not alter PRA, it does result in systemic acidosis, making potassium levels uninterpretable. Future studies will need to address blood, urine, and tissue potassium levels and control for this potentially confounding variable.

The effects of ACEI are not limited to blunting production of AngII. Angiotensin converting enzyme is identical to kininase II, the enzyme that inactivates bradykinin (58). ACEI thus decrease AngII activity and increase bradykinin activity. The latter is an important stimulus for autocrine and paracrine factors that control vasodilatation, including endothelium-derived relaxing factor and prostaglandins (58). Angiotensin-converting enzyme may play other roles as well. *In vitro* it is capable of acting on substance P and LH-releasing hormone, although no *in vivo* role for these activities has been defined (58). Although it seems likely that it is the anti-AngII effects of ACEI that are active in the present study, these other effects of ACEI cannot be ruled out. Further study with more specific AngII receptor blocking agents will be necessary to define the mechanism of furosemide-induced renal growth.

In summary, 6 wk of furosemide treatment in the growing rat resulted in renal hypertrophy, particularly of the glomeruli, without glomerulosclerosis. The greatest change occurred in the cellular component of the mesangium, whether due to mesangial cell proliferation, hypertrophy, or both. Cortical tubular volume was increased by furosemide treatment as well. The glomerular changes in renal structure correlated with PRA and were blocked by concurrent treatment with enalapril, suggesting that AngII mediated glomerular and mesangial hypertrophy in this model. Other effects of furosemide treatment, such as increased distal sodium delivery or hypokalemia, may also have mediated structural changes demonstrated here, especially the tubular hypertrophy, because this latter parameter was not prevented by concurrent enalapril administration. Given the relationship of mesangial growth and glomerulosclerosis, the potential role of diuretics and AngII in progressive renal disease warrants further study.

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