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Effects of chronic treatment with angiotensin converting enzyme inhibitor or an angiotensin receptor antagonist in two-kidney, one-clip hypertensive rats

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Effects of chronic treatment with angiotensin converting enzyme inhibitor or an angiotensin receptor antagonist in two-kidney, one-clip hypertensive rats. The effects of chronic angiotensin II (Ang II) receptor blockade (losartan) or converting enzyme inhibition (enalapril) on blood pressure (BP), urinary albumin excretion ($U_{alb}V$), renal histology and the hemodynamic and excretory function of the clipped and nonclipped kidneys were studied in two-kidney, one-clip (2-K 1-C) rats. One day after clipping the right renal artery, male Wistar rats were divided into three groups receiving: (1) losartan, 20 mg/kg/day ($N = 7$), (2) enalapril, 20 mg/kg/day ($N = 8$), or (3) no treatment (controls, $N = 9$) for three weeks. Both losartan and enalapril treatments maintained conscious BP at comparably lowered levels compared to control animals (116 ± 6 mm Hg and 113 ± 2 mm Hg vs. 188 ± 11 mm Hg, respectively, $P < 0.01$). Treatment also prevented the increase in $U_{alb}V$, observed for the untreated group, three weeks after clipping (1.7 ± 0.5 and 0.7 ± 0.1 mg/24 hr vs. 17.8 ± 7 mg/24 hr, respectively, $P < 0.01$). After three weeks of treatment, acute study of renal function during pentobarbital anesthesia revealed higher values of GFR and RPF and lowered vascular resistance for nonclipped kidneys from the losartan and enalapril groups compared to the corresponding kidneys from control animals. Despite the lower BP of both treated groups, clipped kidney GFR and RPF were unchanged compared to the control group. $U_{alb}V$ for nonclipped kidneys from untreated rats was approximately 5- to 10-fold higher than in the nonclipped kidneys from the treated groups. Histological evaluation revealed evidence of early glomerulosclerosis in the nonclipped kidneys from the untreated but not the treated groups, and decreased indices of glomerular size and mesangial expansion in clipped kidneys for the treated groups compared to the untreated group. These data support a primary role for Ang II modulation of blood pressure and renal function in the nonclipped kidney of the 2-K 1-C rat.

The contribution of the renin-angiotensin system (RAS) to the development and maintenance phase of renovascular hypertension (RVH) has long been recognized. Earlier experiments with pharmacological inhibitors of the RAS, such as angiotensin converting enzyme inhibitors (CEI), prototypic peptide angiotensin II receptor antagonists or renin inhibitors demonstrated the causal role of the RAS in the development and early maintenance phases of hypertension in rat models of renal artery stenosis [1, 2]. Guyton et al have proposed that the normal or nonstenotic kidney should be protective for the development of hypertension in a model of two-kidney single renal artery stenosis hypertension [3]. Several studies have demonstrated that acute blockade of the RAS results in augmented renal hemodynamic and filtration functions of the nonstenotic kidney during conditions when blood pressure is decreased [1, 4, 5]. Consequently, it has been attractive to postulate that effects of angiotensin II (Ang II) cause the nonclipped kidney to participate in the hypertensive process by forcing reabsorption of salt and water that would otherwise be excreted in response to the hypertension-induced pressure diuresis. Although the acute and chronic blood pressure responses and the effects of acute application of angiotensin converting enzyme inhibitors (ACEI) on global renal function during the development of hypertension have been described, the effects of chronic interruption of the RAS on individual renal function for the stenotic and nonstenotic kidneys during the developmental phase of two-kidney, one-clip hypertension (2-K 1-C HT) have not, to our knowledge, been reported. In models of established RVH, acute and chronic administration of ACEI are associated with deleterious effects on post-stenotic kidney structure or function [4, 6–8]. The probability that the use of ACEI in clinical practice is likely to increase, especially in groups at a high risk for occult renovascular disease, dictates that the effects of chronic RAS blockade on renal structure and function especially in the post-stenotic kidney in experimental renovascular disease merits further investigation.

The effects of chronic administration of new specific angiotensin receptor antagonists on the function of each kidney have also not been reported in this model. Losartan (DuP 753) is an orally active AT1 Ang II receptor antagonist which lacks the partial agonist properties of earlier receptor blocking agents [9, 10]. In contrast to the suspected kinin potentiating effects of the converting enzyme inhibitors, losartan offers the ability to inhibit the effects of Ang II activity specifically at the level of the receptor [9].

The purpose of this study was to assess the effects of chronic blockade of the RAS, initiated at the time of clipping, on blood pressure, renal hemodynamic and clearance function and glomerular histology of clipped and nonclipped kidneys of 2-K 1-C HT rats. We compared the effects of chronic blockade of the RAS achieved with enalapril or losartan to no treatment in identically

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prepared animals. Further, to explore the possible role of Ang II in the development of albuminuria in RVH, we compared the effects of three weeks of treatment with the converting enzyme inhibitor or the receptor antagonist on 24-hour urinary albumin excretion and on albumin excretion for each kidney in 2-K 1-C HT.

Methods

Animals and protocol

2-K 1-C Goldblatt hypertension was produced by placing a silver clip (0.2 mm internal diameter) on the right renal artery of male Wistar rats weighing 80 to 120 g (Charles River, Wilmington, MA, USA) under pentobarbital anesthesia (50 mg/kg i.p.). All animals were fed standard rat chow (Wayne Rodent Blox, Teklad, Madison, WI, USA) containing 0.31% sodium and were allowed tap water *ad libitum*. Rats were randomly divided into three groups. Group one rats received losartan 20 mg/kg/day ($N = 7$), and group two animals received enalapril 20 mg/kg/day ($N = 8$). Treatments were delivered in the drinking water from one day after clipping and continued until clearance studies were conducted. Nine additional clipped rats remained untreated to serve as controls (group 3). The dose of losartan was determined from a preliminary study as the dose equipotent to enalapril 20 mg/kg/day in preventing the development of hypertension in this model. Systolic blood pressure (SBP) was measured in awake rats by the tail-cuff method prior to clipping and twice weekly during the study period. All animals were placed in metabolic cages 18 to 24 days after clipping and two 24-hour urine samples were collected for the measurement of urinary albumin excretion.

Renal function

Animals were anesthetized with pentobarbital (50 mg/kg i.p.) 21 to 27 days after clipping and placed on a thermostatically controlled, heated micropuncture table. The rats were then surgically prepared for clearance study. After tracheotomy, three polyethylene catheters were inserted into a jugular vein for infusion of: 10% polyfructosan (Inutest, Laevosan-Gesellschaft, Linz, Austria) and 2% para-aminohippurate (PAH; Merck Sharp & Dohme, West Point, PA, USA) in 0.9% NaCl, and for the supplemental administration of anesthetic. The total infusion rate was 1.8 ml/hr during surgery. The left femoral artery was cannulated for the direct blood pressure measurement and collection of blood samples. Blood pressure was measured continuously with a Statham pressure transducer, and recorded on a polygraph (Grass Instrument Co., Quincy, MA, USA). The left kidney was exposed through a flank incision and the left ureter was cannulated proximally and ligated distal to the cannula. The urinary bladder was cannulated through an abdominal incision for collection of urine samples from the right kidney. Following completion of surgery, the polyfructosan and PAH solution and the 0.9% NaCl were administered as priming volumes of 0.6 ml each, followed by an infusion rate of 0.6 ml/hr (total infusion rate 1.2 ml/hr). One hour was allowed to achieve steady state conditions and then two or three timed (30 min) urine collections were obtained. Blood samples for measurement of plasma polyfructosan and PAH concentrations and hematocrit were taken before and after each urine collection period. Following clearance periods, bolus injections of 25 and 50 ng Ang I were administered intravenously in six to seven rats from each group. Increments in mean arterial pressure (MAP) were used as an index of the magnitude of inhibition of Ang II formation by enalapril or blockade of Ang II receptors by losartan. At the end of the experiment, placement of the clip was confirmed and both kidneys were removed, blotted gently and weighed.

Renal histology

An additional three groups of clipped rats (untreated or treated with losartan or enalapril) were prepared for histological study. Three weeks after clipping, tail-cuff pressures were measured. Rats were then anesthetized, placed on a thermostatically-controlled heated table and the left femoral artery was cannulated for the direct measurement of MAP. Following stabilization, MAP was obtained over a 10 minute period. The aorta was then cannulated above the illiac bifurcation and clamped distal to the diaphragm. Small incisions were quickly made in both renal veins; the kidneys were immediately perfused *in situ*, initially with 0.9% NaCl and then with Bouin's fixative, at pressures approximating renal perfusion pressure. The kidneys were removed and further immersion fixed, routinely processed, paraffin embedded, cut in 5 μm sections and stained with hematoxylin and eosin and PAS. Histological evaluation was performed with the examiner (EL) blinded as to source of the kidney. Changes in glomerular architecture were quantitatively evaluated in 10 to 40 randomly chosen cortical glomeruli in each kidney in which the sectioning plane had passed through the hilum and included the efferent and/or afferent arteriole. In these properly oriented glomeruli two measurements were made at a magnification of 400×. First, the distance from the hilar vessels to the edge of the glomerulus nearest the urinary pole was measured. Second, the greatest glomerular width was measured perpendicular to the first measurement. These two values in microns were multiplied for each glomerulus to give the glomerular size index. Mesangial matrix expansion was evaluated using the criteria of Raij, Azar and Keane [11]. Briefly, expansion was assessed by the accumulation of increased amounts of finely granular periodic acid-Schiff (PAS) positive matrix in the glomerular mesangium. Glomeruli were scored semiquantitatively to evaluate the degree of damage on an arbitrary scale from 0 to 4 according to the percentage of glomerular involvement. Thus 25% involvement of the glomerulus was a score of 1 while 75% involvement represented a score of 3. In addition, the presence of distinct, spherical PAS positive staining deposits was noted.

Analytical procedures

Urine samples were collected under oil in preweighed containers and urine volumes were determined gravimetrically. Polyfructosan and PAH concentrations in plasma and urine were measured by modified semimicro, colorimetric techniques [12, 13]. Glomerular filtration rate (GFR) was determined from the clearance of polyfructosan. Renal plasma flow (RPF) was estimated from PAH clearance adjusted for an average measured extraction ratio of 0.83. Renal blood flow (RBF) filtration fraction (FF) and renal vascular resistance (RVR) were then calculated using standard formulae. Plasma and urine sodium and potassium concentrations were measured with flame photometry. Urinary albumin concentration was measured with an immunoelectrophoresis technique [14] using a sensitive and specific rabbit antiserum to rat serum albumin.

Table 1. Body weight, kidney weight and mean arterial pressure (MAP)

Group	N	Preclip body wt g	Three weeks post-clip			
			Body wt g	Kidney weight g		MAP mm Hg
				Nonclip	Clip	
Control	9	102 ± 4	287 ± 10	1.49 ± 0.05[c]	1.16 ± 0.06	171 ± 6
Losartan	7	99 ± 7	288 ± 9	1.70 ± 0.10[c]	1.11 ± 0.03	109 ± 6[a]
Enalapril	8	100 ± 4	254 ± 4[ab]	1.48 ± 0.06[c]	0.97 ± 0.04[a]	94 ± 4[a]

Data are expressed as mean ± SEM.
[a] $P < 0.01$ compared with control group
[b] $P < 0.01$ compared with losartan group
[c] $P < 0.01$ compared with clipped kidney

Statistical analysis

Data obtained from each experimental group are expressed as the mean ± SEM. Tail-cuff SBP data for each treatment group were analyzed by analysis of variance (ANOVA) for repeated measures. Data obtained for SBP, MAP and clearance parameters were analyzed using one-way ANOVA. Multiple comparisons of the means were subsequently analyzed by Bonferroni/Dunn or Student-Newman-Keuls methods, where appropriate. Data with unequal variances were analyzed using non-parametric Kruskal-Wallis one-way ANOVA, and comparisons between the means were subsequently analyzed by Mann-Whitney test with the Bonferroni modification applied to determine significance. Differences in function between clipped and nonclipped kidney for each treatment group were analyzed by Student's unpaired *t*-tests. Data were analyzed using Statview and SuperAnova (Abacus Concepts, Berkley, CA, USA) and significance was accepted as $P < 0.05$.

Results

Body weight, kidney weight, blood pressure and albumin excretion in conscious rats

Prior to clipping there were no significant differences in body weight among the three groups (Table 1). Three weeks after clipping, the body wt of the control, losartan and enalapril groups were 287 ± 10, 288 ± 9 and 254 ± 4 g, respectively. The body weight of the enalapril group was significantly lower than either the control or losartan groups ($P < 0.01$). Despite the slightly lower growth rate, the rats in the enalapril group remained healthy. In all groups, the weight of the left, nonclipped kidney was greater than that of the clipped kidney ($P < 0.001$). The weight of the right, clipped kidney for the enalapril group was significantly lower than that for the control group ($P < 0.05$), but was not different when corrected for body wt.

Prior to clipping, systolic blood pressure was not different among the three groups. In the control group of 2-K 1-C hypertensive rats, SBP increased from a preclip value of 105 ± 2 mm Hg to 188 ± 11 mm Hg at three weeks (Fig. 1). No significant increases in SBP were found during the three weeks following clipping in either the losartan or enalapril groups. On day 21, SBPs for the losartan and enalapril groups were 116 ± 6 mm Hg and 113 ± 2 mm Hg, respectively. SBPs for the two treated groups did not differ significantly at any time during the three weeks.

Three weeks after clipping, urinary albumin excretion ($U_{alb}V$) was 17.8 ± 6.8 mg/day in the awake hypertensive control group (Fig. 2). Both the losartan and enalapril groups had significantly lower $U_{alb}V$ compared to the control animals (1.7 ± 0.5 mg/day, $P < 0.01$ and 0.7 ± 0.1 mg/day, $P < 0.01$, respectively). Although the average $U_{alb}V$ tended to be lower in the enalapril group than for the losartan group, this difference did not achieve statistical significance.

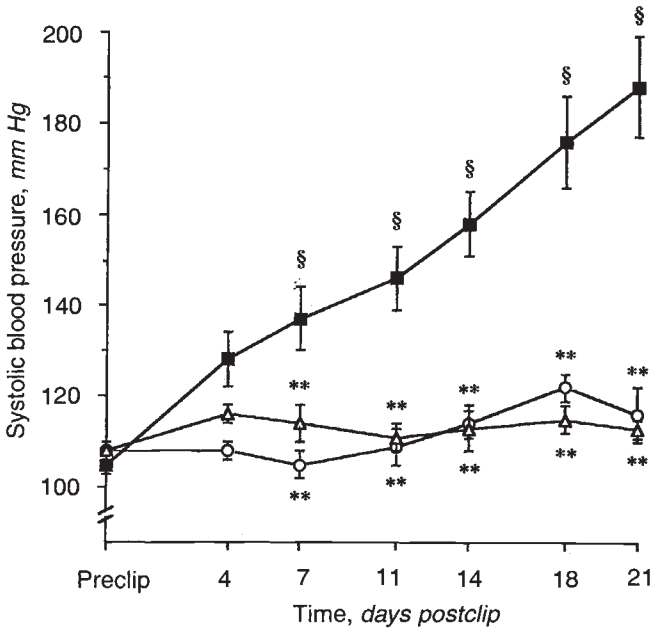

Fig. 1. *Systolic blood pressure (SBP) measured by the tail cuff method in conscious rats prior to and following the placement of a renal artery clip.* SBP of untreated control rats was increased from 7 days postclip (—■—). Chronic treatment with either losartan (—●—) or enalapril (—▲—) prevented the increase in SBP induced by renal artery clipping through three weeks. § $P < 0.01$ compared with preclip, ** $P < 0.01$ compared with control group (comparisons tested by either contrasts or by Bonferroni/Dunn method generated from the appropriate one-way repeated measures ANOVA).

Acute clearance studies—Blood pressure, renal function and renal hemodynamic changes

Mean arterial pressure (MAP) measured directly in anesthetized rats during the clearance studies was 171 ± 6 mm Hg for the untreated, control group. In contrast, MAP for both the losartan and enalapril treated groups was significantly lower (109 ± 6 mm Hg, $P < 0.001$, and 94 ± 4 mm Hg, $P < 0.001$, respectively). There was no significant difference in MAP between the two treated groups.

The average values for GFR were not different between the nonclipped and clipped kidneys of the untreated group. In contrast, GFR was significantly higher in nonclipped compared to clipped kidneys for both treated groups (Fig. 3). The average values for GFR of the nonclipped kidney were significantly higher ($P < 0.01$) for the losartan (1.57 ± 0.13 ml/min) and the enalapril groups (1.39 ± 0.07 ml/min) than for the untreated group (1.02 ± 0.07 ml/min). Interestingly, despite the three weeks of normalized systemic blood pressure by either inhibition of Ang II formation or blockade of Ang II receptors, mean values of GFR for the clipped kidneys of both treated groups were not different from that determined for the corresponding kidneys of the control group. This effect of treatment to increase GFR of the nonclipped

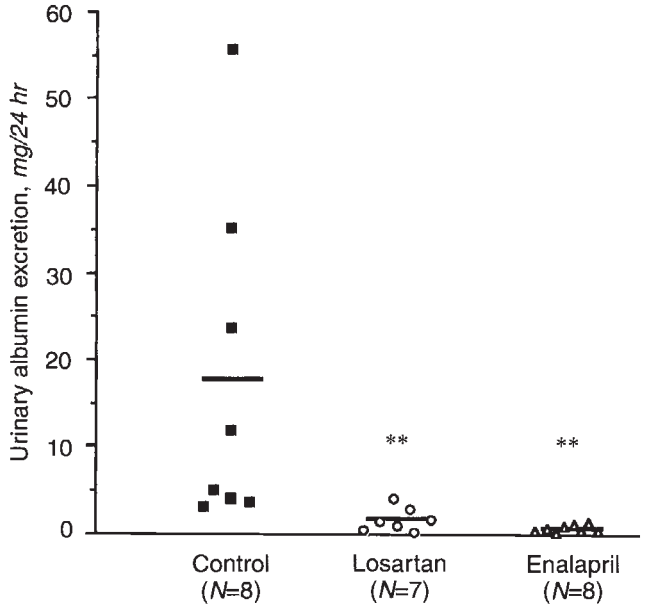

Fig. 2. *Urinary albumin excretion rate of conscious rats three weeks after renal artery clipping.* 2-K 1-C rats treated chronically with either losartan or enalapril had markedly attenuated albuminuria compared with untreated hypertensive rats. ** $P < 0.01$ compared with control group (comparisons using the Mann-Whitney test with the Bonferroni modification).

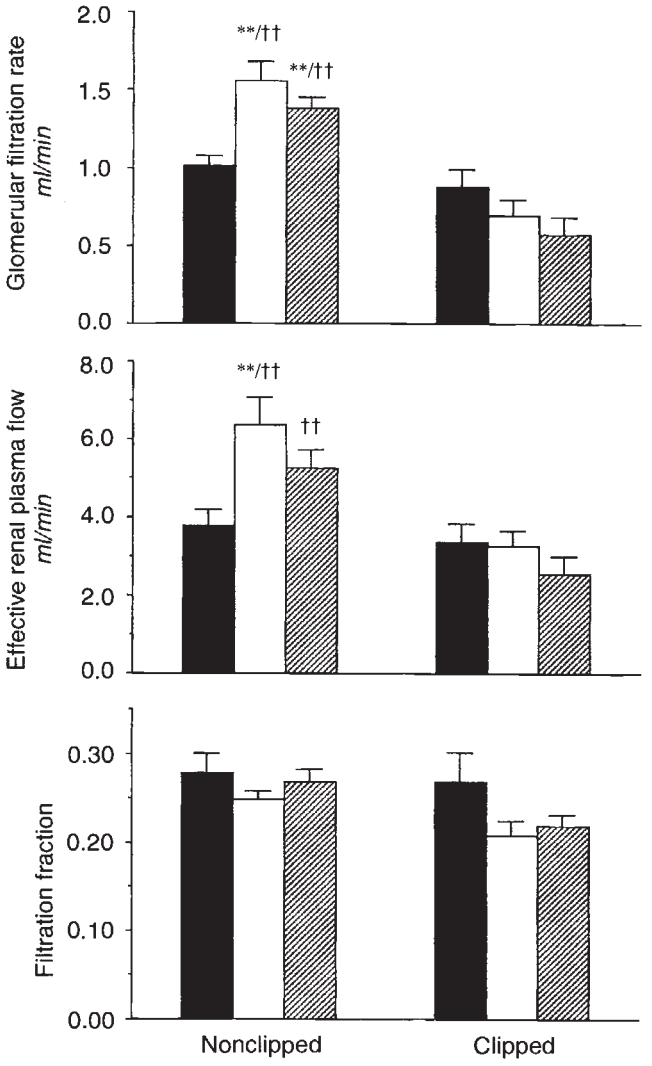

Fig. 3. *Glomerular filtration rate, effective renal plasma flow and filtration fraction of the nonclipped and clipped kidneys from untreated hypertensive and treated 2-K 1-C rats.* The GFR of the nonclipped kidneys from the losartan (□) or enalapril (▨) groups was increased compared to that of the control (■) group. The RPF of the nonclipped kidneys from the losartan group was increased compared to that of the control group. There are no differences in the hemodynamics of the clipped kidney among the three groups. ** $P < 0.01$ compared with control group; †† $P < 0.01$ compared with clipped kidney from the same group (comparisons tested by Student-Newman-Keuls method generated from one way ANOVA).

kidney as well as maintain the GFR of the clipped kidney was also observed when the values for GFR were normalized for differences in kidney weight.

Renal hemodynamic data obtained from the three groups are shown in Figures 3 and 4. The average values for RPF were not different between the nonclipped and clipped kidneys of the untreated group. In contrast, mean RPF values were significantly higher for the nonclipped kidneys compared to clipped kidneys for both treated groups (Fig. 3). RPF for the nonclipped kidney from the losartan group was higher ($P < 0.01$) than that of the nonclipped kidney from the control group. Again, mean values of RPF for the clipped kidneys of both treated groups were not different from that determined for the corresponding kidneys of the control group (3.38 ± 0.47, 3.27 ± 0.39 and 2.56 ± 0.45 ml/min for the untreated, losartan and enalapril groups, respectively). Total RPF did not differ among the three groups. Since nonclipped and clipped kidney weights for the groups differed, RPF data were also examined factored for kidney wt. The mean plasma flow for nonclipped kidneys was significantly larger ($P < 0.05$) in both the losartan and enalapril groups (3.79 ± 0.36 and 3.60 ± 0.34 ml/min/g kidney wt, respectively) compared to that for the control group (2.58 ± 0.30 ml/min/g kidney wt). When factored for kidney wt, mean values of RPF for the clipped kidneys of both treated groups were not different from that determined for the corresponding kidneys of the control group. There were no differences in FF between nonclipped or clipped kidneys among the groups (Fig. 3).

Data for RBF and RVR are shown in Figure 4. For both treated groups, RBF was higher in the nonclipped than the clipped kidneys. The mean RBF of nonclipped kidneys was higher in the losartan group than in the control group (12.12 ± 1.18 and 7.72 ± 0.81 ml/min, respectively, $P < 0.01$). The mean values of RBF for the clipped kidneys did not differ among the three groups. When factored for kidney wt, no significant differences were found for RBF for nonclipped kidneys between the losartan and control groups, and no difference was found between nonclipped and clipped kidney RBF for either the losartan or enalapril groups. In both losartan and enalapril groups, nonclipped kidney RVR was significantly lower than in the control group ($P < 0.01$).

Values for absolute Na^+ excretion from the nonclipped and clipped kidneys of the control group were 155 ± 60 and 32 ± 13 nmol/min ($P < 0.05$), respectively, values not different from those observed for the corresponding kidneys for either treatment group. Although absolute Na^+ excretion ($U_{Na}V$) for nonclipped

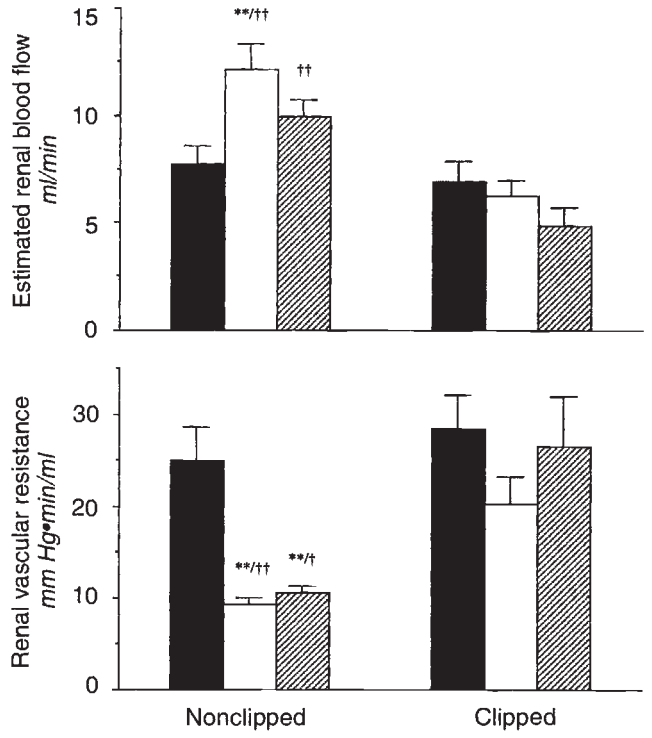

Fig. 4. *Estimated renal blood flow and renal vascular resistance of the nonclipped and clipped kidneys from untreated and treated 2-K 1-C rats.* Losartan (□) or enalapril (▨) induced a marked vasodilation of the nonclipped compared with the nonclipped kidneys of the control (■) rats. This vasodilation was associated with an elevated nonclipped kidney blood flow in the losartan group or the maintenance of blood flow in the nonclipped kidney of rats treated with enalapril. Clipped kidney vascular resistance was not altered by treatment. ** $P < 0.01$ compared with control group; † $P < 0.05$; †† $P < 0.01$ compared with clipped kidney from the same group (comparisons tested by Student-Newman-Keuls method generated from one way ANOVA).

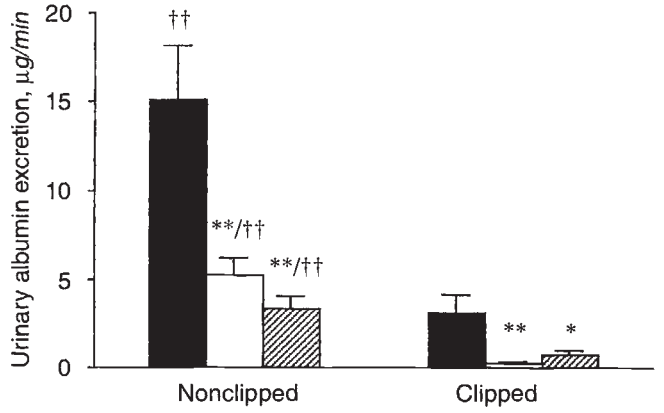

Fig. 5. *Urinary albumin excretion ($U_{alb}V$, μg/min) of nonclipped and clipped kidneys from anesthetized 2-K 1-C rats at the time of acute clearance study (3 weeks postclip).* Losartan (□) and enalapril (▨) induced a significant reduction in $U_{alb}V$ from both kidneys. Symbol (■) is control. $U_{alb}V$ was markedly elevated in nonclipped kidneys compared to the clipped kidneys in all groups. Thus the reduction in $U_{alb}V$ observed in the treated groups was principally due to a reduction in albumin excretion from the nonclipped kidney. * $P < 0.05$ compared with control group; ** $P < 0.01$ compared with control group; †† $P < 0.01$ compared with clipped kidney from the same group (Comparisons tested by Student-Newman-Keuls method generated from one way ANOVA).

or clipped kidneys was not changed in the losartan or enalapril treated groups, the fractional Na^+ excretion from the nonclipped kidney was lower in both the losartan or enalapril treated groups (0.07 ± 0.02 and 0.07 ± 0.02%, respectively compared to 0.12 ± 0.05 for the control group, $P < 0.05$). Treatment with losartan or enalapril was associated with lower U_KV for the clipped kidney (430 ± 100 or 444 ± 110 nmol/min, respectively, vs. 820 ± 150 for the control group, $P < 0.05$), but U_KV by the nonclipped kidney was unaffected. Fractional excretion of K^+ did not differ among the groups (control group, nonclipped kidney 30 ± 6% and clipped kidney 24 ± 4%; losartan group 25 ± 4 and 15 ± 2%, and enalapril group 30 ± 3 and 18 ± 2%, respectively).

Urinary albumin excretion rates ($U_{alb}V$), obtained from the acute clearance experiments during anesthesia, are shown in Figure 5. Nonclipped kidney $U_{alb}V$ was significantly greater than that from the clipped kidney in all groups: 15.1 ± 3.1 μg/min versus 3.1 ± 1.1 μg/min in control group, $P < 0.01$, 5.3 ± 1.0 μg/min versus 0.3 ± 0.1 μg/min in losartan group, $P < 0.001$ and 3.3 ± 0.8 μg/min versus 0.7 ± 0.3 μg/min in enalapril group, $P < 0.01$. $U_{alb}V$ from both nonclipped and clipped kidneys was significantly ($P < 0.01$) less for the losartan and enalapril groups compared to the corresponding kidneys of the control group.

Pressor response to angiotensin I injection

The increments in MAP following bolus injection of 25 and 50 ng Ang I were attenuated by approximately 75% in both losartan and enalapril groups compared to the control group. The ΔMAP responses to either dose of Ang I were not different between the losartan and enalapril groups. The ΔMAP for the untreated group in response to 25 ng Ang I was 20 ± 3 mm Hg compared to 5 ± 2 mm Hg for both the enalapril and losartan groups.

Renal histology

The three additional groups subjected to histological study exhibited SBPs and MAPs which were not different from the groups subjected to the study of renal function of each kidney. The SBPs and MAPs of the rats for histological study were 186 ± 8 and 177 ± 9, 125 ± 12 and 118 ± 3, 121 ± 4 and 108 ± 2 mm Hg for the control, losartan and enalapril groups, respectively.

Indices of glomerular size and mesangial expansion for the nonclipped or clipped kidneys from each of the groups are presented in Table 2. For the untreated group, there were no significant differences in the indices of glomerular size or mesangial expansion between the clipped and nonclipped kidneys. In contrast, for both the treated groups, the indices of glomerular size or mesangial expansion were significantly ($P < 0.01$) lower for clipped compared to nonclipped kidneys. The indices of glomerular size and mesangial expansion determined for the nonclipped kidneys of the losartan or the enalapril treated groups did not differ significantly from the values determined for the untreated group. The indices of glomerular size and mesangial expansion for the clipped kidneys from the losartan treated group were significantly lower compared to those determined for corresponding kidneys of the untreated rats. There was a trend for the indices from the clipped kidneys from the enalapril treated group to be lower compared to the untreated group, but the differences did not achieve statistical significance.

Table 2. Histological indices of glomeruli from untreated 2-K 1-C hypertensive rats, and from 2-K 1-C rats chronically treated with losartan or enalapril

A. Glomerular size index

Kidney	Glomerular size index ($\mu M \times 10^2$)		
	Control	Losartan	Enalapril
Clipped	90.1 ± 4.5 (30/3)	68.1 ± 3.0[c] (30/3)	80.9 ± 4.2 (30/3)
Nonclipped	96.6 ± 4.0 (30/3)	100.5 ± 4.0[a] (30/3)	107.6 ± 5.2[a] (30/3)

B. Mesangial expansion index

Kidney	Mesangial expansion index		
	Control	Losartan	Enalapril
Clipped	1.1 ± 0.1 (120/6)	0.7 ± 0.1[b] (119/6)	0.9 ± 0.1 (120/6)
Nonclipped	1.3 ± 0.1 (120/6)	1.4 ± 0.1[a] (119/6)	1.5 ± 0.1[a] (120/6)

Kidneys were perfused-fixed three weeks after renal artery clipping. Data are presented as mean ± SEM.
[a] $P < 0.01$ compared with clipped kidney from same group
[b] $P < 0.05$ compared with clipped kidney of control group
[c] $P < 0.01$ compared with clipped kidney of control group

In addition to the mesangial expansion (which was manifested as increases in finely granular PAS-positive matrix), we observed discrete PAS-staining spherical deposits in many glomeruli from nonclipped kidneys of untreated control rats (Fig. 6). These discrete PAS-staining spherical deposits were usually large when located in the hilar regions but tended to be smaller in the perpheral capillary loops. The deposits were not necessarily coincident with mesangial expansion. Losartan treatment significantly reduced, and enalapril treatment abolished, the presence of the spherical deposits without significantly affecting mesangial expansion. In contrast to the nonclipped kidneys, glomeruli from the clipped kidneys did not show any evidence of discrete PAS-staining deposits, irrespective of the group to which the rats belonged.

Discussion

This study assessed the effects of angiotensin receptor blockade or ACE inhibition on blood pressure, renal function, albumin excretion and renal histology in both clipped and nonclipped kidneys of two-kidney, one-clip hypertensive rats. As reported previously, chronic administration of ACEI, introduced soon after clipping, prevents the development of systemic hypertension in 2-K 1-C hypertensive rats [15, 16]. Our new finding, that similar antihypertensive effects were obtained with losartan, indicates that AT1 receptor-mediated effects of Ang II are critical to the initial development of hypertension, and implies that ACEI prevents the development of elevated blood pressure chiefly by inhibiting conversion of Ang I to Ang II. Consequently, these observations do not support a major role for kinin potentiation in the antihypertensive effects of ACEI in this model. Whereas the measurements of SBP obtained in awake rats from the enalapril and losartan groups were almost identical, under anesthesia, MAP in the enalapril group fell to somewhat lower levels than in the losartan-treated rats. The growth rate in the enalapril group also appeared slightly lower than that in the losartan group. Whether either or both of these slight inter-group differences reflect inequality in the extent of RAS inhibition achieved, or class-associated differences between the agents, is unclear. In either case, the differences are small and unlikely to be important to the main aspects of this study.

Treatment, either with enalapril or losartan, while preventing any significant rise in blood pressure, was associated with augmentation of both GFR and RPF of the nonclipped kidney when compared to the corresponding kidney in untreated rats. This finding contrasts with the recent report of decreased GFR of nonclipped kidneys following chronic treatment with enalapril in rats with established renovascular hypertension [17]. Augmentation of renal hemodynamic function in the nonclipped kidney may, in part, be due to inhibition of the effects of Ang II within the kidney which, in addition to the effects of Ang II on systemic vascular resistance, are thought to contribute to the generation of hypertension in 2-K 1-C rats [reviewed in 1]. Measurements of components of the RAS in the nonclipped kidney reveal that, whereas renin levels are markedly suppressed [8], Ang II levels appear to be significantly elevated [18]. Altered physiological behavior in the nonclipped kidney, such as blunting of autoregulation and pressure-natriuresis responses, may result from specific actions of Ang II. Acute inhibition of the RAS in 2-K 1-C rats, either by ACEI or Ang II receptor antagonist administration, results in significant increases in RBF, GFR and renal excretory function in the nonclipped kidney despite profound contemporaneous decreases in blood pressure [4, 8, 19]. These findings are consistent with inhibition of known acute intrarenal effects of Ang II, for example, to increase renal vascular resistances at both pre- and post-glomerular segments, to decrease K_f, and to increase tubular fluid reabsorption [20, 21]. Our present findings of increased RPF and GFR in nonclipped kidneys of the treated groups suggest that the effects of chronic inhibition of the RAS are sustained and, to some degree, parallel the effects of acute inhibition. In contrast to the increased fractional Na^+ excretion from the nonclipped kidney following acute inhibition of the RAS, chronic treatment with losartan or enalapril in the present study resulted in a lower FE_{Na^+} from the nonclipped kidney. The lower FE_{Na^+} for the nonclipped kidneys of the losartan and enalapril treated groups may reflect a tubular mechanism to chronically regulate urinary Na^+ excretion in the face of the augmented GFR and increased filtered load of Na^+.

In the present study two distinct forms of PAS-positive material were observed in glomeruli from nonclipped kidneys of untreated control 2-K 1-C hypertensive rats: (i) diffuse, finely-granular staining identified as mesangial matrix expansion and (ii) discrete more densely staining spherical deposits representing deposition of hyalin. Although both of these forms of PAS-positive material may represent early glomerular damage, the deposition of hyalin probably represents an early manifestation of glomerulosclerosis [22]. Losartan or enalapril greatly reduced the distinct glomerular hyalin deposits and prevented the development of proteinuria in the nonclipped kidney. These findings suggest that blockade of the RAS in 2-K 1-C rats either prevents or delays the evolution of glomerular changes associated with the development of sclerotic lesions. Although our protocol did not include untreated normotensive rats for comparison, approximately 38% of the glomeruli from nonclipped kidneys of untreated 2-K 1-C hypertensive rats

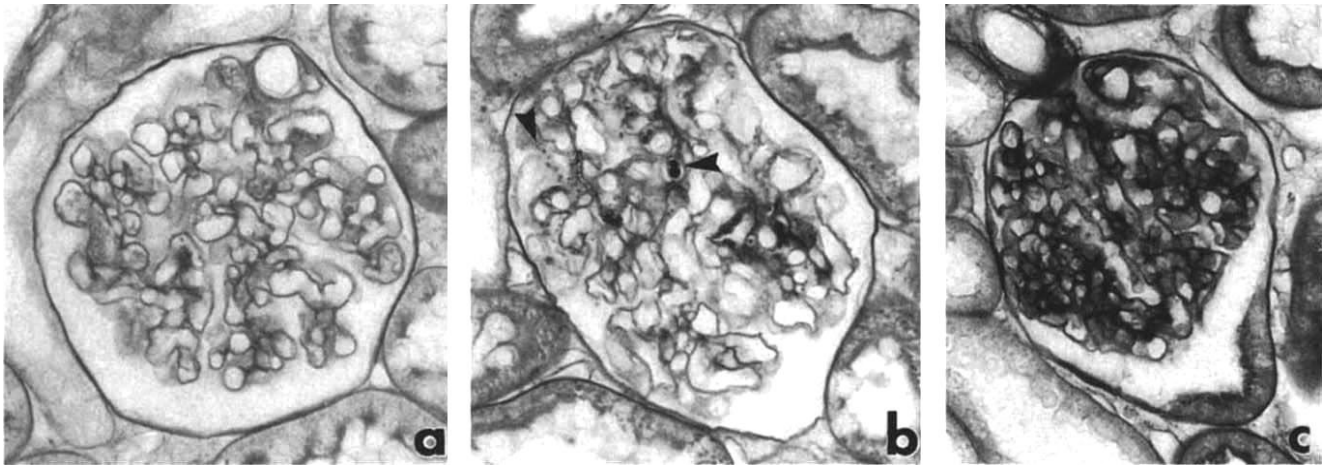

Fig. 6. *Light micrographs of paraffin-embedded kidney sections stained with PAS.* Glomeruli from (**a**) clipped kidney of untreated rats show normal appearing capillary loops and mesangium, (**b**) nonclipped kidney of untreated rats show distinct circular-shaped mesangial deposits of varying sizes. Glomerulus (**c**) from nonclipped kidney of enalapril treated rat shows extensive staining of the mesangium including the hilar region (mesangial expansion grade = 3). No distinct spherical deposits were observed in glomeruli from nonclipped kidneys of enalapril treated rats. Magnification 1300× for all panels.

had moderate to severe accumulation (50% or greater involvement of the glomerulus) of diffuse PAS-staining material, suggesting that the development phase of this model is accompanied by increased elaboration of mesangial matrix. Interestingly, a similar degree of expansion of mesangial matrix was observed in glomeruli from the nonclipped kidneys of rats in both treatment groups. Expansion of mesangial matrix may be a forerunner of glomerular damage induced by glomerular hypertension [22] or by the effects of angiotensin II. However, in the present study, expansion of mesangial matrix occurred under conditions of significant RAS blockade and the complete abrogation of hypertension. These findings suggest that mesangial matrix expansion, without the coexistence of hypertension and/or angiotensin II, does not appear to be detrimental to glomerular functional integrity during the early phase following renal artery clipping.

In the present study, chronic ACEI was not associated with significant deleterious effects on clipped kidney function. This finding contrasts with reports of severe impairment of clipped kidney function in response to acute administration of ACEI to 2-K 1-C hypertensive rats [4, 6–8]. Before drawing conclusions regarding this apparent discrepancy, it should be emphasized that the acute or chronic effects of RAS inhibition have previously only been studied after hypertension was established in this model. Acute reductions in blood pressure from hypertensive levels result in impaired RBF, reduced GFR and decreased excretory function of the clipped kidney. In some settings, where RAS activity is presumably augmented by prior volume depletion, the effects of acute ACEI may be so severe that acute renal failure results. Such decreases in post-stenotic kidney function observed after RAS inhibition have been attributed either to perfusion pressure being decreased to levels below the autoregulatory threshold as a result of profound decreases of systemic BP, or to loss of specific intrarenal effects of Ang II, such as, specific maintenance of post-glomerular arteriolar tone. Our data clearly show that when RAS inhibition is introduced before the development of hypertension, renal function in the post-stenotic kidney is comparatively well maintained. This observation is even more remarkable when the conditions under which the clearance data were obtained are considered. Under anesthesia, MAP was measured at levels close to (losartan group) or frankly below (enalapril group) the usual lower limit of GFR autoregulation. From published data [5, 23] and our own observations [Mackenzie and Ploth, unpublished observations], it is reasonable to assume that during anesthesia post-stenotic perfusion falls well below the usual lower limit of autoregulation in both the enalapril and losartan treated groups. The fact that function of the post-stenotic kidney is well maintained under the conditions of this study has two important implications: (1) renal homeostatic mechanisms for maintaining GFR and RPF may be more effective when adapting to gradual rather than sudden changes in systemic perfusion pressure, and (2) an intact RAS does not appear to be essential to the long-term maintenance of GFR in the clipped kidney. In particular, these observations do not support a role for Ang II-induced preferential efferent arteriolar constriction in the maintenance of GFR.

The clipped kidneys from the untreated hypertensive rats appeared free of structural damage to glomeruli. These observations are in accordance with the findings of Wilson and Byrom [summarized in 24] that the clipped kidneys of 2-K 1-C hypertensive rats do not show evidence of glomerular lesions. Interestingly, in the present study, the clipped kidneys from rats treated with losartan or enalapril did not show evidence of ischemic glomerular damage that would be expected to follow inadequate post-stenotic perfusion. These findings are at variance with earlier reports that chronic administration of ACEI to 2-K 1-C hypertensive rats is associated with fibrosis and atrophy of the clipped kidney, amounting to "pharmacologic nephrectomy" in one study [7]. Our data on the function and structure of the clipped kidney may be interpreted as supporting the notion that, whereas sudden introduction of RAS inhibition may be associated with acute and possibly permanent impairment in GFR and RPF, chronic administration or possibly gradual administration of ACEI, avoiding initial abrupt decreases in blood pressure, is less likely to have adverse effects on the function and structure of the post-stenotic kidney. Further, a recent report suggests that the phenomenon of

acute impairment in renal function with ACEI may be avoided if the agent is introduced gradually, in a step-wise fashion [25].

Mild to severe proteinuria has been observed with renovascular hypertension in humans [26, 27] and rats [28] and the magnitude of proteinuria can be reduced by treatment with ACEI in man [27, 29]. However, treatment with enalapril did not reduce albuminuria in rats with RVH when it was initiated six weeks post-clipping [28], suggesting that in this model proteinuria may represent fixed structural damage of the glomerular barrier rather than a reversible or functional abnormality. One of the goals of the present study was to determine whether ACEI or Ang II receptor blockade would prevent the development of albuminuria if started prior to the onset of hypertension. We found that enalapril or losartan completely attenuated the albuminuria observed three weeks after clipping in the untreated rats. In both treated groups, $U_{alb}V$ was similar to that measured in normal rats of comparable size in our laboratory (0.5 to 1.0 mg/day). The anti-albuminuric response to losartan and enalapril parallel those reported in other models of chronic progressive renal disease [30–32].

An additional original observation in the present study is the effect of ACEI or losartan on the albumin excretion from the individual nonclipped and clipped kidneys. Although the difference in albumin excretion rate between the control and either of the two treated groups was decreased during anesthesia, significant differences in albumin excretion between treatment and control groups remained. In the untreated hypertensive rats, the albumin excretion rate from the nonclipped kidney exposed to the hypertensive perfusion pressure was 5- to 10-fold greater than that from the clipped kidney, suggesting that the nonclipped kidney is the principal source of the albuminuria in 2-K 1-C hypertensive rats. The attenuation of albuminuria by losartan or enalapril treatment indicated reduction in albumin excretion from both the nonclipped and clipped kidneys, although the absolute decrease was greater from the nonclipped kidney. Further, the substantially different absolute albumin excretion rates between the nonclipped and clipped kidneys was maintained in the enalapril and losartan groups. It has been suggested that Ang II induces the increased protein excretion observed in other models of renal disease [33, 34]. The present finding that there is a marked difference in $U_{alb}V$ between the nonclipped and clipped kidneys during inhibition of ACE or blockade of Ang II would suggest that the reduction in albumin excretion was not due to the inhibition of the direct effects of Ang II on glomerular protein permeability, but may reflect abrogation of systemic hypertension or other effects.

In summary, we observed similar efficacy of the antihypertensive effect of chronic CEI and angiotensin receptor blockade in 2-K 1-C hypertensive rats. Following three weeks of maintained normal systemic BP in the treated groups, renal function of the nonclipped kidney was generally increased; the function of the clipped kidney was unchanged compared to the respective kidneys of untreated, hypertensive control animals. While both treatment protocols reduced albumin excretion from nonclipped and clipped kidneys and appeared to prevent the development of early signs of glomerulosclerosis in nonclipped kidneys, neither treatment was associated with adverse effects on clipped kidney structure as evidenced by histologic evaluation. These observations support the concept that, despite normalized blood pressure resulting from prolonged blockade of the RAS, function and histology of the clipped kidney are preserved with impressive efficiency.

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