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F N Hutchison, ... , S K Webster, A A Jaffa

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### Research Article

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# Altered Renal Kallikrein and Renin Gene Expression in Nephrotic Rats and Modulation by Converting Enzyme Inhibition

Florence N. Hutchison, Sarah K. Webster, and Ayad A. Jaffa

Divisions of Nephrology and Endocrinology, Departments of Internal Medicine and Pharmacology, Medical University of South Carolina, Charleston, South Carolina 29425; and Ralph H. Johnson Veterans Affairs Medical Center, Charleston, South Carolina 29403

## Abstract

Urinary kallikrein excretion (UKE) is decreased in rats with passive Heymann nephritis (PHN), but increases after converting enzyme inhibition (CEI). Although CEI potentiates bradykinin activity, neither the effect of CEI on kallikrein secretion nor the abnormal renal kallikrein metabolism in PHN has been examined previously. To determine the mechanism by which CEI increases UKE, normal rats and PHN received enalapril, 40 mg/kg per d orally for 4 d. UKE was 85% lower in PHN than in normals and increased in both groups after CEI, although UKE in PHN remained significantly less than in normals. Kallikrein mRNA was significantly lower in PHN compared to normals but not in PHN treated with CEI and did not change in normal rats. Renin mRNA was significantly lower in PHN, and was stimulated by CEI only in normals. Renal kallikrein and renin content were not different and were not altered by CEI. Both kallikrein and renin genes appear to be transcriptionally suppressed in rats with PHN and the depressed kallikrein mRNA levels can be reversed by CEI. The modest increase in UKE despite normalization of kallikrein mRNA after CEI suggests that there is also a posttranscriptional defect in synthesis and/or secretion of kallikrein. (*J. Clin. Invest.* 1993; 92:1073–1079.) Key words: passive Heymann nephritis • proteinuria • kallikrein • renin • kininase II

## Introduction

Converting enzyme inhibitors (CEIs)<sup>1</sup> are effective antihypertensive agents that are capable of limiting the progression of chronic renal disease and reducing proteinuria in the nephrotic syndrome. The effect of CEIs on proteinuria and preservation of renal function appears to be specific to CEIs as a class and is not simply a result of control of hypertension (1, 2). Converting enzyme (kininase II, EC 3.4.15.1) is a ubiquitous dipeptidylpeptidase which hydrolyzes dipeptides from the COOH-terminal of several peptide substrates, including angiotensin I and bradykinin (3). Inhibition of synthesis of the vasoconstrictive peptide angiotensin II from angiotensin I and inhibition of degradation of the vasodilatory peptide bradykinin to inactive me-

tabolites both have been demonstrated to contribute to the antihypertensive action of the converting enzyme inhibitors (4), however, the hormonal mechanism by which these agents beneficially influence renal function and modify proteinuria is not known.

We previously demonstrated that the capacity of CEI to reduce proteinuria in nephrotic rats is dependent on the presence of an intact kallikrein-kinin system (5). We also found that urinary kallikrein excretion was decreased in untreated rats with passive Heymann nephritis relative to the values typically measured in this lab for normal rats (6). Treatment with CEI caused a small but significant increase in urinary kallikrein excretion as well as a decrease in albuminuria, while treatment with the angiotensin II receptor antagonist, losartan, affected neither urinary kallikrein excretion nor albuminuria. The increase in urinary kallikrein excretion with CEI suggested that these agents are capable of stimulating kinin activity, not only by inhibiting degradation of kinin, but by increasing secretion of kallikrein, an effect of CEI not previously recognized.

Although it is clear that the renal kallikrein-kinin system is capable of modifying renal blood flow and urinary sodium excretion, the role of this autacoid system in the regulation of renal function in health or in disease is poorly understood. Kallikrein is synthesized as the inactive precursor and is secreted both as the active enzyme and as the inactive zymogen, which can be activated by hydrolysis after secretion (7, 8). Bradykinin is generated from the hydrolysis of kininogen by active kallikrein and is subsequently degraded by the action of neutral endopeptidase, converting enzyme, or carboxypeptidase (9). Histochemical studies have localized renal kallikrein to cells of the cortical connecting tubule which approximate the afferent arteriole (10). Since kallikrein released from the basolateral surface may enter the interstitium and gain access to glomerular structures, as is believed to occur with renin, and both B1 and B2 kinin receptors have been identified in the glomerulus (11, 12), endogenous renal production of kallikrein may influence glomerular function directly.

The abnormalities of kallikrein excretion observed in nephrotic rats and the effect of CEI on urinary kallikrein excretion could be a result of modified secretion or synthesis of kallikrein. The present study was performed to further characterize the abnormalities of the kallikrein-kinin system in rats with passive Heymann nephritis, to determine the mechanism responsible for the reduced kallikrein excretion in nephrotic rats, to determine the mechanism by which CEI modifies urinary kallikrein excretion in nephrotic rats, and to determine whether CEI exerts a similar affect on renal kallikrein metabolism in normal rats.

## Methods

24 male Sprague Dawley rats weighing 140–160 g were given a single intraperitoneal injection of sheep FX1A antiserum to induce passive

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Address correspondence to Dr. Florence N. Hutchison, Chief, Dialysis Unit, Veterans Affairs Medical Center, 109 Bee Street, Charleston, SC 29403.

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1. Abbreviations used in this paper: CEI, converting enzyme inhibitor; PHN, passive Heymann nephritis; UKE, urinary kallikrein excretion.

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Heymann nephritis, a model of human membranous glomerulonephritis (13). Five rats were injected with a similar volume of saline to serve as normal controls. 12 d after injection of antisera, all rats were weighed and blood pressure was measured by tail cuff plethysmography without anesthesia. Rats were placed in metabolic cages and urine was collected daily for 6 d for determination of urinary albumin and kallikrein excretion rates. Rats were provided with standard rat chow containing 21% protein and with tap water ad libitum. 12 of the nephrotic rats were given enalapril, 40 mg/kg per d, in the drinking water for the last 4 d of collections.

After completing the urine collections, blood pressure was again measured and all rats were anesthetized with Na pentobarbital. A polyethylene catheter was inserted into the infrarenal aorta and 2 ml blood was drawn into iced tubes containing 50  $\mu$ l 15% potassium EDTA for measurement of plasma renin activity (PRA). The kidneys then were perfused antegrade with ice-cold saline until blanched. The kidneys were excised and weighed, and the right kidney was placed in ice-cold saline and frozen at  $-70^{\circ}\text{C}$  for determination of renin and kallikrein content. In five rats from each group the left kidney was snap-frozen in liquid nitrogen for extraction of RNA.

In a second experiment eight male Sprague Dawley rats weighing 200–250 g were placed in metabolic cages for 6 d for urine collections as described previously and four rats were treated with enalapril, 40 mg/kg per d for the last 4 d of collections. On the sixth day all rats were weighed and blood pressure was measured. The rats were then anesthetized, blood collected, and the kidneys prepared as in the first experiment for measurement of renal renin and kallikrein content and extraction of RNA.

**Chemistry methods.** The right kidney was bisected and each half weighed. For extraction of renal renin, one-half kidney was thawed and refrozen three times, homogenized in 0.9% saline with 1% EDTA, pH 4.9, using a polytron, and centrifuged at 35,000  $g$  (14). The supernatant was aspirated and the pellet resuspended and recentrifuged, and the second supernatant was combined with the first. Renin was subsequently determined directly from the supernatant after addition of plasma from anephric rats and PRA from plasma by radioimmunoassay using a kit (Biotecx Lab, Inc., Friendswood, TX). Protein in the supernatant of the renal homogenate was measured by the Lowry method (15) and renin content was normalized to the protein content of the renal homogenate.

Renal kallikrein was extracted as previously described (16) from the remaining portion of the right kidney. In brief, tissue was homogenized in 1.5 ml of phosphate-buffered saline, pH 7.4, using a teflon glass homogenizer. Sodium deoxycholate was added to the homogenate for a final concentration of 0.5%. The homogenate was incubated at  $4^{\circ}\text{C}$  for 1 h and centrifuged at 27,000  $g$  for 30 min. The resulting supernatant was used for analysis of tissue kallikrein and total protein. Active kallikrein was measured in urine and tissue supernatant by radioimmunoassay using a monoclonal antibody that specifically recognizes the active enzyme (17). Total kallikrein is measured by radioimmunoassay using the same antibody after trypsin activation of the sample.

Albumin in urine and sera was measured by rocket immunoelectrophoresis using rabbit antisera to rat serum albumin (18).

**RNA extraction and Northern blot hybridization.** Kidney tissue was minced and then homogenized with a polytron in buffer containing 4 M guanidine thiocyanate, 0.3 M sodium acetate, and 0.1 M 2-mercaptoethanol. The homogenate was ultracentrifuged over cesium chloride (5.7 M cesium chloride, 80 mM sodium acetate) and the resulting RNA pellet was dissolved in diethyl pyrocarbonate-treated water. The concentration of extracted RNA was determined spectrophotometrically by absorbance at 260 nm. Total RNA extracted by this procedure had an  $A_{260}/A_{280}$  ratio of  $1.98 \pm 0.01$ .

An oligonucleotide probe with the sequence: 5'-TCCAATCCG-TCAGGTGATG-3' and that is specific for the true kallikrein gene was used to measure kallikrein mRNA content in kidney (19). The renin cDNA (pRen 44. ceb) was isolated from rat kidney gt-10 cDNA library

and subcloned in the plasmid pGEM4 (20). This insert constitutes  $\sim 1,425$  bp and was a gift of Dr. K. R. Lynch, University of Virginia, Charlottesville, VA.  $\beta$ -actin cDNA is  $\sim 540$  bp and was a gift of Dr. J. Norris, Medical University of South Carolina, Charleston, SC. All cDNA probes were labeled with  $^{32}\text{P}$  using a nick-translation kit (GIBCO Bethesda Research Laboratories, Bethesda, MD) to achieve a specific activity of  $1\text{--}2 \times 10^8$  cpm/ $\mu\text{g}$  DNA. A G-50 spin column was used to remove unincorporated label. The oligonucleotide probe was end-labeled with [ $^{32}\text{P}$ ]ATP to a similar specific activity.

RNA hybridization was performed as described by Thomas (21). Total RNA, 30  $\mu\text{g}$ , in 10  $\mu\text{l}$  100% formamide, 4  $\mu\text{l}$  37% formaldehyde, and 2  $\mu\text{l}$  10 $\times$  MOPS buffer (200 mM 4-morpholinopropanesulfonic acid, 40 mM sodium acetate, 5 mM EDTA, pH 7.0) was denatured at  $65^{\circ}\text{C}$  for 15 min and then run on an agarose gel containing 2.2 M formaldehyde in 1 $\times$  MOPS buffer. Total RNA was transferred from the gel to Nytran membrane filters by a Possiblot Pressure Transducer (Stratagene Corp., La Jolla, CA) for at least 2 h. The filters were then crosslinked in an ultraviolet Stratalinker (Stratagene Corp.) and prehybridized at  $60^{\circ}\text{C}$  in a solution containing 6 $\times$  SSC, 5 $\times$  Denhardt's solution, 1% SDS, and 100  $\mu\text{g}/\text{ml}$  denatured salmon sperm DNA. Hybridization was performed at  $60^{\circ}\text{C}$  for 16–18 h with the nick-translated cDNA probes described above. Hybridization with the oligonucleotide probe was performed at  $42^{\circ}\text{C}$ . Hybridized membranes with the nick-translated cDNA probes were washed three times at room temperature in 3 $\times$  SSC, 0.1% SDS, and twice in 3 $\times$  SSC, 0.1% SDS at  $60^{\circ}\text{C}$  for 15 min each wash. Membranes hybridized with the oligonucleotide probe were washed two times at room temperature in 3 $\times$  SSC, 0.1% SDS, and twice at  $42^{\circ}\text{C}$  in 0.2 $\times$  SSC, 0.1% SDS. Autoradiographs of the membranes were made and quantitated in arbitrary units of optical density using a Joyce Loebl microdensitometer.  $\beta$ -actin mRNA was quantitated in the same samples as an internal standard.

**Statistics.** Statistical analysis was performed by analysis of variance with post hoc paired or unpaired  $t$  test for normally distributed data or Kruskal Wallance rank analysis for non-normally distributed data. Values are presented as the mean  $\pm$  SEM. A single value for urinary albumin excretion for each animal was derived as the mean urinary albumin excretion rate for the two 24-h baseline collections obtained before administration of enalapril.

## Results

Blood pressure decreased significantly from  $127 \pm 6$  to  $101 \pm 6$  mmHg in the nephrotic rats treated with enalapril ( $P < 0.02$ ) but was constant in the nephrotic control group ( $135 \pm 6$  and  $134 \pm 2$  mmHg) and in the normal control group. Weight gain was similar in each of the three experimental groups (Table I).

Urinary albumin excretion rates were compared before and after 4 d of treatment with CEI. There was no difference between the two groups of nephrotic rats prior to treatment with enalapril (untreated:  $418 \pm 43$  mg/d vs. treated:  $533 \pm 45$  mg/d), but albuminuria decreased to  $268 \pm 46$  mg/d after CEI treatment and was significantly lower compared to either the pretreatment excretion rate ( $P < 0.001$ ) or to the untreated nephrotic control group ( $493 \pm 81$  mg/d on day 4,  $P < 0.02$ ). Albuminuria in both nephrotic groups was more than 400-fold greater than in the normal control animals.

Urinary excretion of both active and total kallikrein was significantly lower in the nephrotic control group compared to the normal animals (Table I), but tissue content of active and total kallikrein was not different. Although urinary excretion of total kallikrein was significantly higher and active kallikrein tended to be higher in nephrotic rats treated with enalapril compared to the untreated nephrotic rats, urinary active and

Table I. Effect of Induction of Passive Heymann Nephritis and Converting Enzyme Inhibition on Urinary and Renal Kallikrein and the Renal Renin-Angiotensin System

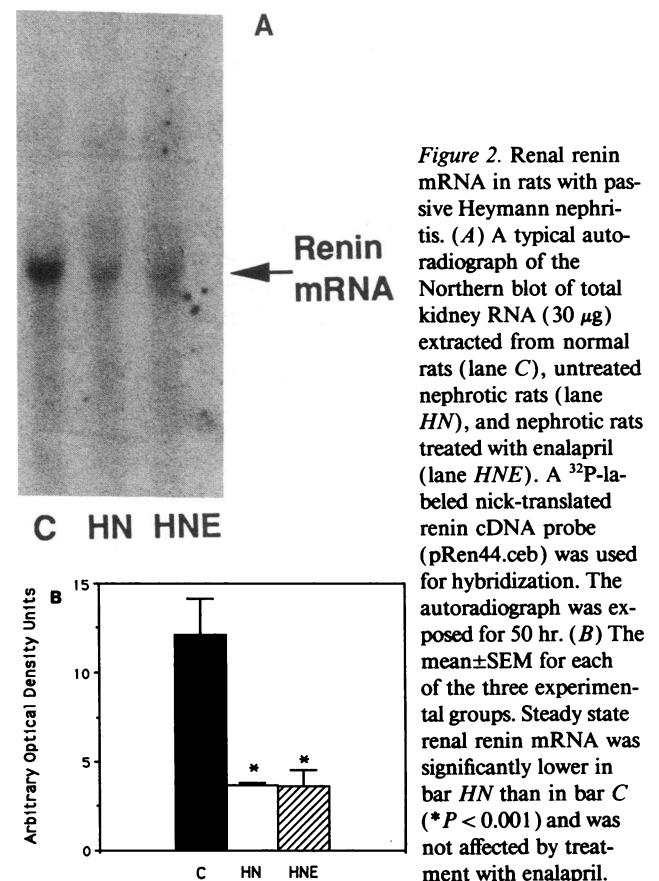
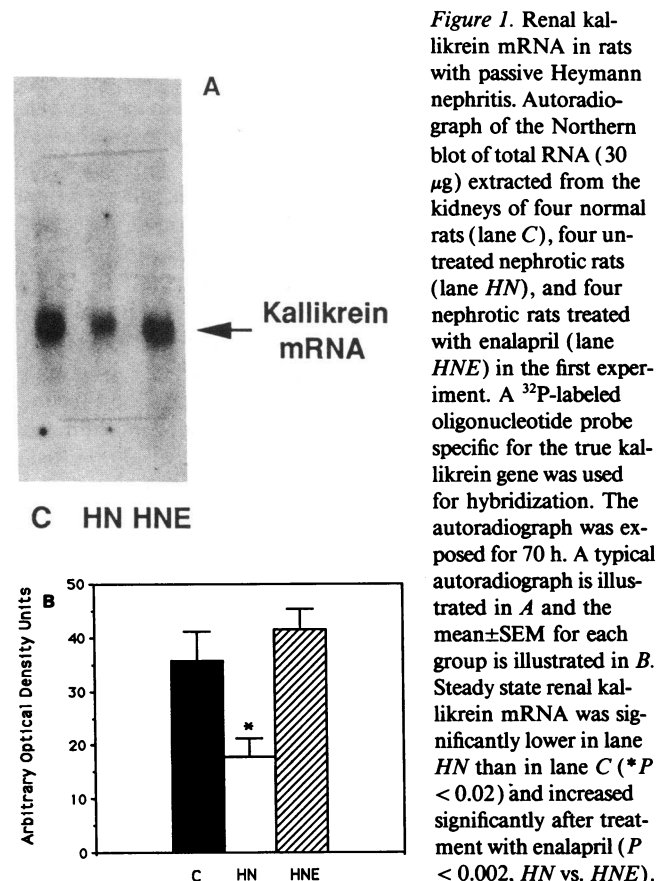
Group	Day	Wt	UKK <sub>act</sub>	UKK <sub>total</sub>	Renal KK <sub>act</sub>	Renal KK <sub>total</sub>	PRA	Renal renin
		g	μg/d	ng/mg prot	ng/mg prot	ng/mg prot	ng/ml per h	μg/mg prot/h
Normal	0	286±9	121.3±11.7	173.1±13.8				
	4	303±11*	128.8±9.3	177.5±10.8	25.1±1.8	38.2±2.3	9.8±2.1	1.34±0.10
PHN	0	273±6	14.3±3.3*	35.9±6.2*				
	4	295±7*	18.2±4.9*	40.2±8.3*	26.1±3.4	39.8±3.3	13.7±1.0	1.19±0.19
PHNE	0	268±5	17.1±3.1*	45.6±7.1*				
	4	288±8*	27.7±8.0*	58.6±8.9* <sup>‡</sup>	22.8±3.6	40.6±4.2	19.4±1.5* <sup>‡</sup>	1.25±0.18

\*  $P < 0.05$  vs. day 0 within a group,  $^{\dagger} P < 0.05$  vs. normal group on the same day,  $^{\S} P < 0.05$  vs. untreated nephrotic group (PHN). (Wt, body weight; UKK<sub>act</sub>, urinary active kallikrein excretion rate; UKK<sub>total</sub>, urinary total kallikrein excretion rate; Renal KK<sub>act</sub>, active renal kallikrein; Renal KK<sub>total</sub>, total renal kallikrein; PRA, plasma renin activity).

total kallikrein excretion rates remained significantly lower than in the normal rats (Table I). Values for renal active and total kallikrein content were not different among the three groups (Table I).

Steady state renal kallikrein mRNA was decreased significantly in the nephrotic control group relative to the normal control group (Fig. 1). Kallikrein mRNA content in the kidneys of nephrotic rats treated with enalapril was significantly greater than in the untreated nephrotic group and was not statistically different from the normal control animals.

PRA was increased significantly only in the group treated with enalapril (Table I). In contrast, renal renin content was not different among any of the three groups. Renal renin mRNA was significantly lower in the untreated nephrotic group compared to normal controls (Fig. 2). Treatment of nephrotic rats with CEI had no effect on renin mRNA content, which remained significantly decreased relative to the normal control group and was not different from the untreated nephrotic animals. Renal  $\beta$ -actin mRNA content was not different among the three experimental groups (Fig. 3).



The results of the second experiment in which normal rats were treated with the CEI, enalapril, are detailed in Table II and Fig. 4. Body weights were similar in both groups at the beginning and end of the study period. Likewise systolic blood pressure was not different between the two groups prior to CEI (untreated:  $129 \pm 7$ , treated  $128 \pm 5$  mmHg). After 4 d of CEI, blood pressure was  $124 \pm 2$  mmHg in the treated group and  $135 \pm 8$  mmHg in the untreated group, but this difference was not statistically significant.

Initial rates of urinary total kallikrein excretion were not different in either the experimental or control group from that measured in normal animals in the first experiment (Table II). Urinary total kallikrein excretion increased significantly after 4 d of CEI treatment, but did not change in the untreated control group. Renal kallikrein content, both active and total, was not different between the treated and untreated groups. In contrast to the rats with passive Heymann nephritis studied in the first experiment, enalapril had no effect on steady state renal kallikrein mRNA content (Fig. 4).

As expected, PRA was significantly higher in rats treated with CEI compared to the untreated group (Table II). Renal renin content also increased significantly in the group of normal rats treated with enalapril. Likewise renin mRNA was ten-fold higher in the normal rats treated with CEI compared to the control group (Fig. 4).  $\beta$ -actin mRNA was not different between the CEI-treated and untreated normal rats.

## Discussion

The present study was performed to characterize the kallikrein-kinin system in rats with passive Heymann nephritis and to determine the mechanism responsible for the lower urinary kallikrein excretion rate we observed previously in this nephrotic model. We confirmed our previous observation that urinary kallikrein excretion was significantly depressed in rats with passive Heymann nephritis compared to normal animals. The decrease in urinary kallikrein excretion rate was accompanied by a decrease in the level of kallikrein mRNA in the kidney, suggesting that the decreased excretion of this peptide was due, at least in part, to reduced kallikrein gene transcription or to decreased stability of transcribed kallikrein mRNA. Despite the significantly reduced levels of renal kallikrein mRNA and the low urinary kallikrein excretion rate, renal tissue kallikrein content was not diminished. Based on these data one cannot discern whether the decrease in kallikrein mRNA is the primary event responsible for the lower rate of urinary kallikrein excretion. It is possible that the decreased urinary kallikrein excretion rate is due solely to decreased synthesis of the kallikrein peptide resulting from the reduced availability of kallikrein mRNA. However, it is also possible that a defect in kallikrein secretion may result in intracellular accumulation of kallikrein and suppress transcription of the kallikrein gene. Although we did not find an increase in renal kallikrein content, if one assumes that secretion of kallikrein into the interstitium is decreased in parallel with urinary kallikrein excretion, then the normal tissue kallikrein levels, which are the sum of both intracellular and interstitial kallikrein, may actually reflect an increase in intracellular kallikrein content. Thus, elevated intracellular kallikrein levels might potentially be responsible for suppressing expression of the kallikrein gene.

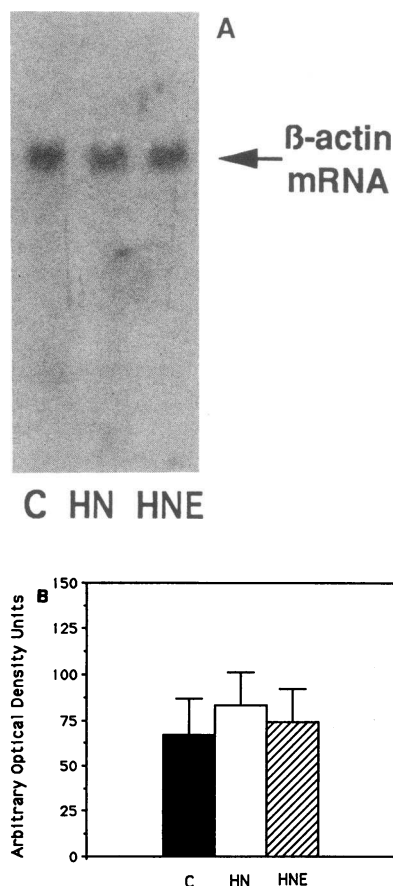
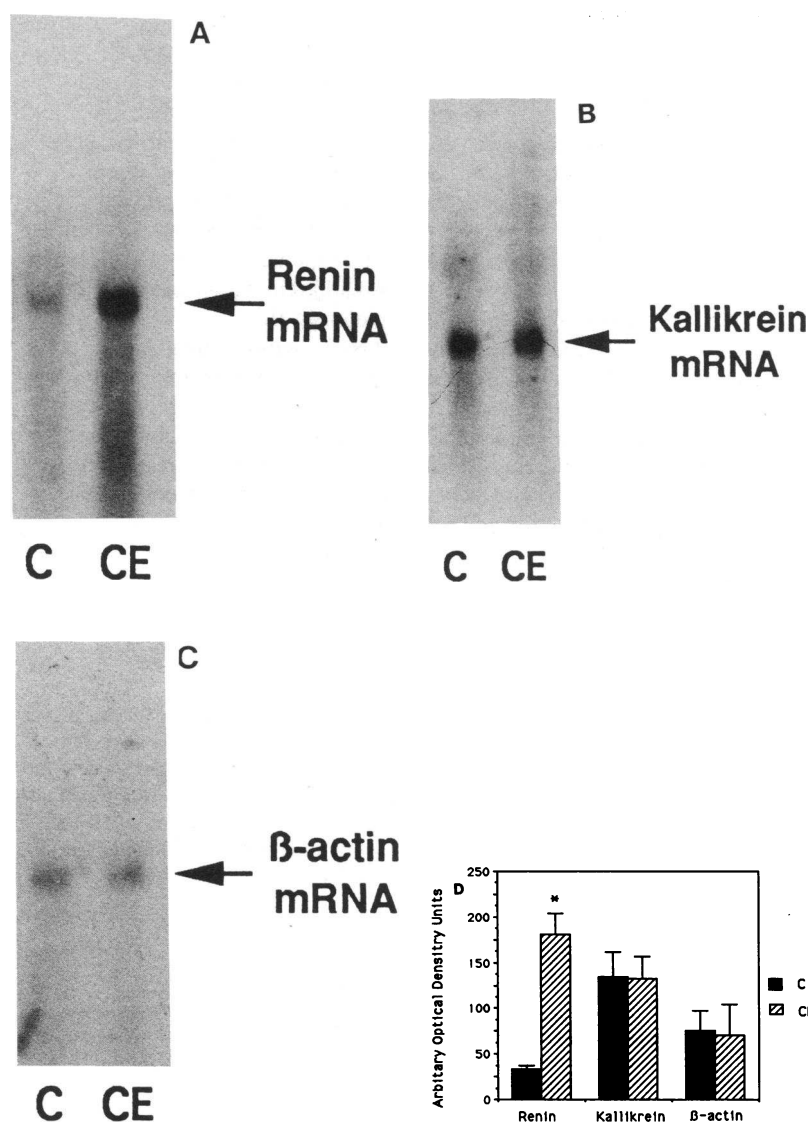


Figure 3. Renal  $\beta$ -actin mRNA in rats with passive Heymann nephritis. A shows a typical autoradiograph of a Northern blot of total kidney RNA (30  $\mu$ g) extracted from normal controls (lane C), untreated nephrotic rats (lane HN), and nephrotic rats treated with enalapril (lane HNE). A  $^{32}$ P-labeled nick-translated  $\beta$ -actin probe was used for hybridization and the autoradiograph was exposed for 48 hr. The mean values for each experimental group are depicted in B. There were no differences in renal  $\beta$ -actin content among the three groups.

Our results are consistent with those of Glasser and Michael (22) who reported a decrease in urinary kallikrein excretion in rats with nephrotic syndrome induced by antiglomerular basement membrane antiserum or by puromycin aminonucleoside. In each case, the decrease in urinary kallikrein excretion preceded or occurred concomitantly with the development of proteinuria and was related inversely to the magnitude of proteinuria. As the proteinuria resolved, urinary kallikrein excretion increased toward normal, suggesting a possible interaction between the renal kallikrein-kinin system and the development and resolution of proteinuria. Nakamura et al. (23) also found that plasma kinin-like activity was low and kininase II (converting enzyme) activity was elevated in nephrotic humans and that these abnormalities resolved as the disease remitted. Although it is possible that these observations of decreased activity of the kallikrein-kinin system in nephrotic subjects may represent a parphenomenon resulting from associated changes in volume status, diuretics, or sodium intake, or that proteinuria per se impairs kallikrein excretion, the close association between proteinuria and decreased kallikrein and kinin activity and the results of our earlier studies (5, 6) suggest a more direct interaction for the kallikrein-kinin system in the regulation of proteinuria.

CEIs have been demonstrated to potentiate kinin activity in normal subjects, but their effect on the renal kallikrein-kinin system in nephrotic subjects has not been explored previously. In the present study treatment with the CEI, enalapril, caused a significant increase in urinary kallikrein excretion as well as a



**Figure 4.** Renal kallikrein, renin, and  $\beta$ -actin mRNA in normal rats treated with enalapril. (A–C) Autoradiographs of Northern blots of total RNA (30  $\mu$ g) extracted from the kidneys of normal rats (lanes C) and normal rats treated with enalapril (lanes CE) and hybridized to the kallikrein oligonucleotide probe (B) or to renin (A) or  $\beta$ -actin (C) cDNA probes. Autoradiographs were exposed for 72, 48, and 48 h, respectively. The mean values for each experimental group are illustrated in D. Enalapril had no effect on steady state levels of renal kallikrein mRNA, but induced a significant increase in renal renin mRNA ( $P < 0.001$ ). There was no difference in steady state levels of renal  $\beta$ -actin mRNA.

decrease in albuminuria in the nephrotic rats, although the urinary kallikrein excretion rate remained substantially lower than that measured in normal rats. The increase in urinary kallikrein excretion was most likely due to an increase in translation of renal kallikrein mRNA since the steady state level of kallikrein mRNA was significantly greater in nephrotic rats

treated with the CEI compared to untreated nephrotic rats and renal kallikrein content was not changed. Since the CEI was not administered until after the onset of proteinuria, one may conclude that the higher kallikrein mRNA level was not a result of differences in the severity of glomerulonephritis between the two groups. Additionally, the increase in kallikrein mRNA

**Table II.** Effect of Converting Enzyme Inhibition on Renin and Kallikrein Metabolism in Normal Rats

Group	Day	Wt	UKK <sub>total</sub>	Renal KK <sub>act</sub>	Renal KK <sub>total</sub>	PRA	Renal renin
		g	$\mu$ g/d	ng/mg prot	ng/mg prot	ng/ml per h	$\mu$ g/mg prot/h
Control	0	223 $\pm$ 4	199.7 $\pm$ 20.8				
	4	292 $\pm$ 3*	210.7 $\pm$ 18.6	30.0 $\pm$ 2.2	46.1 $\pm$ 2.3	10.3 $\pm$ 0.9	1.00 $\pm$ 0.11
Enalapril	0	225 $\pm$ 3	206.8 $\pm$ 6.5				
	4	295 $\pm$ 3*	250.1 $\pm$ 25.0*	24.7 $\pm$ 2.8	43.7 $\pm$ 3.2	19.6 $\pm$ 1.1*	1.06 $\pm$ 0.16

\*  $P < 0.05$  vs. day 0 within a group,  $\dagger P < 0.05$  vs. same-day control group. (Wt, body weight; UKK<sub>act</sub>, urinary active kallikrein excretion rate; UKK<sub>total</sub>, urinary total kallikrein excretion rate; Renal KK<sub>act</sub>, active renal kallikrein; Renal KK<sub>total</sub>, total renal kallikrein; PRA, plasma renin activity).

seems to be a specific effect of CEI in the nephrotic rats, since CEI did not alter steady state levels of renal renin or  $\beta$ -actin mRNA in this group and had no effect on kallikrein mRNA in the normal rats. As was the case with the untreated nephrotic rats, it is not possible to distinguish whether the increased kallikrein mRNA content was due to increased gene transcription or to decreased degradation of kallikrein mRNA.

The essentially normal level of kallikrein mRNA produced by CEI treatment was accompanied by only a modest increase in urinary kallikrein excretion, implying the existence of persistent defects in synthesis and/or secretion of kallikrein in the nephrotic rats that were not entirely corrected by the CEI. Interestingly, neither the decrease in renal kallikrein mRNA after induction of nephritis nor the increase in renal kallikrein mRNA after CEI treatment altered renal kallikrein content, suggesting that renal kallikrein tissue levels are tightly regulated in a balance between synthesis and secretion.

The effects of CEI on kallikrein metabolism in normal animals were examined in the second experiment. In contrast to the nephrotic rats, CEI had no appreciable effect on renal kallikrein mRNA content. Although renal kallikrein content was not altered, urinary kallikrein excretion increased significantly, suggesting that the CEI stimulated synthesis and/or secretion of kallikrein in the normal animals. Thus, the capacity of CEI to increase kallikrein mRNA content appears to be restricted to the nephrotic rats with their very low basal kallikrein mRNA levels, but the effect of CEI to increase urinary kallikrein excretion rate is expressed regardless of basal excretion rate or the presence of proteinuria.

Since CEIs clearly modify the activity of the renin-angiotensin system and may interact directly with the kallikrein-kinin system, we also examined the effect of induction of passive Heymann nephritis and CEI on PRA, tissue renin content, and steady state renal renin mRNA content. Although PRA and renal renin content were not altered in rats with passive Heymann nephritis, renal renin mRNA content was significantly reduced compared to normal rats. The maintenance of normal PRA and tissue renin levels suggest that synthesis of renin from available mRNA may be accelerated or that there may be increased peripheral conversion of inactive to active renin in these nephrotic rats.

Administration of the CEI to normal animals increased both PRA and renal renin mRNA levels, consistent with reports from other investigators (24). Treatment of the nephrotic rats with the CEI resulted in an increase in PRA, but did not affect either renal renin content or renin mRNA content. We have previously demonstrated that the dose of enalapril used in these studies effectively blocks the pressor response to large exogenous doses of angiotensin I in rats with passive Heymann nephritis (5). Additionally, in the present study blood pressure and urinary albumin excretion rate both dropped significantly after treatment with the CEI. Thus, the lack of an increase in renin mRNA in the nephrotic rats cannot be ascribed to inadequate inhibition of converting enzyme. Furthermore, since kallikrein mRNA increased in response to CEI and  $\beta$ -actin mRNA was not affected by CEI, the failure of renin mRNA to increase in response to CEI was not due to a generalized suppression of gene transcription, but must be a specific consequence of the nephritis on the renin-angiotensin system.

In normal animals the increase in plasma renin activity after CEI is accompanied by an increase in renin mRNA, as

was the case in the present study, and is believed to result from a loss of the tonic negative feedback from angiotensin II. However, kallikrein has been reported to increase renin release (25) and to convert inactive renin to the active peptide (26), and has been proposed to play an important role in the normal regulation of renin. Since urinary kallikrein excretion is increased by administration of CEI, it is possible that the increase in plasma renin activity observed in the nephrotic rats is the result of renin release and/or peripheral conversion of inactive to active renin induced by kallikrein rather than by loss of negative feedback from angiotensin II.

In summary, the present study demonstrates that the activity of the renal kallikrein-kinin and renin-angiotensin systems both are impaired at the level of gene transcription in rats with passive Heymann nephritis. Treatment with a CEI normalizes renal kallikrein mRNA levels and partially restores urinary kallikrein excretion as well as reducing urinary albumin excretion, but does not modify the deficit of renal renin mRNA.

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