Kidney International, Vol. 33 (1988), pp. 787–791

Albuminuria causes lysozymuria in rats with Heymann nephritis

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Albuminuria causes lysozymuria in rats with Heymann nephritis. To determine if changes in dietary protein intake alter renal excretion of small molecular weight proteins in passive Heymann nephritis, 21 rats with passive Heymann nephritis were fed 8.5% protein for 12 days after injection with antiserum. Dietary protein intake was then increased to 40% in 10 rats (LP-HP) while 11 rats remained on 8.5% protein (LP-LP). Lysozymuria ($U_{lys}V$) increased from 66.5 ± 31.0 mcg/day to 457.5 ± 98.0 mcg/day ($P < 0.001$) after five days in LP-HP, but was unchanged in LP-LP. Albuminuria ($U_{alb}V$) increased only in LP-HP, from 168 ± 23 mg/day to 447 ± 45 mg/day ($P < 0.001$). Urinary lysozyme excretion correlated with $U_{alb}V$ ($r = 0.737$, $P < 0.001$), and changes in $U_{lys}V$ correlated with changes in $U_{alb}V$ ($r = 0.657$, $P < 0.01$). To determine whether the increase in $U_{lys}V$ was the direct effect of the change in diet, enalapril 40 mg/kg/day was administered to prevent the increase in $U_{alb}V$ that occurs when these rats are fed a high protein diet. Twelve rats were fed 8.5% (LP) and 10 were fed 40% protein (HP) from the time of injection with antiserum. Six LP (LPE) and five HP (HPE) received enalapril. $U_{lys}V$ was 873 ± 391 mcg/day in HP and nearly undetectable in the other three groups. $U_{alb}V$ was significantly greater in HP (368 ± 60 mg/day) compared to the other three groups (114 ± 16 in LP, 136 ± 44 in HPE, 95 ± 21 in LPE). A third group of nephrotic rats, maintained on a constant diet of 21% protein had enalapril added to their drinking water. $U_{lys}V$ decreased from 49 ± 9 mcg/day to less than 2 mcg/day ($P < 0.001$) and $U_{alb}V$ decreased from 516 ± 67 to 183 ± 32 mg/day ($P < 0.001$). Both $U_{lys}V$ and $U_{alb}V$ remained unchanged in untreated rats. Lysozyme, an enzyme normally entirely reabsorbed by the kidney, is found in the urine of rats with passive Heymann nephritis, and increases when dietary protein intake is increased. High protein diets increase $U_{lys}V$ only in as much as $U_{alb}V$ is increased, and when $U_{alb}V$ is reduced by use of an angiotensin converting enzyme inhibitor in the presence of a high protein diet $U_{lys}V$ is reduced in a parallel fashion, suggesting that albuminuria itself decreases the capacity of the renal tubule to reabsorb lysozyme.

Urinary protein excretion has characteristically been classified as being primarily of glomerular or of tubular origin [1, 2]. Most small molecular weight proteins are filtered freely by the glomerulus and are then reabsorbed and efficiently catabolized by the renal tubules [3, 4]. The filtration of low molecular weight proteins is so great relative to inulin, that an increase in glomerular permeability to large macromolecules, such as albumin or IgG, would not be expected to increase the quantity of these small peptides delivered to the glomerular ultrafiltrate. An increase in the urinary excretion of low molecular weight proteins should result instead from a reduction in the capacity of the renal tubule to reabsorb them [1–5]. Hardwicke and Squire [6] demonstrated that intravenous infusion of albumin into nephrotic patients resulted in an increase in both urinary IgG and albumin excretion. Increased IgG excretion was attributed to saturation of renal reabsorptive capacity by the increased filtered load of albumin. However, IgG is restricted by the glomerular basement membrane on the basis of size [7] and an alteration in glomerular size selectivity as a result of plasma volume expansion could increase its renal clearance, without requiring a reduction in its tubular reabsorption. Furthermore, plasma volume expansion that takes place with infusion of albumin might alter glomerular permselectivity in such a manner as to nonselectively increase the renal clearance of high molecular weight macromolecules, including that of both albumin and IgG [8]. Indeed, Earley et al [9] were able to increase the renal clearance of albumin in nephrotic patients by the intravenous infusion of either albumin or dextran.

Alterations in the urinary excretion of low, rather than high molecular weight proteins are more likely to result from changes in tubular protein reabsorption, since their renal clearance is not increased by changes in glomerular permselectivity. Lysozyme is found in very low concentration in the urine of normal humans [1, 2] and is nearly undetectable in the urine of normal rats. Preliminary studies in our laboratory found lysozyme to be present in the urine of rats rendered nephrotic by intravenous injection of adriamycin, and the urinary excretion of this enzyme was affected by dietary protein intake [10]. Although proximal tubular injury occurs in the kidney of rats with active Heymann nephritis [11], no such damage has been reported in rats with passive Heymann nephritis [12]. Urinary albumin excretion can be manipulated in passive Heymann nephritis by modification of dietary protein content [13], or by the use of angiotensin converting inhibitors [14]. It is therefore possible to alter urinary albumin excretion without the intravenous infusion of protein. In the following study albuminuria was altered by manipulation of dietary protein intake and by the use of the angiotensin converting enzyme inhibitor enalapril, in order to determine whether changes in the urinary excretion of lysozyme would parallel changes in urinary albumin excretion.

Received for publication May 29, 1987
and in revised form November 20, 1987

Methods

Animals

Protocol 1. Effect of increasing dietary protein intake on $U_{lys}V$ in rats with established proteinuria.

Twenty-one 120 g, male Sprague-Dawley rats (Bantin Kingman Farms, Fremont, California, USA) were each injected intraperitoneally with sheep antibody to FX-1A (4 ml/kg) to produce passive (heterologous) Heymann nephritis [11, 15]. Animals were housed in a temperature regulated, light/dark cycled room and fed a diet containing 8.5% protein as casein (Purina Purified Protein Diet 5769, Ralston Purina Co., Richmond, Indiana, USA) for 10 days after the injection with antiserum. Glomerular filtration rate (GFR) was measured as the renal clearance of [^{51}Cr]ethylene diamine tetraacetic acid (EDTA), using implanted osmotic pumps (Alza Corp., Palo Alto, California, USA) [15] starting on day thirteen. The chromium was obtained as the chloride (New England Nuclear, Boston, Massachusetts, USA) and was reacted with excess sodium EDTA (100 mM) at pH 6.0 to form the chromium chelate. Eleven days after the administration of antiserum, each animal was anesthetized with sodium pentobarbital (20 mg/kg) and an osmotic infusion pump—0.2 ml volume—containing 500 μCi of [^{51}Cr]EDTA was inserted subcutaneously in the back of the neck. Rats were placed in metabolic cages, and after allowing two days for acclimatization, daily 24-hour urines were collected from each animal for four consecutive days. Blood, 250 μl, was collected from the tail vein of each animal without anesthesia each day. On day 14, dietary protein intake was increased to 40% in 10 animals (LP-HP). The change in diet was accomplished by placing the animals on Purina purified protein diet 5779, which isocalorically replaced carbohydrate—a 50/50 mixture of dextrose and dextrin, with casein. The two diets contain identical quantities of Na, K, P, and fat, and are supplemented with methionine. Eleven animals remained on the 8.5% protein diet as time controls (LP-LP). Glomerular filtration rate was not measured in three animals in the LP-LP group. Urine and serum samples were counted in a gamma counter (Searle 1185, Searle Analytics Inc. Des Plaines, Illinois, USA) and GFR calculated as the renal clearance of [^{51}C]EDTA.

Protocol 2. Use of an angiotensin converting enzyme inhibitor to separate the effect of dietary protein intake from that of $U_{alb}V$ on lysozymuria.

In this protocol dietary protein intake was fixed at the time of injection with anti-serum, before the development of albuminuria. Twenty-two male, Sprague-Dawley rats were each injected with antiserum, as described in protocol 1, and then placed on either the 8.5% (LP) or the 40% (HP) protein diet. We have previously shown that enalapril, an angiotensin converting enzyme inhibitor, will prevent the increase in urinary albumin excretion that occurs in rats with passive Heymann nephritis when they are fed the high protein diet [14]. By adding enalapril to the drinking water of animals fed a high protein diet, the direct effect of dietary protein content on urinary lysozyme excretion could be separated from effects of albuminuria on urinary lysozyme excretion. Enalapril, 40 mg/kg, was therefore added to the drinking water of six rats on the 8.5% protein diet (LPE), and five of the rats eating 40% protein (HPE). Rats were placed in metabolic cages for collection of urine on day 13 after injection with antiserum.

Protocol 3. Effect of angiotensin converting enzyme inhibition on lysozymuria, independent of dietary manipulations.

In order to determine whether reduction in urinary albumin excretion would lead to a parallel reduction in urinary lysozyme excretion in the absence of any dietary change, dietary protein intake was fixed at 21% at the time of injection with antiserum and enalapril was then added to the drinking water. Thirteen rats (120 g, male Sprague-Dawley) were injected with antiserum and placed in Purina diet 5755 containing 21% protein. This diet is isocaloric to both the 8.5% and 40% protein diets and contains an identical mineral and fat composition. On day 12 following injection with antiserum, osmotic infusion pumps containing [^{51}Cr]EDTA were placed subcutaneously as described above, and rats were placed in metabolic cages. Urine was collected daily, and blood was obtained from the tail vein, as above, for three consecutive days for basal measurements. Following these measurements, enalapril (40 mg/kg) was added to the drinking water of seven animals. The remaining animals served as time controls. Urine was collected for the next three days, and blood taken daily.

Laboratory measurements

Albumin was measured in both serum and urine using immunoelectrodiffusion [16] as previously described [13]. Lysozyme was measured by the method of Schill and Schumacher [17] using hen's egg lysozyme (Sigma Chemical Co. St. Louis, Missouri, USA) as a standard.

Statistics

Analysis of normally distributed data was by either Student's paired *t*-test or by analysis of variance. Non-normally distributed data were analyzed by Kruskal Wallis one-way analysis of variance by ranks [18].

Results

Urinary lysozyme excretion ($U_{lys}V$) was 66.5 ± 31.0 mcg/day during the period when rats were fed an 8.5% protein diet in Protocol 1 (Fig. 1), and increased to 457.5 ± 98.0 mcg/day ($P < 0.001$) by day 5 after dietary protein intake was increased in LP-HP, but remained unchanged in LP-LP (Fig. 2). Albuminuria ($U_{alb}V$) increased in LP-HP from 168 ± 23 mg/day on day 0 to 447 ± 45 mg/day ($P < 0.001$) by day 5 (Fig. 1), while remaining unchanged in LP-LP (Fig. 2). Within this group of animals, $U_{lys}V$ correlated with $U_{alb}V$ ($r = 0.737$, $P < 0.001$, $N = 42$; Fig. 3), and changes in $U_{lys}V$ correlated with changes in $U_{alb}V$ ($r = 0.657$, $P < 0.01$, $N = 21$). GFR (the renal clearance of [^{51}Cr]EDTA) was 0.85 ± 0.05 during the low protein period, and was 0.73 ± 0.05 during the high protein period in LP-HP (NS). Similarly, GFR remained unchanged during the two periods of measurements in LP-LP as well (0.81 ± 0.07 during the first period of measurement and 0.72 ± 0.07 during the second period of measurement).

Protocol 2 was performed to determine whether the change in $U_{lys}V$ was a direct effect of the change in diet, or instead a result of the change in $U_{alb}V$. The angiotensin converting enzyme inhibitor, enalapril was added to the drinking water two days after injection with antiserum, prior to the onset of albuminuria. Urinary albumin excretion was significantly greater in HP compared to the other three groups (Table 1), and $U_{lys}V$ was also significantly greater in the nephrotic rats fed a 40% protein

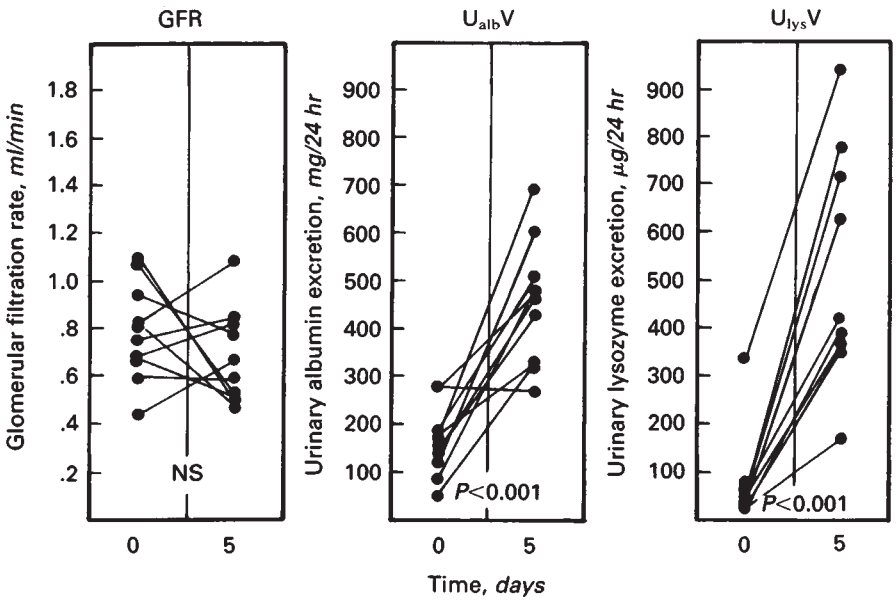

Fig. 1. *The effect of an increase in dietary protein intake from 8.5% to 40% on glomerular filtration rate (GFR), urinary albumin excretion ($U_{alb}V$), and urinary lysozyme excretion $U_{lys}V$ in rats with Heymann nephritis.*

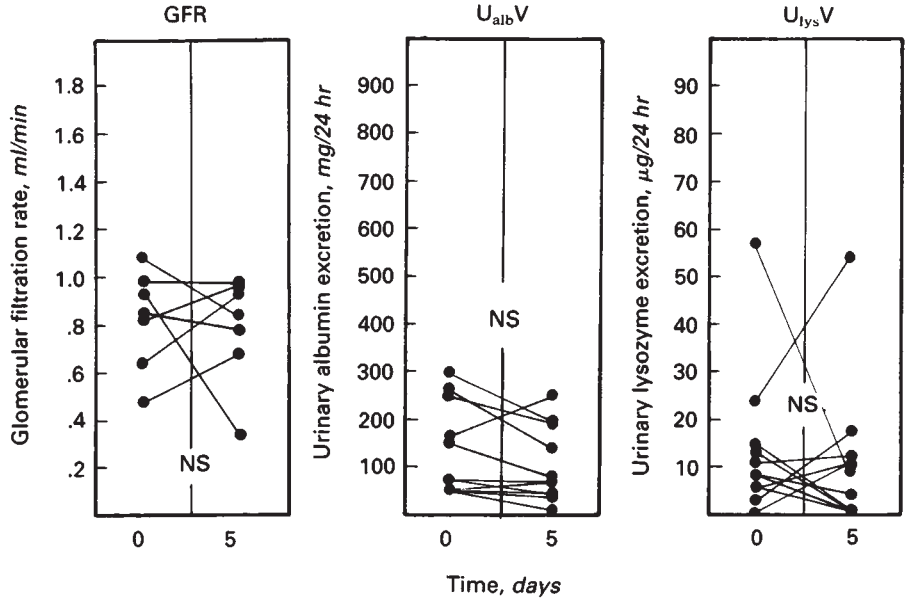

Fig. 2. *Glomerular filtration rate, $U_{alb}V$ and $U_{lys}V$ in rats with Heymann nephritis maintained on an 8.5% protein diet.*

diet when compared to any of the other three groups of animals. Indeed, urinary lysozyme was undetectable in nearly all of the animals in each of the other three groups (Table 1).

Protocol 3 was performed in order to remove the variable of dietary protein content from issue. The administration of enalapril to nephrotic rats maintained on a constant (21%) protein diet resulted in a prompt and reproducible reduction in urinary albumin excretion as well as a parallel decrease in urinary lysozyme excretion (Table 2). Glomerular filtration rate was not reduced by administration of enalapril. Nephrotic animals that remained untreated during this period maintained a constant urinary loss of both albumin and lysozyme and glomerular filtration rate did not change in this group of animals (Table 2).

Discussion

We have clearly shown that urinary lysozyme excretion is increased in rats with passive Heymann nephritis, and is proportional to the urinary albumin excretion. Lysozyme excretion is increased in parallel with that of albumin after dietary protein is increased, and is reduced in parallel with the reduction in urinary albumin excretion caused by administration of the angiotensin converting enzyme inhibitor, enalapril. It is

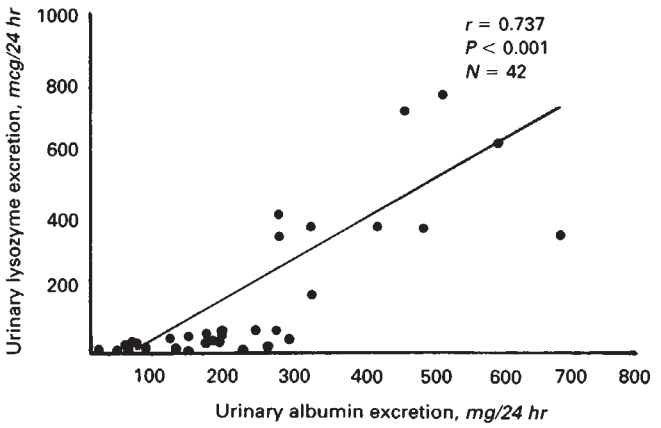

Fig. 3. *Relationship between urinary albumin and lysozyme excretion in rats with Heymann nephritis.*

Table 1. Effect of dietary protein content and of angiotensin converting enzyme inhibition on urinary lysozyme and albumin excretion in nephrotic rats

Experimental group	Urinary albumin excretion *mg/day*	Urinary lysozyme excretion *mcg/day*
8.5% Protein ($N = 6$)	114 ± 16	13.1 ± 11.2
8.5% Protein plus enalapril ($N = 6$)	95 ± 21	<2
40% Protein ($N = 5$)	368 ± 60[b]	873 ± 392[a]
40% Protein plus enalapril ($N = 5$)	136 ± 44	<2

[a] $P < 0.001$ with respect to each of the other three groups
[b] $P < 0.05$ with respect to each of the other three groups

Table 2. Effect of angiotensin converting enzyme inhibition on urinary lysozyme and albumin excretion in nephrotic rats during consumption of a constant 21% protein diet

	Enalapril treated $N = 7$		Untreated $N = 6$	
Time	Day 1	Day 6	Day 1	Day 6
Urinary albumin excretion *mg/day*	516 ± 67	183 ± 32	404 ± 57	374 ± 45
P	< 0.001		NS	
Urinary lysozyme excretion *mcg/day*	49 ± 9	<2	42 ± 33	32 ± 23
P	< 0.001		NS	
GFR *ml/min*	1.21 ± 0.07	1.14 ± 0.08	1.15 ± 0.16	1.07 ± 0.1
P	NS		NS	

Measurements of the urinary excretion of lysozyme, albumin, and glomerular filtration rate during a baseline period (day 1) and three days after the addition of enalapril (40 mg/kg) to the drinking water (day 6). Rats ate a 21% protein diet. Enalapril was added on day 3.

unlikely that dietary protein intake directly alters the ability of the proximal tubule to reabsorb lysozyme to the extent observed here, since the increase in urinary lysozyme excretion, as well as the increased excretion of albumin caused by the high protein diet, could be prevented by administration of enalapril. The alterations in urinary albumin excretion that occur in rats with passive Heymann nephritis resulting from the use of angiotensin converting enzyme inhibitors or from dietary manipulations are most likely the result of altered glomerular permselectivity to large macromolecules. The renal clearance of lysozyme is unlikely to be measurably increased by such a process, since lysozyme is filtered by the glomerulus with an efficiency of between 80 and 100% that of inulin, even in the absence of glomerular injury [19].

Net charge and charge density are major determinants of the kinetics of uptake of protein within the proximal tubule [20]. Lysozyme is a highly cationic protein. It is possible that the effect of albuminuria on the renal excretion of anionic proteins might be different than that demonstrated here for lysozyme. While this point must be tested experimentally, derivatized cationic proteins are usually reabsorbed more avidly than are their anionic counterparts [20]. Although the ability of the proximal tubule to efficiently reabsorb filtered protein may be less than optimal in passive Heymann nephritis as a direct result of interaction of the antibody with the proximal tubule cells, there is no strong evidence that a general defect in tubular solute reabsorption occurs in this model [15], nor is there any clear mechanism whereby a general defect in proximal tubular function would be markedly worsened by ingestion of a high protein diet, and corrected or prevented by administration of an angiotensin converting enzyme inhibitor.

Proteinuria has classically been divided into glomerular proteinuria, resulting from an increase in the filtered load of protein, or tubular proteinuria, resulting from decreased tubular reabsorption of protein [1, 2]. Emphasis has been focused on the differences between "glomerular" and "tubular" proteinuria as being quantitative, rather than qualitative; that is, both large and small molecular weight proteins appear in the urine in either condition, but the proportion of small molecular weight to large molecular weight proteins is much greater in the presence of tubular disease [1, 2]. The increase in urinary excretion of large molecular weight proteins, predominantly albumin, in the presence of tubular disease is due to the fact that albumin, as well as small molecular weight proteins, is reabsorbed by the proximal tubule. Tubular disease results in the disruption of the reabsorption of the small amount of albumin that is filtered by the normal glomerulus with resulting increase in its urinary excretion. If patients with a variety of renal diseases are considered together, no clear relationship is discernible between urinary protein excretion and the urinary excretion of the specific small molecular weight protein, lysozyme, although there is a striking correlation between the

urinary appearance of lysozyme and a variety of other small molecular weight proteins [2]. In patients with renal tubular disease the urinary excretion of specific low molecular weight proteins correlate with total urinary protein excretion, and within the population of patients with glomerular disease urinary lysozyme excretion increases in proportion to increasing total urinary protein [2]. These observations have led to the correct conclusion that low molecular weight proteinuria results from damage to the proximal tubule, and to the less well founded conclusion that diseases of glomerular origin are not associated with an increase in the renal excretion of low molecular weight proteins.

We have demonstrated that lysozymuria may occur in rats with passive Heymann nephritis, and its occurrence is in proportion to the magnitude of albuminuria. These findings are in accord with those of Straus, who demonstrated that an injection of ovalbumin reduced proximal tubular uptake of horseradish peroxidase in the rat [21]. We can only speculate as to the mechanism responsible for this phenomenon. Reabsorption of protein by the proximal tubule is known to occur by two separate processes. Proteins are either bound to receptors and internalized, or reabsorption occurs by fluid phase endocytosis [22]. The reabsorbed proteins are then transported to lysosomal vesicles where they are catabolized [20]. The membrane that engulfed the endocytosed protein is then returned to the luminal surface. It is possible that the massive increase in albumin presented to the cells of the proximal tubule for reabsorption in some way limits the recycling of plasma membrane necessary for endocytosis of proteins, and impairs the reabsorption of other proteins that are normally reabsorbed completely.

Acknowledgments

Sheep anti-FX-1A antiserum used to produce proteinuria was a kind gift from Dr. William Couser. This work was supported in part by the research service of the United States Veterans Administration. A portion of this work has been published in abstract form in *Clin Res* 35:174A, 1987.

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