Characterization of proteinuria and tubular protein uptake in a new model of oral L-lysine administration in rats

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Intravenous infusion of basic amino acids is used experimentally and pharmacologically to prevent renal proximal tubular uptake of filtered proteins. Intravenously injected L-lysine is rapidly cleared from plasma and the effect on tubular protein reabsorption is transient. To obtain a more sustained effect, we developed a model of oral L-lysine administration and characterized this model by analyzing urinary protein excretion and proximal tubule uptake of filtered proteins. Rats placed in metabolic cages were treated with 20 mmol/kg/6 h of L-lysine, glycine, or water. Urines were analyzed for proteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, immunoblotting, and radioimmunoassay. Proximal tubule uptake of proteins and expression of apical membrane receptors were investigated by immunocytochemistry. *In vitro* uptake and receptor expression were studied using a yolk sac cell line. L-lysine administration produced increased urinary excretion of a large number of proteins while the effect on tubular accumulation of selected proteins was variable. L-lysine treatment induced changes in the localization of two receptors responsible for tubular endocytosis of filtered proteins. In conclusion, oral L-lysine treatment induced proteinuria, in particular albuminuria, as efficiently as previous reports on intravenous infusion. The effect on tubular protein accumulation was variable suggesting differential effects on tubular reabsorption and degradation of filtered proteins. Changes in tubular protein handling were accompanied by changes in the localization of the endocytic receptors, megalin, and cubilin. *In vitro* experiments supported the *in vivo* observations. The findings suggest that L-lysine may affect receptor trafficking in addition to possible effects on the direct binding of ligands to the receptors.

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Tubular uptake of filtered protein is mediated by proximal tubule endocytosis. This process involves the initial binding to endocytic receptors at the luminal surface of the proximal tubule cells. Previous studies have established two multiligand receptors, megalin and cubilin, as responsible for the endocytic uptake of a large number of different proteins and peptides, including lipoproteins, hormones, nutrients, and enzymes.[1,2] Both receptors are heavily expressed at the luminal surface of proximal tubule cells and other absorptive epithelia, including yolk sac epithelial cells. Inhibition of proximal tubule reabsorption of proteins is an important experimental approach for the study of glomerular permeability and tubular function. Also inhibition of renal uptake has been introduced to protect the kidney from filtered, nephrotoxic agents, for example radiolabeled peptides and antibody fragments used experimentally as targeted anti-cancer drugs.[3–9] Basic amino acids, notably L-lysine, inhibit the reabsorption of proteins in the proximal tubule whereas neutral or acidic amino acids do not.[10,11] Inhibition of proximal tubule endocytosis of plasma proteins and peptides by L-lysine infusion has been evaluated in several experimental studies[12–14] primarily based on analysis of the excretion of specific proteins in the urine.

The present study characterizes a new model of orally administered L-lysine to inhibit tubular protein uptake. We have characterized the effect of L-lysine treatment on tubular protein handling by comparing, qualitatively and quantitatively, the urinary protein excretion and cellular uptake of selected, endogenous proteins, including albumin, vitamin D-binding protein (DBP), retinol-binding protein (RBP), transferrin, and β_2-microglobulin. Urinary protein excretion was characterized by one- and two-dimensional gel electrophoresis, immunoblotting, and radioimunoassay whereas tubular uptake of proteins and receptor expression were analyzed by immunocytochemistry. Futhermore, megalin- and cubilin-expressing yolk sac BN16 cells were exposed to L-lysine and the effects on albumin endocytosis and the expression of endocytic receptors were analyzed.

RESULTS

Oral L-lysine treatment

Rats generally tolerated oral L-lysine treatment although one L-lysine-treated rat was excluded after 24 h of L-lysine treatment because of diarrhea. One rat exhibited heavy proteinuria prior to the administration of glycine and was excluded.

Oral L-lysine treatment resulted in a significant, sixfold increase in plasma L-lysine concentration compared to water treated rats (Table 1) and caused a significant, 46- and 33-fold increase in urinary L-lysine excretion after 24 and 30 h of treatment, respectively, compared to baseline levels (Table 2). Plasma levels of glycine were increased fivefold in the glycine-treated rats (Table 1) resulting in a significant 12-fold increase in urinary glycine excretion after 24 h of treatment (Table 2). No significant changes in the plasma levels of sodium, potassium, glucose, or creatinine were observed, either in L-lysine- or glycine-treated rats. Glycine treatment caused a significant increase in plasma urea (Table 1) and urinary urea excretion after 24 h of treatment compared to baseline levels (Table 2). L-Lysine treatment induced an increase in urine output from 12.5 to 25 μl/min after 24 h treatment (Table 2). Oral L-lysine treatment did not result in any significant changes in the urinary excretion of sodium, creatinine, or urea (Table 2). A urine glucose dip-stick test was negative in all rats (Table 2). Electron microscopy of the glomerulus and proximal tubule cells showed no ultrastructural changes after L-lysine treatment (Figure 1a and b).

Table 1 | Plasma analysis in rats treated orally for 30 h with L-lysine (n=4), glycine (n=4), or water (n=5)

	L-Lysine treatment	Glycine treatment	Water treatment
L-Lysine (mM)	5.92±1.69[a]	1.16±0.23	0.99±0.21
Glycine (mM)	0.35±0.03	2.42±0.50[b]	0.49±0.09
Creatinine (μM)	42.2±3.59	37.8±2.36	36.8±3.42
Urea (mM)	7.98±2.60	10.20±0.43[c]	7.02±0.76
Sodium (mM)	133±19	155±20	143±11
Potassium (mM)	3.83±0.40	3.71±0.33	3.68±0.34
Glucose (mM)	6.94±0.95	9.24±2.30	8.63±0.76

Analyzed by one-way analysis of variance with *post hoc* *t*-test and Bonferroni correction. $P<0.05$ is considered significant.
Values are expressed as mean±s.d.
[a]P=0.001 vs glycine treatment and P=0.0003 vs water treatment.
[b]P=0.0002 vs L-lysine treatment and P=0.00006 vs water treatment.
[c]P=0.0001 vs water treatment.

Table 2 | Results of urine analysis in rats before, after 24, and 30 h of oral treatment with L-lysine (n=4) or glycine (n=4)

	L-lysine treatment		
	Before	0–24 h	24–30 h
Urine output (μl/min)	12.5±1.6	25.0±4.2[a]	18.9±2.2
L-Lysine (μmol/min)	0.07±0.03	3.23±1.23[b]	2.31±0.88[b]
Glycine (μmol/min)	0.50±0.21	0.26±0.10	0.20±0.09
Albumin (μg/min)	0.30±0.18	1.08±0.15[c]	1.74±0.26[d]
Creatinine (nmol/min)	46.0±2.7	31.4±7.8	41.1±±11.9
Urea (μmol/min)	7.8±0.7	7.0±1.2	7.9±1.9
Sodium (μmol/min)	0.80±0.13	1.41±0.34	1.04±0.34
Glucose	Negative	Negative	Negative
	Glycine treatment		
	Before	**0–24 h**	**24–30 h**
Urine output (μl/min)	12.2±4.2	14.7±4.6	13.5±5.3
L-Lysine (μmol/min)	0.06±0.03	0.08±0.06	0.03±0.02
Glycine (μmol/min)	0.45±0.14	5.61±1.20[e]	2.14±1.37
Albumin (μg/min)	0.49±0.25	0.51±0.24	0.43±0.26
Creatinine (nmol/min)	48.9±6.3	44.3±5.5	58.7±12.3
Urea (μmol/min)	9.0±1.5	11.9±2.1[f]	13.2±3.5
Sodium (μmol/min)	0.94±0.19	0.84±0.24	0.96±0.33
Glucose	Negative	Negative	Negative

Values are expressed as mean±s.d.
P-values vs before treatment: [a]P=0.009; [b]P=0.014; [c]P=0.0001; [d]P=0.005; [e]P=0.004; [f]P=0.006.
Analyzed by one-way ANOVA with *post hoc* paired *t*-test and Bonferroni's correction. $P<0.05$ is considered significant.

Urinary protein analysis

One-dimensional electrophoresis and silver staining of urine from rats receiving water, glycine, or L-lysine revealed extensive proteinuria in L-lysine-treated rats and several protein bands were identified exclusively in urine from L-lysine-treated rats (Figure 2, lanes 9–12). There was no difference in urinary protein content following glycine treatment (Figure 2, lanes 5–8) compared to water treatment (Figure 2, lanes 1–4). Thus, in the following rats treated with glycine will be referred to as controls.

Urine pooled from four control or four L-lysine-treated rats was analyzed by two-dimensional gel electrophoresis (Figure 3). Oral L-lysine treatment induced excretion of a large variety of primarily acidic proteins (Figure 3b) not identified in the control urine (Figure 3a). Immunoblotting allowed the identification of several of these spots as immunoreactive for albumin or DBP (Figure 3b) and thus representing these proteins or fragments thereof. Haptoglobin, hemopexin, and angiotensinogen were identified by mass spectrometry (Figure 3b). None of the latter proteins have previously been identified in L-lysine-induced proteinuria

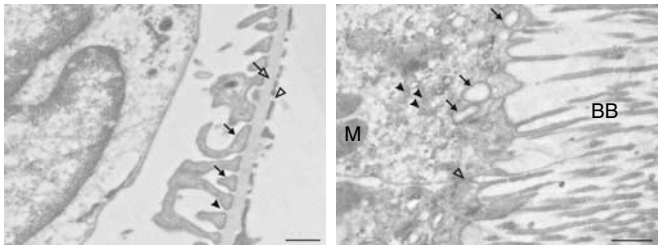

Figure 1 | Renal ultrastructure following oral L-lysine treatment. (**a**) Electron micrographs of glomerular basement membrane, and (**b**) proximal tubule cell from rat treated with L-lysine for 30 h. (**a**) An intact filtration barrier can be identified including fenestrations of the endothelium (white arrowhead), an intact basement membrane (white arrow), and filtration slits (black arrowhead) between the foot processes of the podocyte (black arrows). (**b**) The ultrastructure of the proximal tubule cell also appears normal with endocytic vacuoles (black arrows), dense apical tubules (black arrowheads), and tight junction (white arrowhead). BB: brush border; M: mitochondria. Bars equal 1 μm.