Lysine Fluxes across the Jejunal Epithelium in Lysinuric Protein Intolerance

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A B S T R A C T Lysinuric protein intolerance (LPI) is one of a group of genetic diseases in which intestinal absorption of the diamino acids lysine, arginine, and ornithine is impaired. In LPI, the clinical symptoms are more severe than in the kindred disorders. The mechanism of lysine absorption was, therefore, investigated in vitro on peroral jejunal biopsy specimens in seven patients with LPI and 27 controls. The lysine concentration ratio between cell compartment and medium was significantly higher in the LPI group (mean ± SEM, 7.17±0.60) than in the controls (5.44±0.51). This was also true for the intracellular Na concentration (LPI, 73.6±10.8 mM; controls 42.3±3.7 mM). The rate of unidirectional influx of lysine across the luminal membrane was Na dependent and was the same in the two groups. In the absence of an electrochemical gradient, net transepithelial lysine secretion was observed in LPI. This was entirely the result of a 60% reduction of the unidirectional flux from mucosa to serosa. Calculation of unidirectional fluxes revealed the most striking difference at the basolateral membrane, where the flux from cells to serosa was reduced by 62% and the corresponding permeability coefficient reduced by 71%. A progressive reduction in short-circuit current appeared in the epithelia of all four patients with LPI tested after addition of 3 mM lysine. Thus, LPI appears to be the first disease in which a genetically determined transport defect has been demonstrated at the basolateral membrane.

INTRODUCTION

Excessive urinary excretion of the diamino acids lysine, arginine, and ornithine (with or without cystine) characterizes a group of genetic disorders whose clinical manifestations differ considerably (1). In fact, French-Canadian patients with “hyperdibasicaminoaciduria” display no constant symptoms at all (2). In cystinuria, a tendency to develop urinary tract stones is probably the sole clinical problem (1). Protein-tolerant patients with hyperdiaminoaciduria (3) may have gastrointestinal complaints, impaired growth, and mental retardation. In contrast, patients with lysinuric protein intolerance (LPI) have severe symptoms, including hyperammonemia and diarrhea (4).

In most of these diseases there is a defect in the transport of diamino acids, not only in the proximal renal tubules but also in the jejunal epithelium. In both LPI and cystinuria, oral loads have revealed subnormal absorption of diamino acids (5-11). However, puzzling differences in diamino acid transport appear between cystinuria and LPI: (a) in jejunal biopsies, lysine accumulation against a concentration gradient is impaired in cystinuria (12-14) but not in LPI (15), (b) absorption of the diamino acids from dipeptides is normal in cystinuria (16) but subnormal in LPI (17), and (c) transport in the liver is also defective in LPI but presumably normal in cystinuria (18, 19).

This study confirms in vitro that the transport defect in LPI is localized to the basolateral membrane of the epithelial cell. The defect is associated with an increased concentration of intracellular sodium and an unsteady short-circuit current while lysine is present in the incubation medium.

METHODS

Seven patients with LPI (1.1-34 yr) were studied. Two were Moroccan sibs whose parents were not consanguineous. Both had failed to thrive after weaning, suffered from vomiting.

1 Abbreviations used in this paper: c, cell compartment; C/M, ratio of the intracellular to extracellular L-lysine or Na concentration; Isc, short-circuit current; J, flux; LPI, lysinuric protein intolerance; m, mucosal bathing solution; P, permeability coefficients; s, serosal bathing solution.

Received for publication 4 October 1979 and in revised form 25 January 1980.
and later from protein aversion. Both displayed hepato- and splenomegaly, osteoporosis, short stature (heights -2.5 and -4.0 SD at the age of 10 mo), and hyperammonemia after meals. The concentrations of lysine, arginine, and ornithine were subnormal in plasma but increased in urine. The other patients were Finnish. Three of them were included in an earlier report (4). The two other patients were unrelated children with the symptoms and findings pathognomonic of LPI (4).

All patients were free of gastrointestinal symptoms at the time of the study. Treatment with amino acid supplement (20) was stopped at the latest 2 d before the intestinal biopsy. All pieces of jejunum were histologically normal.

The 27 control subjects (0.2-39 yr) fulfilled the criteria of absence of diarrhea, normal jejunal histology, and a normal urinary aminoacidogram. The children were biopsied on clinical grounds, but the two adult controls were entirely healthy. Some of the control data have already been published (14). All subjects and/or their guardians had given informed consent to the biopsy.

Biopsy procedure. After a 6-h fast, a jejunal biopsy was taken near the ligament of Treitz with a Carey capsule (21) under fluoroscopic control. Part of the biopsy specimen was examined histologically. The rest was used for transport studies.

In vitro transport studies

Intracellular Na and lysine concentrations. These concentrations were measured as previously described (14) using pieces of jejunal epithelium and the immediately underlying tissue without the muscular layer (22). In brief, the tissue was shaken at 37°C for 60 min in 10 ml of Ringer or Na-free solution containing 3 mM Lysine, 1.5 μCi of L-[14C]lysine (New England Nuclear, Boston, Mass.), 1 μCi of 32Na (Commissariat à l’Energie Atomique, France), and 12.5 μCi of [3H]polyethylene glycol (4,000 mol wt) (New England Nuclear). All measurements were made at the 3-mM lysine concentration, which is sufficient for reliable measurement of transepithelial fluxes and Na dependence. The affinity constant (K) values reported for the Na-dependent fluxes are 3 mM in rat jejunum (23) and 10 mM in rabbit ileum (24). The Ringer solution contained, in millimolars, 140 Na, 5.2 K, 1.2 Ca, 1.2 Mg, 120 Cl, 25 HCO3, 2.4 HPO4, and 0.4 H2PO4 (pH 7.4). The Na-free solution was obtained by replacing NaCl with choline chloride, and NaHCO3 with KHCO3. Consequently, the potassium concentration of the solution was ~30 mM. After incubation for 60 min, the tissue pieces were washed in cold 0.3 M mannitol, blotted gently, weighed, and extracted in 2 ml of 0.1 N nitric acid. Aliquots of the eluate were measured by liquid scintillation photometry. Results are given as the ratio of the intracellular to extracellular (C/M) L-lysine or Na concentration. According to bidimensional radiochromatography, L-[14C]lysine was not metabolized during the incubation, as has been documented (24). For comparison, the C/M for glucose was measured in five biopsies at 10 mM glucose concentration.

Lysine influx. Lysine influx from the incubation medium across the luminal membrane into the cell (Jme) was determined as previously described (14). After a 30-min preincubation in Ringer’s solution, the accurately timed (~60 s) lysine influx was measured in the presence and absence of Na. The extracellular space was estimated with [3H]polyethylene glycol.

Transepithelial fluxes. The biopsy specimen was mounted between two lucite chambers as a flat sheet on filter paper (22). The exposed area was 12.57 mm². The tissue was bathed on both sides with identical Ringer solutions (1.5 ml), which were circulated, oxygenated, and maintained at 37°C. The solutions bathing the mucosal and serosal sides were connected via agar bridges to calomel electrodes for measurement of the transepithelial potential difference, and to Ag-AgCl electrodes for passing current through the system. Short-circuit current (Isc) was recorded in the absence of potential difference after appropriate correction for fluid resistance between the potential difference-sensing bridges. It was expressed as p eq equivalent per hour per square centimeter to make it comparable with the lysine fluxes. After 5 min of steady Isc, 3 mM of L-lysine was added on both sides and 20 μCi of L-[3H]lysine on the mucosal side. When the appearance of 3H into the opposite chamber reached a steady rate (5 min), the flux from mucosa to serosa (Jms) was determined by collecting 20 μl samples from the serosal reservoir at 5-min intervals and replacing them by the same amount of “cold” Ringer-lysine solution. After four flux periods, the bathing solutions were removed, the chambers washed and filled with cold Ringer plus 3 mM L-lysine solution. 2 μCi of L-[3H]lysine was added on the serosal side to allow similar determinations of flux from serosa to mucosa (Jsm) during four periods.

Calculations. The fluxes (J) between the mucosal bathing solution (m), the cell compartment (c), and the serosal bathing solution (s) were calculated as follows:

\[J_{\text{net}} = J_m - J_s.\] (1)
\[J_m = J_{mc} \times J_{cs}.\] (2)
\[J_m = J_{sc} \times J_{cm}.\] (3)
\[J_s = J_{cm} / (J_{mc} / J_{ms}) - 1.\] (4)
\[J_{sc} = J_{cs} - J_{net}.\] (5)

Apparent permeability coefficients (P) across the membrane were calculated as

\[P = J/\text{[lysine]}.\] (7)

The intracellular Na and lysine concentrations, the transepithelial fluxes Jms and Jsm, and the influx at the luminal membrane Jme were all obtained experimentally (see Results). Because not all measurements were obtained from each biopsy specimen, calculations were based on the mean values for each group.

Student’s t test and nonparametric tests (Mann-Whitney U test or Wilcoxon one-sample test) were used to compare means and ranges.

RESULTS

Intracellular lysine and Na concentrations (Fig. 1). When Na was present in the incubation solution, the

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\(^2\) The relationship between the current (I) and the ionic fluxes (J) is calculated as follows:

\[J = \frac{I}{F},\]

\[F = 96,500 \, \mu C/\mu e q.\]

The exposed surface area = 0.1257 cm². Thus,

\[J (\mu e q/h \, cm²) = \frac{I_{\text{net}} (\mu A) \times 3,600}{96,500 \times 0.1257 \times 3.37}.\]
mean C/M for lysine was significantly higher ($P < 0.05$) in patients (mean ± SEM, 7.17 ± 0.60; range 5.37–10.96) than in controls (5.44 ± 0.51; range 4.03–10.29). In the absence of Na, no difference was found (patients: 3.17 ± 0.68; range 0.94–6.70; controls: 2.69 ± 0.34; range 1.17 ± 4.84) and the ratio was significantly smaller ($P < 0.01$) in both groups.

In the presence of 3 mM lysine, the intracellular Na concentration was higher in patients (73.6 ± 10.8; range 43.4–113.4 mM) than in controls (42.3 ± 3.7; range 21.0–59.4 mM).

With 10 mM glucose in the incubation solution, the C/M for glucose of the five patients (3.85 ± 0.63) did not differ from that of 23 control children (3.97 ± 0.23) (22). $J_{mc}$ (Fig. 2). When Na was present in the incubation solution, the $J_{mc}$ values of the two groups did not differ significantly (patients: 1.45 ± 0.34, range 0.77–2.68; controls: 1.11 ± 0.21, range 0.54–2.54 μmol/h cm²), nor did they differ in the absence of Na (patients: 0.60 ± 0.09, range 0.36–1.02; controls: 0.57 ± 0.07, range 0.28–0.82 μmol/h cm²). The lysine $J_{mc}$ was equally Na dependent in the two groups.

Transepithelial lysine fluxes (Fig. 3). In the control group, the two unidirectional lysine fluxes across the whole epithelium were not significantly different. Consequently, the net flux was minimal. In contrast, in the epithelia of the patients, the net result was lysine secretion. This was entirely the result of a 60% lower rate in $J_{ms}$ ($P < 0.01$), with no difference in $J_{sm}$. Because the fluxes and the apparent permeability at the basolateral membrane could not be determined experimentally, but were calculated from Eqs. 4–6, we fur-
ther reexamined the relationship between the transmural unidirectional fluxes and the unidirectional fluxes across the luminal and basolateral membranes (Eqs. 2 and 3). The reduction of \( J_{ms} \) with no change in \( J_{sm} \) could obviously be related to a decrease in \( J_{mc} \) or in \( J_{cs} \). Because the \( J_{mc} \) values were found, experimentally, not to differ significantly in the two groups, the alteration in net transepithelial fluxes in patients is clearly the result of the 62% reduction in \( J_{cs} \). Comparison of the apparent permeability coefficients at each membrane (Table I) indicated a 71% reduction in cell-to-serosa permeability (\( P_{cs} \)) in the patients, but no difference in the other three permeability coefficients.

\( I_{sc} \) (Fig. 4). On the same pieces of tissues, we also recorded the \( I_{sc} \). In the control specimens, 3 mM lysine stimulated \( I_{sc} \) by 0.18 μeq/h cm². This remained stable throughout the 60-min observation period. In all four specimens from the patients, we found a progressive decrease in \( I_{sc} \) after addition of lysine. A negative reversed current appeared after 30 min. The overall decrease during the 60-min recording was 0.71 μeq/h cm². However, the isotopic lysine fluxes remained constant throughout the experiment in epithelia from both controls and patients.

**DISCUSSION**

Our results indicate that, in LPI, the lysine transport defect is located at the basolateral membrane in cells of the jejunal epithelium. As compared with controls, there was a 62% reduction in the \( J_{cs} \) and a 71% decrease in the corresponding permeability coefficient. Such a defect is entirely consistent with the smaller \( J_{ms} \) and the greater lysine accumulation ratio found in LPI in the presence of Na. In an earlier study, Kekomäki (15) measured C/M at 0.1 mM in five patients with LPI, one control, and one cystinuric subject. The ratios were 6.0 (mean) (range 4.0–6.9), 3.9, and 1.8, respectively. In an intestinal two-lumen perfusion study, he did not find any difference between two patients and one cystinuric subject in the rate of disappearance of 3H-labeled lysine from the jejunal lumen. His results in LPI can now be accurately explained by the basolateral transport defect. The present in vitro findings are also compatible with the lack in the patients of an increase in plasma lysine concentration after oral loads of lysine dipeptides (17). Thus, LPI appears to be the first disease in which a genetically determined transport abnormality has been demonstrated at the basolateral membrane.

Lysine malabsorption in LPI and in cystinuria is the result of very different mechanisms. In cystinuria, the defect is located at the luminal membrane, as shown by impaired Na-dependent lysine accumulation (12–14), reduced lysine permeability at the luminal membrane (14), and normal in vivo lysine absorption from dipeptides (16). In contrast, in LPI the Na-dependent lysine transport at the luminal membrane is intact, as indicated by the accumulation of lysine observed in the presence of Na and the unaltered lysine influx across this membrane (Figs. 1 and 2). Nothing is yet known about the precise location of the transport defect in other types of hyperdiaminouric aciduria and related diseases.

The present results justify speculation as to the nature of the basic defect in LPI. Very little is known about the mechanism of transport across the basolateral membrane. However, recent findings indicate that

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**Table I**

Calculated Apparent Permeability Coefficients across the Luminal and Basolateral Membranes in the Jejunal Biopsies

<table>
<thead>
<tr>
<th></th>
<th>( P_{mc} )</th>
<th>( P_{rm} )</th>
<th>( P_{cs} )</th>
<th>( P_{sc} )</th>
<th>( P_{mc}/P_{rm} )</th>
<th>( P_{sc}/P_{cs} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>0.369</td>
<td>0.073</td>
<td>0.0085</td>
<td>0.067</td>
<td>5.05</td>
<td>0.13</td>
</tr>
<tr>
<td>LPI*</td>
<td>0.484</td>
<td>0.075</td>
<td>0.0205</td>
<td>0.057</td>
<td>6.45</td>
<td>0.04</td>
</tr>
</tbody>
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* Note the low exit permeability from the cell across the basolateral membrane.
sugars and amino acids are translocated by a facilitated transport system that is independent of Na (25–27). This would make the maximum $J_{net}$ across the basolateral membrane a function of the lysine concentrations in the cell, $[\text{Lys}]_c$, and the solution bathing the serosa, $[\text{Lys}]_b$, and also the Michaelis-Menten transport constants of the two sides of the membrane, $K_c$ and $K_s$. Thus, assuming that $J_{max}$ across the basolateral membrane are equal for the two directions,

$$J_{net} = J_{max} \left[ \frac{[\text{Lys}]_c}{K_c + [\text{Lys}]_c} - \frac{[\text{Lys}]_b}{K_s + [\text{Lys}]_b} \right] .$$

In normal human jejunal epithelium, as in the ileal epithelium of the rabbit (24), lysine transport displays an asymmetry, expressed by the fact that $P_{es} < P_{se}$ (Table I) or that $K_c < K_s$. This asymmetry may be connected with the positive charge on lysine. In LPI, the present results suggest a decrease in $K_c$.

Our findings on the role of Na in lysine transport in the intestinal epithelium are in accord with those reported earlier (24). The absence of Na from the incubation medium affected the accumulation ratio of lysine as much in the patients as in the controls. However, the higher intracellular Na concentration and the decreasing $I_{se}$ observed in LPI in the presence of Na are puzzling. According to the Na gradient hypothesis, a less steep gradient should lead to an increased efflux of lysine across the luminal membrane (28). The lack of a significant difference between the luminal influxes in specimens from patients and controls, together with the existence of net transepithelial secretion in the patients, suggests that the efflux is increased in LPI, although we were unable to demonstrate this. We may also legitimately infer that the rise in intracellular Na concentration is associated with a decreased transserosal potential difference (29), which would lower the $K_c$ of the basolateral membrane. However, further experiments are required to elucidate the origin of the Na disturbances and their effects on other Na-independent solutes.

In LPI, the reduced cell-to-serosa efflux of lysine and also, presumably, of the other diamino acids, arginine and ornithine, might be responsible for impaired in vivo absorption of these amino acids in the free form and as short oligopeptides. It is tempting to speculate that the transport defect in the proximal tubules of the kidney is similar (30). Furthermore, in LPI, the liver also shows the defect in diamino acid transport, suggesting a relation between the transport mechanism at the liver plasma membrane and the basolateral mem-

ACKNOWLEDGMENTS

The authors are indebted to Mrs. J. M. Perttunen and Miss M. Dreyfus for skillful assistance in the preparation of the manu-

script, and to Mrs. K. Helanto and Mrs. M. Vias for secretarial help.

This study was supported by grant CRL 771267 from the Institut National de Santé et de Recherches Médicales, grants from the Foundation for Pediatric Research, Finland, the Sigrid Jusélius Foundation, Finland, and the Association of Finnish Life Insurance Companies.

REFERENCES


