Polyamines Stimulate Lysosomal Cystine Transport*

Adam J. Jonas‡, Linda J. Symons, and Rebecca J. Speller
From the Department of Pediatrics, University of Texas Medical School at Houston, Houston, Texas 77030

Lysosomal cystine transport is a carrier-dependent process that, in isolated lysosomes, is stimulated by proton gradients, membrane potential, and millimolar concentrations of divalent cations. The importance of these regulatory factors in vivo is not well established. Polyamines were found to stimulate cystine transport in Percoll gradient purified rat liver lysosomes with spermidine ≈ putrescine = cadaverine > spermine in order of effectiveness. Maximal stimulation was achieved with 500 μM spermidine. The effects of optimal concentrations of polyamines and divalent cations on cystine transport were not additive. Spermidine stimulated cystine efflux from lysosomes of cultured human diploid fibroblasts, but had no effect on lysosomes of cystinotic fibroblasts which have defective cystine transport. Spermidine did not accumulate within lysosomes in exchange for cystine, had no effect on lysosomal pH, had only slight effects on the lysosomal membrane potential, and had little effect on either methionine or tyrosine efflux. Polyamines are cellular cytoplasmic components that, in physiologic concentrations, stimulate lysosomal cystine transport.

Lysosomal cystine transport is a carrier-mediated process that is quite specific for cystine and is essential for the cellular recycling of free cystine obtained by lysosomal proteolysis (1–6). When transport is impaired, as in the human inherited disorder cystinosis, large amounts of lysosomal cystine accumulate (1, 3). Studies of lysosomes isolated from either rat liver or cultured fibroblasts have shown that transport is dependent upon proton gradients as well as the membrane potential (3–5, 7). The interplay of these regulatory factors in intact cells is unclear, although it has been demonstrated that cystine transport in cultured fibroblasts is diminished when intracellular ATP levels are lowered (8). Presumably, this occurs because the lysosomal proton gradient cannot be maintained by the activity of the lysosomal proton pumping ATPase under conditions of substrate limitation. Divalent cations in millimolar concentrations also regulate cystine transport in isolated lysosomes, but they do not appear to stimulate transport through effects on either lysosomal membrane potential or lysosomal pH (1, 2, 5). Interestingly, their stimulatory effects on transport are blocked by the ionophore A23187 (5). One possible explanation for these findings is that divalent cations regulate the binding of cystine to the carrier.

Polyamines are abundant intracellular organic cations that are present in concentrations comparable to that of glutathione (9–12). They are subject to enzymatic regulation and have been implicated in a variety of cellular processes including protein synthesis, calcium flux, amino acid transport, and activation of protein kinases (9, 11, 13–18). It has been demonstrated that polyamines bind to acidic membrane components such as phosphatidylserine and, depending upon conditions, may promote either membrane stabilization or membrane fusion (19, 20). Since polyamines have a role in membrane processes and divalent cation flux, they were examined for their effects on lysosomal amino acid transport.

MATERIALS AND METHODS AND RESULTS

Our studies indicate that polyamines stimulate lysosomal cystine transport when divalent cation concentrations are suboptimal. At optimal concentrations, the effects of magnesium and polyamine are not additive. Both cationic species stimulate transport in a manner that is independent of the electrochemical potential. Although experiments with structurally related compounds indicate that a net positive charge is essential for the effects of polyamines, other factors such as chain length or charge distribution may also contribute to their actions, as suggested by the results obtained with the highly cationic polyamine, spermine.

There are several possible explanations for the effects of polyamines on lysosomal cystine transport. One possibility is that polyamines are involved in an exchange reaction with cystine across the lysosomal membrane. Polyamines have been reported to traverse membranes through counter-transport with system A neutral amino acids (16). While bi-directional cystine counter-transport has been demonstrated in lysosomal preparations, we found no indication of polyamine counter-transport with cystine. A second possibility is that polyamines open selective pores in the lysosomal membrane. However, this appears unlikely since polyamines do not induce a discernible lysosomal proton leak, have no effect on methionine efflux, and do not affect cystine efflux from cystinotic lysosomes. A third alternative is that the effects of polyamines on transport are mediated by calcium flux. Polyamines increase calcium flux across membranes by a postulated cation-exchange reaction with bound calcium (13). In

* This work was supported by Basil O'Connor Starter Research Grant 5-424 from the March of Dimes Birth Defects Foundation and National Institutes of Health Grant DK37483. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Dept. of Pediatrics, The University of Texas Health Science Center at Houston, 6431 Fannin St., Houston, TX 77030.

1 Portions of this paper (including "Materials and Methods," "Results," Figs. 1–3, and Tables 1–V) are presented in miniprint form at the end of this paper. The abbreviation used is: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 87M-752, cite the authors, and include a check or money order for $2.50 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
mouse renal proximal tubular cells, amino acid and hexose transport are stimulated in response to these calcium fluxes (15). Two experimental findings argue against this type of action. Stimulation of cystine efflux by spermidine is unaffected by the presence of EDTA and highly cationic spermine is a weak stimulator of cystine transport. Polyamine-mediated effects on calcium flux can be mimicked using the ionophore A23187 (15). Previous work showing that divalent cation effects on lysosomal cystine transport are abolished by A23187 is also inconsistent with this hypothesis (5).

Thus it appears that the most likely mechanism of action is that polyamines regulate the lysosomal cystine carrier by binding to specific acidic sites on either the carrier or on the lysosomal membrane.

Polyamines are present intracellularly in millimolar concentrations and, in the case of spermidine, are more effective stimulators of lysosomal cystine transport than divalent cations. Intracellular levels of polyamines, which are regulated by both transport and enzymatic processes, increase remarkably during cell growth (10, 28). Not only do total levels of polyamines change during growth, but the relative amounts of different polyamine species vary as well. The ratio of spermine to spermidine increases as the cells become confluent and exhibit quiescence (10). These two species are the least and most effective polyamine stimulators of cystine transport, respectively. Polyamines should thus be given consideration as components of an in vivo regulatory system of lysosomal amino acid transport.

Intracellular regulation of lysosomal cystine transport by polyamines has some intriguing implications. Lysosomes, as lysosome may be the only intracellular location of this amino acid, can be appreciable concentrations of amino acids. As demonstrated previously in rat liver, due to its vesicular structure, the system of lysosomal amino acid transport.

**Results**

**Effect of spermidine on lysosomal cystine transport was assessed fluorometrically (5). A cell line of BHK-21 Chinese hamster fibroblasts was used as the amount of enzyme necessary to bring about one unit of substrate per unit time was the same in the presence of spermidine as in its absence (15).

**Acknowledgment**—We would like to thank Dr. Julie E. Noble for her aid with the manuscript.

**References**


**Supplementary Material to: Polyanines Stimulate Lysosomal Cystine Transport**

**Adam J. Jonas, Linda J. Symans, and Rebecca J. Speller**

**Materials and Methods**

**Materials:** All chemicals were obtained from Sigma Chemical Company and were of the highest purity available. Coon’s F11 and fetal bovine serum were from Irvine Scientific Co. 14C-cystine and 14C-lysine were obtained from the American Radiolabeled Chemicals. A23187 and 2-mercaptoethanol were from ICN. Female Sprague-Dawley rats (250g) were obtained from Timco.

**Preparation of lysosomes:** Lysosomes were prepared from rat liver, using differential centrifugation and Percoll density gradient centrifugation (15). Lysosomes were prepared from cultured fibroblasts by differential centrifugation (11). Lysosomes were prepared from cultured fibroblasts by differential centrifugation (11).

**Cell culture:** Human diploid fibroblast lines were established from skin biopsies and were grown in an atmosphere of 10% CO2 using Coon’s F11 medium supplemented with 10% fetal bovine serum (22).

**Lysosomal loading:** Lysosomes were loaded with amino acids by exposing them to F11 medium at 25°C to 20 μM amino acid methyl ester which had been synthesized using methionine S-methyltransferase loading. After washing twice with 15 volumes of cold isotonic buffer and resuspended in 1 ml of 0.25 M sucrose, 20 μM spermidine, pH 7.2 at 4°C, lysosomal contents were analyzed by this method chromatography to ensure complete hydrolysis of the methyl ester before the start of experiments (15).
Polyamines and Lysosomal Cystine

Figure 1. Lysosomal cystine efflux and polyamines

Polyamines had little negatively charged compound

Although tyrosine Polyamines had SLOLI~~ to

Electrochemical potential: Polyamines had

Iysosomal (Figure

Experimental conditions and statistical analysis were as described in Table I. All additions were previously buffered to pH 7.0 with NaOH.

Table II. Effect of charge on cystine efflux

<table>
<thead>
<tr>
<th>Additive</th>
<th>Efflux pmol/3 Hr/min</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>27±3.2</td>
<td>----</td>
</tr>
<tr>
<td>2M spermidine</td>
<td>46±4.6</td>
<td>.001</td>
</tr>
<tr>
<td>2M MgCl₂</td>
<td>15±3.1</td>
<td>.001</td>
</tr>
<tr>
<td>2M spermidine</td>
<td>52±5.5</td>
<td>.005</td>
</tr>
</tbody>
</table>

Lysosomes were incubated for 10 minutes at 37°C in 0.25 M sucrose, 20 mM Hepes, pH 7.0, with 0.2 mg/ml human term albumin. Samples were in The Ricia label associated with lysosomes increased approximately 10 times over 10 minutes incubation period. Lysosomal radiolabel following incubation period, levels of lysosomal radiolabel, following incubation were similar whatever lysosomes were previously filled with cystine or not (Table IV). Thus, spermidine does not appear to exchange with lysosomal stores of cystine.

Table IV. Lysosomal uptake

<table>
<thead>
<tr>
<th></th>
<th>Efflux pmol/3 Hr/min</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>unloaded</td>
<td>2,000±100</td>
<td>----</td>
</tr>
<tr>
<td>cystine loaded</td>
<td>1,000±200</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>5,700±150</td>
<td>----</td>
</tr>
<tr>
<td>2,000±200</td>
<td>5,800±200</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Uptake was determined over 16 minutes at 37°C in 0.25 M sucrose, 20 mM Hepes, pH 7.0. Cytosolic uptake lysosomes had been exposed to 20 µM unlabeled cystine dissociated over as in Methods. Statistical analysis per formed using Student’s t test.

Table V. Fibroblasts (lysosomal cystine efflux)

<table>
<thead>
<tr>
<th>Fibroblasts</th>
<th>Efflux pmol/3 Hr/min</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal</td>
<td>27±5.5</td>
<td>----</td>
</tr>
<tr>
<td>2M spermidine</td>
<td>46±2.1</td>
<td>.005</td>
</tr>
<tr>
<td>cystinotic</td>
<td>0.1±0.12</td>
<td>----</td>
</tr>
<tr>
<td>2M spermidine</td>
<td>0.2±0.22</td>
<td>.1</td>
</tr>
</tbody>
</table>

Experiments were performed with partially purified lysosomes as in Table I. Statistical analysis performed using Student’s t test.