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Polyamines are absorbed through a y^+ amino acid carrier in rat intestinal epithelial cells

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Summary. Due to the similarity in transport characteristics of polyamines and the y^+ basic amino acid system, we hypothesized that both substrates could be moving through a common carrier site. Competitive and cross inhibition experiments in intestinal epithelial cells revealed the possibility of a common transport site. N-ethylmalemide (NEM) inhibited both lysine and putrescine transport, confirming that both were carried by a y^+ transporter. Overexpressing the y^+ transporter CAT-1 in a polyamine transport-deficient cell line, CHO-MG, did not reconstitute polyamine-transport. Thus, polyamines are not traveling through CAT-1. To determine if lysine is carried by a polyamine transport site, an antizyme-overexpressing cell line was used. Antizyme overexpression decreased polyamine uptake by 50%; in contrast, lysine transport was unaffected. Therefore, lysine is not traveling through a polyamine transport site. It appears that polyamines and lysine are likely traveling through a common unknown y^+ transport site.

Keywords: Basic amino acid – Cationic amino acid – y^+ transport – Antizyme – Polyamine transport – Putrescine

Introduction

The polyamines putrescine (put), spermidine (spd), and spermine (spm) are small, linear, cationic molecules that are essential for cell growth and proliferation and are found in all cells in nature. Both plant and animal cells contain millimolar concentrations of polyamines making polyamines a part of the normal diet in man. Additionally, they have been identified in mammary gland milk and as a component of many enteral feeding solutions (for review Seidel and Scemama, 1997). Polyamines can be either synthesized in cells from ornithine or transported into cells through specific transport sites. Polyamine synthesis can be pharmacologically inhibited by the drug difluoromethylornithine (DFMO), an inhibitor of one of the polyamine biosynthetic enzymes, ornithine decarboxylase. Even in the presence of DFMO, however, cells still maintain adequate intracellular polyamine levels due to their ability to transport polyamines from the extracellular compartment. The extracellular source of polyamines is in turn derived in large part from dietary sources. Thus, it is of interest to characterize, identify and ultimately clone the human polyamine carrier sites (Pegg, 1988; Seiler and Dezeure, 1990; Seiler et al., 1996, 1998; Seidel and Scemama, 1997).

The amino acid/polyamine/organocation (APC) superfamily is one of the largest transport groups in nature. It is has approximately 250 sequenced transporters that can be further subdivided into 10 distinct families containing transporters with common structural characteristics which absorb similar substrates (Jack et al., 2000; Saier, 2000). The APC superfamily contains many carrier types including both symporters and antiporters. In addition, transporters can be Na^+-dependent, Na^+-independent, or display both components. Two members of the APC superfamily are the basic amino acid/polyamine antiporter (APA) family and the cationic amino acid (CAT) family (Jack et al., 2000; Saier, 2000). The CAT family is the only ubiquitously distributed subset of the APC superfamily (Jack et al., 2000; Palacin et al., 1998; Deves and Boyd, 1998; Closs, 1996); at least one member of the CAT family has been identified in all tissues tested thus far. The APA family has been cloned or identified in prokaryotes, yeast, and fungi, but to date the polyamine transporters have not been cloned from a mammalian source (Igarashi and Kashiwagi, 1996; Kashiwagi et al., 2000; McNemar et al., 2001). However, polyamine transport sites have been characterized kinetically in a variety of mammalian cell types (Seiler et al., 1996, 1998). In gastrointestinal

epithelial cells, polyamine transport is especially important due to the mM concentration of polyamines in the gastrointestinal lumen (Osborne and Seidel, 1990). In the small intestinal epithelial cell line, IEC-6, two sodium-independent polyamine transport sites have been identified in the apical membrane domain allowing for vectoral movement of polyamines from gut lumen to blood. One site has a high affinity but low capacity for polyamine transport, while the other has a low affinity but high capacity (Scemama et al., 1993).

Like polyamines, the basic amino acids, lysine (lys), arginine (arg), and ornithine (orn) are positively charged at neutral pH. Unlike polyamines, the transporters for basic amino acids have been clearly characterized and cloned from mammalian cells. In the 1960's, Christensen classified the saturable, high affinity, sodium independent transporter of cationic amino acids as y^+ (Christensen, 1964; White and Christensen, 1982; Torras-Llort et al., 1996; Rojas and Deves, 1999). The y^+ system is composed of multiple members of the CAT family. At least one transporter from the CAT family is expressed in all mammalian tissues tested thus far. CAT-1 is expressed in all tissues tested, with the exception of liver, and transports basic amino acids in a Na^+-independent manner with K_m values in the low μM range (Deves and Boyd, 1998; Closs, 2002, 1996; Palacin et al., 1998; Malandro and Kilberg, 1996).

The structural similarity of polyamines and basic amino acids, the wide tissue expression pattern of transport, and the fact that both substrates are moved with a high affinity in the absence of sodium, led to the question of identifying a common transport site for both substrates. Preliminary experiments demonstrated that basic amino acids specifically inhibited polyamine transport. Therefore, the hypothesis was tested that one or more of the polyamine transporters could, in fact, be one of the CATs, specifically CAT-1, or vice-versa.

Methods and materials

To determine the physiologic concentration of amino acids in the small intestine, several 6 cm length segments of jejunal contents were collected from two, 24 hr fasted, male Sprague-Dawley rats (225 g) two hrs post re-feeding with normal rat chow. Briefly, lumenal contents were acid precipitated in a final concentration of 10% TCA and 100 μL of the supernatant were injected onto a cation exchange resin column with post-column DMSO-based ninhydrin colorimetric detection systems as previously described (Possaine et al., 1991).

IEC-6 cells (ATTC, Manassas, VA), rat small intestinal epithelial cells, were used at passage 17–23 and cultured in Delbecco's Modified Eagles Medium (DMEM, Sigma, St. Louis, MO) supplemented with 5% fetal bovine serum (FBS) (HyClone, Logan, UT) in a 37°C incubator with 5% CO_2. For uptake assays, cells were plated at 60,000 cells per 35 mm dish. Substrates were dissolved in Tyrode's solution (composition in mM: 149.2 Na^+, 2.7 K^+, 1.8 Ca^{++}, 1.05 Mg^{++}, 145.3 Cl^-, 11.7 HCO_3^-, 0.4 $H_2PO_4^-$, 0.5 EGTA, and 5.0 glucose) containing Tris buffer, pH 7.4, and heated to 37°C. All reagents, amino acids, and polyamines were purchased from Sigma. [^{3}H]put (41 Ci/mmol), [^{3}H]spd (44.5 Ci/mmol), or [^{3}H]lys (81 Ci/mmol) were purchased from NEN/PerkinElmer, Boston, MA. Cells were incubated in 1900 μL of Tyrode's containing varying concentrations of substrate and 100 μL solution of either 0.6 μCi [^{3}H]put, 0.5 μCi [^{3}H]spd, or 0.5 μCi [^{3}H]lys for 30 min then washed twice with Tyrode's solution maintained at 4°C. Cells were dissolved in 1 mL 0.1N NaOH for 30 min and 100 μL 1N HCl were added to each dish to neutralize the solution. 500 μL were then removed from each dish and placed into vials containing 4.5 mL of scintillation fluid and radioactivity was determined by scintillation spectroscopy.

Cells treated with 10 μM N-(6-aminohexyl)-5-chloro-1-naphthalelesulfonamide (W-7) (Sigma), a CaM antagonist, or Ca^{++}-free Tyrode's (equimolar replacement of $CaCl_2$ with NaCl and 0.5 mM EGTA) were then incubated for 15 min because Ca^{++}-free conditions tended to disrupt cell adhesion. Specific transport was calculated by subtracting non-specific uptake (uptake of radiolabeled put, spd, or lys in the presence of 30 mM of the same cold substrate) from total uptake (uptake of radiolabeled put, spd, or lys only).

EXOD-1 and Az-2 cells are stable cell lines derived from mouse FM3A cells. EXOD-1 cells are used as the parental cell control. Az-2 cells are EXOD-1 cells that have been stably transfected with the pMAMneo plasmid containing a dexamethasone inducible promoter to the Z1 antizyme cDNA, the active form of antizyme (Kameji et al., 1993; Suzuki et al., 1994). EXOD-1 and Az-2 cells graciously supplied by K. Igarashi (Chiba University, Chiba, Japan) were cultured in DMEM supplemented with 2% FBS, 5 mM DFMO (Merrell Dow, Cincinnati, OH), and maintained in 0.5 mg/ml G418 (Sigma) in a 37°C incubator with 5% CO_2. For uptake assays, cells were plated in 10% FBS to promote cell adherence. Az-2 cells were treated with 1 μM dexamethasone (Sigma) for 24 hrs prior to the experiment to induce antizyme (Az) expression. Cell counts were performed before and after experiments to normalize data between duplicate experiments and cell types.

Chinese hamster ovarian (CHO) cells and their polyamine transport deficient derivative (CHO-MG) cells (Mandel and Flintoff, 1978; Byers et al., 1989) graciously provided by WF Flintoff (New London, Ontario) were cultured in DMEM supplemented with 5% FBS in a 37°C incubator with 5% CO_2. CHO-MG cells were plated at approximately 50% confluency and transfected with the pCNA3 plasmid containing mCAT-1 cDNA (Kim et al., 1991), supplied by JM Cunningham (Howard Hughes Medical Institute, Boston, MA), using a calcium phosphate precipitation protocol (Aususel et al., 1996). Cells were maintained in normal culture conditions for 48 hrs, and then treated with 500 μM G418 for 14 days to select for positive transfectants. Stably transfected CHO-MG cells were then used for uptake assays as described above.

For Western blots, three confluent 100 mm dishes were washed twice with 10 mL phosphate buffered saline (Sigma), collected, and sonicated 3 times for 5 sec to disrupt cell membranes. To determine protein concentration, the Bradford assay was performed using gamma globulin as the protein standard (Aususel et al., 1996). All lanes were loaded with 120 μg of protein. Antizyme antibody (Mitchell et al., 1996) was graciously supplied by JL Mitchell (Northern Illinois University, Dekalb, Illinois) and used at a concentration of 1:1000. Actin (Santa Cruz Biotechnology, Santa Cruz, CA) was used as a loading control. All figures were created using the computer program Prism 3.0 (GraphPad Software, Inc, San Diego, CA). Statistical analyses were performed using Microsoft Excel (Microsoft Office 2002). Data were compared using Student t-test and significance was defined as $p \leq 0.05$.

Results

The postprandial concentration of amino acids in rat duodenal contents was measured by HPLC. Table 1 illustrates that the majority of amino acids are present in high μM concentrations with several at or approaching a concentration of 1 mM. For example, lysine and arginine concentrations were 943 μM and 428 μM, respectively. This laboratory has previously demonstrated that the concentration of polyamines in the gastrointestinal lumen is in the low mM range (Osborne and Seidel, 1990). Therefore, the concentration of amino acids and polyamines used in transport assays to determine specific uptake was set at 30 mM to insure complete inhibition of transport during uptake assays. Under these conditions, specific uptake of both polyamines and amino acids was consistently >95% of total uptake.

Table 1. Intestinal postprandial concentration of various amino acids. Contents of 6 cm jejunal segments were collected 2 hrs post-feeding and HPLC used to determine amino acid concentrations. N = 2 rats, 1 in duplicate

Amino acid	Concentration (μM)
Alanine	508
Arginine	428
Glutamine	952
Glycine	303
Histidine	53.5
Leucine	263
Lysine	943
Methionine	230
Phenylalanine	150
Proline	12.6
Serine	1060
Valine	168

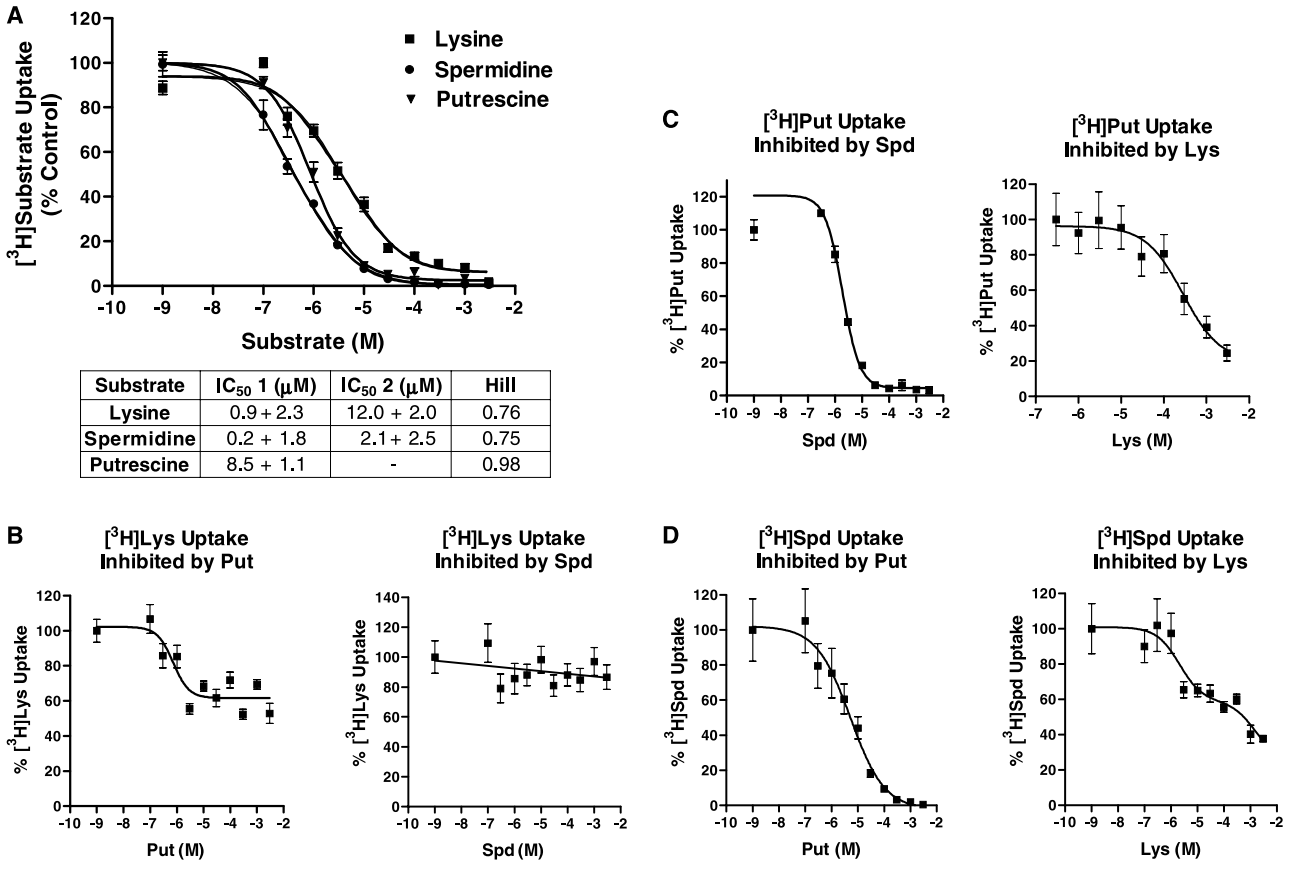
Table 2. Percent inhibition of total [^{3}H]put or [^{3}H]lys uptake. IEC-6 cells were incubated with (A) 30 mM amino acid and 0.6 μCi [^{3}H]put for 30 min or (B) 30 mM put or spd and 0.5 μCi [^{3}H]lys for 30 min. Cellular uptake of [^{3}H]put or [^{3}H]lys was measured by scintillation spectroscopy. Data are expressed as percent inhibition of [^{3}H]put or [^{3}H]lys uptake. N = 3. Each experiment was performed in duplicate

Substrate (30 mM)	% Inhibition of [^{3}H]put uptake
Basic amino acids	
Arginine	84 ± 0.55
Lysine	94 ± 0.33
Neutral amino acids	
Alanine	2.2 ± 4.4
Leucine	0 ± 0.35
Proline	3.5 ± 3.6
Uncharged polar amino acids	
Glutamine	19 ± 5.6
Substrate (30 mM)	**% Inhibition of [^{3}H]lys uptake**
Polyamine	
Putrescine	77.4 ± 6.2
Spermidine	61.7 ± 3.7

Transport of both putrescine and lysine was linear over the 1.25 hrs uptake period (data not shown). Uptake experiments were performed for 30 min, well within the linear phase of transport. The basic amino acids lysine and arginine (30 mM) inhibited polyamine transport by 94% and 84%, respectively (Table 2). Other classes of amino acids produced little to no inhibition of polyamine transport, suggesting that basic amino acids specifically inhibit polyamine uptake. Therefore, polyamines and cationic amino acids appear to be binding to a common site or transporter. In the converse experiment, putrescine or spermidine (30 mM) inhibited approximately 75% and 60% of lysine transport, respectively (Table 2). Thus, a significant fraction of lysine transport is polyamine sensitive.

Competitive inhibition curves were constructed for lysine, spermidine, and putrescine (Fig. 1A). Analysis of the inhibition curves suggested that spermidine and lysine appear to recognize two transport sites. The Hill slope is 0.75 for spd transport, and the IC_{50} values are $0.2 \pm 1.8\,\mu$M and $2.1 \pm 2.5\,\mu$M. The Hill slope is 0.76 for lys transport, and the IC_{50} values are $0.9 \pm 2.3\,\mu$M and $12 \pm 2.0\,\mu$M. In contrast, put is transported by a single carrier with a Hill slope of 0.98 and an IC_{50} value of $8.5 \pm 1.1\,\mu$M. These polyamine transport data are in agreement with results previously published by (Scemama et al., 1993).

A series of cross inhibition experiments were performed to determine whether polyamines and lysine are transported through similar or distinct transporters. Putrescine at a maximal concentration of 3 mM inhibited approximately 50% of lysine uptake, while spermidine produced only minimal inhibition of lysine transport (Fig. 1B). These data suggest the possibility that putrescine and lysine could be traveling through a common transporter but that spermidine is not a physiologic substrate for this common site. Spermidine completely inhibited putrescine transport, while 3 mM lysine inhibited roughly 80% of putrescine, again suggesting a common site for polyamine and basic amino acid transport (Fig. 1C). Finally, 3 mM putrescine completely inhibited spermidine uptake and lysine inhibited approximately 60% of spermidine uptake (Fig. 1D). Thus, lysine could be moving through one of the possible spermidine sites. In summary, spermidine is transported through two sites whereas putrescine recognizes only a single site. The polyamines can be transported through all three carriers



Substrate	IC_{50} 1 (μM)	IC_{50} 2 (μM)	Hill
Lysine	0.9 + 2.3	12.0 + 2.0	0.76
Spermidine	0.2 + 1.8	2.1 + 2.5	0.75
Putrescine	8.5 + 1.1	-	0.98

Fig. 1. Specific competitive inhibition transport curves in IEC-6 cells. (**A**) Competitive inhibition curves. Specific [^{3}H]substrate uptake was inhibited by same substrate. Concentration of unlabeled inhibitor ranged from 0.1 μM to 3 mM. Each point represents the mean of three experiments, each performed in triplicate. (**B**) Polyamines inhibit [^{3}H]lys uptake. Concentration of put or spd ranged from 0.1 μM to 3 mM. (**C**) Spd and lys inhibit [^{3}H]put uptake. Concentration of spd or lys ranged from 0.1 μM to 3 mM. (**D**) Put and lys inhibit [^{3}H]spd uptake. Concentration of put or lys ranged from 0.1 μM to 3 mM. Cellular uptake of [^{3}H]substrate was measured by scintillation spectroscopy. Each point represents the mean of two experiments each performed in triplicate for B–D

and are able to completely inhibit each others uptake. In addition, there are two lysine transporters, one of which also appears to transport polyamines.

Previous data from this laboratory have shown polyamine transport to be calcium/calmodulin (Ca^{++}/CaM) sensitive (Groblewski et al., 1992; Scemama et al., 1993). Figure 2 shows that both polyamine and basic amino acid transport is Ca^{++}/CaM sensitive in IEC-6 cells. The removal of extracellular Ca^{++} using Ca^{++}-free Tyrode's solution, inhibited 66% of putrescine transport, 27% of spermidine transport, and 42% of lysine in IEC-6 cells (Fig. 2A). Treating cells with 10 μM W-7, a calmodulin antagonist, inhibited 96% of putrescine uptake, 85% of spermidine, and 88% of lysine uptake (Fig. 2B). Clearly, the transport of both types of substrates has a Ca^{++}/CaM-dependent component.

N-ethylmalemide (NEM) is considered to be an accepted inhibitor of the y^+ lysine transport system and is often used to differentiate between the y^+ and the y^+L systems of transport (Deves et al., 1993; Torras-Llort et al., 1996; Pan et al., 1995). Therefore, NEM was used to determine if lysine and or polyamines were being transported through a y^+ transporter. NEM inhibited 56% of lysine transport and a surprising 92% of putrescine transport in IEC-6 cells (Fig. 3) confirming that both lysine and polyamine transport were occurring via a y^+ transporter. NEM inhibition of lysine transport was also measured in CHO and CHO-MG cells to determine if there was a decrease in lysine transport in the polyamine transport deficient CHO-MG cells as compared to their parental CHO cells. NEM inhibited 84% of lysine uptake in CHO and 31% in CHO-MG cells (Fig. 3). The 53% discrepancy in lysine transport indicates that significantly less lysine is being transported through the y^+ system in the polyamine transport deficient CHO-MG cells, as compared to the parental cell line. Therefore, lysine could

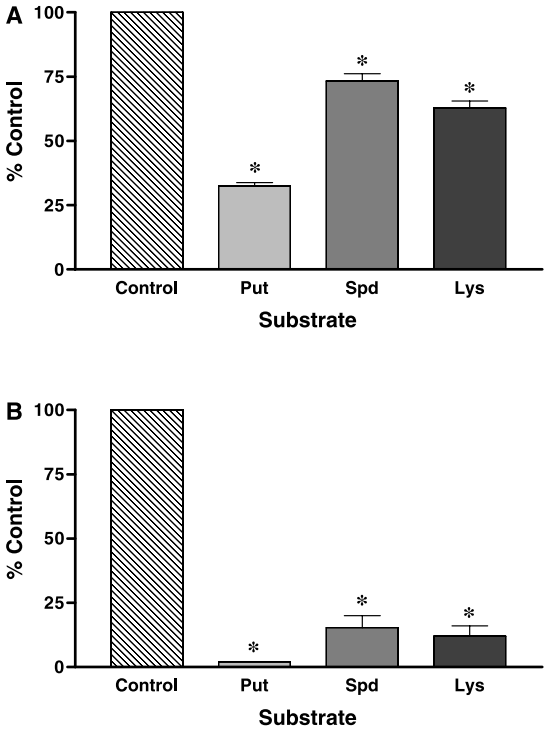


Fig. 2. Specific transport of polyamines and lysine is Ca^{++}/CaM sensitive. (**A**) Removal of extracellular Ca^{++} (Ca^{++}-free Tyrode's) inhibits polyamine and lysine transport. (**B**) Treatment with the calmodulin antagonist W-7 (10 μM) inhibits polyamine and lysine transport. IEC-6 cells were incubated in either Ca^{++}-free Tyrode's solution or W-7(10 μM) and either 0.5 μCi [^{3}H]lys or 0.6 μCi [^{3}H]put or 0.5 μCi [^{3}H]spd for 15 min. Cells were rinsed, dissolved, and cellular [^{3}H]substrate uptake was measured by scintillation spectroscopy. Each bar represents an $n = 3$. Each experiment performed in duplicate. Data are expressed as percent control of [H^3]substrate uptake. 100% control for [H^3]put was 66 fmol/10^6 cells-hr. 100% control for [H^3]spd was 71 fmol/10^6 cells-hr. 100% control for [H^3]lys was 31 fmol/10^6 cells-hr. $^*p \leq 0.5$

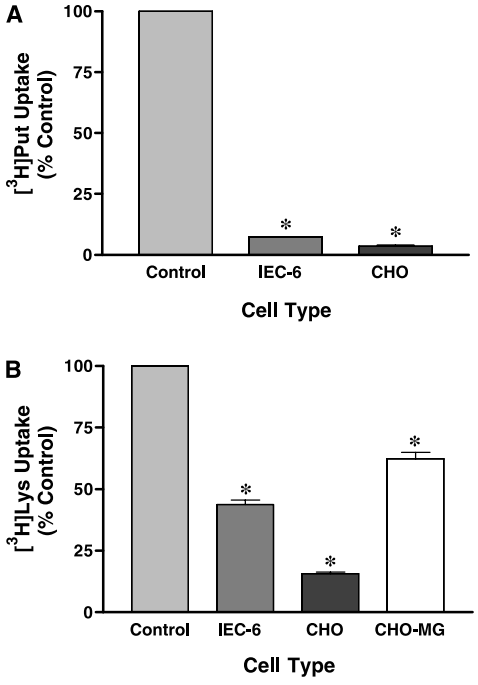


Fig. 3. NEM inhibits specific putrescine and lysine transport. IEC-6 cells, wild-type parental CHO cells, or polyamine transport deficient CHO-MG were incubated with NEM (0.2 mM) and (A) 0.6 μCi [H^3]put or (B) 0.5 μCi [H^3]lys for 30 min. Cells were rinsed, dissolved, and cellular uptake was measured by scintillation spectroscopy. Each bar represents the mean of four experiments. Data are expressed as percent control of [H^3]put or [H^3]lys uptake. $^*p \leq 0.5$

possibly be traveling through a polyamine carrier site in the CHO cells that is absent in the CHO-MG.

Transfection experiments were performed using a plasmid expressing the mCAT-1 cDNA to definitively determine if polyamines are traveling through the mCAT-1 transporter. Experiments were performed in the polyamine transport deficient cell line CHO-MG overexpressing mCAT-1. CHO-MG cells transport lysine at one-third the rate of the parental CHO cell line but do not transport polyamines. When lysine uptake experiments were performed in the stable transfectants, lysine transport doubled as compared to CHO-MG cells alone, confirming that the overexpressed mCAT-1 was functional (Fig. 4A). When putrescine and spermidine transport were measured in the CHO-MG cells transfected with mCAT-1, there was no increase in polyamine transport (Fig. 4B, C). Thus, while the parental CHO cell line transported both lysine and polyamines, expression of mCAT-1 in CHO-MG cells restored lysine transport as expected but not polyamine transport as hypothesized (Fig. 4B, C). These data provide definitive evidence that polyamines are not carried through the mCAT-1 transporter.

Since the polyamine transporter has not been cloned, transfection experiments using the polyamine transporter cDNAs could not be performed. However, antizyme (Az) is an intracellular protein that binds the polyamine transporter and inhibits polyamine uptake (Sakata et al., 1997, 2000). Az-2 cells containing a dexamethasone-inducible antizyme overexpression vector were employed to determine if antizyme overexpression would inhibit both polyamine and lysine uptake. If antizyme were to inhibit lysine transport it could be concluded that lysine is carried through a polyamine site. The immunoblot in Fig. 5 shows that Az overexpression is induced by 24 hr treatment with 1 μM dexamethasone. Nascent antizyme protein occurs in two forms; a larger 26 kD inactive form and a shorter, frameshifted, active form of 21 kD. Az-2 cells are stable transfectants containing the functional shorter form of antizyme. In the control lane of Fig. 5 both forms of antizyme are faintly detected. However, when the cells were treated with 1 μM dexamethasone

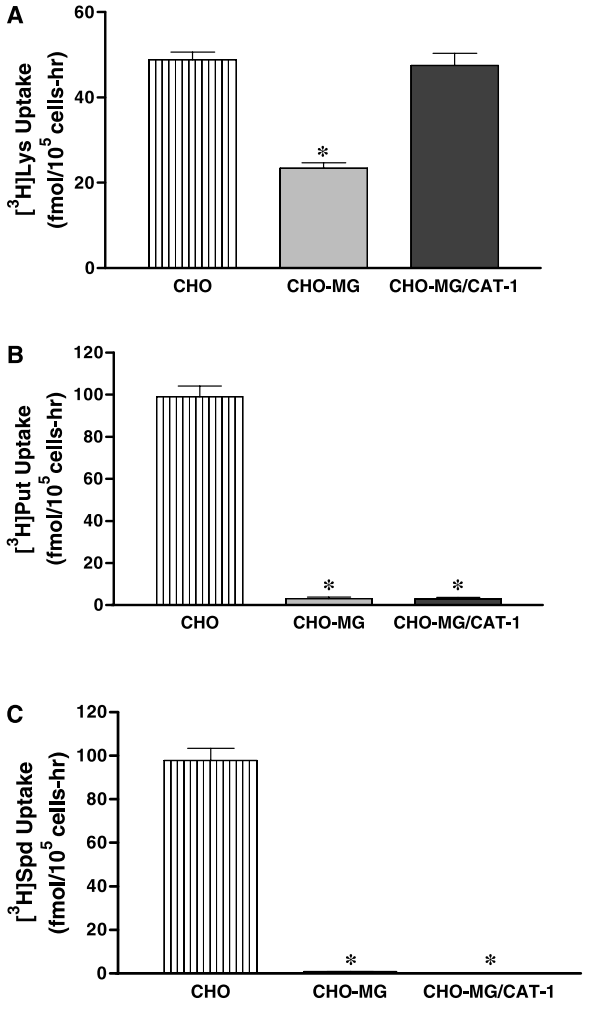


Fig. 4. Specific [^{3}H]Lys, [^{3}H]Put, or [^{3}H]Spd uptake in CHO, CHO-MG, and CHO-MG/mCAT-1 cells. CHO-MG cells were transfected with mCAT-1 cDNA and positive clones were isolated by G418 selection. (**A**) [^{3}H]lys, (**B**) [^{3}H]put, and (**C**) [^{3}H]spd uptake was measured in all three cell types. Each bar represents the mean of six experiments. $^*p \leq 0.5$

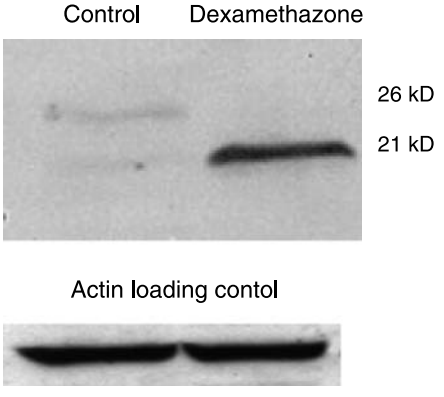


Fig. 5. Dexamethasone induces antizyme expression in Az-2 cells. Az-2 cells were treated with dexamethasone (1 μM) for 24 hrs and protein was collected and compared with protein from untreated control cells. The 26 kD band is endogenous, inactive Az protein, and the 21 kD band is the active protein overexpressed in Az-2 cells. Actin is shown for the loading control

for 24 hr, there was a large increase in the 21 kD, active form of antizyme.

When putrescine and spermidine uptake were measured in dexamethasone treated Az-2 cells overexpressing antizyme, polyamine transport dropped approximately 50% when compared to untreated Az-2 cells (Fig. 6A, B) confirming that a functional antizyme protein was being produced in the stable transfectants and that polyamine uptake was Az sensitive. However, when lysine transport was measured during Az overexpression, there was no

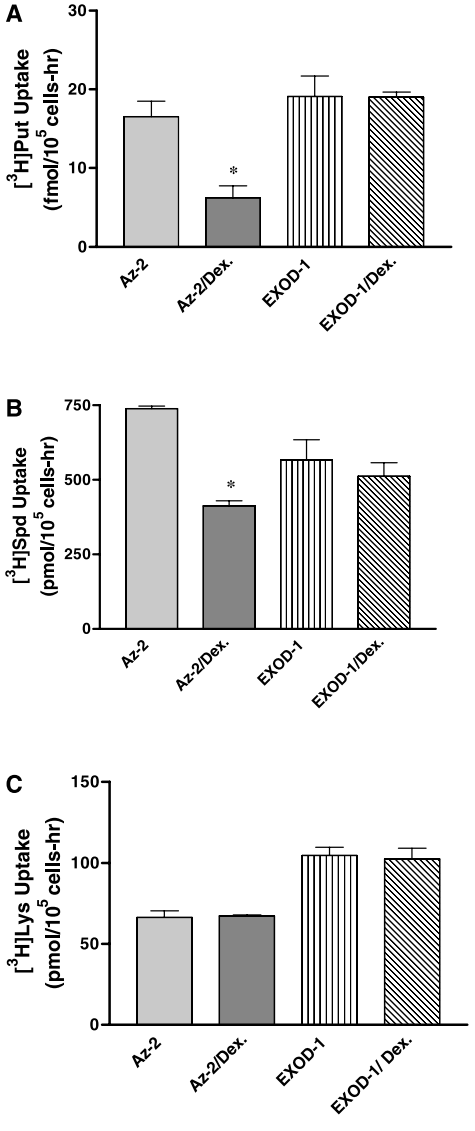


Fig. 6. [^{3}H]Put, [^{3}H]Spd, or [^{3}H]Lys uptake in Az-2 and EXOD-1 cells. EXOD-1 and Az-2 cells were treated with 1 μM dexamethasone for 24 hrs to induce antizyme overexpression in Az-2 cells and as a control in EXOD-1. All 4 cell groups were incubated with (**A**) 0.6 μCi [^{3}H]put, (**B**) 0.5 μCi [^{3}H]spd, or (**C**) 0.5 μCi [^{3}H]lys for 30 min. Cells were rinsed, dissolved, and [^{3}H]substrate uptake was measured. Each bar represents an $n = 3$. Each experiment performed in duplicate with similar results. $^*p \leq 0.5$

change in lysine transport (Fig. 6C). Therefore, lysine does not appear to be transported through an antizyme sensitive polyamine transport site. In control experiments, treatment of EXOD-1 cells with dexamethasone did not inhibit polyamine transport demonstrating the inhibition of polyamine transport in Az-2 cells was not due to dexamethasone treatment itself.

Discussion

Polyamines and lysine appear to compete for a common transporter as evidenced by the cross inhibition experiments. The transport of both polyamines and lysine is Ca^{++}/CaM and NEM sensitive, also suggesting a potential common transporter for both substrates. However, the y^+ transporter CAT-1 is not the common carrier. CAT-1 cDNA expression in a polyamine transport deficient cell line reconstituted lysine but not polyamine transport. Polyamines and lysine are also not traveling through an antizyme sensitive polyamine transporter as antizyme expression inhibited polyamine transport but was without effect on lysine transport.

In 1991, Medina et al. found that polyamines and cationic amino acids travel through similar transporters. Putrescine (55 μM) inhibited 47% of the uptake of L-ornithine (0.5 mM), a basic amino acid, in Ehrlich-cell membrane vesicles. By 30 minutes 99% of ornithine uptake was inhibited. The K_m for ornithine transport was 0.32 mM, within the range described for y^+ system transport. Two Na^+-independent pathways for uptake of ornithine were identified, and putrescine inhibited transport through both sites (Medina et al., 1991). To our knowledge, this is the only research to date demonstrating a common transport site for both cationic amino acids and polyamines.

Osborne and Seidel established that the postprandial polyamine concentration in the GI lumen was approximately 2 mM, well above the K_m values for polyamine transport, thus leading to the conclusion that intestinal polyamine transporters are saturated following ingestion of a meal (Osborne and Seidel, 1990). Table 1 shows the postprandial concentration of various amino acids. Most amino acids are present in high micromolar concentrations, specifically lysine and arginine. The K_m values for y^+ transporters range from 70 μM to 250 μM depending on the source (Deves and Boyd, 1998; Closs, 1996, 2002; Malandro and Kilberg, 1996; Palacin et al., 1998). Figure 1A shows that the IC_{50} values for lysine transport in IEC-6 cells are 1 μM and 12 μM. Since the gut concentration of lysine is 943 μM, significantly higher than the reported K_m values or the IEC-6 IC_{50} values for lysine transport, then it is reasonable to assume that all lysine transport sites are fully saturated during the postprandial period.

The competitive inhibition and cross inhibition curves in Fig. 1 were done to determine the possible overlap in intestinal transport of polyamines and lysine. There appear to be two distinct lysine transporters, one of which may also carry putrescine. There is only one putrescine site; this carrier is inhibited by both spermidine and lysine. Finally, there are two transport sites for spermidine. Spermidine transport is inhibited completely by putrescine but only partially by lysine. Therefore, there could be common transport site for the three substrates.

Removal of extracellular calcium by using a modified Ca^{++}-free Tyrode's solution produced a significant drop in both lysine and putrescine transport. The calmodulin antagonist W-7 also significantly inhibited the transport of both lysine and polyamines confirming that polyamine transport is Ca^{++}/CaM sensitive and revealing the novel finding that lysine transport also appears to have a Ca^{++}/CaM component. These data further support the hypothesis that there is a common transport site for both substrates. NEM is an accepted inhibitor for distinguishing lysine transport through the y^+ system. Since NEM inhibited 92% of putrescine transport in IEC-6 cells, it is probable that polyamines are specifically being carried through a y^+ transporter, and not one of the other systems capable of transporting basic amino acids. CAT-1 seemed the most likely candidate; it is expressed in small intestine and its K_m values for lysine are in the micromolar range, similar to the K_m values for polyamine uptake in intestinal epithelial cells. However, overexpression of the lysine carrier CAT-1 in the polyamine transport deficient cell line CHO-MG did not reconstitute polyamine uptake. Based on the ability of NEM to inhibit polyamine transport, the possibility of polyamines traveling through another member of the y^+ system should be considered. It should also be noted that NEM is an agent that breaks sulfhydryl bonds, but its ability to specifically inhibit lysine transport in IEC-6 cells suggests that the inhibition of polyamine transport by NEM is also specific. The NEM inhibition of polyamine transport appears to be just as selective as the inhibition of lysine transport is selective for y^+ transporters.

Polyamines could, therefore, be traveling through another CAT protein. There are three other known CAT transporters, CAT-2A, -2B, and -3. CAT-2A can be eliminated for multiple reasons. First, it is expressed only in skin, liver, and muscle especially post-trauma. CAT-2A also transports cationic amino acids with K_m values in the low mM range, which contradicts the K_m values for lysine presented in Fig. 1. CAT-3 can also be eliminated

because it is expressed mainly in the brain with no expression in the GI system. CAT-2B transports cationic amino acids with the K_m constants comparable to the micromolar range found for CAT-1. CAT-2B also has a wider expression pattern than CAT-2A and -3; it is expressed to high levels in lung and testis. It is also present in activated macrophages, ovary, skeletal muscle, skin, activated splenocytes, stomach, thymocytes, and uterus; however, no CAT-2B isoform was detected in the small intestine and only minimal expression was observed in large intestine (MacLeod, 1996; Malandro and Kilberg, 1996; Palacin et al., 1998; Closs, 1996; Deves and Boyd, 1998).

Although many of the competitive inhibition and calcium dependency experiments led us to conclude a common transport site exists for polyamines and the basic amino acids, overexpression of CAT-1 in CHO-MG cells did not recover polyamine transport eliminating it as the possible common transport site. Likewise, antizyme overexpression did not inhibit lysine transport demonstrating that lysine is probably not being carried through an antizyme sensitive polyamine transporter. In summary, the most plausible explanation of a common carrier is that polyamines are traveling through an unidentified y^+ lysine transport site.

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