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Na^+-independent Lysine Transport in Human Intestinal Caco-2 Cells

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Abstract. The nature of transepithelial and cellular transport of the dibasic amino acid lysine in human intestinal epithelial Caco-2 cells has been characterized. Intracellular accumulation of lysine across both the apical and basolateral membranes consists of a Na^+-independent, membrane potential-sensitive uptake. Na^+-independent lysine uptake at the basolateral membrane exceeds that at the apical membrane. Lysine uptake consists of both saturable and nonsaturable components. Na^+-independent lysine uptake at both membranes is inhibited by lysine, arginine, alanine, histidine, methionine, leucine, cystine, cysteine and homoserine. In contrast, proline and taurine are without inhibitory effects at both membranes. Fractional Na^+-independent lysine efflux from preloaded epithelial layers is greater at the basolateral membrane and shows trans-stimulation across both epithelial borders by lysine, arginine, alanine, histidine, methionine, and leucine but not proline and taurine. Na^+-independent lysine influx (10 μM) in the presence of 10 mM homoserine shows further concentration dependent inhibition by lysine. Taken together, these data are consistent with lysine transport being mediated by systems $b^{o,+}$, y^+ and a component of very low affinity (nonsaturable) at both membranes. The relative contribution to lysine uptake at each membrane surface (at 10 μM lysine), normalized to total apical uptake (100%), is apical $b^{o,+}$ (47%), y^+ (27%) and the nonsaturable component (26%), and basal $b^{o,+}$ (446%), y^+ (276%) and the nonsaturable component (20%). Northern analysis shows hybridization of Caco-2 poly(A)$^+$RNA with a human rBAT cDNA probe.

Key words: Amino acid transport — Brush-border membrane — Intestine — Epithelium — Caco-2 cell

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Introduction

The ability of intestinal enterocytes to absorb nutrients such as amino acids depends on the expression of discrete transporters at both the apical and basolateral borders of the epithelial cell. Recently the use of the expression cloning system involving *Xenopus laevis* oocytes has allowed the molecular identification of some of these transporters (Bertran et al., 1994; McGivan & Pastor-Anglada, 1994; Van Winkle, 1993). One such transporter originally cloned from the kidney of both rat [NBAT or D2, renal neutral and basic amino acid transporter (Wells & Hediger, 1992; Yan et al., 1992)] and rabbit [rBAT, renal basic amino acid transporter (Bertran et al., 1992*a*)] is associated with transport of dibasic (arginine and lysine) and certain neutral amino acids (including cysteine and cystine). Homologous cDNA for rBAT has also been cloned from human renal cortex (Bertran et al., 1993; Lee et al., 1993). rBAT mRNA is present in small intestine mucosae (Magagnin et al., 1992) and NBAT-related protein is expressed in brush borders (Pickel et al., 1993). When injected into *Xenopus laevis* oocytes, the associated transport properties are similar to those described for system $b^{o,+}$ (Bertran et al., 1992*a*), originally described in mouse blastocysts (Van Winkle, Campione & Gorman, 1988).

The rBAT protein is not the only one involved in dibasic amino acid transport. The ecotropic murine leukaemia virus receptor has been identified as the dibasic amino acid transport system y^+ (mCAT) (Kim et al., 1991; Wang et al., 1991). An hepatic form (mCAT-2) is a low-affinity, high-capacity transporter in contrast to mCAT-1 (high affinity) which is widely distributed in nonhepatic tissues (Closs et al., 1993). Both systems y^+ and $b^{o,+}$ share the ability to transport neutral amino acids such as cysteine and homoserine but y^+ may only transport neutral amino acids in the presence of Na^+, whereas

$b^{o,+}$ may transport such amino acids both in the presence and absence of Na^+ (Magagnin et al., 1992).

The Caco-2 cell system has proved a suitable model system for intestinal epithelial permeability studies (Hidalgo, Raub & Borchardt, 1989) of both nutrients [peptides and amino acids (Thwaites et al., 1993*a;b;* 1994*b*; Thwaites, Hirst & Simmons, 1994*a*)] and pharmaceutical products (Thwaites et al., 1994*a*; 1995). The purpose of the present study has been to define the ability of human intestinal Caco-2 cells to transport the dibasic amino acid lysine. We show Na^+-independent transport of lysine sensitive to membrane potential and to inhibition by both dibasic and neutral amino acids including homoserine. These observations suggest that system $b^{o,+}$ is involved in lysine transport at both the apical and basal borders of Caco-2 intestinal cells. A Na^+-independent and homoserine-insensitive component of lysine uptake is further inhibited by lysine suggesting that system y^+ also participates in lysine uptake at both epithelial surfaces. Poly $(A)^+$RNA isolated from Caco-2 cells induces transport of the dibasic amino acid arginine when injected into *Xenopus laevis* oocytes (*data not shown*). Northern analysis shows hybridization of Caco-2 poly$(A)^+$RNA with a human rBAT cDNA probe.

Materials and Methods

Materials

L-[U-^{14}C]lysine (specific activity 75 mCi/mmol) and L-[4,5-^{3}H]lysine (specific activity 85 Ci/mmol) were obtained from Amersham. Lysine and other amino acids were from Sigma and were the L-isomer unless stated otherwise. All other chemicals were from Merck. Cell culture consumables were from Life Technologies.

Cell Culture

Caco-2 cells (passage number 111–121) were cultured as described previously (Thwaites et al., 1993*a*; 1993*b*). Cell monolayers were prepared by seeding at high density ($4.4–5.0 \times 10^5$ cells.cm^2) onto 12 or 24.5 mm diameter tissue culture inserts [Transwell polycarbonate filters (Costar)]. Cell monolayers were maintained at 37°C in a humidified atmosphere of 5% CO_2 in air. Cell confluence was estimated by microscopy and determination of transepithelial resistance. Radiolabeled fluxes for lysine were performed 18–25 days after seeding and 18–24hr after feeding.

Transepithelial Lysine Fluxes and Intracellular Accumulation

Measurements were performed essentially as described previously (Thwaites et al., 1993*b*; 1994*b*). Briefly, cell monolayers were extensively washed (4 times in 500 ml of modified Krebs buffer (of composition (all mmol/l), NaCl 140, KCl 5.4 $CaCl_2$ 2.8, $MgSO_4$ 1.2, NaH_2PO_4 0.3, KH_2PO_4 0.3, HEPES 10, glucose 10 (pH to 7.4 at 37°C with Tris base))) or Na^+-free Krebs buffer where appropriate (as above but choline Cl replacing NaCl and NaH_2PO_4 *omitted*), and placed in fresh 6-well plates, each well containing 2 ml of prewarmed modified Krebs or Na^+-free Krebs buffer (pH 7.4). Aliquots of fresh Krebs buffer or Na^+-free Krebs (pH 7.4) were then placed in the apical chamber. Choline media did not contain detectable Na^+ (<0.1 mM). Radiolabeled lysine was used at tracer concentrations (0.2 μCi/ml) with lysine added to give a final concentration of 100 μM (or as stated). [^{3}H]Lysine was used to trace apical-to-basal flux (J_{a-b}) while [^{14}C]lysine was used to trace basal-to-apical flux (J_{b-a}). Epithelial layers were then incubated for 60 min at 37°C after which 200 μl samples were taken from apical and basal solutions for determination of transepithelial fluxes. After aspiration of remaining radioactive incubation solutions, cell monolayers were then washed by sequentially transferring tissue culture inserts through 4 beakers each containing 500 ml of ice-cold Na^+-free Krebs buffer (pH 7.4) to remove any extracellular radiolabel, and removed from the insert. Cell monolayer-associated radiolabel was determined by scintillation counting. Cellular accumulation of lysine is expressed as mM (or as a cell to medium (C/M) ratio). Cell height was determined by confocal imaging of intact cell layers and this value was used to estimate intracellular water. No allowance was made for osmotically inactive space, thus intracellular concentrations are likely to be underestimated. The amount of lysine incorporated into intracellular protein during the incubation period was assessed by measuring the amount of trichloroacetic acid (TCA) extractable activity. After incubation and washing, epithelial layers were extracted with 1 ml of 1% TCA overnight. After extraction TCA soluble and insoluble activity was determined. In choline media at 10 μM lysine (where the C/M ratio is maximal) 75 ± 2 and 69 ± 4% (n = 12) of [^{3}H]/[^{14}C]lysine activity entering the cell monolayers from apical and basolateral incubations, respectively, was TCA soluble.

Measurement of Lysine Influx and Efflux across Apical and Basolateral Cell Borders

Influx

Cell monolayers were washed by sequential transfer through 4 beakers containing 500 ml Na^+-free Krebs (pH 7.4) at 37°C and placed in fresh 6-well plates containing 2 ml prewarmed Na^+-free Krebs (pH 7.4) in both apical and basolateral compartments. Uptake was initiated by replacing the apical and/or basal solution with an experimental solution containing [^{3}H] or [^{14}C]lysine (0.2 μCi/ml, 10 μM). After incubation (1–60 min) the apical and basal solutions were rapidly aspirated and the cell monolayer was washed by sequential transfer through 4 beakers containing 500 ml ice-cold Na^+-free Krebs (pH 7.4). Cell-associated radioactivity was determined as above.

Efflux

Cell monolayers were washed in Krebs (composition as above) and then preloaded with [^{3}H]lysine (1 μCi/ml; 100 μM) in Krebs for 2 hr at 37°C. Cell monolayers were then extensively washed in ice-cold Na^+-free Krebs before being placed in dishes containing 2 ml of Na^+-free Krebs (basolateral solution), 2 ml of identical solution being added to the apical compartment (both prewarmed to 37°C). Efflux was measured simultaneously across both apical and basolateral cell borders by successive addition and collection of 2-ml aliquots of Na^+-free Krebs from both apical and basal compartment for each 10-min efflux period. The effects of external amino acids upon [^{3}H]lysine efflux were tested after 20-min control efflux by switching from amino acid-free (control) media to that containing 20-mM unlabeled amino acids in both apical and basal compartments. Efflux data are expressed as fractional loss

(×100) in each time period across both apical and basolateral membranes.

Isolation of Caco-2 Poly(A)$^+$RNA

Poly(A)$^+$RNA was isolated from 4×10^8 Caco-2 cells (grown as confluent monolayers in stock Roux flasks for 3–4 weeks) using the Invitrogen FastTrack mRNA isolation kit according to the manufacturer's instructions. Typical yield was 60 μg poly(A)$^+$RNA. Caco-2 mRNA was stored at −80°C until required.

Northern Analysis of Caco-2 Poly(A)$^+$RNA

Caco-2 poly(A)$^+$RNA (5 μg) and rabbit renal rBAT (2.2.kB) cRNA (10 ng) were denatured, and loaded and fractionated on a 1% agarose/formaldehyde gel. The gels were transferred to a GeneScreen nylon membrane by a vacuum procedure. Blots were prehybridized and hybridized in 50% formamide. After hybridization, blots were washed four times in 2× standard saline citrate (SSC)/0.1% SDS at room temperature and then twice for 30 min in 2× SSC/0.1% SDS at 42°C in a gently swirling water bath (50 rev/min). The ^{32}P-labeled cDNA probe for human rBAT was a 2.2kb *Bam*H1 fragment lacking 148bp at the 5′-end of the complete human rBAT cDNA (Bertran et al., 1993).

Statistics

Results are expressed as mean ± SEM. Statistical comparison of mean values were made using one-way analysis of variance (ANOVA) where multiple comparisons were made with a Dunnett's multiple comparison test. Constants for Michaelis-Menten kinetics (with and without a linear component) were calculated by nonlinear regression with the method of least-squares (FIG-P, Biosoft). Choice of the model which best-fit the data was made by an F test of the residual sum of squares between model fits (Dunham & Ellory, 1981).

Results

Transepithelial Lysine Transport and Intracellular Accumulation

In the presence of Na^+, transepithelial lysine fluxes are associated with a variable asymmetry. At 100 μM, in two separate cell batches apical-to-basal ($J_{a\text{-}b}$) transport (8.8 ± 0.2 ($n = 3$) and 1.2 ± 0.01 ($n = 4$) nmol.cm^{-2}.h^{-1}) was 4.21 and 0.98 fold that of basal-to-apical ($J_{b\text{-}a}$) transport; net transport (J_{net}) being 6.6 ± 0.2 ($n = 3$) and -0.13 ± 0.11 ($n = 4$) nmol.cm^{-2}.h^{-1}, respectively. At 10 μM lysine, $J_{a\text{-}b}$ was 0.51 ± 0.03 ($n = 7$) nmol.cm^{-2}.h^{-1} for two cell batches, the asymmetry was 2.6-fold and J_{net} was 0.31 ± 0.03 nmol.cm^{-2}.h^{-1}.

Complete replacement of medium NaCl by choline Cl did not inhibit apical-to-basal ($J_{a\text{-}b}$) lysine transport. At 10 μM lysine, $J_{a\text{-}b}$ was 0.47 ± 0.04 nmol.cm^{-2}.h^{-1} ($n = 8$) (not significantly different from paired data above) while J_{net} was reduced but not abolished (0.18 ± 0.04 nmol.cm^{-2}.h^{-1}).

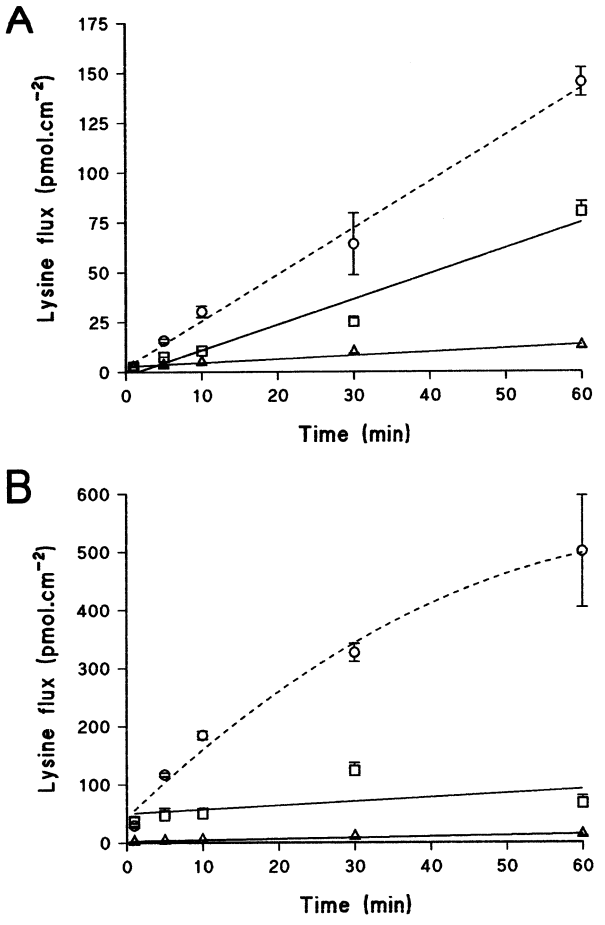

Fig. 1. Time dependence of lysine uptake across apical (*A*) or basolateral (*B*) surfaces of Caco-2 human intestinal epithelia. (○) Total uptake, (□) uptake in the presence of 10 mM homoserine, (△) uptake in the presence of both 10 mM homoserine and 10 mM lysine. Solid lines are linear regression lines or are fitted by eye. Data are the mean ± SEM of three epithelial layers per data point.

The time course of cellular lysine influx in Na^+-free medium (at 10 μM) is shown in Fig. 1. Cellular lysine uptake across the apical membrane is linear up to 1hr. The magnitude of total initial cellular lysine influx (5 min) across the basal membrane (1.40 ± 0.037 nmol.cm^{-2}.h^{-1} ($n = 3$) exceeds that across the apical membrane (0.19 ± 0.01 nmol.cm^{-2}.h^{-1}). Cellular accumulation of lysine above medium levels is evident across both apical and basal cell borders after 1 hr incubation (Fig. 1; Table 1,2). At 100 μM lysine (in Na^+-containing media) there is a 2.04 ± 0.20 ($n = 7$) and 4.20 ± 0.18-fold accumulation across the apical and basal cell borders, respectively. On replacement of medium Na^+ by choline there is a marked increase in lysine intracellular accumulation across both cell borders (Fig. 2; Table 2). In Na^+-free media, equimolar replacement of choline Cl by KCl results in a pronounced reduction in lysine ac-

Table 1. Inhibition of transepithelial lysine fluxes, and cellular accumulation across either the apical or basolateral membrane by dibasic and neutral amino acids

Amino acid	Transepithelial lysine flux (nmol · cm^{-2} · h^{-1})		Cellular lysine accumulation (mM)	
	J_{a-b}	J_{b-a}	Apical	Basolateral
Control	2.44 ± 0.38	2.64 ± 0.24	0.77 ± 0.04	2.09 ± 0.016
Lysine	1.65 ± 0.26	0.85 ± 0.26[b]	0.04 ± 0.00[b]	0.09 ± 0.00[b]
Arginine	1.10 ± 0.05	0.90 ± 0.21[b]	0.03 ± 0.00[b]	0.19 ± 0.01[b]
Alanine	3.32 ± 1.33	3.30 ± 0.64	0.13 ± 0.01[b]	0.51 ± 0.05[b]
Histidine	1.95 ± 0.20	1.20 ± 0.23[a]	0.11 ± 0.00[b]	0.47 ± 0.01[b]
Methionine	1.15 ± 0.22	1.37 ± 0.15[a]	0.09 ± 0.00[b]	0.61 ± 0.03[b]
Leucine	2.10 ± 0.51	0.82 ± 0.10[b]	0.07 ± 0.02[b]	0.60 ± 0.03[b]
Proline	3.17 ± 0.04	2.45 ± 0.37	1.13 ± 0.12[b]	3.17 ± 0.16[b]
Taurine	2.12 ± 0.27	2.55 ± 0.25	0.71 ± 0.04	1.87 ± 0.08

Experiments were performed in Na^+-free conditions with lysine present in both apical and basolateral chambers at 100 μM. Inhibitor amino acids (20 mM) were added to both apical and basolateral cell surfaces. Data are the mean ± SEM (n = 4 epithelial layers). Levels of significance of mean difference *vs.* control values were analyzed by ANOVA with multiple comparisons being made using Dunnetts Test ([a]$P < 0.05$, [b]$P < 0.01$).

Table 2. Inhibition of transepithelial lysine fluxes and cellular accumulation across either the apical or basolateral membrane by homoserine (in Na^+-containing and Na^+-free media), cysteine and cystine

Amino acid	Transepithelial lysine flux (nmol · cm^{-2} · h^{-1})		Cellular lysine accumulation (mM)	
	J_{a-b}	J_{b-a}	Apical	Basolateral
Na^+ media				
Control (7)	0.51 ± 0.03	0.19 ± 0.00	0.05 ± 0.00	0.09 ± 0.00
10 mM HS (6)	0.29 ± 0.08	0.19 ± 0.01	0.02 ± 0.00[b]	0.03 ± 0.00[b]
Choline media				
Control (8)	0.53 ± 0.08	0.29 ± 0.02	0.15 ± 0.01[a]	0.48 ± 0.02[a]
10 mM HS (7)	0.36 ± 0.03	0.29 ± 0.03	0.02 ± 0.00[c]	0.12 ± 0.01[c]
Diamide (4)	0.67 ± 0.13	0.30 ± 0.00	0.19 ± 0.01	0.39 ± 0.04
Cystine (8)	0.36 ± 0.07	0.40 ± 0.06	0.03 ± 0.01[c]	0.18 ± 0.08[c]
DTT (4)	0.40 ± 0.04	0.22 ± 0.00	0.16 ± 0.01	0.33 ± 0.03
Cysteine (4)	0.35 ± 0.09	0.20 ± 0.00	0.01 ± 0.00[c]	0.08 ± 0.01[c]

Apical and basolateral lysine concentration were 10 μM. Inhibitor amino acids were added to both apical and basolateral cell surfaces. Homoserine was present at 10 mM, while cysteine was at 10 mM (in the presence of 0.1 mM dithiothreitol (DTT)), cystine was at 200 μM with 0.1 mM diamide. Control data for DTT and diamide are shown. Data are the mean ± SEM (n = 4–8 epithelial layers). Levels of significance of mean difference *vs.* appropriate control values were analyzed by ANOVA with multiple comparisons being made using Dunnetts Test: [a]$P < 0.01$ (choline control *vs.* Na^+-media control); [b]$P < 0.01$; [c]$P < 0.001$.

cumulation across both cell borders (Fig. 2). This behavior is consistent with a Na^+-independent electrical membrane potential-sensitive lysine uptake mechanism at both apical and basal membranes.

Concentration Dependence of Lysine Transport and Intracellular Accumulation

Figure 3*a* shows the concentration dependence of the transepithelial fluxes J_{a-b}, J_{b-a} and J_{net}. It is clear that in Na^+-free media the extent of net transepithelial lysine transport is minimal (there is no significant absorption nor secretion). Inspection of the concentration-dependence of the transepithelial bidirectional fluxes indicates an absence of saturation. Kinetic analysis of J_{a-b} using either linear (L), rectangular hyperbola (saturable) (S) or linear plus rectangular hyperbola (L + S) models indicate that the latter model best fits the data (residual sum of squares (L) 616.3, (S) 928.8 and (L + S) 107.1, respectively, $F = 7.7$ $P < 0.05$ for L *vs.* L + S). The

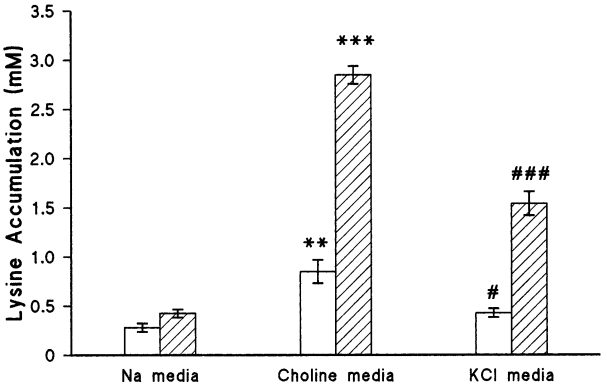

Fig. 2. Intracellular lysine accumulation from apical or basolateral cell solutions. Accumulation from the apical (open columns) or basal (hatched columns) medium. Na^+-free Krebs was prepared as indicated in Materials and Methods. To obtain KCL media, 50 mM KCl replaced choline Cl isosmotically. Significance of difference of choline media from Na^+ media (**, $P < 0.01$; ***, $P < 0.001$); significance of difference of KCl media from choline media (#, $P < 0.05$; ###, $P < 0.001$).

linear component (slope for $J_{a\text{-}b}$ = 16.5 ± 0.5 nmol.cm^{-2}.h^{-1}/mM) comprises the greater proportion of transepithelial flux (e.g., at 5 mM lysine) whereas the saturable component ($J_{a\text{-}b}$, V_{max} = 36.9 ± 7.2 nmol.cm^{-2}.h^{-1}) is minor. A similar analysis may be made for $J_{b\text{-}a}$ (residual sum of squares for L, S and L + S were 1930.5, 439.4 and 266.9, respectively, F = 8.36, $P < 0.025$ for improvement of model for L *vs.* L + S). The linear component of $J_{b\text{-}a}$ (16.5 ± 1.7 nmol.cm^{-2}.h^{-1}/mM) was identical to that for $J_{a\text{-}b}$, while the saturable component was again a minor component. This data is consistent with a substantial portion of transepithelial flux resulting from passive movement (perhaps via a paracellular route) at concentrations >5 mM lysine.

Since transepithelial lysine flux will consist of both flux across the paracellular pathway and across 2 membranes in series, it is likely that the kinetics of uptake across the apical and basal cell borders will provide more information concerning the cellular pathway. Figure 3*b* shows that intracellular accumulation of lysine in Na^+-free media is greater across the basal cell border. For cellular uptake across the basal cell border, the data are not best fit by a single saturable process (S, residual sum of squares 33.2), rather the data are more adequately described by a saturable component together with a smaller linear component (L + S, residual sum of squares, 5.0, F = 8.1, $P < 0.05$ S *vs.* L + S). The apparent K_m values (mM) for apical, basal and total intracellular lysine accumulation are similar (0.36 ± 0.07, 0.42 ± 0.12 and 0.38 ± 0.09, respectively) but are likely to represent ''lumped'' values reflecting the operation of at least two membrane transports (*see below*). The existence of a nonsaturable component implies the existence of a very low affinity pathway (possibly a passive leak pathway). The extremely low affinity (leak) pathway is relatively more important for lysine transport across the apical membrane.

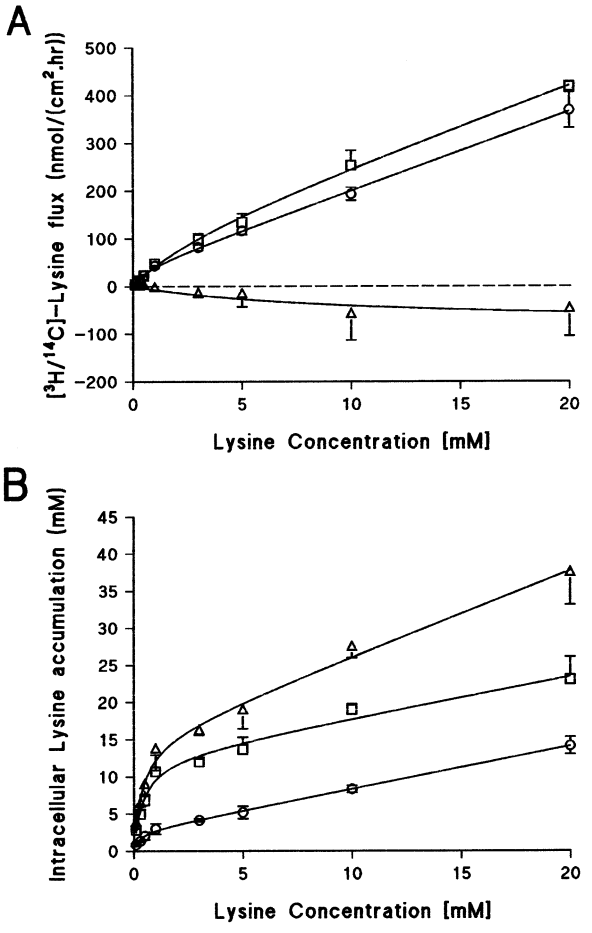

Fig. 3. Concentration dependence of lysine transepithelial transport and cellular accumulation in Caco-2 epithelia. Determinations were made in Na^+-free media in which lysine concentrations were equal in both apical and basal solutions. Results are mean ± SEM, n = 3–4 cell layers per concentration. (*A*) Transepithelial flux, (○) apical-to-basal transport ($J_{a\text{-}b}$), (□) basal-to-apical transport ($J_{b\text{-}a}$), and (△) net lysine transport ($J_{net} = J_{a\text{-}b}\text{-}J_{b\text{-}a}$). The solid lines for $J_{a\text{-}b}$ and $J_{b\text{-}a}$ show nonlinear least square fits of the data to $J = (V_{max} \cdot L/K_m + L) + mL$, where L is the lysine concentration, and m is the slope of a linear flux component (*see text*). For $J_{a\text{-}b}$ r^s = 0.999, for $J_{b\text{-}a}$ r^2 = 0.994. (*B*) Cellular accumulation, from basolateral (□) and apical (○) surfaces. Total cellular lysine accumulation (△) is also shown. The solid lines for cellular lysine accumulation show nonlinear least square fits of the data to $A = (V_{max} \cdot L/K_m + L) + mL$, where L is the lysine concentration, and m is the slope of a linear flux component. For basal accumulation K_m = 0.42 ± 0.12 mM, V_{max} = 12.7 ± 1.2 mmol/l cell water/hr and m = 0.56 ± 0.08 (r^2 = 0.984). For apical accumulation K_m = 0.36 ± 0.07 mM, V_{max} = 2.6 ± 0.2 mmol/l cell water/hr and m = 0.57 ± 0.02 (r^2 = 0.998).

Inhibition of Lysine Transport and Intracellular Accumulation

Table 1 shows the pattern of inhibition of transepithelial fluxes and of lysine accumulation (at 1 hr) by dibasic

amino acids and by certain neutral amino acids in Na^+-free media. The dibasic amino acids lysine and arginine inhibited cellular accumulation of lysine across both the apical and basal cell borders to low levels. Alanine, histidine, methionine, and leucine were all effective at inhibition of lysine accumulation at both cell borders but the intracellular levels of lysine uptake were elevated with respect to that seen with arginine and lysine. Proline and taurine were not effective as inhibitors. The relative inhibitory effects of amino acids on transepithelial lysine fluxes were not as marked as upon intracellular accumulation (Table 1). This is most likely due to the presence of a significant paracellular component to transepithelial lysine flux (above).

Table 2 shows that cystine (200 μM) in the presence of diamide is effective as an inhibitor of lysine accumulation across both the apical and basolateral membranes in Na^+-free media (compared to Na^+-free media containing diamide only). Similarly cysteine in the presence of DTT is also an effective inhibitor of lysine accumulation at both epithelial surfaces. No significant effect of inhibitors on transepithelial fluxes are observed at the lysine concentrations used (10 μM).

Dibasic amino acid transport may be mediated by both systems y^+ and $b^{o,+}$ (Magagnin et al., 1992). To assess the contribution of such transporters to lysine transport in Caco-2 cells the effect of homoserine (10 mM) on lysine accumulation was tested in both Na^+-containing and Na^+-free media (Table 2). Homoserine (10 mM) is an effective inhibitor of lysine accumulation at both the apical and basal surfaces in Na^+-free media. Since system y^+ shows inhibition by neutral amino acids only in the presence of Na^+, the inhibition seen by homoserine in these conditions may be attributed to functional expression of $b^{o,+}$ at both the apical and basal membranes of human intestinal Caco-2 cells. Figure 1 shows in Na^+-free conditions that lysine (10 mM) in the presence of 10 mM homoserine is capable of further inhibiting radiolabeled lysine influx (10 μM) in the presence of 10 mM homoserine alone at both the apical and basolateral cell borders. The existence of a neutral amino acid insensitive component to lysine uptake at both apical and basal surfaces in Na^+-free conditions which shows further inhibition by dibasic amino acids (Fig. 1 and Table 1) argues for an additional component of lysine transport in addition to that accounted for by $b^{o,+}$.

Figure 4 shows the lysine concentration dependence of the homoserine-insensitive lysine influx component measured in Na^+-free conditions at the apical and basolateral surfaces of Caco-2 cells. Basolateral influx is best fit by a simple Michaelis-Menton kinetics with a K_m of 0.56 ± 0.14 mM, and a V_{max} of 2.11 ± 0.12 $nmol.cm^{-2}.5min^{-1}$ (residual sum of squares was increased when a linear component introduced). In contrast, apical influx is best fit by a saturable component (K_m of 1.11 ± 0.40 mM and V_{max} of 0.54 ± 0.10 $nmol.cm^{-2}.h^{-1}$) together with a linear component of 0.22 $nmol.cm^{-2}.h^{-1}$/mM (residual sum of squares for L, S, and L + S models were 68,317, 23275 and 2422, respectively, F = 7.3 for S improved by L + S, $P < 0.025$). Thus apical influx consists of a saturable process of higher affinity and an extremely low affinity (leak) pathway.

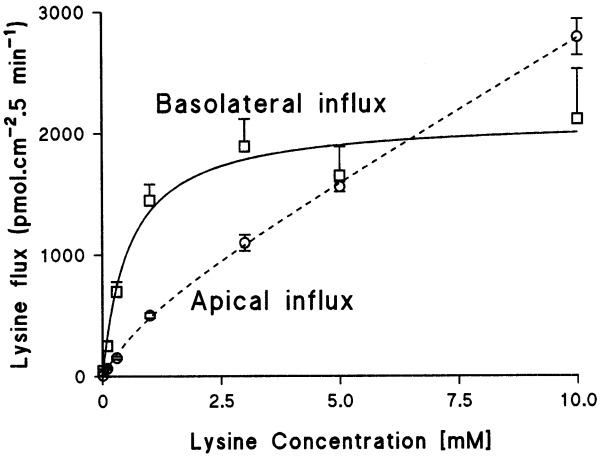

Fig. 4. Lysine concentration dependence of the Na^+-independent, homoserine (10 mM)-insensitive component of lysine uptake. Uptakes were measured at 5 min, (□) basolateral and (○) apical uptake, respectively. Data are the mean ± SEM of three epithelial layers per data point.

Stimulation of Lysine Exchange Efflux by External Amino Acids in Na^+-free Media

Expression of the rBAT protein in *Xenopus laevis* oocytes is associated with the exchange of dibasic and some neutral amino acids in Na^+-free conditions (Busch et al., 1994; Coady et al., 1994). We have tested whether lysine efflux across both the apical and basal membranes of Caco-2 epithelia may be trans-stimulated by extracellular amino acids. Table 3 shows that the fractional rate of lysine efflux across the basolateral membrane exceeds that across the apical membrane by 2.8-fold [fractional efflux across the apical border was 2.3 ± 0.3 (n = 36), that across basolateral border was 6.6 ± 1.0 (n = 36)]. External lysine, arginine, alanine, histidine, methionine, and leucine all result in a significant increase in the rate of fractional loss across both the apical and basolateral surfaces (Table 3). In contrast, proline and taurine are without effect at both membranes (Table 3). This pattern of transstimulation of lysine exchange efflux is entirely consistent with the observed pattern of inhibition of lysine uptake at each cellular border (Table 1). Of note is the contrasting action of alanine at the apical and basal

Table 3. Fractional efflux of lysine across the apical and basolateral borders of Caco-2 epithelia. Epithelial monolayers were preloaded with [^{3}H]lysine (100 μM total concentration) for 2 hr

Time (min)	Efflux (% total loaded)				
	T_{0-10}	T_{10-20}	T_{20-30}	T_{30-40}	T_{40-50}
Control					
Apical	6.9 ± 1.0	3.8 ± 0.3	3.5 ± 0.5	2.5 ± 0.6	2.6 (n = 2)
Basal	11.8 ± 1.3	10.8 ± 1.0	8.4 ± 0.5	4.3 (n = 2)	3.8 (n = 2)
+ Lys					
Apical	5.7 ± 0.5	3.2 ± 0.4	7.3 ± 1.0	6.1 ± 0.5	4.8 ± 0.2
Basal	12.0 ± 1.8	11.1 ± 1.8	64.9 ± 4.9	18.9 ± 3.7	6.9 ± 0.7
+ Arg					
Apical	5.5 ± 0.1	3.1 ± 0.1	5.3 ± 0.1	4.8 ± 0.4	4.5 ± 0.4
Basal	14.3 ± 1.1	9.2 ± 0.5	68.0 ± 3.4	20.3 ± 0.5	8.8 ± 1.6
+ Ala					
Apical	5.2 ± 0.5	2.3 ± 0.2	17.6 ± 0.7	9.8 ± 0.3	5.7 ± 0.5
Basal	7.3 ± 0.3	5.9 ± 0.4	25.3 ± 0.6	28.7 ± 1.0	18.9 ± 0.4
+ Pro					
Apical	4.1 ± 0.4	2.0 ± 0.2	1.5 ± 0.1	1.2 ± 0.1	1.0 ± 0.1
Basal	6.5 ± 0.6	5.2 ± 0.7	4.8 ± 0.3	3.7 ± 0.1	3.7 ± 0.4
+ Tau					
Apical	3.3 ± 0.1	1.5 ± 0.0	1.5 ± 0.1	1.1 ± 0.1	1.4 ± 0.1
Basal	6.2 ± 0.2	3.8 ± 0.1	3.9 ± 0.1	2.8 ± 0.3	3.9 ± 0.3
+ Leu					
Apical	3.3 ± 0.1	1.6 ± 0.2	5.1 ± 0.3	3.2 ± 0.2	2.5 ± 0.1
Basal	5.9 ± 0.3	4.5 ± 0.1	59.7 ± 1.4	20.6 ± 2.6	6.3 ± 0.5
+ His					
Apical	3.4 ± 0.2	1.6 ± 0.1	5.8 ± 0.3	3.6 ± 0.1	3.0 ± 0.1
Basal	6.1 ± 0.2	4.4 ± 0.2	64.5 ± 0.5	14.2 ± 0.6	6.2 ± 0.2
+ Met					
Apical	3.6 ± 0.2	1.8 ± 0.2	6.9 ± 0.5	3.6 ± 0.3	3.0 ± 0.3
Basal	6.2 ± 0.5	4.7 ± 0.3	49.4 ± 2.6	24.9 ± 2.3	10.4 ± 0.4

Efflux was measured in sequential 10-min periods (*see* Materials and Methods). At T = 20 min, efflux media were switched from amino acid-free (control) media to those containing 20 mM unlabeled amino acids as indicated. Data are the means ± SEM of 4 separate monolayers (except where stated otherwise).

borders; the relative stimulation of lysine efflux at the apical membrane by alanine exceeds that seen with other substrates for system $b^{o,+}$. The opposite pattern is observed at the basal membrane. This may indicate a difference between the transporter expressed at the apical membrane with respect to the basal membrane or additional transport pathways for alanine per se at the apical membrane which influence lysine efflux.

Molecular Verification of rBAT Expression in Caco-2 Epithelial Cells

The Northern blot of Caco-2 poly(A)$^+$RNA after hybridization with a human rBAT cDNA probe (Fig. 5) shows two signals of 2.2–2.3 kB and 3.7–3.9 kB. This is similar to that obtained from mRNA obtained from rabbit epithelia (Markovich et al., 1993). It is known that both mRNA transcripts are translated to an identical protein with identical functional characteristics in rabbit kidney

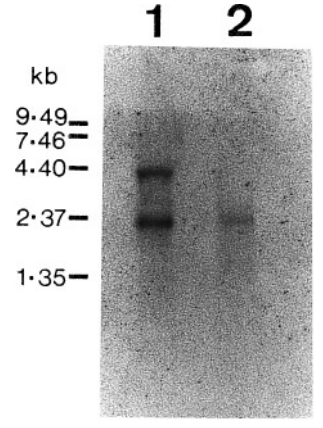

Fig. 5. Northern blot analysis of expression of rBAT by Caco-2 intestinal epithelia. Caco-2 poly(A)$^+$RNA (5 μg) and rabbit renal rBAT (2.2.kB) cRNA (10 ng) were fractionated on a 1% agarose gel and transferred to nitocellulose. Human renal rBAT probe hybridized to Caco-2 poly(A)$^+$RNA (lane 1) and rabbit renal rBAT cRNA (lane 2).

(Markovich et al., 1993). It is likely that the human homologue of rBAT is expressed in Caco-2 cells.

Discussion

In studies of isolated rat intestinal cells by Reiser & Christiansen (1973*a*), the concentrative Na^+-independent uptake of the dibasic amino acid lysine was documented. Cellular uptake was linear for extended influx times up to 10 min. In addition, trans-stimulation of lysine transport was noted with intracellular alanine suggesting that certain neutral amino acids could interact with the transporter (Reiser & Christiansen, 1973*b*). The current data showing concentrative Na^+-independent accumulative transport of the dibasic amino acid lysine across both apical and basolateral surfaces are similar to that reported for isolated enterocytes. In the present data we show that in Na^+-free conditions lysine influx is increased compared to Na^+-media and that Na^+-free, high K^+-media reduce lysine uptake compared to Na^+-free media consistent with a membrane-potential sensitive uptake of lysine. The extended uptake of lysine does not show equilibration even after 1 hr indicating that the intracellular compartment acts as a sink for lysine uptake, uptake at the basolateral surface exceeding that across the apical surface.

A number of different cDNAs (rBAT, Bertran et al., 1992*a*; murine leukaemia virus receptor, Kim et al., 1991; CAT-1/2, Closs et al., 1993; 4F2hc antigen, Bertran et al., 1992*b*) induce dibasic amino acid transport when in vitro transcribed cRNAs are expressed in *Xenopus* oocytes. The associated transport properties resemble the properties of system $b^{o,+}$ (rBAT), system y^+ (MuLVr, CAT-1), or mixtures of $b^{o,+}$ and y^+, or y^+L (4F2hc). Currently there is a great deal of discussion as to whether the rBAT/D2/NBAT cDNA encodes for a component (activator or subunit) of an amino acid transporter or for a complete transport mechanism (Bertran et al., 1994; Van Winkle, 1993). Recently, Wang & Tate (1995) have demonstrated that the NBAT-related protein (85 kDa) exists as a heterodimer with a 50 kDa protein in rat kidney and jejunal brush-border membranes, rabbit kidney brush-border membranes and *Xenopus laevis* oocyte membranes. System $b^{o,+}$ may, therefore, consist of two membrane components, the 85 kDa protein (rBAT/D2/NBAT) and the unidentified 50 kDa protein (Wang & Tate, 1995).

The relative contribution of the amino acid transporters $b^{o,+}$ and y^+ to the intestinal transport of dibasic amino acids in intact preparations is not certain. Magagnin et al. (1992) have determined that mRNA isolated from rabbit intestinal mucosae may induce both $b^{o,+}$ and y^+ activity when expressed in *Xenopus laevis* oocytes. Hybrid-depletion experiments using rBAT antisense cDNA revealed two components to arginine uptake in oocytes. The mRNA-induced component of arginine uptake which was resistant to rBAT-hybrid depletion was inhibited by an excess of L-homoserine but only in the presence of Na^+. In choline medium, homoserine was without effect of this component. This characteristic is typical of system y^+.

For Caco-2 human intestinal cells at least three components are evident in lysine uptake at both the apical and basolateral membranes. There are both homoserine-sensitive and insensitive components to lysine influx in both Na^+-containing and Na^+-free media. The ability of homoserine, cystine, cysteine, alanine, histidine, methionine and leucine, but not proline and taurine to inhibit lysine influx in Na^+-free media is consistent with the expression of system $b^{o,+}$ at both epithelial surfaces. The evidence for expression of system $b^{o,+}$ at both epithelial surfaces is strengthened further by the data on amino acid efflux which show that those amino acids capable of cis-inhibition of lysine influx are also capable of trans-stimulation of lysine efflux in Na^+-free conditions. We have confirmed by Northern blot analysis that a highly homologous mRNA to rBAT is expressed in Caco-2 epithelial cells. In addition to system $b^{o,+}$ the homoserine-insensitive component shows further inhibition by lysine consistent with expression of system y^+ at both epithelial surfaces. Pan, Malandro & Stevens (1995) have investigated the uptake of arginine in Caco-2 cells grown on plastic culture dishes. However, under these conditions the relative uptake at apical and basolateral membranes cannot be assessed. These authors show that arginine uptake into Caco-2 cells is mediated in part by system y^+ and that Caco-2 cells express mCAT-1 mRNA. In the present study, the kinetics of transport in the presence of homoserine at each epithelial surface differ markedly (we have fitted the data for the apical surface to a saturable component of higher affinity and a very low affinity (linear) component). Van Winkle, Kakuda & MacLeod (1995) have shown that mCAT-1 and mCAT-2 expressed in *Xenopus laevis* oocytes are associated with multiple kinetics when arginine transport is analyzed using electrophysiological means. The data shown for Caco-2 cells could indicate the cellular coexistence of two forms of the y^+ transporter of different affinities. The data from oocytes suggest the possibility that single mCAT proteins may exist in kinetically different forms or that their transport characteristics may be altered by accessory proteins. In Caco-2 cells alternatively spliced products of a single protein may exist or the different environment of the protein in the apical or basolateral domain could influence the kinetic properties. Further experiments are required to resolve this issue. System y^+L (a high-affinity, low-capacity transporter that shows high-affinity inhibition of lysine transport by neutral amino acids only in the presence of Na^+) may play a role in intestinal lysine transport, as identified in the placenta

(Eleno, Deves & Boyd, 1994; Furesz, Moe & Smith, 1995), although its quantitative significance may be limited. Injection of 4F2hc cRNA into *Xenopus laevis* oocytes leads to an increase in system y^+ L-like activity (Van Winkle, 1993). However, 4F2hc cRNA-injected oocytes showed no uptake of cystine either in the presence or absence of Na^+ (Bertran et al., 1992*b*).

In the present study in Caco-2 cell monolayers, in addition to saturable components of higher affinity there is also evidence for lysine uptake of very low affinity at both epithelial surfaces. This is of quantitative importance for uptake at the apical surface. Whether this uptake represents membrane permeation via cation-selective channel pathways is unclear. There is precedent in volume-activated Cl^- channels being able to mediate amino acid loss from intracellular locations in volume regulatory decrease (Roy, 1995). Finally, we have made an assessment of the relative contribution of systems $b^{o,+}$, y^+ and a nonsaturable component to lysine uptake at each membrane surface. At 10 μM lysine the fluxes [normalized to total apical uptake (100%)] across the apical membrane represent $b^{o,+}$ (47%), y^+ (27%) and a nonsaturable component (26%), and across the basolateral surface $b^{o,+}$ (446%), y^+ (276%) and a nonsaturable component (20%). The current data have been derived from fully differentiated cells grown as epithelial layers on permeant matrices. Pan et al. (1995) show that the magnitude of arginine uptake in Caco-2 cells declines as confluence is reached. An interpretation of these data could be that the basolateral surface becomes inaccessible. Our own data show that transport across the basolateral surface exceeds that across the apical surface in epithelial layers of Caco-2 cells grown on permeant matrices. With respect to the magnitude of the difference between apical and basolateral surface, it is uncertain whether this represents a difference in membrane area between apical and basolateral domains. It should be noted that the nonsaturable component of lysine uptake is of a similar magnitude across both apical and basolateral surfaces.

It is now clear that mutations in the rBAT-related protein are associated with the clinical condition of cystinuria (Calonge et al., 1994) in which disordered renal (and intestinal) transport of dibasic amino acids and cystine occurs (Calonge et al., 1994). Injection of mutated human rBAT cRNA in *Xenopus* oocytes shows depressed uptake of cystine and dibasic amino acid transport compared with transport in oocytes injected with wild type rBAT cRNA (Calonge et al., 1994). An intestinal defect in lysine absorption has been characterized in cystinuric patients. In isolated peroral biopsies the Na^+-dependent accumulation of lysine across the brush border was reduced without change in the Na^+-independent lysine accumulation (Coicadan et al., 1980). Our present observations suggest that multiple transport pathways mediate uptake at the brush-border and that a Na^+-dependent defect would not be predicted.

For Caco-2 epithelia in open circuit conditions the spontaneous p.d. is small (0.1 mV basal solution electropositive, *unpublished data*, N.L. Simmons). Thus net transepithelial lysine movements will not be primarily determined by such gradients despite the paracellular component. In the absence of other external gradients of amino acids, concentrative cellular uptake of lysine gives variable levels of net lysine transepithelial transport which are relatively insensitive to medium Na^+ replacement. It is clear that for transepithelial absorption to occur, exit across the basolateral membrane must occur. Amino acid exchange fluxes mediated by both $b^{o,+}$ and y^+ must contribute to efflux across this membrane. Since exchange fluxes are rheogenic (Ahmed et al., 1995; Busch et al., 1994; Coady et al., 1994), lysine efflux from cytosol (absorption) would be driven by the uptake of neutral amino acids across the basolateral membrane. In the enterocyte this net direction of neutral amino acid flow would only occur when amino acids were not being accumulated from the lumen (i.e., postprandially). Our present data show that in Caco-2 human intestinal cells system $b^{o,+}$-like activity is predominantly at the basolateral membrane, and is likely to contribute to exchange efflux of lysine.

Ultrastructural localization of rBAT and NBAT-related protein(s) in rat renal epithelial cells shows localization to the microvillus membrane of proximal straight tubules (Furriols et al., 1993; Pickel et al., 1993). In rat intestinal enterocytes the microvilli were less frequently immunoreactive, with submucosal neurons being strongly immunoreactive (Pickel et al., 1993) Thus in the intestine, the relative expression at apical and basolateral domains, perhaps with low levels of protein, remains unclear. Further work will need to establish the spatial distribution of lysine transport mechanisms on human intestinal enterocytes in vivo.

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References

Ahmed, A., Peter, G.J., Taylor, P.M., Harper, A.A., Rennie, M.J. 1995. Sodium-independent currents of opposite polarity evoked by neutral and cationic amino acids in neutral and basic amino acid transporter cRNA-injected oocytes. *J. Biol. Chem.* **270:**8482–8486

Bertran, J., Magagnin, S., Werner, A., Markovich, D., Biber, J., Testar, X., Zorzano, A., Kühn, L.C., Palacin, M., Murer, H. 1992*b*. Stimulation of system y^+-like amino acid transport by the heavy chain of the human 4F2 surface antigen in *Xenopus laevis* oocytes. *Proc. Natl. Acad. Sci. USA* **89:**5606–5610

Bertran, J., Testar, X., Zorzano, A., Palacin, M. 1994. A new age for

plasma membrane amino acid transporters. *Cell Physiol. Biochem.* **4:**217–241

Bertran, J., Werner, A., Chillaron, J., Nunes, V., Biber, J., Testar, X., Zorzano, A., Estivill, X., Murer, H., Palacin, M. 1993. Expression cloning of a human renal cDNA that induces high affinity transport of L-cystine shared with dibasic amino acids in *Xenopus* oocytes. *J. Biol. Chem.* **268:**14842–14849

Bertran, J., Werner, A., Moore, M.L., Stange, G., Markovich, D., Biber, J., Testar, X., Zorzano, A., Palacin, M., Murer, H. 1992*a*. Expression cloning of a cDNA from rabbit kidney cortex that induces a single transport system for cystine and dibasic neutral amino acids. *Proc. Natl. Acad. Sci. USA* **89:**5601–5605

Busch, A.E., Herzer, T., Waldegger, S., Schmidt, F., Palacin, M., Biber, J., Markovich, D., Murer, H., Lang, F. 1994. Opposite directed currents induced by the transport of dibasic and neutral amino acids in *Xenopus* oocytes expressing the protein rBAT. *J. Biol. Chem.* **269:**25581–25586

Calonge, M.J., Gasparini, P., Chillaron, J., Chillon, M., Gallucci, M., Rousaud, F., Zelante, L., Testar, X., Dallapiccola, B., Di Silverio, F., Barcelo, P., Estivill, X., Zorzano, A., Nunes, V., Palacin, M. 1994. Cystinuria caused by mutations in rBAT, a gene involved in the transport of cystine. *Nature Genetics* **6:**420–425

Closs, E.I., Albritton, L.M., Kim, J.W., Cunningham, J.M. 1993. Identification of a low affinity, high capacity transporter of cationic amino acids in mouse liver. *J. Biol. Chem.* **268:**7538–7544

Coady, M.J., Jalal, F., Chen, X., Lemay, G., Berteloot, A., Laponte, J.Y. 1994. Electrogenic amino acid exchange via the rBAT transporter. *FEBS Lett* **356:**174–178

Coicadan, L., Heyman, M., Grasset, E., Desjeux, J.-F. 1980. Cystinuria: reduced lysine permeability at the brush border of intestinal membrane cells. *Pediat. Res.* **14:**109–112

Dunham, P.B., Ellory, J.C. 1981. Passive potassium transport in low potassium sheep red cells: dependence upon cell volume and chloride. *J. Physiol.* **318:**511–530

Eleno, N., Deves, R., Boyd, C.A.R. 1994. Membrane potential dependence of the kinetics of cationic amino acid transport systems in human placenta. *J. Physiol.* **479:**291–300

Furesz, T.C., Moe, A.J., Smith, C.H. 1995. Lysine uptake by human placental microvillus membrane: comparison of system y^+ with basal membrane. *Am. J. Physiol.* **268:**C755–C761

Furriols, M., Chillaron, J., Mora, C., Castello, A., Bertran, J., Camps, M., Testar, X., Vilaro, S., Zorzano, A., Palacin, M. 1993. rBAT, related to L-cystine transport is localized to the microvilli of proximal straight tubules, and its expression is regulated in kidney by development. *J. Biol. Chem.* **268:**27060–27068

Hidalgo, I.J., Raub, T.J., Borchardt, R.T. 1989. Characterisation of the human colonic carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. *Gastroenterology* **96:**736–749

Kim, J.W., Closs, E.L., Albritton, L.M., Cunningham, J.M. 1991. Transport of cationic amino acids by the mouse ecotropic retrovirus receptor. *Nature* **352:**725–728

Lee, W.-S., Wells, R.G., Sabbag, R.V., Mohandas, T.K., Hediger, M.A. 1993. Cloning and chromosomal localization of a human kidney cDNA involved in cystine, dibasic and neutral amino acid transport. *J. Clin. Invest.* **91:**1959–1963

Magagnin, S., Bertran, J., Werner, A., Markovich, D., Biber, J., Palacin, M., Murer, H. 1992. Poly(A)$^+$RNA from rabbit intestinal mucosa induces $b^{o,+}$ and y^+ amino acid transport activities in *Xenopus laevia* oocytes. *J. Biol. Chem.* **267:**15384–15390

Markovich, D., Stange, G., Bertran, J., Palacin, M., Werner, A., Biber, J., Murer, H. 1993. Two mRNA transcripts (rBAT-1 and rBAT-2) are involved in system $b^{o,+}$-related amino acid transport. *J. Biol. Chem.* **268:**1362–1367

McGivan, J.D., Pastor-Anglada, M. 1994. Regulatory and molecular aspects of mammalian amino acid transport. *Biochem. J.* **299:**321–334

Pan, N., Malandro, M., Stevens, B.R. 1995. Regulation of system y^+ arginine transport capacity in differentiating human intestinal Caco-2 cells. *Am. J. Physiol.* **268:**G578–G585

Pickel, V.M., Nirenberg, M.J., Chan, J., Mosckovitz, R., Udenfriend, S., Tate, S.S. 1993. Ultrastructural localisation of a neutral and basic amino acid transporter in rat kidney and intestine. *Proc. Natl. Acad. Sci. USA* **90:**7779–7783

Reiser, S., Christiansen, P.A. 1973*a*. The properties of Na^+-dependent and Na^+-independent lysine uptake by isolated intestinal epithelial cells. *Biochim. Biophys. Acta* **307:**212–222

Reiser, S., Christiansen, P.A. 1973*b*. Exchange transport and amino acid charge as the basis for Na^+-independent lysine uptake by isolated intestinal epithelial cells. *Biochim. Biophys. Acta* **307:**223–233

Roy, G. 1995. Amino-acid current through anion channels in cultured human glial cells. *J. Membrane Biol.* **147:**35–44

Thwaites, D.T., Brown, C.D.A., Hirst, B.H. Simmons, N.L. 1993*a*. Transepithelial glycylsarcosine transport in intestinal Caco-2 cells mediated by expression of H^+-coupled carriers at both apical and basal membranes. *J. Biol. Chem.* **268:**7640–7642

Thwaites, D.T., Cavet, M., Hirst, B.H., Simmons, N.L. 1995. Angiotension-converting enzyme (ACE) inhibitor transport in human intestinal epithelial (Caco-2) cells. *Br. J. Pharmacol* **114:**981–986

Thwaites, D.T., Hirst, B.H., Simmons, N.L. 1994*a*. Substrate specificity of the di/tripeptide transporter in human intestinal epithelia (Caco-2): identification of substrates that undergo H^+-coupled absorption. *Br. J. Pharmacol.* **113:**1050–1056

Thwaites, D.T., McEwan, G.T.A., Brown, C.D.A., Hirst, B.H., Simmons, N.L. 1993*b*. Na^+-independent, H^+-coupled transepithelial β-alanine absorption by human intestinal Caco-2 cell monolayers. *J. Biol. Chem.* **268:**18438–18441

Thwaites, D.T., McEwan, G.T.A., Brown, C.D.A., Hirst, B.H., Simmons, N.L. 1994*b*. L-Alanine absorption in human intestinal cells driven by the proton electrochemical gradient. *J. Membrane Biol.* **140:**143–151

Van Winkle, L.J. 1993. Endogenous amino acid transport systems and expression of amino acid transport proteins in *Xenopus* oocytes. *Biochim. Biophys. Acta* **1154:**157–172

Van Winkle, L.J., Campione, A.L., Gorman, J.M. 1988. Na^+-independent transport of basic and zwitterionic amino acids in mouse blastocysts by a shared system and by processes which distinguish between these substrates. *J. Biol. Chem.* **263:**3150–3156

Van Winkle, L.J., Kakuda, D.K., MacLeod, C.L. 1995. Multiple components of transport are associated with murine cationic amino acid transporter (mCAT) expression in *Xenopus* oocytes. *Biochim. Biophys. Acta* **1233:**213–216

Wang, H., Kavavaugh, M.P., North, R.A., Kabat, D. 1991. Cell surface receptor for ecotropic murine retroviruses is a basic amino acid transporter. *Nature* **352:**729–731

Wang, Y., Tate, S.S. 1995. Oliomeric structure of a renal cystine transporter: implications in cystinuria. *FEBS Lett.* **368:**389–392

Wells, R.G., Hediger, M.A. 1992. Cloning of a rat kidney cDNA that stimulates dibasic and neutral amino acid transport and has sequence similarity to glucosidases. *Proc. Natl. Acad. Sci. USA* **89:**5596–5600

Yan, N., Mosckovitz, R., Udenfriend, S., Tate, S.S. 1992. Distribution of mRNA of a Na^+-independent neutral amino acid transporter cloned from rat kidney and its expression in mammalian tissues and *Xenopus laevis* oocytes. *Proc. Natl. Acad. Sci. USA* **89:**9982–9985