J. Membrane Biol. 171, 55–62 (1999)

The Journal of
Membrane
Biology

Identification of Three Cationic Amino Acid Transporters in Placental Trophoblast: Cloning, Expression, and Characterization of hCAT-1

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Received: 19 January 1999/Revised: 14 May 1999

Abstract. The concentrative transfer of amino acids from maternal to fetal blood is essential to fetal growth and metabolism. Cationic amino acids are transported across the placental microvillous and basal membranes by multiple pathways which act to mediate maternal/fetal transport. To identify the cationic amino acid transporters of human placenta, total RNA was harvested from cultured trophoblast and from the BeWo choriocarcinoma cell line, b30 clone, and used for reverse transcription (RT) and polymerase chain reaction (PCR). Primers based on published sequences identified expression of mRNAs for hCATs-1, -2B, and -4. RT-PCR yielded a 2.1 kb hCAT-1 cDNA which was cloned. hCAT-1 cRNA injection into *Xenopus laevis* oocytes stimulated saturable lysine uptake (K_m ~100 μM). In the presence of Na^+, uptake was inhibited by leucine, homoserine, and alanine but not by valine and glutamate. These transport characteristics are comparable to those of system y^+ in placental basal membrane, but differ from those of the same system in microvillous membrane. The identification, cloning, and characterization of multiple human placental cationic amino acid transporters has the potential to facilitate molecular investigation of transport by the maternal- and fetal-facing membranes of placental trophoblast and increase understanding of the mechanism of transplacental amino acid transfer.

Key words: Fetal nutrition — Lysine uptake — System y^+ — hCAT-2B — hCAT-4

Introduction

Fetal growth and development depend on the continuous transfer of amino acids from maternal to fetal blood across the syncytiotrophoblast [31]. The movement of cationic amino acids across the microvillous (maternal-facing) and basal (fetal-facing) plasma membranes is mediated by a group of sodium-independent mechanisms [14, 16, 34–36]. In human placental membranes and related cell culture models, we and others have identified the high capacity system y^+ and the low capacity, high affinity systems $b^{0,+}$ and y^+L [17–21]. Functional differences in the processes by which cationic amino acids interact with these two membranes are likely to provide the basis for vectorial maternal/fetal transport [20]. In particular, the differential interaction of the high capacity transport mechanisms of these membranes with neutral amino acids in the presence of sodium may be expected to enhance uptake at the microvillous membrane and facilitate transplacental transfer to the fetus [20].

In mammalian tissues four cDNAs are known to code for proteins mediating high capacity cationic amino acid transport [2, 12, 15, 26, 32]. These have been termed CAT-1, CAT-2, CAT-3, and CAT-4. The protein products of all four of these cDNAs mediate high capacity cationic amino acid transport. At present the CAT-3 sequence is known only in the mouse and rat. The variably detailed published characterizations of the CAT transporters do not permit direct comparison with the transport activities of the two placental membranes. In particular, interaction with neutral amino acids in the presence of sodium, an established characteristic of system y^+ [10, 11, 39], has not been investigated in the expressed cDNAs.

To elucidate the molecular basis of placental membrane cationic amino acid transport activity we undertook to identify, clone and express the hCAT transporter cDNAs present in two cultured human placental trophoblast cell models. We have used both trophoblast directly cultured from human placenta and the b30 BeWo choriocarcinoma cell line, which is known to differenti-

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ate in vitro [40] and to transport neutral amino acids in a manner similar to human placenta [22]. This paper describes the identification of three hCAT genes in these cell models, the cloning and expression of hCAT-1, and the comparison of its expressed transport activity with that of isolated placental basal membrane.

Materials and Methods

Materials

L-[^{3}H]lysine (specific activity 87.4 Ci/mmol) was obtained from NEN-DuPont (Boston, MA). Opti-Fluor was from Packard Instruments (Downers Grove, IL). Chemically defined fetal bovine serum (FBS) was obtained from Hyclone Laboratories (Logan, Utah). Essential amino acids were obtained from Hazelton Biologics (Lenexa, KS). Minimal essential medium, vitamins and DH5α cells were from Gibco BRL (Gaithersburg, MD). Gentamicin and nonessential amino acids were from the Washington University Tissue Culture Support Center. GeneAmp RNA PCR kit was from Perkin Elmer (Foster City, CA). ABI Prism™ Dye Terminator Cycle Sequencing Ready Reaction Kit was from Perkin Elmer (Foster City, CA). Collagenase (Type 1) was obtained from Worthington Biochemical (Freehold, NJ). Other chemicals were obtained from Sigma (St. Louis, MO) or Aldrich (Milwaukee, WI). *Xenopus laevis* frogs were purchased from *Xenopus* I (Ann Arbor, MI). All human and animal studies have been approved by the Washington University School of Medicine Review Boards.

Cell Preparation and Culture

The b30 BeWo clone was derived from the original parent BeWo line by limiting dilution [40]. Cells were maintained in 25 cm^2 flasks and cultured in MEMB media [22, 25] containing 10% FBS, gentamicin (0.5 mg/ml) and penicillin (100 units/ml)/streptomycin (100 μg/ml).

Cellular trophoblasts were isolated by trypsin digestion followed by separation on a Percoll gradient as described previously [22]. Cells were immediately frozen in 10% dimethyl sulfoxide after isolation and stored in liquid nitrogen for later culture [22].

Isolation of RNA

Total RNA was isolated from cultured BeWo and trophoblast cells with the RNA STAT-60 kit (Tel-Test "B", Friendswood, TX). The procedure uses a single phase solution of phenol and guanidinium thiocyanate [9]. Upon addition of chloroform, the homogenate separates into two phases. The RNA remains in the aqueous phase while DNA and proteins are in the organic phase. The RNA was precipitated by addition of isopropanol, washed with 70% ice-cold EtOH, and finally resuspended in diethyl pyrocarbonate (DEPC) treated water [30]. After measurement of OD at 260 nm, the RNA was stored at −80°C. Integrity was tested on 1% nondenaturing Seakem LE agarose gel (FMC Bioproducts, Rockland, ME).

Reverse Transcription (RT)

RT was performed using random hexamers as primers. Final volume was 20 μl with 1 μg of total RNA from either BeWo or trophoblast cells. The reaction mixture contained 1 mM of each deoxynucleoside triphosphate (dNTP), 1 U/μl RNase inhibitor, 5 mM $MgCl_2$, 2.5 U/μl Murine leukemia virus (MuLV) reverse transcriptase, 2.5 μM random hexamers in 50 mM KCl and 10 mM Tris-HCl (pH 8.3). It was first incubated at room temperature for 10 min, and then at 42°C for 20 min. The mixture was then heated at 99°C for 5 min and cooled on ice for 5 min to inactivate the transcriptase.

Polymerase Chain Reaction (PCR)

PCR was performed in the same tubes as RT, in 100 μl total volume. Final concentrations were 2 mM $MgCl_2$, 0.2 mM of each dNTP, and 2.5 U/100 μl Ampli Taq DNA polymerase in the 50 mM KCl and 10 mM Tris-HCl buffer (pH 8.3). For generation of full-length cDNAs, a PE 9600 thermocycler (Perkin Elmer, Foster City, CA) was programmed as follows: 95°C for 105 sec (denaturation), 35 cycles at 55°C for 30 sec (annealing), 74°C for 2 min 30 sec (extension), 94°C for 30 sec (hold). Finally the samples were incubated at 72°C for 7 min for the final extension. An abbreviated two step cycle was used to generate shorter (300–600 nt) fragments.

To identify the transporters present, primers were constructed based on published sequences. For hCAT-1 these were forward primer: nt 386-403 (CTGGCCTGTGCTATGGCG) and reverse primer: nt 753-735 (GCCCAGGACCAGGACGTTA) (human lymphocyte (H13), [41]). For hCAT-2 these were forward primer: nt 1015-1032 (TTGCAACAACTGGTGAAG) and reverse primer: nt 1414-1397 (TTCAGGTCAAACAGAAAGG) (human hepatoma, HepG2 [12]). This fragment has a unique *Bam*H I site (nt 1257) which is present only in isoform 2B and not in 2A. For hCAT-4 (human placental library, [32]) these were forward primer: nt 1526-1544 (TTGGCGTTATGTTGGCCTC) and reverse primer: nt 1845-1846 (CGCTGGTTCTCCTTGCTATG) [32].

A 2.1 kb (full-length) hCAT-1 sequence was generated using forward primer: nt 28-49; (GGCTTGGATTCTGAAACCTTCC) and reverse primer: nt 2157-2138; (TGCAGTGAGGGTGTGGACGC) [41].

Cloning and Transformation

The 2.1 kb cDNA hCAT-1 sequence generated by RT-PCR was gel purified using low melting point (LMP) agarose (Gibco BRL, Gaithersburg, MD) and a Qiaex II gel extraction kit (Qiagen, Valencia, CA). The purified cDNA was then ligated to pGEM-T Easy vector (Promega Corporation, Madison, WI) in the presence of T4 DNA ligase by overnight incubation at 4°C. A 2 μl portion of the ligation mixture was added to 100 μl DH5-α cells and incubated for 1 hr on ice. The cells were incubated for 45 sec at 42°C, and immediately cooled on ice for 2 min. A 400 μl portion of LB (Luria-Bertani) medium [30] at 37°C was added to the cells, which were then incubated at 37°C for 1 hr with shaking at 225 rpm. The cells were plated on two agar plates containing X-gal/IPTG and Carbenicillin. The plates were incubated at 37°C overnight. Several white colonies were picked for growth overnight in LB broth containing carbenicillin at 37°C with shaking at 225 rpm. The plasmid DNA was isolated using the Wizard Plus miniprep DNA purification kit (Promega Corporation, Madison, WI) for alkaline lysis of the bacteria [30] and the separation of plasmid by resin. The plasmid DNA was then digested with *Eco*R I incubating 1 to 2 hr to yield the two fragments. Appropriate clones were sequenced.

In Vitro Transcription

Plasmid DNA containing 2.1 kb hCAT-1 cDNA was linearized with *Sal* I leaving the hCAT-1 gene under the control of T7 RNA polymerase promotor. The cRNA was synthesized by in-vitro transcription

using the mMessage-mMachine kit (Ambion, Austin, TX) containing cap analogue ($m^7G(5')ppp(5')G$). The reaction mixture containing 2 μl 10× reaction buffer, 10 μl 2× ribonucleotide mixture (15 mM ATP, CTP, UTP and 3 mM GTP + 12 mM Cap analogue), 2 μl 10× T7 RNA polymerase enzyme mix, 1 μg linearized hCAT-1 cDNA, and RNase free water in 20 μl, was incubated at 37°C for 2 to 3 hr. A 1 μl portion of RNase free DNase I (2 U/μl) was added and incubation continued at 37°C for 15 min to eliminate residual template DNA. The reaction was stopped by adding 115 μl RNase free water + 15 μl NH_4OAc, extracted once with phenol/$CHCl_3$ and again with $CHCl_3$. The organic phase was re-extracted with 50 μl water. All the aqueous media were combined and the cRNA was precipitated by adding equal volume of isopropanol and incubating at −20°C for 30–45 min, followed by centrifugation. The cRNA pellet was resuspended in RNase-free water and OD_{260} was measured.

Sequencing

Sequencing of plasmid DNA containing hCAT-1 insert was performed by fluorescence based cycle sequencing using ABI Prism Dye Terminator Cycle Sequencing kit (Perkin Elmer, Foster City, CA). A tube containing 8 μl terminator ready reaction mix, hCAT-1 plasmid DNA (400–500 ng), primer (3.2 pmole), and distilled water in a volume of 20 μl, was placed in the PE9600 thermocycler (Perkin Elmer, Foster City, CA). The thermocycler was programmed as follows: 25 cycles at 96°C for 10 sec, 50°C for 5 sec, and 60°C for 4 min. The extension product was first precipitated as suggested in the manual (Protocol I), with 3 M Na-acetate, pH 5.2 and 95% EtOH. The pellet was rinsed with 70% EtOH. After carefully aspirating all the alcohol solution it was dried under vacuum for half an hour. The samples were analyzed with the ABI 373 DNA sequencer (Perkin Elmer, Foster City, CA) in a facility reserved for this purpose. The resultant sequences were analyzed using the GCG program [13].

Expression in *Xenopus* Oocytes

A small piece of ovarian lobe was dissected from *Xenopus laevis* under anesthesia [23] and incubated in Ca^{2+}-free medium (92 mM NaCl, 1 mM KCl, 2 mM $NaHCO_3$ and 15 mM Hepes/Tris, pH 7.4) containing 2 mg/ml collagenase type 1 for 90 min at room temperature. After removing the oocytes from the medium that contained collagenase they were placed in sterile modified Barth's saline (92 mM NaCl, 1 mM KCl, 2 mM $NaHCO_3$, 0.32 mM $Ca(NO_3)_2$, 0.61 mM $CaCl_2$, 0.8 mM $MgSO_4$ and 15 mM Hepes/Tris, pH 7.4) supplemented with gentamicin (50 μg/ml) and albumin (5 g/L) and were allowed to recover overnight at 4°C. The following day the oocytes were injected with 46 nl (50–75 ng) of hCAT-1 cRNA. Injected oocytes were maintained at 18°C for the desired number of days in sterile modified Barth's saline [1, 4, 6].

Uptake of [^{3}H]lysine into oocytes was measured at room temperature [3, 18]. Seven to 10 oocytes were incubated for 30 min [4, 18, 24] in the desired medium (92 mM NaCl or CholineCl, 1 mM KCl, 2 mM $NaHCO_3$ or $KHCO_3$, 0.32 mM $Ca(NO_3)_2$, 0.61 mM $CaCl_2$, 0.80 mM $MgSO_4$, and 15 mM Hepes/Tris, pH 7.4). Uptake was terminated by washing the oocytes six times with ice-cold 20 mM phosphate-buffered NaCl (200 mM). Each oocyte was then incubated in 200 μl 10% SDS [18, 23] for 30 min with gentle shaking. Opti-Fluor scintillation fluid (5 ml) was added for determination of radioactivity in a liquid scintillation counter (Pharmacia LKB Nuclear, Gaithersburg, MD). Control (nonstimulated) uptake was determined using either water-injected or noninjected oocytes which we and others found to give equivalent values [1, 8]. Stimulated uptake was calculated by subtracting control from total uptake.

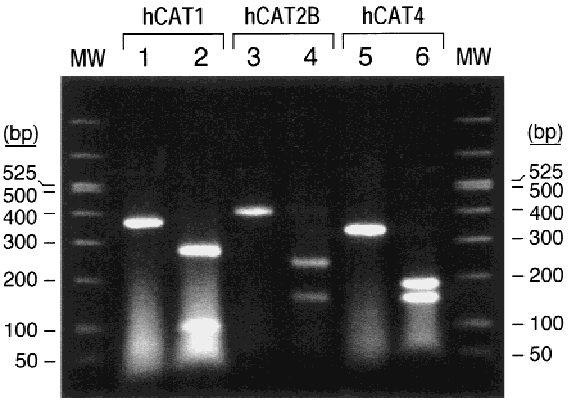

Fig. 1. Identification of CAT isoforms. RT-PCR was performed with BeWo RNA and primers for PCR were chosen from published hCATs-1, -2B, and -4 sequences (Materials and Methods). The products were separated with electrophoresis on 2% agarose gel. Lane 1: 368 bp hCAT-1 cDNA; Lane 2: *Bcl* I cut of hCAT-1 cDNA to yield 267 and 101 bp fragments; Lane 3: 400 bp hCAT-2B cDNA; Lane 4: *Bam*H I cut of hCAT-2B cDNA to yield 243 and 157 bp fragments; Lane 5: 340 bp hCAT-4 cDNA; Lane 6: *Nco* I cut of hCAT-4 cDNA 184 and 156 bp fragments. Similar results were obtained using trophoblast RNA.

Kinetic and Statistical Analyses

The Henri-Michaelis-Menten equation describing the relationship of saturable activity to concentration with an added diffusion term was fit to uptake data using the RS/1 program [5] on a VAX computer. Inhibition data were analyzed similarly to determine a K_i value for competitive inhibition [21]. The program finds the least-squares solution by the Marquardt-Levenberg method of iteration.

Results

Identification of Three hCAT cDNAs in Trophoblast Cell Models

Messenger RNAs for three hCAT species were identified in both trophoblast and BeWo cell models by RT-PCR using primers based on the human sequences of hCAT-1, hCAT-2, and hCAT-4. In each case a product of the appropriate chain length was seen on gel electrophoresis after RT-PCR (Fig. 1, lanes 1, 3, and 5). Digestion with appropriate restriction enzymes yielded products of the expected length (lanes 2, 4, and 6). Digestion of hCAT-2 with *Bam*H I demonstrated the unique restriction site characteristic of the 2B isoform. These results were obtained with both trophoblast and BeWo RNA. Using primers based on the rCAT-3 sequences we attempted to identify a cDNA related to CAT-3 in BeWo and trophoblast cells. No cDNA was present although control rat brain RNA yielded a product of the expected size (*data not shown*).

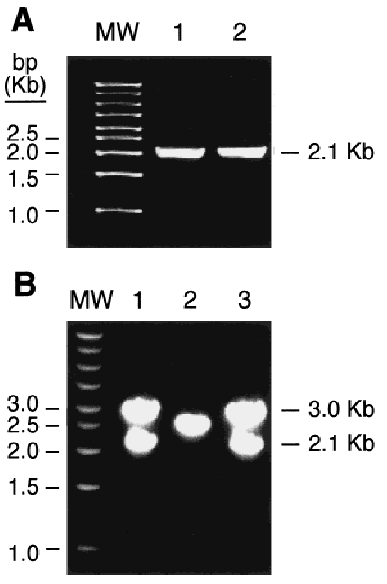

Fig. 2. Reverse transcription (RT)-PCR product representing a full-length human cationic amino acid transporter (hCAT-1) from BeWo RNA. (*A*) The major 2.1 kb product (lanes 1 and 2), was subjected to gel purification for cloning. (*B*) Gel electrophoresis of cloned hCAT-1 after *Eco*R I digestion. Lanes 1 and 3 show two clones. The bands visualized represent the removed insert cDNA (2.1 kb) and the residual vector DNA (3 kb). Lane 2 shows vector alone without an insert.

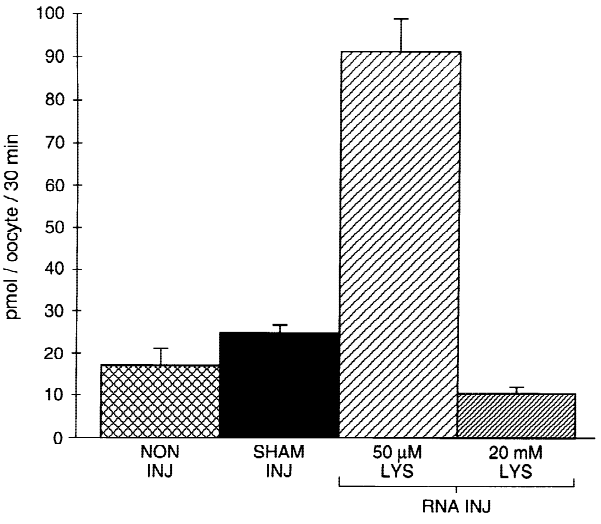

Fig. 3. Lysine uptake in *Xenopus* oocytes injected with placental hCAT-1 cRNA (~70 ng). Oocytes were injected with cloned BeWo hCAT-1 (RNA inj), water (SHAM inj), cloned BeWo hCAT-1 (20 mM) or not injected (NON inj). Uptake of 50 μM L-[^{3}H] lysine was measured at 22°C for 30 min. Each bar is the mean of lysine uptake in 9–10 oocytes. Right-handed bar shows uptake of L-[^{3}H] lysine in the presence of 20 mM unlabeled L-lysine. hCAT-1 cRNA injection produced a mean stimulation of approximately 4.5-fold ($n = 4$).

Cloning and Sequencing of Trophoblast hCAT-1

The pair of primers specified in Materials and Methods yielded a 2.1 kb cDNA product (Fig. 2*A*, lanes 1 and 2) from both trophoblast and BeWo RNA. After gel purification this cDNA was cloned into the pGEM-T Easy vector and DH5α cells were transformed with plasmid DNA. Clones were identified by X-gal/IPTG blue/white screening and digested with *Eco*R1 (Fig. 2*B*, lanes 1 and 3) to confirm the presence of the 2.1 kb insert. The sequences of both trophoblast and BeWo cDNAs (GenBank #AF078107) were identical to that of the H13 human lymphocyte hCAT-1 [41].

Stimulation of Transport by Cloned Protein

Purified BeWo mRNA (~70 ng in 46 nl) was injected into *Xenopus laevis* oocytes. Optimal transporter expression was obtained with a 3 to 5 day period of incubation after RNA injection and a 30 min incubation for uptake measurement. Under these conditions the cloned placental transporter stimulated L-lysine (50 μM) uptake fivefold (Fig. 3). Addition of 20 mM L-lysine demonstrated complete saturability of the stimulated uptake. Lysine transport rates obtained with water-injected oocytes were similar to those of uninjected oocytes (Fig. 3).

Concentration Dependence of hCAT-1 Stimulated L-lysine Uptake in *Xenopus* Oocytes

Uptake of L-[^{3}H]lysine (1 μM) was inhibited by increasing concentrations of nonradioactive L-lysine in *Xenopus* oocytes. A one system model fitted to concentration dependence data yielded K_m and V_{max} values of 98 ± 23 μM and 347 ± 66 pmol/oocyte/30 min (Fig. 4). These parameters agree with the K_m value of 100 μM previously reported in undifferentiated BeWo cells for System 1 by our laboratory [37]. Attempts to fit the data with a 2-site model failed to improve the quality of the fit.

Inhibition of hCAT-1 Stimulated L-lysine Uptake in *Xenopus* Oocytes

Inhibition by neutral amino acids in the presence of Na^+ is an established characteristic of system y^+ in placental and other membranes [38] but had not been investigated in cloned hCAT transporters. We therefore studied the effects of various amino acids (2 mM) on L-lysine (20 μM) uptake in the presence and absence of Na^+. In the absence of Na^+, uptake was not inhibited by L-leucine, L-homoserine and L-alanine (Fig. 5*A*, solid bars). In the presence of Na^+, uptake was inhibited completely by these three amino acids (Fig. 5*B* solid bars). L-Glutamate and L-valine did not inhibit in the presence of Na^+. Inhibition of uptake by the cloned transporter was

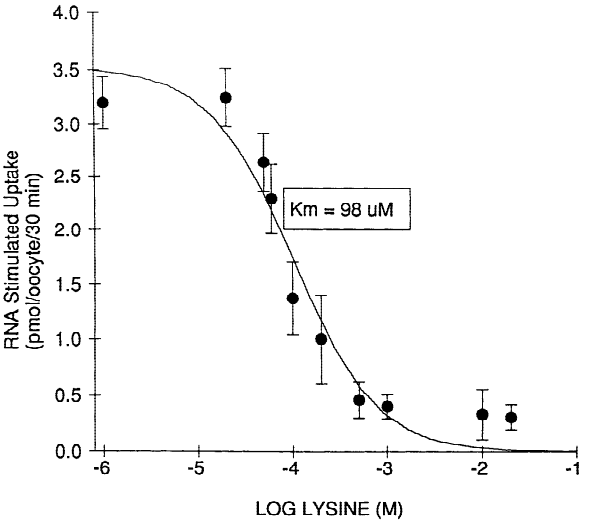

Fig. 4. Concentration dependence of cloned BeWo hCAT-1 uptake in *Xenopus* oocytes. *Xenopus* oocytes were injected with mRNA (~70 ng) and the concentration dependence of 1 μM L-[^{3}H] lysine uptake was measured in medium containing NaCl. Each point is the mean of hCAT-1 cRNA stimulated lysine uptake in 7 to 10 oocytes (Uptake in noninjected oocytes has been subtracted). A model with one saturable component was fit with K_m = 98 ± 23 μM and V_{max} = 347 ± 66 pmol/oocyte/30 min. Data are means ± SE of 4 experiments.

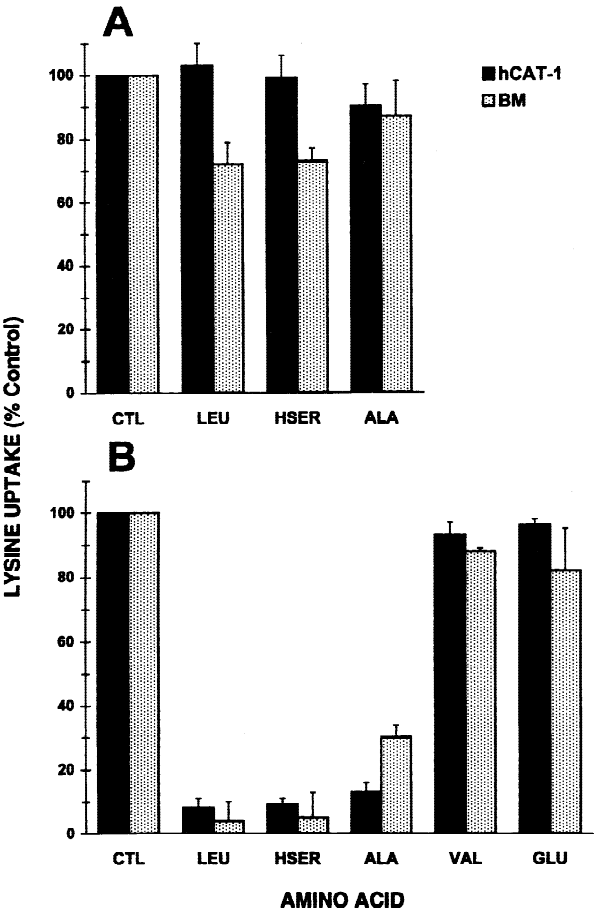

Fig. 5. (*A* and *B*) Inhibition of lysine uptake in *Xenopus* oocytes stimulated by hCAT-1 cRNA compared with that of human placental basal membrane (BM) both in the absence (Fig. 5*A*) and presence (Fig. 5*B*) of Na^+. Oocyte uptake (solid bars) was measured with 20 μM L-[^{3}H] lysine at 22°C for 30 min in hCAT-1 cRNA injected and control oocytes. The figure shows inhibition of hCAT-1 cRNA stimulated, substrate inhibitable uptake. Each bar is the mean of lysine uptake in 7 to 10 oocytes. Data are means ± SE of 4 (Fig. 5*A*) and of 3 to 8 experiments (Fig. 5*B*). CTL = uptake in the absence of inhibitors (CTL uptake = 58 ± 12 pmol/oocyte/30 min). BM data (stippled bars) is taken from Furesz, Moe and Smith [19].

comparable to that in basal membrane previously reported by our laboratory (stippled bars) [19].

Because leucine is known to interact with high affinity with the cationic transport system $b^{0,+}$ and y^+L, we investigated the concentration dependence of its inhibition of lysine uptake by the expressed hCAT-1 transporter. At both 20 μM and 1 μM lysine, relatively high concentrations of leucine were required for 50% inhibition (K_i values apparently 115 and ~313 μM) (Fig. 6 and data not shown).

Discussion

This paper demonstrates the expression of mRNAs for three cationic amino acid transporters in human placental trophoblast and describes the amino acid interaction of one of these. The first two cDNAs, hCAT-1 and hCAT-2B, code for membrane proteins mediating the high capacity activity functionally identified as system y^+ [12]. The third cDNA, that of hCAT-4 [32], has only recently been identified and the transporter it codes for has not been characterized in any detail. We were unable to look effectively for the potential presence of a CAT-3 cDNA since the human sequence is not available. The presence of multiple CAT transporters gives the trophoblast the potential to vary interaction with substrates and inhibitors in different cellular locations.

The characteristics of uptake mediated by the cloned hCAT-1 transporter expressed in *Xenopus laevis* oocytes closely resemble those of cationic amino acid uptake by system y^+ in the BeWo cell model and in basal membrane isolated from human placenta [19]. The K_m value of the cloned transporters, 98 μM, is essentially the same as that of uptake by placental basal membrane (200 μM), and undifferentiated BeWo cells (98 μM) [19, 37]. Additionally, uptake by both the basal membrane [19] and the expressed cloned transporter are strongly inhibited by neutral amino acids in the presence of sodium. These properties strongly suggest that the cloned transporter is a mediator of uptake in the placenta and cultured trophoblast cells and is likely to be present in the basal membrane.

The known properties of the cloned transporter ex-

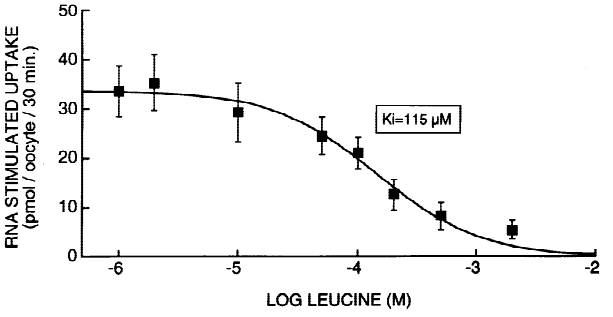

Fig. 6. Concentration dependence of inhibition by L-leucine of L-[^{3}H]lysine (20 μM) uptake in the presence of Na^+ in *Xenopus* oocytes stimulated by hCAT-1 cRNA. The K_m value used in fit was from Fig. 4. Each point is the mean of hCAT-1 cRNA stimulated lysine uptake in 7 to 10 oocytes (uptake in noninjected oocytes has been subtracted). A model with one saturable component was fit with K_i = 115 ± 18 μM and V_{max} = 199 ± 7 pmol/oocyte/30 min. Data are means ± SE of 4 experiments.

pressed in oocytes differ in important respects from those of system y^+L. System y^+L is a high affinity transporter (K_m 17 μM and 44 μM in placental microvillous membrane and erythrocytes), whereas the K_m of the cloned transporter is 100 μM [16, 20]. In the same two membranes, system y^+L is inhibited in the presence of sodium by leucine at low micromolar concentrations (K_i 10 μM in placental microvillous membrane and 22 μM in erythrocytes) [16, 20, 21]. Inhibition of the cloned hCAT-1 transporter in the presence of Na^+ requires substantially higher concentrations of leucine (K_i ~115 and 313 μM at 20 and 1 μM lysine). The molecular characteristics of the two systems also differ. System y^+L has been reported to be related to a heterodimer of the 4F2hc antigen protein and a recently cloned "hLAT" cDNA [27, 33]. Coexpression of these two cDNAs in oocytes produces a transporter which interacts sensitively with leucine. System y^+ activity is produced by expression of a single cDNA [12] with a sequence substantially different than that of hLAT (nucleotide sequence similarity 35%).

In considering the interactions of systems y^+ and y^+L with substrates and inhibitors it is important to take into account the cell membranes used, the potential presence of both systems in the same membrane and the influence of the conditions used on the system responsible for the observed activity [15, 29]. Some investigations using intact membranes indicate that substantial inhibition of system y^+ requires millimolar concentrations of neutral amino acids in the presence of sodium [10, 11, 29]. Various observations in intact membranes, have used substrate concentrations in the millimolar range. Under these conditions, system y^+L will be saturated and inhibition will likely reflect the properties of system y^+ [20, 21, 29, 39]. Other investigations have used leucine and sodium to inhibit system y^+L and study system y^+, a procedure which makes it impossible to study any interaction of leucine with system y^+ [20, 21, 29]. Ongoing investigations are increasingly demonstrating molecular heterogeneity of both system y^+L [27, 33] as well as system y^+ [12, 15, 26, 32]. To our knowledge the present investigation is the first or one of the first to describe inhibition of the activity of an expressed CAT cDNA by sodium and neutral amino acids. The K_i values observed with the expressed transporter are somewhat lower than those of system y^+ in microvillous membrane [20] or the erythrocyte [10, 29], but are greater than those of system y^+L. Clearly more study will be necessary to understand the role of the individual CAT transporters in the interaction of system y^+ as it occurs in the intact placental membranes with leucine and other neutral amino acids.

The expression of multiple CAT genes in human placental trophoblast suggests a potential mechanism for some of the differences in high capacity cationic amino acid uptake by the microvillous and basal membranes. Although similarities between the substrate affinities of some expressed transporters are apparent [12], detailed knowledge of characteristics such as interaction with neutral amino acids has not been reported. We have previously shown inhibition of system y^+ uptake by neutral amino acids in the presence of sodium to be much stronger in basal than in microvillous membrane [19, 20]. Alanine, phenylalanine and leucine [7, 28] inhibit basal membrane uptake [19–21] and CAT-1 uptake. Other long chain neutral amino acids such as serine and threonine are likely to interact similarly. The concentrations [7, 28] of these amino acids in maternal and fetal blood and within the placenta or its syncytiotrophoblast are extremely difficult to determine accurately. The available imprecise estimates do not permit detailed consideration of *cis* and *trans* interactions of individual amino acids [7, 28]. The data do indicate that the combined concentrations of the above amino acids will be inhibitory *in utero.* Strong inhibition of hCAT-1 in the basal membrane would favor lysine uptake at the microvillous membrane and facilitate its transfer from maternal to fetal circulation. Additional characterization of the hCAT isoforms expressed in trophoblast should help elucidate the relationship of their properties to lysine and arginine uptake by its maternal- and fetal-facing plasma membranes and maternal/fetal transport of cationic amino acids.

We thank Drs. Mike Mueckler and Lawrence Salkoff, Ms. Carol Makepeace and Mr. Aguan Wei for helping us to learn oocyte techniques and Drs. Arnold Strauss and Peter Rotwein and Mr. Harold Sims and Ms. Beverly Gibson for their help in cloning. We also appreciate the help of Michael Zahner, William Thom and Dr. Ellen Heath-Monnig with various aspects of this study. We thank Ms. Barbara Hartman for her help in preparing the manuscript. This work was supported by a grant from the National Institute of Child Health and Human Development, HD-07562.

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