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Characterization of amino acid transport systems in human placental brush-border membrane vesicles

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Brush-border microvillous plasma membrane vesicles were prepared from human full-term placental syncytiotrophoblasts and purified 33-fold from the homogenate with reference to a membrane marker enzyme, alkaline phosphatase (EC 3.1.3.1). Transport of α-(methylamino)isobutyrate by the membrane vesicles was stimulated in the presence of an Na^+ gradient from the outside to the inside of the vesicles. The initial rate of uptake in a 10-s period was enhanced with increasing concentration of Na^+ in the external medium. The level of α-(methylamino)isobutyrate transported into the vesicles reached a maximum 1 min after the start of incubation at 37°C, and then decreased with time due to efflux. Extrapolation to infinite medium osmolarity showed no uptake, indicating transport of α-(methylamino)isobutyrate into membrane vesicles. The initial rate of uptake was dependent on temperature and pH: the highest rate occurred at 37°C and the optimal pH was 8.0. When the α-(methylamino)isobutyrate concentration was varied, the initial rate of uptake dependent on an Na^+ gradient (out > in) obeyed Michaelis-Menten kinetics with K_m and V_{max} values of 1.07 mM and 3.23 nmol/10 s per mg of protein, respectively. Cross-inhibition patterns indicated that at least three Na^+-dependent and two Na^+-independent carrier-mediated pathways existed in the human placental brush border. One Na^+-dependent pathway interacted with all substrates tested. Another Na^+-dependent route interacted with L-proline, α-(methylamino)isobutyrate, and L-methionine, while a third pathway was selective for L-methionine. One Na^+-independent pathway was selective for L-cysteine, while the other pathway interacted with all substrates tested.

Introduction

The placenta is the primary site of absorption of nutrients required for development of the fetus, involving the uptake of nutrients from the maternal circulation across the brush-border membrane of the syncytiotrophoblast, diffusion through the cytoplasm, and exit into the fetal circulation across the basolateral membrane. The brush-border membranes are known to contain specific transport systems. Brush-border membrane vesicles from human full-term placental syncytiotrophoblasts have been used to study the transport of amino acids including α-aminoisobutyric acid [1], L-proline [2], and L-alanine and L-leucine [3], all of which are stimulated by an Na^+ gradient from the outside to the inside of the vesicles. Glucose, on

Abbreviations Hepes, 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid; BCH, 2-aminonorbornane 2-carboxylic acid

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the other hand, is transported into the membrane vesicles by facilitated diffusion and is not affected by an Na^+ gradient [4]. Although amino acid transport systems in intestinal [5–7] and renal [8] brush-border membranes have been extensively studied, very little has been clarified about transport systems in the placental brush-border membrane. Therefore, using brush-border membrane vesicles prepared and purified from human full-term placental syncytiotrophoblasts, we investigated the properties of α-(methylamino)isobutyrate transport, and in the present paper we describe the amino acid transport systems characterized on the basis of a cross-inhibition profile of several amino acids.

Materials and Methods

Isolation of membrane vesicles Membrane vesicles were prepared from human full-term placenta by a modified procedure of the method of Smith et al. [9], in which all operations were carried out below 4°C. Placentae were obtained within 10 min from vaginal or cesarean section deliveries at full term, and chilled on ice. The fetal membrane was removed, the placenta was cut into cotyledons, and the decidual surface was removed. The tissue was washed three times with ice-cold 250 mM sucrose containing 10 mM Tris-Hepes buffer (pH 7.5) and 0.25 mM $CaCl_2$, cut into small pieces, and then suspended in 200 ml of the same buffer. After stirring for 30 min using a magnetic stirrer, the suspension was filtered through a single layer of cotton gauze. The filtrate was then centrifuged for 10 min at $800 \times g$ to remove unbroken fragments of the tissue. The supernatant was collected and then $CaCl_2$ was added at a concentration of 1 mM, followed by centrifugation for 10 min at $10\,500 \times g$. The supernatant obtained was centrifuged in turn for 40 min at $25\,000 \times g$. The pellet thus obtained was suspended in 3 ml of 5 mM Tris-Hepes buffer (pH 7.5) containing 0.5 mM EDTA and then homogenized with a 25-gauge needle. The homogenate was applied to the top of a discontinuous sucrose gradient which was composed of 5 ml each of 30%, 40%, 50% and 60% (w/v) sucrose solutions containing 5 mM Tris-Hepes buffer (pH 7.5), and then centrifuged for 120 min at $70\,000 \times g$. After centrifugation, the interface between the 30% and 40% sucrose layers was collected, diluted with 5 mM Tris-Hepes buffer (pH 7.5), and then centrifuged for 60 min at $110\,000 \times g$. The final pellet was suspended in 2 mM Tris-Hepes buffer (pH 7.5) containing 300 mM D-mannitol and 0.1 mM $MgSO_4$ to give a final protein concentration of approx. 4 to 6 mg/ml. This membrane vesicle preparation showed a degree of alkaline phosphatase enrichment 33-times greater than that of the starting homogenate, while acid phosphatase showed a one-fifth decrease in its specific activity. The transport activity was observed to be intact even after the vesicles had been stored for 8 weeks at −70°C.

Uptake method All assays of transport activity were carried out at a substrate concentration of 20 μM. In the case of α-(methylamino)isobutyrate transport, a double-isotope medium containing α-[1-^{14}C](methylamino)isobutyrate (25 μCi/ml) and L-[^{3}H]glucose (75 μCi/ml) was used for determining the specific transport of α-(methylamino)isobutyrate. Corrections for simple diffusion and non-specific trapping of substrate were made by subtracting the amount of L-glucose associated with each sample. The membrane vesicles (approx. 200 to 300 μg of membrane protein) were incubated in medium containing 300 mM D-mannitol, 2 mM Tris-Hepes buffer (pH 7.5), 0.1 mM $MgSO_4$ and labeled substances. Other additions are described in the figure legends. The uptake of the substrate was terminated by diluting the sample with a 40-fold excess of an ice-cold buffer composed of 150 mM NaCl, 50 mM $MgCl_2$, 30 mM D-mannitol and 10 mM Tris-Hepes buffer (pH 7.5). The diluted sample was immediately filtered through a Millipore cellulose filter (0.45 μm) and washed with 3 ml of the same ice-cold buffer Radioactivity retained on the filter was counted by liquid scintillation α-(Methylamino)-isobutyrate uptake was proportional to membrane vesicle concentration up to a protein concentration of 9 mg/ml.

Potassium-loaded vesicles Membrane vesicles were preloaded with 100 mM K^+ by washing three times in 100 mM KCl, 0.1 mM $MgSO_4$, 100 mM mannitol and 2 mM Tris-Hepes (pH 7.5), followed by preincubation in the same medium for 30 min at 25°C.

Treatment with N-ethylmaleimide. An aliquot of freshly prepared *N*-ethylmaleimide solution was added to the membrane suspension to give the indicated final concentrations of *N*-ethylmaleimide, and the suspension was incubated for 10 min at 25 °C. The reaction was terminated by the addition of ice-cold 5 mM Tris-Hepes (pH 7.5) containing 10 mM dithiothreitol, and unreacted *N*-ethylmaleimide was removed by centrifugation for 60 min at 110000 × *g*. The resulting membrane pellet was washed twice with 5 mM Tris-Hepes (pH 7.5) containing 10 mM dithiothreitol and the final pellet was suspended in 2 mM Tris-Hepes (pH 7.5) containing 300 mM D-mannitol and 0.1 mM $MgSO_4$. In protection experiments, membrane vesicles were first mixed with 10 mM α-(methylamino)isobutyrate and 50 mM NaCl before the addition of *N*-ethylmaleimide.

Protein estimation. The protein concentration of the vesicle preparation was determined using the method of Lowry et al. [10] using bovine serum albumin as a standard.

Chemicals. All reagents were of the highest purity commercially available. All the labeled amino acids were purchased from New England Nuclear, α-(methylamino)isobutyrate, *N*-ethylmaleimide, and valinomycin were from Sigma Chemical Co., and 2-aminonorbornane 2-carboxylic acid (BCH) was from Aldrich Chemical Co.

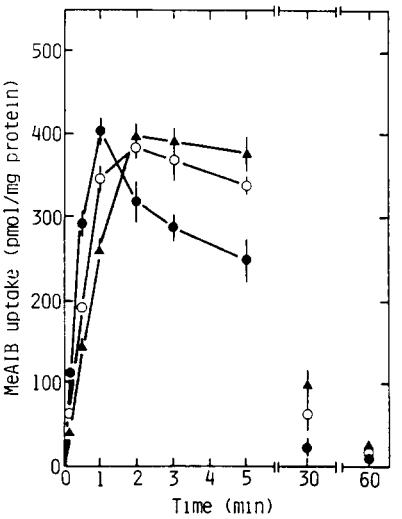

Fig. 1 Time course of α-(methylamino)isobutyrate uptake by brush-border membrane vesicles of human placenta in the presence of an Na^+ gradient (out > in) Membrane vesicles were suspended in a medium containing 300 mM D-mannitol, 0 1 mM $MgSO_4$, and 2 mM Tris-Hepes (pH 7 5) α-(Methylamino)isobutyrate (MeAIB) uptake was initiated by adding 50 μl of the membrane suspension (4–6 mg protein/ml) to 60 μl of an incubation medium composed of 36 7 μM α-[1-^{14}C](methylamino)isobutyrate, 36 7 μM L-[^{3}H]glucose, 50 mM D-mannitol, 0 1 mM $MgSO_4$, 20 mM Tris-Hepes (pH 7 5) and 220 mM NaCl Both the membrane suspension and the incubation medium were preincubated independently at the temperature indicated for 5 min before mixing, followed by further incubation at each temperature. 25 °C (▲), 30 °C (○), 37 °C (●). Each point represents the mean ± S D for four experiments.

Results

1. Properties of α-(methylamino)isobutyrate transport systems

1.1. Time course of Na^+ gradient-dependent α-(methylamino)isobutyrate uptake

The uptake of α-(methylamino)isobutyrate by the membrane vesicles as a function of incubation time with varying temperature is shown in Fig. 1. The presence of an Na^+ gradient toward the inside from the outside of the vesicle stimulated α-(methylamino)isobutyrate uptake. The uptake reached a maximum level after 1 min of incubation at 37 °C and then decreased with time to a steady-state level, a phenomenon known as 'overshoot', indicating secondary active transport of α-(methylamino)isobutyrate into membrane vesicles. Na^+ gradient-independent uptake of α-(methylamino)isobutyrate was below 2 pmol/mg protein at any period, indicating that Na^+-independent uptake was negligible. The initial rate of α-(methylamino)isobutyrate uptake was increased as temperature increased, and the uptake reached a maximum level earlier at higher temperature, followed by a decrease with time to the same level.

1.2. Effect of medium osmolarity on α-(methylamino)isobutyrate uptake

The uptake of α-(methylamino)isobutyrate was inversely proportional to medium osmolarity, and no uptake was apparent upon extrapolation to infinite medium osmolarity (Fig. 2). This indicated that α-(methylamino)isobutyrate was transported into a vesicular space, rather than being adsorbed by the vesicles.

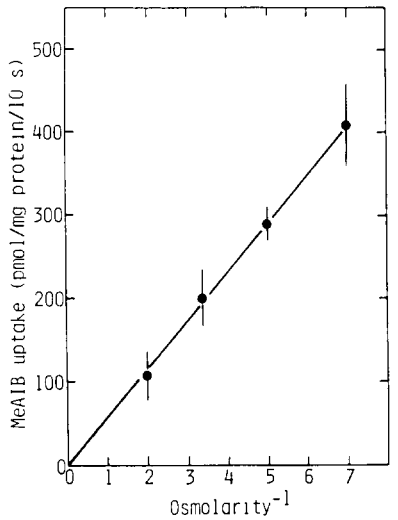

Fig 2 Effect of medium osmolarity on α-(methylamino)isobutyrate uptake α-(Methylamino)isobutyrate (MeAIB) uptake was measured 10 s after incubation in a medium containing 20 μM α-[1-^{14}C](methylamino)isobutyrate, 0 1 mM $MgSO_4$, 2 mM Tris-Hepes (pH 7 5), and 25 mM NaCl at final concentrations and D-mannitol was added to the medium to give the indicated osmolarities Each point represents the mean ± S D for four experiments

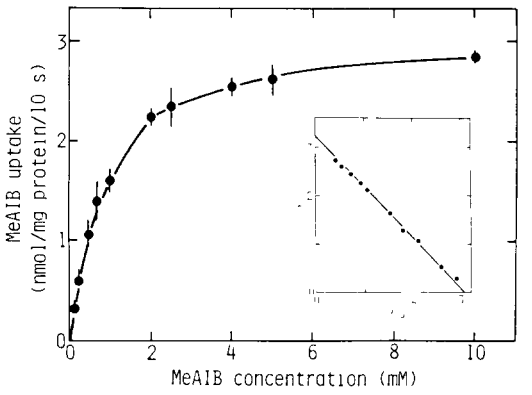

Fig 3 Effect of α-(methylamino)isobutyrate concentration on the initial rate of α-(methylamino)isobutyrate uptake The uptake over a 10-s period was measured in medium containing α-(methylamino)isobutyrate (MeAIB) at the indicated concentrations, 50 mM D-mannitol, 0 1 mM $MgSO_4$, 20 mM Tris-Hepes (pH 7 5) and 120 mM NaCl To obtain the indicated concentrations of the substrate, α-[1-^{14}C](methylamino)-isobutyrate (100 μCi/ml) was diluted with unlabeled α-(methylamino)isobutyrate Each point represents the mean ± S D for four experiments

1.3. Effect of α-(methylamino)isobutyrate concentrations on the initial rate of uptake

The effect of different concentrations of α-(methylamino)isobutyrate on the initial rate of uptake is illustrated in Fig. 3. The uptake rate showed a saturable hyperbolic curve that obeyed Michaelis-Menten kinetics. Eadie-Hofstee plots corresponding to the Na^+ gradient-dependent uptake rates showed a straight line (inserted figure). The calculated values of K_m and V_{max} for the Na^+ gradient-dependent transport of α = (methylamino)isobutyrate were 1.07 mM and 3.23 nmol/10 s per mg of protein, respectively.

1 4 Effect of Na^+ concentrations on the rate of α-(methylamino)isobutyrate uptake

The effect of external Na^+ concentrations on the initial rate of α-(methylamino)isobutyrate uptake was determined (Fig. 4). Concentrations of D-mannitol were adjusted so as to maintain medium iso-osmolarity. Na^+ in the external medium stimulated α-(methylamino)isobutyrate transport in a concentration-dependent manner. No uptake could be detected in the absence of an

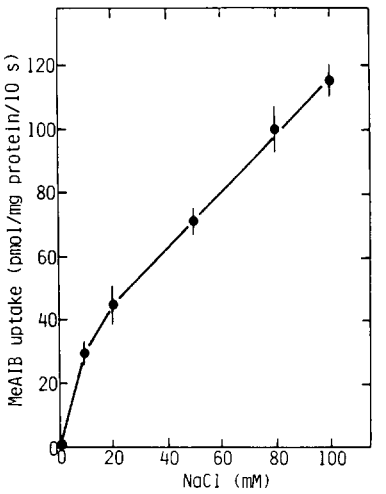

Fig 4 Effect of NaCl concentration on the initial rate of α-(methylamino)isobutyrate uptake The uptake over a 10-s period was determined under the same conditions as those described in the legend of Fig. 1, except for changes in final NaCl concentration (0–100 mM) Osmolarity of the medium was maintained at a constant level by adjusting the added concentration of D-mannitol Each point represents the mean ± S D for four experiments MeAIB, α-(methylamino)isobutyrate

Na^+ gradient, indicating that α-(methylamino)isobutyrate is co-transported into vesicles with Na^+.

1.5. Effect of cations on α-(methylamino)isobutyrate transport

In order to investigate the effect of cations on α-(methylamino)isobutyrate transport, the uptake was determined in the presence of NaCl, KCl, LiCl, CsCl, choline chloride or D-mannitol as the control. The uptake was specifically stimulated by Na^+, and only Li^+ was able to replace Na^+ in part. K^+, Cs^+ and choline had no stimulatory effect on the uptake (Fig. 5)

1.6. Effect of anions on α-(methylamino)isobutyrate transport

The effect of anions on the uptake of α-(methylamino)isobutyrate was determined (Fig. 6). Both the initial rate of uptake and the peak value of overshoot in the presence of permeable SCN^- were greater than those in the presence of Cl^- In contrast, both these values were smaller in the presence of SO_4^{2-}, which is impermeable. The effect of anions on Na^+-dependent α-(methylamino)isobutyrate uptake thus seemed to be related to differences in the anion diffusion potential across the membrane, supporting the assumption that an electrochemical potential due to an Na^+ gradient is the direct driving force for active transport of α-(methylamino)isobutyrate.

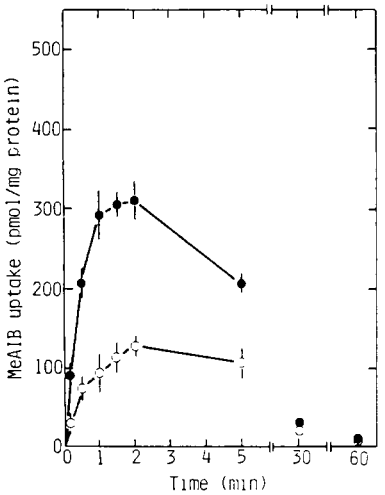

Fig 5 Effect of cations on the time course of α-(methylamino)isobutyrate uptake The uptake was measured under the same conditions as those described in the legend of Fig 1, except for replacement of NaCl with an equal concentration of either choline chloride, KCl, LiCl, or CsCl NaCl (●), LiCl (○) The level of uptake is represented as the mean ± S D. for four experiments. MeAIB, α-(methylamino)isobutyrate

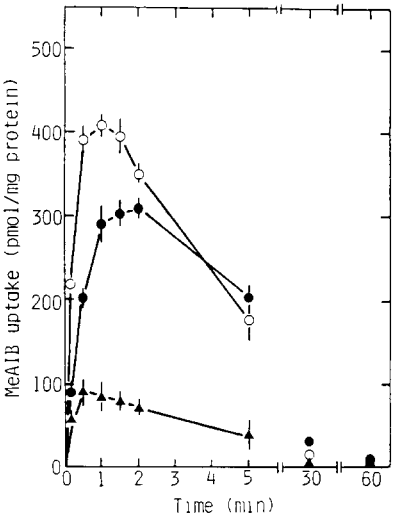

Fig 6. Effect of anions on the time course of α-(methylamino)isobutyrate uptake The uptake was determined under the same conditions as those described in the legend of Fig. 1, except for replacement of 220 mM NaCl with either 220 mM NaSCN or 110 mM Na_2SO_4 NaCl (●), NaSCN (○), Na_2SO_4 (▲) The level of uptake is represented as the mean ± S D for four experiments. MeAIB, α-(methylamino)isobutyrate

1.7. Effect of valinomycin on Na^+ gradient-dependent uptake of α-(methylamino)isobutyrate

In order to further investigate the effect of an electrochemical potential across the membrane on Na^+-dependent α-(methylamino)isobutyrate uptake, K^+-preloaded (in > out) membrane vesicles were used. When valinomycin was added outside the preloaded vesicles to enhance the electrochemical potential in the presence of an Na^+ gradient, the uptake was further enhanced. Valinomycin had no effect on Na^+-dependent α-(methylamino)isobutyrate uptake into vesicles which were not preloaded with K^+ (data not shown). These findings support the view described above that α-(methylamino)isobutyrate is co-transported with Na^+ into the vesicles by an Na^+ electrochemical gradient.

1.8 Effect of pH on the initial rate of α-(methylamino)isobutyrate uptake

The effect of external pH on the initial rate of α-(methylamino)isobutyrate uptake was determined. The optimal pH was 8.0 and an H^+ gradient toward the inside (pH 7.5) from outside (pH 5.5–7.0) the vesicle did not stimulate α-(methylamino)isobutyrate uptake (data not shown).

1.9. Effect of N-ethylmaleimide on Na^+-dependent α-(methylamino)isobutyrate transport

The effect of a sulfhydryl modifying reagent, *N*-ethylmaleimide, on the initial rate of α-(methylamino)isobutyrate transport was determined. *N*-Ethylmaleimide inactivated α-(methylamino)isobutyrate transport in a concentration-dependent manner. The half-maximal inhibition was observed at 360 μM *N*-ethylmaleimide/mg of protein and 90% inhibition occurred at 2 mM *N*-ethylmaleimide/mg of protein. Preincubation of the vesicles with 10 mM α-(methylamino)isobutyrate and 50 mM Na^+ gave slight but significant protection against the *N*-ethylmaleimide inactivation of transport (data not shown). These results suggested that sulfhydryl groups are essential for the transport of α-(methylamino)isobutyrate.

2 Analysis of amino acid transport systems

In order to analyze the amino acid transport system, we selected seven amino acids in addition to α-(methylamino)isobutyrate: the neutral amino acids, L-cysteine, L-methionine, L-glycine and L-leucine; a basic amino acid, L-lysine; an acidic amino acid, L-glutamic acid; and an imino acid, L-proline. We first measured the diffusional, Na^+-dependent, and Na^+-independent components of the initial rate of uptake, and then performed cross-inhibition tests using these amino acids as both substrates and inhibitors.

2.1. Initial rate of amino acid uptake

Table I shows the initial rate of amino acid uptake measured under various conditions, as indicated in the legend. An Na^+ gradient-dependent uptake of a given amino acid was expressed by subtracting the uptake in the presence of 120 mM KCl ($V_{(K)}$) from that in the presence of 120 mM NaCl ($V_{(Na)}$). An Na^+ gradient-independent and carrier-mediated uptake was expressed by subtracting the diffusional component (V_D) from the uptake in the presence of 120 mM KCl. The diffusional component of a substrate was determined by measuring the influx in the presence of 50 mM unlabeled substrate. Uptake time was appropriately short in order to measure the initial rate of uptake of each amino acid.

Under the conditions described, α-(methylamino)isobutyrate was transported only Na^+-dependently; when K^+ was substituted for Na^+, α-(methylamino)isobutyrate uptake was reduced to the passive diffusion level. This suggested the absence of any α-(methylamino)isobutyrate transport catalyzed by an Na^+-independent carrier.

TABLE I

INITIAL RATES OF AMINO ACID UPTAKE

The uptake levels of eight amino acids were measured. Membrane vesicles were suspended in a medium containing 300 mM D-mannitol, 0.1 mM $MgSO_4$ and 2 mM Tris-Hepes (pH 7.5). Amino acid uptake was initiated by adding 50 μl of the membrane suspension to 60 μl of an incubation medium composed of 36.7 μM labeled substrate, 50 mM D-mannitol, 0.1 mM $MgSO_4$, 20 mM Tris-Hepes (pH 7.5) and 220 mM NaCl ($V_{(Na)}$) or 220 mM KCl ($V_{(K)}$). The diffusional component (V_D) was determined by measuring the total influx of labeled substrate in the presence of 50 mM unlabeled substrate. Incubation time for uptake measurements was 10 s. Values are mean ± S.D. for four experiments. MeAIB, α-(methylamino)isobutyrate.

	Initial rates of uptake (pmol/10 s per mg protein)							
	MeAIB	Pro	Met	Cys	Gly	Glu	Lys	Leu
$V_{(Na)}$	129.9±4.3	35.2±4.8	70.8±3.2	75.7±4.2	14.3±1.6	23.4±2.2	56.6±5.0	64.7±3.1
$V_{(K)}$	1.1±0.3	9.3±0.8	39.0±2.9	40.0±3.0	10.3±0.9	12.0±1.0	57.8±5.4	66.3±2.9
V_D	1.3±0.3	3.5±0.2	15.2±1.1	13.4±1.8	3.8±0.2	2.8±0.6	14.2±0.9	5.8±0.8
Na^+-dependent uptake	128.8±4.1	25.9±4.2	31.8±2.1	35.7±3.9	4.0±0.8	11.4±1.8	0.0±0.2	0.0±0.1
Na^+-independent uptake	0.0±0.2	5.8±1.8	23.8±2.7	26.6±1.3	6.5±1.4	9.2±0.8	43.6±4.8	60.5±2.8

Uptake of L-lysine and L-leucine, on the other hand, were not accelerated by an inwardly directed Na^+ gradient. Uptake of L-proline was largely Na^+-dependent (73.6%) with a small Na^+-independent fraction which was greater than a diffusional component. This suggested the presence of an Na^+-independent carrier mechanism for L-proline transport in addition to the Na^+-dependent system. In the case of L-methionine, L-cysteine, L-glycine and L-glutamic acid, Na^+-dependent uptake was nearly equal to each respective Na^+-independent uptake, suggesting the presence of both Na^+-dependent and Na^+-independent systems for these amino acids.

2.2. Competitive inhibition of amino acid uptake

Amino acid uptake at 20 μM was measured in the presence of amino acid inhibitor at a concentration of 30 mM, in the presence ($V_{i(Na)}$) or absence ($V_{i(K)}$) of Na^+. The percentage inhibition of an Na^+-dependent carrier-mediated uptake was calculated using the relation $100 - 100([V_{i(Na)} - V_{i(K)}]/[V_{(Na)} - V_{(K)}])$. The percentage inhibition of an Na^+-independent uptake was calculated using the relation $100-100([V_{i(K)} - V_D]/[V_{(K)} - V_D])$. Table II summarizes the percentage inhibition of the Na^+-dependent and Na^+-independent uptake components of each substrate.

2.2 1. Competitive inhibition of Na^+-dependent amino acid uptake. L-Methionine completely inhibited the carrier-mediated uptake of all substrates, while L-methionine uptake was inhibited partially by other amino acids. L-Proline and α-(methylamino)isobutyrate inhibited L-methionine uptake by 72.8% and 63.2%, respectively; L-cysteine, L-glycine and L-glutamic acid inhibited within a range of between 29% and 40%. These results suggested that a fraction of L-methionine transport might be mediated by an L-methionine-specific system which was unable to interact with the other amino acids tested.

L-Proline and α-(methylamino)isobutyrate produced complete inhibition of L-cysteine, L-glycine and L-glutamic acid transport, while L-cysteine, L-glycine and L-glutamic acid inhibited only a fraction (39.8%–48.3%) of the Na^+-dependent L-

TABLE II

CROSS-INHIBITION OF Na^+-DEPENDENT AND Na^+-INDEPENDENT AMINO ACID UPTAKE

The uptake over a 10-s period was measured under the same conditions as those described in the legend of Table I, except for the addition of 30 mM unlabeled amino acid and the osmolarity of the medium was adjusted to a constant value by the addition D-mannitol Values are mean ± S D. of percentage inhibition calculated using the equation in the text for four experiments Uptake values (pmol/10 s per mg protein) in the absence of inhibitor for Na^+-dependent and Na^+-independent uptake are, respectively α-(methylamino)isobutyrate (MeAIB), 123 4 and 0, Pro, 24 2 and 3 8, Met, 30 1 and 21 4, Cys, 34 0 and 25 2, Gly, 3 6 and 5 1, Glu, 10 6 and 8 6, Lys, 0 and 40.2; Leu, 0 and 58 2

Substrate	Percentage inhibition, inhibitor								
	MeAIB	Pro	Met	Cys	Gly	Glu	Lys	Leu	BCH
A Na^+-dependent uptake									
MeAIB	100 0±0 3	98 9±0 6	99 2±0 0	39 8±4.8	48 3±2 9	43.5±3.8	4 8±2 6	8 3±1 1	1 3±1 0
Pro	96 8±1 2	98 9±0 2	100.0±0 0	48 2±5 2	39 4±3 6	40.1±4 6	5 0±3 8	7 8±2 8	2 8±0 8
Met	63 2±2 1	72 8±1 3	100 0±0.2	39.2±2.0	29 4±4 0	40.2±3 9	4 3±1 2	2 6±1 0	3 2±0 2
Cys	89 3±1 4	93 4±0 9	97 9±0 6	98.3±0.2	86 3±1 1	96 5±0 8	8 2±1 8	3 0±0 9	4 8±0 6
Gly	96 8±0 2	93 8±1 2	89 6±0 2	93.2±0.8	94 3±0 8	86 5±0 9	6 2±0 4	4 9±0 6	6 3±1 2
Glu	93 4±0 3	91 5±0 7	88 9±1 3	86 4±0.6	91 4±0 9	96 3±1.2	6 9±0 8	5 2±1 8	2 9±1 1
B Na^+-independent uptake									
Pro	1 3±0 6	96 3±0.6	87 5±0 6	97 5±0 8	89 9±0 1	93.8±0 8	98 8±0 7	98 8±1 6	98 8±1 1
Met	2 0±1 1	96 7±0.2	99 7±0 3	88 9±0.9	91 4±0 8	93.4±0.4	98 3±0 8	93 3±3 2	97 9±2 9
Cys	0 3±0 4	23 4±1.2	26 8±3.8	98.2±1.1	34 2±3 8	30.1±2 9	19 8±2 6	27 9±4 6	28 3±6 9
Gly	3 1±0 6	89.4±0 9	98 1±1.1	91.4±0.2	93 5±2 1	94.2±3.6	96 4±1 3	98.1±1 2	97 3±1 8
Glu	3 0±1 9	93.4±1 2	96 4±0.9	95.4±0.6	98 3±1 3	96 4±1 0	94 5±2 5	93 2±0 9	96 3±2 0
Lys	0 6±0 0	98 5±1 8	96 9±0 8	89 0±1 8	90 9±3 4	87 4±0 8	98 9±3 6	86 9±1 1	94 5±3 2
Leu	3 2±1 0	95 1±1 1	85 3±1.3	100 0±0 2	99 8±0 9	89.1±0 7	96 2±0 1	99 8±0 9	91 6±1 6

proline and α-(methylamino)isobutyrate transport. Therefore, it appears that a fraction, roughly 40%–50%, of the L-proline and α-(methylamino)isobutyrate fluxes may be mediated by a system which is capable of interacting with L-methionine, L-cysteine, L-glycine and L-glutamic acid. The remainder of the L-proline and α-(methylamino)isobutyrate fluxes are mediated by a system which interacts with L-methionine, but not with L-cysteine, L-glycine and L-glutamic acid.

Transport of L-cysteine, L-glycine and L-glutamic acid showed complete mutual inhibition, suggesting that these substrates possibly share a common carrier. L-Lysine, L-leucine and BCH, which were transported only Na^+-independently, were not effective in inhibiting Na^+-dependent uptake for any of the tested substrates.

2.2.2. Competitive inhibition of Na^+-independent amino acid uptake. As shown in Table II, Na^+-independent uptake of L-cysteine was partially inhibited by any of the inhibitors tested, except for L-cysteine alone. However, L-cysteine inhibited the total uptake of any other substrate. These results suggested that a fraction of L-cysteine uptake was mediated by an L-cysteine-specific Na^+-independent system which was not interacting with any other amino acid tested. The inhibition of transport of other amino acids was complete, suggesting the presence of a common Na^+-independent amino acid transport pathway. α-(Methylamino)isobutyrate, transported only Na^+-dependently, was unable to inhibit the Na^+-independent transport of any amino acid tested.

Discussion

It is important to explore the regulatory mechanisms of nutrient transport across the brush-border membrane of placental syncytiotrophoblasts, since they may control fetal development. This paper describes: first, a modified method for the purification of brush-border membrane vesicles from human full-term placenta; second, the properties of α-(methylamino)isobutyrate transport; and third, the transport pathways for amino acids in human placental brush-border membrane vesicles. The membrane vesicles obtained by a modified method were purified 33-fold with reference to the activity of a brush-border marker enzyme, which is a higher degree of purity than those described previously [11–13]. Such a pure preparation was considered suitable for kinetics studies and analysis of the amino acid transport systems since the initial rate of uptake was determined correctly.

Christensen classified the amino acid transport systems of a wide variety of nonepithelial cell types into six fundamental categories A, ASC, L, y+, β, and glycine transport systems [14]. However, the amino acid transport systems characterized in the rabbit jejunal [6] and rabbit kidney [8] brush-border membranes appear to be distinct from the classical Na^+-dependent amino acid transport systems found in nonepithelial cells and in the basal lateral plasma membranes of intestinal epithelial cells [15]. In previous investigations of placental amino acid transport systems [1,2,16,17], the uptake characteristics of only one amino acid were studied by inhibition with various other amino acids, and the uptake time was too long to allow determination of the initial rate of uptake. In the present study, however, we performed transport studies to delineate the amino acid transport systems in human full-term placental brush-border membrane vesicles using various amino acids as both substrates and inhibitors.

In the presence of an Na^+ gradient (out > in), α-(methylamino)isobutyrate uptake showed a typical 'overshoot' phenomenon after reaching a maximal uptake level 1 min after the start of incubation. This 'overshoot' of the uptake resulted from an electrochemical gradient formed by the Na^+ gradient, and its subsequent loss with time due to Na^+-substrate co-transport. This finding was supported by an anion replacement experiment (Fig. 6) and an experiment using K^+-preloaded membrane vesicles: when valinomycin was added outside the preloaded vesicles to enhance the electrochemical potential in the presence of the Na^+ gradient, the uptake 'overshoot' was further enhanced. The initial rate of α-(methylamino)isobutyrate uptake was greatly reduced at lowered extravesicular pH, which is one of the characteristics of the A system. Similar evidence has been presented for the transport of other amino acids into brush-border membrane vesicles prepared from placenta [1–3], intestine [5], and kidney [18].

In order to delineate the amino acid transport system in human full-term placental brush-border

membranes, we determined the percentage inhibition by various amino acids of both Na^+-dependent and Na^+-independent carried-mediated uptake (Table II). It was found that there are at least three Na^+-dependent carrier-mediated amino acid transport pathways.

The observed cross-inhibition of Na^+-dependent amino acid uptake suggests that, first, the amino acids tested share a common Na^+-dependent carrier, since substrate amino acids showed a significant degree of mutual cross-inhibition. The Na^+-dependent uptake of L-cysteine, L-glycine and L-glutamic acid was totally supported by this system. The partial inhibition (29.4%–48.3%) of α-(methylamino)isobutyrate, L-proline and L-methionine uptake by L-cysteine, L-glycine and L-glutamic acid indicates that a fraction of the uptake of α-(methylamino)isobutyrate, L-proline and L-methionine is handled by this common amino acid carrier. This suggests the presence of an additional Na^+-dependent pathway serving L-proline, α-(methylamino)isobutyrate and L-methionine. This Na^+-dependent common pathway probably corresponds to the classical A system, since α-(methylamino)isobutyrate, a model substrate for the A system, totally inhibited the uptake mediated by this pathway.

Second, L-proline and α-(methylamino)isobutyrate showed total mutual inhibition, and displayed the same patterns of inhibition and sensitivity to the amino acids tested. They produced complete inhibition of L-cysteine, L-glycine and L-glutamic acid uptake and partial inhibition (63.2%–72.8%) of L-methionine uptake. In addition to the partial inhibition by L-cysteine, L-glycine and L-glutamic acid of L-proline and α-(methylamino)isobutyrate uptake, the uptake was inhibited totally by L-methionine. These results suggested that L-proline and α-(methylamino)isobutyrate fluxes are mediated by two pathways, one being the common Na^+-dependent pathway described above, and the other being common to L-methionine.

Third, L-methionine uptake was incompletely inhibited by all the amino acids tested, and showed complete inhibition only by itself. This suggested the presence of an Na^+-dependent pathway specific for L-methionine.

The data in Table I suggest the presence of an Na^+-independent carrier-mediated amino acid transport pathway in human placental brush-border membranes. This is supported by the cross-inhibition data shown in Table II, which suggest the existence of two pathways. One system is a common Na^+-independent amino acid transport pathway, which supports the total transport of all amino acids tested except for partial transport of L-cysteine. The second system mediates a major fraction of the L-cysteine influx, which was not inhibited by any other amino acid tested. Therefore, 70%–80% of the Na^+-independent L-cysteine uptake is supported by the second system and 20%–30% by the common pathway. BCH, a model substrate that appears to have an affinity solely for the classical L system [19], showed the same inhibition patterns as the test inhibitors, except for α-(methylamino)isobutyrate and L-cysteine. A similarity between the classical L system and the common Na^+-independent system presented here is shown by the fact that α-(methylamino)isobutyrate showed no inhibition of either of the systems. These observations suggest that this common pathway may correspond to the classical L system.

Smith et al. [15] demonstrated the existence of three transport systems in human full term placenta by using a competitive inhibition technique, and these systems correspond to the A, ASC and L systems. Asai et al. [16] also observed three amino acid transport systems, A, ASC and L, in human placental brush-border membranes. By use of our more purified brush-border membrane vesicles, we concluded that there are at least three distinct Na^+-dependent and two distinct Na^+-independent amino acid transport systems in human full-term placental brush borders. The common Na^+-dependent pathway first described may correspond to the classical A system, while the other two Na^+-dependent pathways do not conform to the classical ASC system, since the second system is sensitive to α-(methylamino)isobutyrate and the third is not sensitive to L-cysteine. Stevens et al. [6] reported that rabbit jejunal brush-border membrane transports amino acids via at least three Na^+-dependent carrier systems: the NBB system, the PHE system and the IMINO system. Mircheff et al. [8] also characterized similar systems in the rabbit renal brush-border mem-

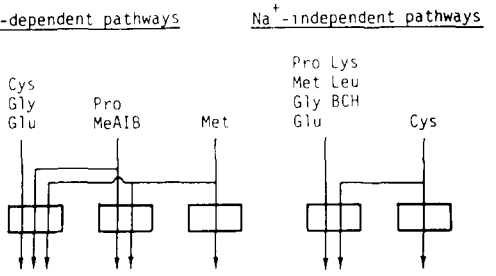

Fig 7 A schematic representation of the amino acid transport pathways in human placental brush-border membrane The boxes represent transport-catalyzing systems and the arrows passing through the boxes indicate that transport occurs MeAIB, α-(methylamino)isobutyrate

brane, with some exceptions. The second and third systems dependent on Na^+ presented in this paper are similar to the IMINO system and the PHE system, respectively. The IMINO system exclusively transports L-proline and α-(methylamino)-isobutyrate, while the PHE system primarily handles L-phenylalanine and L-methionine. The common Na^+-dependent pathway in placental brush border does not correspond to the NBB system, since the latter system does not interact with α-(methylamino)isobutyrate.

In the case of Na^+-independent systems, one system is similar to the classical L system, while the other is sensitive solely to L-cysteine. This L-cysteine-specific Na^+-independent system has not been reported previously in any other cell types

The amino acid transport pathways observed in human placental brush-border membrane are schematically illustrated in Fig. 7. The identification and purification of these carriers, which will be necessary in order to further explore the amino acid transport systems present in the brush-border membrane of human placenta, are currently under way.

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