Biochimica et Biophysica Acta, 1021 (1990) 169–174
Elsevier

BBAMEM 74718

Characterization of amino acid transport systems in human placental basal membrane vesicles

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(Received 4 July 1989)

Key words: Amino acid transport; Cross-inhibition analysis; Basal membrane vesicle; (Human placenta)

The amino acid transport systems have been characterized in basal membrane vesicles prepared from human full-term placental syncytiotrophoblasts. Transport of amino acids across basal membranes occurred via passive diffusion and Na^+-independent and Na^+-dependent carrier-mediated systems. Passive diffusion was responsible for a substantial fraction of transport. L-Glutamate and α-(methylamino)isobutyrate were transported only Na^+-independently, while the transport of L-alanine was dependent solely on an Na^+ gradient from the outside to the inside of the vesicles. L-Methionine, L-leucine, glycine and L-proline transport were supported by both Na^+-independent and Na^+-dependent systems. L-Lysine transport was decreased in the presence of cations, an inwardly directed Na^+ gradient was much more effective than a K^+ gradient at slowing L-lysine transport. A cross-inhibition analysis of these amino acids indicates that at least three Na^+-independent and five Na^+-dependent carrier-mediated systems exist in the human placental syncytiotrophoblast basal membranes. One Na^+-independent system interacts with all substrates tested. Another Na^+-independent system carries glycine, L-methionine, L-leucine and L-lysine; it is sensitive to L-glutamate, but not to L-proline or α-(methylamino)isobutyrate. The third system is selective for L-lysine, which is inhibited by L-methionine, glycine and L-leucine, but inaccessible to L-glutamate, L-proline and α-(methylamino)isobutyrate. One Na^+-dependent system carries L-alanine, glycine, L-methionine and L-leucine, and it is sensitive to L-proline. The second system mediates transport of L-alanine, glycine, L-methionine and L-proline, but is not sensitive to L-leucine. The third system carries L-alanine, glycine and L-proline, and is inaccessible to L-methionine and L-leucine. The fourth system is responsible for L-methionine and L-leucine; it is sensitive to L-alanine and glycine, but not to L-proline. The fifth system is selective for L-proline.

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 ciruclation across the basal membrane. Brush-border membrane vesicles from human full-term placental syncytiotrophoblasts have been used extensively to study the transport into the epithelium of amino acids [1–4], dipeptides [5], glucose [6] and ions [7]. Recently, we reported that at least three Na^+-dependent and two Na^+-independent carrier-mediated pathways for amino acids exist in the human placental brush-border [4]. In other other epithelial tissues including small intestine [8–11] and renal cortex [12,13], amino acid transport systems in both brush-border membranes [9,12] and basolateral membranes [8,10,11,13] have been extensively studied with isolated membrane vesicles. However, no full report has yet appeared on amino acid transport in placental basal membranes. Therefore using basal membrane vesicles prepared from human full-term placental syncytiotrophoblast, in the present paper we have measured the diffusional, Na^+-dependent and Na^+-independent components of the initial rate of uptake of several amino acids and have gone on to perform cross-inhibition studies using these amino acids as both substrates and inhibitors in an attempt to characterize the amino acid transport systems present in this membrane. These studies indicate marked differences in the distribution of amino acid transport systems between the apical and basal surface of the human syncytiotrophoblast.

Abbreviation: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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Materials and Methods

Preparation of basal membrane vesicles. Basal membrane vesicles were prepared from freshly obtained human full-term placenta according to the method of Kelley et al. [14]. Membrane vesicles were finally 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 basal membrane vesicle preparation showed a degree of dihydroalprenol binding activity enrichment 27-times greater than that of the villous tissue homogenate, while alkaline phosphatase showed no increase in its specific activity. The purity of this membrane vesicle preparation is similar to an original report [14]. There was an inverse correlation between L-[^{3}H]lysine uptake at equilibrium and external osmolarity. Thus it appears that amino acid uptake represents transport into the intravesicular space, rather than nonspecific binding to the membrane. The transport activity was observed to be intact even after the membrane vesicles had been stored for 6 weeks at −20 °C. These observations suggest that this membrane vesicle preparation is useful in transport study of the basal plasma membranes of human placental syncytiotrophoblasts.

Uptake studies. The uptake was carried out according to the procedure as described previously [4]. The uptake was initiated by adding 50 μl of the membrane suspension (approx. 200 to 300 μg of membrane protein) to 60 μl of an incubation medium composed of 36.7 μM labelled substrate, 50 mM D-mannitol, 0.1 mM $MgSO_4$, 20 mM Tris-Hepes (pH 7.5) and 190 mM NaCl or 190 mM KCl. Other additions are described in the table or figure legends. Both the membrane suspension and the incubation medium were preincubated independently at 37 °C for 5 min before mixing, followed by further incubation at 37 °C. The uptake of substrate was terminated by diluting the sample with 40-fold excess of an ice-cold buffer composed of 150 mM NaCl, 50 mM $MgSO_4$, 30 mM D-mannitol and 10 mM Tris-Hepes buffer (pH 7.5). The diluted sample was immediately filtered through a Millipore cellulose filter (0.65 μm) and washed once with 3 ml of the same ice-cold buffer. The radioactivity of labelled substrate retained on the filter was counted by liquid scintillation.

Protein estimation. Protein concentration of the vesicle preparation was determined by the method of Lowry et al. [15] using bovine serum albumin as a standard.

Chemicals. All the labelled amino acids were purchased from Amersham International (Amersham, U.K.). All other chemicals were of the highest purity commercially available.

Results

1. Initial rate of amino acid uptake

In order to analyze the amino acid transport systems, we selected nine representative amino acids: neutral amino acids, glycine, L-alanine, L-leucine and L-methionine; a basic amino acid, L-lysine; an acidic amino acid, L-glutamate; and imino acids, L-proline and α-(methylamino)isobutyrate. We first investigated the time course of uptake of each of these amino acids (data not shown). In each case, the uptake of 20 μM labelled substrate measured under Na^+ gradient or K^+ gradient conditions was a linear function of time for at least the first 30 s of incubation. Therefore, uptake measured at 30 s of incubation was used to estimate the initial rate of amino acid influx.

We then measured the diffusional, Na^+-dependent and Na^+-independent components of the initial rate of uptake. Table I summarizes the initial rate of amino acid uptake measured under various conditions, as indicated in the legend. An Na^+ gradient-dependent uptake

TABLE I

Initial rates of amino acid uptake

The uptake of the nine amino acids indicated was measured at a final amino acid concentration of 20 μM. 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 labelled substrate, 50 mM D-mannitol, 0.1 mM $MgSO_4$, 20 mM Tris-Hepes (pH 7.5) and 190 mM NaCl ($V_{(Na)}$) or 190 mM KCl ($V_{(K)}$). The diffusional component (V_D) was determined by measuring the total influx of labelled substrate in the presence of 50 mM unlabelled substrate. Incubation time for uptake measurements was 30 s. Values are mean ± S.D. for three experiments. MeAIB, α-(methylamino)isobutyrate.

	Initial rates of uptake (pmol/30 s per mg protein)							
	Ala	Met	Leu	Gly	Lys	Glu	Pro	MeAIB
$V_{(Na)}$	12.48 ± 0.63	16.77 ± 0.24	14.37 ± 0.32	14.29 ± 0.65	17.90 ± 1.97	6.83 ± 0.25	9.30 ± 1.05	5.11 ± 0.79
$V_{(K)}$	4.87 ± 0.57	8.49 ± 0.58	10.94 ± 0.92	10.94 ± 0.70	25.86 ± 1.86	6.62 ± 0.17	6.74 ± 0.52	4.95 ± 0.38
V_D	4.75 ± 1.00	4.99 ± 0.47	9.08 ± 0.49	8.46 ± 0.45	3.41 ± 0.30	2.50 ± 0.54	3.32 ± 0.41	2.91 ± 0.10
Na^+-dependent uptake	7.61 ± 0.63	8.28 ± 0.24	3.43 ± 0.32	3.35 ± 0.65	–	0.21 ± 0.25	2.56 ± 1.05	0.16 ± 0.79
Na^+-independent uptake	0.12 ± 0.57	3.50 ± 0.58	1.86 ± 0.92	2.48 ± 0.70	22.45 ± 1.86	4.12 ± 0.17	3.42 ± 0.52	2.04 ± 0.38

TABLE II

Cross-inhibition of Na^+-independent amino acid uptake

The uptake over a 30-s period was measured under the same conditions as those described in the legend of Table I, except for the addition of 30 mM unlabelled amino acid. The osmolarity of the medium was adjusted appropriately by addition of D-mannitol. Values are mean ± S.D. for four experiments of percentage inhibition calculated using the equation in the text. MeAIB, α-(methylamino)isobutyrate.

Substrate	Percentage inhibition; inhibitor						
	Met	Leu	Gly	Lys	Glu	Pro	MeAIB
Met	91.8 ± 4.8	89.1 ± 8.4	91.2 ± 3.9	97.1 ± 6.4	109.8 ± 13.4	13.1 ± 3.5	15.4 ± 6.8
Leu	105.6 ± 3.9	97.3 ± 6.8	104.0 ± 9.6	96.6 ± 2.8	114.2 ± 14.7	19.8 ± 4.6	29.8 ± 3.9
Gly	96.2 ± 8.2	92.8 ± 9.6	99.8 ± 3.8	100.6 ± 3.6	90.2 ± 9.8	29.6 ± 3.9	34.6 ± 6.7
Lys	91.6 ± 6.2	93.2 ± 2.8	88.2 ± 3.1	101.2 ± 5.6	14.8 ± 6.3	9.8 ± 3.6	13.1 ± 2.9
Glu	96.0 ± 5.6	95.2 ± 4.3	89.0 ± 3.6	86.8 ± 5.0	106.2 ± 9.8	96.8 ± 2.8	94.1 ± 3.1
Pro	85.1 ± 6.2	95.0 ± 3.8	94.2 ± 5.3	94.0 ± 3.6	91.8 ± 4.8	86.3 ± 3.1	94.5 ± 5.9
MeAIB	90.3 ± 3.9	89.6 ± 4.5	88.6 ± 10.5	92.3 ± 9.7	99.9 ± 6.1	108.6 ± 7.3	113.6 ± 6.8

rate of a given amino acid was defined a the difference in uptake rate between that measured in the presence ($V_{(Na)}$) and that in the absence of sodium ($V_{(K)}$). An Na^+ gradient-independent yet carrier-mediated uptake rate was defined by subtracting the diffusional component (V_D) from the uptake in the presence of 100 mM KCl. The diffusional component was determined by measuring the influx of labelled amino acid in the presence of 50 mM unlabelled substrate. It is notable that substantial uptake of certain amino acids occurred by diffusion with no apparent carrier mediation: This was particularly marked for the hydrophobic amino acid L-leucine and also for glycine and was substantialy smaller for the charged amino acids L-lysine and L-glutamate as well as for the imino acids.

The uptake of L-alanine was dependent solely on an Na^+ gradient, suggesting the absence of any transport catalyzed by an Na^+-independent carrier. The carrier-mediated uptake of L-methionine and L-leucine was mainly Na^+-dependent; substitution of Na^+ by K^+ reduced the total uptake of these amino acids. However, the total uptake in the absence of an Na^+ gradient was greater than diffusion, suggesting the presence of a small contribution from an Na^+-independent carrier-mediated system for these amino acids. Uptake of both L-glutamate and α-(methylamino)isobutyrate, on the other hand, was not accelerated by an inwardly directed Na^+ gradient. This suggested the absence of any L-glutamate and α-(methylamino)isobutyrate transport catalyzed by an Na^+-dependent carrier. In the case of L-proline and glycine, Na^+-dependent and Na^+-independent uptake were nearly equal, suggesting the presence of both Na^+-dependent and Na^+-independent carrier-mediated systems for these two amino acids. Uptake of L-lysine at 20 μM was decreased by the presence of an Na^+ gradient from the outside to the inside of the vesicles.

2. Inhibition of amino acid uptake

Amino acid uptake at 20 μM was measured in the presence of different inhibitor amino acids each used at a concentration of 30 mM, either in the presence of an

TABLE III

Cross-inhibition of Na^+-dependent amino acid uptake

The uptake over a 30-s period was measured under the same conditions as those described in the legend of Table I, except for the addition of 30 mM unlabelled amino acid. The osmolarity of the medium was adjusted appropriately by addition of D-mannitol. Values are means ± S.D. for four experiments of percentage inhibition calculated using the equation in the text.

Substrate	Percentage inhibition; inhibitor				
	Ala	Gly	Met	Leu	Pro
Ala	101.7 ± 2.4	89.7 ± 2.2	57.2 ± 5.2	60.1 ± 11.6	95.8 ± 2.7
Gly	91.5 ± 2.4	101.7 ± 2.9	68.5 ± 3.9	38.5 ± 4.8	87.8 ± 8.5
Met	86.5 ± 4.2	90.2 ± 6.3	98.6 ± 5.8	55.2 ± 1.7	48.5 ± 5.2
Leu	94.6 ± 0.4	101.2 ± 5.4	89.8 ± 2.5	96.9 ± 3.4	26.8 ± 1.7
Pro	68.8 ± 4.5	61.5 ± 2.8	72.2 ± 6.7	−4.7 ± 9.0	97.2 ± 7.9

inwardly directed K^+ ($V_{i(K)}$) or Na^+ gradient ($V_{i(Na)}$). The percentage inhibition of an Na^+-independent and an Na^+-dependent carrier-mediated uptake was calculated using the relationship $100 - 100([V_{i(K)} - V_D]/[V_{(K)} - V_D])$ and $100 - 100([V_{i(Na)} - V_{i(K)}]/[V_{(Na)} - V_{(K)}])$, respectively.

2.1. Inhibition of Na^+-independent amino acid uptake. The percentage inhibition of the Na^+-independent component of amino acid uptake is summarized in Table II. L-Methionine, L-leucine, glycine and L-lysine uptake were mutually totally inhibitable and partially (9.8%–43.6%) inhibited by L-proline and α-(methylamino)isobutyrate. L-Glutamate produced complete inhibition of L-methionine, L-leucine and glycine uptake and partial (14.8%) inhibition of L-lysine uptake. Transport of L-glutamate, L-proline and α-(methylamino)isobutyrate was inhibited totally by each of the amino acid inhibitors shown in Table II. L-Alanine is omitted from Table II because it was transported solely via an Na^+-dependent route and was not effective in inhibiting Na^+-independent uptake for any of the substrates tested (data not shown).

2.2. Inhibition of Na^+-dependent amino acid uptake. As shown in Table III, cross-inhibition tests were performed using five amino acids which were transported significantly by Na^+-dependent systems. Transport of L-alanine and glycine showed complete mutual inhibition, and were inhibited partially (38.5%–68.5%) by L-methionine and L-leucine and completely by L-proline. L-Methionine uptake was totally inhibited by L-alanine, glycine and L-methionine itself and partially, 55.2% and 48.5%, by L-leucine and L-proline, respectively. Total uptake of L-leucine was inhibited by L-alanine, glycine, L-methionine and L-leucine itself and 26.8% of total uptake was inhibited by L-proline. L-Proline uptake was inhibited completely only by L-proline itself and partially (61.5%–72.2%) by L-alanine, glycine and L-methionine, but not at all by L-leucine. L-Proline as an inhibitor produced complete inhibition of L-alanine and of glycine uptake and partial inhibition of influx of L-methionine and L-leucine.

Discussion

It is important to explore the mechanisms of amino acid transport across the placental syncytiotrophoblast, since this is the first step in its absorption for the developing fetus and may be one of the regulatory steps for fetal development. We have reported the amino acid transport systems at the brush-border side of human full-term placental syncytiotrophoblast on the basis of a cross-inhibition profile using membrane vesicles [4]. We now apply the same strategy in an attempt to delineate the amino acid transport systems at the basal side of this epithelium.

The basal membrane vesicles used in the present study were purified 27-fold with respect to dihydroalprenolol binding activity, a basal plasma membrane marker of syncytiotrophoblast [14], while a brush-border membrane marker enzyme [1–4], alkaline phosphatase, showed no increase in its specific activity. Furthermore, the brush-border membranes contain solely an Na^+-coupled mechanism for α-(methylamino)isobutyrate transport [4], however there is no indication of an Na^+-stimulated component for α-(methylamino)isobutyrate transport in the basal membrane vesicles. These two lines of evidence exclude the possibility that Na^+-dependent uptake of certain amino acids by the basal

TABLE IV

Amino acid transport systems in human placental basal membrane

A. Simple diffusion			
B. Na^+-independent systems			
System	Transports	Other interacting amino acids	Non-interacting amino acids
1	Methionine Glycine Leucine Lysine Glutamate Proline Methyl-AIB	none	none
2.	Methionine Glycine Leucine Lysine	Glutamate	Proline Methyl-AIB
3	Lysine	Methionine Glycine Leucine	Glutamate Proline Methyl-AIB
C. Na^+-dependent systems			
System	Transports	Other interacting amino acids	Non-interacting amino acids
1	Proline	none	Alanine Glycine Methionine Leucine
2	Alanine Glycine Proline	none	Methionine Leucine
3	Alanine Glycine Methionine Leucine	Proline	none
4	Alanine Glycine Methionine Proline	none	Leucine
5	Methionine Leucine	Alanine Glycine	Proline

membrane preparation is caused by contamination with brush-border membranes.

The amino acid transport systems characterized on the basis of a cross-inhibition profile in the present study are summarized in Table IV, in which they are broadly classified into three categories: (A) 'passive' diffusion, (B) Na^+-independent carrier-mediated systems, and (C) Na^+-dependent carrier-mediated systems (presumably secondarily active).

(A) 'Passive' diffusion

In placental syncytiotrophoblast basal plasama membranes, it is apparent that even at low concentration (20 μM) 'passive' diffusion is responsible for significant transmembrane flux of amino acids (Table I). In our previous study of amino acid uptake by placental brush-border membrane vesicles [4], the 'passive' diffusional component was considered to be unimportant. The relatively leaky nature of the basal plasma membrane may play a physiological role in allowing accumulated amino acids to diffuse relatively readily from syncytiotrophoblast into fetal circulation. However, for certain amino acids such as L-methionine and L-lysine the value of V_D is greater in the brush-border than in the basal membrane: this excludes the possibility that the high values of V_D of the basal membranes for amino acids such as α-(methylamino)isobutyrate, glycine and L-leucine are merely a consequence of non-specific damage to the membrane during vesicle preparation. It seems possible that such variations in V_D between apical and basal membrane reflect underlying difference in membrane structure and lipid composition.

(B) Na^+-independent carrier-mediated systems

The observed cross-inhibition of Na^+-independent amino acid uptake indicates that the amino acids which we have tested share a common Na^+-independent carrier, designated system 1 in Table IV, since substrate amino acids showed a significant degree of mutual cross-inhibition. The Na^+-independent uptake of L-glutamate, L-proline and α-(methylamino)isobutyrate was totally inhibited by any of the inhibitors tested. L-Glutamate produced complete inhibition of L-methionine, L-leucine and glycine and partial inhibition of L-lysine uptake, while L-proline and α-(methylamino)isobutyrate inhibited only a fraction of L-methionine, L-leucine, glycine and L-lysine influx. These results suggest that, first, Na^+-independent uptake of L-glutamate, L-proline and α-(methylamino)isobutyrate is totally supported by this common system, and second, the presence of an additional Na^+-independent pathway serving L-methionine, glycine, L-leucine and L-lysine.

L-Methionine, L-leucine and glycine showed total mutual inhibition, and displayed identical patterns of inhibition and sensitivity to the amino acids tested. They produced complete inhibition of all the amino acids tested. In addition to the partial inhibition by L-proline and α-(methylamino)isobutyrate, their uptake was inhibited totally by L-lysine and L-glutamate. L-Lysine completely inhibited the Na^+-independent uptake of all substrates, while L-lysine uptake was inhibited by L-methionine, L-leucine, glycine and L-lysine itself and partially by L-glutamate, L-proline and α-(methylamino)isobutyrate. The results with L-glutamate as inhibitor indicate that the additional pathway responsible for L-methionine, glycine, L-leucine and L-lysine transport must itself be partitioned into two separate systems, one of which is sensitive to L-glutamate (designated system 2 in Table IV); the other insensitive to L-glutamate (designated system 3). System 2, however, cannot account for a significant fraction of L-glutamate flux since uptake of L-glutamate was inhibited totally by L-proline and α-(methylamino)isobutyrate through the system 1. System 3, although inhibited by L-methionine, glycine or L-leucine cannot carry a significant fraction of the fluxes of these three amino acids since these are completely inhibited by L-glutamate. Therefore it seems probable that a fraction of L-lysine transport is mediated by an L-lysine specific Na^+-independent system. L-Lysine transport was lowered in the presence of cations as compared with in their absence; an inwardly directed gradient of Na^+ was much more effective than K^+ at slowing L-lysine transport (Table I). We are currently studying the transport of this amino acid transport in more detail [16].

(C) Na^+-dependent carrier-mediated systems

The data in Table I suggests the presence of Na^+-dependent carrier-mediated amino acid transport in basal as well as in brush-border membranes of human placental syncytiotrophoblast. This is supported by the cross-inhibition data shown in Table III, which suggests the existence of five systems.

A component of L-proline uptake was insensitive to any of the other amino acids tested, while L-proline produced complete inhibition of L-alanine and glycine transport and partial inhibition of L-methionine and L-leucine. This suggests the presence of an L-proline specific transport system which is designated system 1 in Table IV.

L-Alanine and glycine showed total mutual inhibition and displayed the same patterns of inhibition and sensitivity to the other amino acids tested. They produced complete inhibition of L-methionine and L-leucine and partial inhibition of L-proline uptake. In addition to the total inhibition by L-proline, their uptake was inhibited partially by L-methionine and L-leucine. These results suggest that the flux both of L-alanine and glycine may be partitioned into three components; one of which (designated system 2) is capable of interacting with L-proline but with neither L-methionine nor L-leucine;

the second (designated system 3) is sensitive to L-proline, L-methionine and L-leucine, however, no significant fraction of L-proline can be carried by this system, since L-proline flux was not directly inhibited by L-leucine; the third system (designated system 4) is responsible for transport of L-methionine and L-proline as well but does not interact with L-leucine. This follows since part of the L-methionine flux is insensitive to L-leucine inhibition.

System 5, transporting L-methionine and L-leucine, is insensitive to L-proline and does not mediate significant flux of L-alanine and glycine since these fluxes themselves were not directly inhibited by L-methionine and L-leucine.

Our analysis leads to the conclusion that there exist at least three distinct Na^+-independent and five distinct Na^+-dependent carrier-mediated amino acid transport systems in human full-term placental basal membranes. The common Na^+-independent pathway, system 1 in Table IV, does not correspond to the classical L system, since α-(methylamino)isobutyrate can interact with this system [17], indeed system 2 appears to be more similar to the L system. Na^+-independent system, 3, which carried L-lysine but not any of the other amino acids which we have studied, may conform to y^+, the Na^+-independent cationic amino acid transport system [17]. Recent studies in the perfused guinea-pig placenta have provided evidence for the existence of a y^+-like amino acid transport system mediating the uptake of L-lysine across the basal membrane [18]. The five Na^+-dependent amino acid transporting systems we have delineated in the present study appear to be distinct from the classical Na^+-dependent systems found in nonepithelial cells [19] and in rat jejunal basal lateral plasma membranes [8]. The A system has a broad specificity and also transport α-(methylamino)isobutyrate, while none of these five systems can interact with α-(methylamino) isobutyrate. Sodium-dependent systems 2, 3 ad 4 all differ from the classical ASC system since the latter system excludes glycine. Stevens et al. [9] reported that rabbit jejunal brush-border membrane transports amino acids via at least three Na^+-dependent carrier-mediated systems: the NBB system, the PHE system and the IMINO system and somewhat similar systems are found in human placental brush-border membranes [4]. The NBB system transports most neutral amino acids, as do Na^+-dependent systems 2, 3 and 4 in Table IV. Na^+-dependent system 1 and system 5 are similar to the IMINO and the PHE system, respectively. The IMINO system exclusively transports imino acids, while the PHE system primarily handles L-phenylalanine and L-methionine.

The Na^+-independent carrier-mediated transporting systems as well as 'passive' diffusion driven by the amino acid concentration gradients would be sufficient for the basal membrane exit step in the overall process of active amino acid transport from maternal to fetal circulation. The existence of Na^+-dependent amino acid transport systems in the basal membranes may be significant in the carriage of amino acids from the fetal circulation into the syncytiotrophoblast during the regulation of fetal plasma amino acids.

Note added in proof: (Received 5 December 1989)

Since this paper was submitted Hoeltzli and Smith [20] have published data on L-alanine transport in placental basal membranes. In contrast to this study they found a significant Na^+-independent transport pathway for this amino acid. The reasons for this discrepancy are not apparent.

Acknowledgements

We are grateful to Dr. J.C. Bustamante for his advice in improving the technique to prepare the basal membrane vesicles and the MRC for financial assistance.

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