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# TRANSPORT SPECIFICITY FOR NEUTRAL AND BASIC AMINO ACIDS AT MATERNAL AND FETAL INTERFACES OF THE GUINEA-PIG PLACENTA

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SUMMARY

1. The unidirectional influx of amino acids into the guinea-pig syncytiotrophoblast was measured using a single circulation paired-tracer dilution technique which allows separate characterization of both fetal and maternal interfaces. An *in situ* preparation perfused through the fetal circulation was used to examine the fetal side, while an isolated preparation perfused through both the fetal and maternal circulations was used to study both interfaces simultaneously.

2. On the fetal side the maximal uptake ($U_{max}$) determined at tracer concentrations was high for the short-chain neutral amino acid alanine (76%) and the long-chain neutrals, leucine (75%), phenylalanine (90%) and tyrosine (82%) and for the basic amino acid lysine (65%). In contrast, $U_{max}$ was negligible for $\alpha$-methylaminoisobutyric acid and taurine, a $\beta$-amino acid.

3. The uptake of alanine and phenylalanine on the fetal side was inhibited by both short-chain (alanine, serine, cysteine) and long-chain (phenylalanine, methionine, leucine) neutral amino acids. D-alanine had no effect on L-alanine uptake whereas D-phenylalanine significantly inhibited that of L-phenylalanine. Diaminobutyric acid, lysine and arginine were effective inhibitors of alanine uptake but had no effect on phenylalanine uptake.

4. On the maternal side uptake of alanine, phenylalanine and lysine was measured. Over a wide range of concentrations self-inhibition of alanine influx was similar to the cross-inhibition observed with phenylalanine. In contrast, the influx of phenylalanine, which was strongly self-inhibited, was only partially cross-inhibited by alanine.

5. Influx of alanine and phenylalanine was measured at various perfusate concentrations and was found to be saturable on both maternal and fetal sides. The data were fitted to a single hyperbola and, on the maternal side, the $K_m$ for alanine ($10.3 \pm 2.7$ mM, mean $\pm$ S.E., $n = 3$) was three-fold higher than the value measured for phenylalanine ($3{\cdot}1 \pm 0{\cdot}8$ mM). On the fetal side the $K_m$ values for alanine ($8{\cdot}4 \pm 1{\cdot}4$ mM, $n = 4$) and phenylalanine ($11{\cdot}9 \pm 1{\cdot}9$ mM, $n = 3$) were similar.

6. The uptake of alanine, phenylalanine and lysine appeared to be highly sodium-dependent accounting for 40–70% of the total influx. However, the inhibited fractions were found to be different on the two sides of the placenta.

7. The results of uptake, cross-inhibition and $Na^+$-dependency experiments suggest

the presence of an alanine-serine-cysteine (ASC) type system and a leucine (L) type system with markedly overlapping specificities at both the fetal and maternal interfaces. Separate kinetic characterization of a two carrier system was not possible under the conditions of these experiments. However, kinetic parameters for the over-all transport of alanine and phenylalanine were measured.

## INTRODUCTION

Most amino acids are transferred from either the fetal or maternal circulation across the placenta (Eaton & Yudilevich, 1981) and transplacental gradients are established in favour of the fetus (Christensen & Streicher, 1948; Reynolds & Young, 1971). However, the transport mechanisms for influx and efflux of amino acids at both surfaces of the trophoblast are still to be elucidated (see review, Young, 1981). Three transport systems appeared to be involved in the uptake of neutral amino acids at the maternal interface in human placental villi (Enders, Judd, Donohue & Smith, 1976). These corresponded to Christensen's A-, ASC- and L-systems (see Christensen, 1979). Transport kinetics for the non-metabolizable amino acid, 2-aminoisobutyric acid, AIB, have been reported in human villous fragments (Smith, Adcock, Teasdale, Meschia & Battaglia, 1973), in placental slices (Miller & Berndt, 1974) and in maternal membrane vesicles (Ruzycki, Kelley & Smith, 1978). In a recent study Boyd & Lund (1981) measured the kinetic parameters for L-proline uptake into human placenta brush-border vesicles.

The unidirectional influx, and rapid efflux, of an amino acid into the tissue compartment from either the fetal or maternal circulation of the perfused guinea-pig placenta has been investigated by Eaton & Yudilevich (1981). Their study made use of a single circulation paired-tracer dilution technique previously used to investigate placental transport of glucose (Yudilevich, Eaton, Short & Leichtweiss, 1979), lactate (Leichtweiss & Schröder, 1981) and prostaglandins and other substrates (Yudilevich, Eaton & Mann, 1981). Fifteen naturally occurring amino acids were shown to be transported across maternal and fetal sides of the syncytiotrophoblast in the intact dually perfused preparation. In a preliminary communication the kinetics of L-phenylalanine influx across the fetal side were reported (Yudilevich & Eaton, 1980). We here examine the specificity of L-alanine and L-phenylalanine transport at both sides of the trophoblast of the intact guinea-pig placenta. Cross-inhibition tests of alanine and phenylalanine uptake suggested that the transport of neutral amino acids was mediated by at least two systems similar to the ASC- and L-carriers described in other cell membranes. The absence of methyl-AIB uptake suggested that an A-type system was not present. A preliminary communication of part of this work has been presented to the Physiological Society (Eaton, Mann & Yudilevich, 1981).

## METHODS

### *Animal preparations*

*Isolated dually perfused guinea-pig placenta.* Details of this preparation were originally described by Leichtweiss & Schröder (1971) and subsequently modified by Yudilevich *et al.* (1979). White Dunkin–Hartley guinea-pig dams of about 60 days gestation were tranquillized with 10 mg diazepam (Valium, Roche) injected intraperitoneally and anaesthetized with sodium pentobarbitone

15–20 mg $kg^{-1}$ (Nembutal, Abbott Laboratories) via a permanent cannula in a limb vein. The uterus was exteriorized into a bath filled with physiological saline at 37 °C and sprayed with a 1 mg $ml.^{-1}$ solution of nifedipine (Adalat, Bayer) to relax the uterine muscles. One of the placentae was isolated with a pair of annular perspex clamps. On the fetal side the vitelline vessels were tied and cut, an umbilical artery and umbilical vein were cannulated, perfusion was started and the fetus was then removed. A single maternal artery and vein were then cannulated and perfusion started. The remaining maternal vessels were tied and cut and the preparation was transferred to a small organ bath maintained at 37 °C.

*'In situ' singly perfused placenta.* This preparation has also been previously described (Money & Dancis, 1960; Reynolds & Young, 1971). As before, the dam was anaesthetized and a fetus exteriorized via small abdominal and uterine incisions. The cord vessels were cannulated and the fetus was removed. The maternal circulation remained intact, while the fetal circulation was artificially perfused.

Placentae were perfused using a peristaltic pump and perfusion rates were 3 ml. $min^{-1}$ into both circulations of the isolated preparation and 2 ml. $min^{-1}$ into the fetal circulation of the *in situ* preparation. Perfusion pressures were generally less than 50 mmHg and in the isolated preparation were usually similar on the two sides.

*Perfusates*

The control perfusate was a Krebs–Ringer solution of the following composition (mM): NaCl, 118·1; KCl, 4·8; $KH_2PO_4$, 1·2; $MgSo_4.7H_2O$, 1·2; $NaHCO_3$, 25·0; $CaCl_2.2H_2O$, 2·5, and contained 40 g $l.^{-1}$ Dextran 40 (Sigma Chemical Co.). and 1 g $l^{-1}$ bovine serum albumin (Cohn Fraction V, Sigma). Glucose (5·5 mM) was only added to the perfusates used for the isolated preparation. In the kinetic experiments a specific unlabelled amino acid was added to the control perfusate at a known concentration. In experiments designed to test the sodium-dependency of amino acid transport the NaCl and $NaHCO_3$ in the perfusate were removed and replaced by equimolar quantities of buffered Trizma HCl (Sigma). In these experiments 1 mM-adenosine (Sigma) was added to both sodium-free and control perfusates to maintain maximal vasodilatation in the preparation (Jones, Mann & Smaje, 1980).

All perfusates were maintained at 37 °C and gassed with 95% $O_2$–5% $CO_2$ to a pH of 7·3–7·4.

*Paired-tracer dilution experiment*

In the dually perfused placenta a 100 $\mu$l. bolus of a tracer mixture was rapidly (1–2 sec) injected into the arterial inflow of one of the circulations. This was immediately followed by the collection of thirty successive four-drop samples from the venous outflow of the injection side. A last sample was then accumulated for a further 4 min. Concurrently, a series of four-drop samples and a 4 min sample or a single 6 min collection was made from the contralateral (acceptor) side circulation. In the *in situ* preparation a bolus injection into the fetal artery was followed by similar sampling only from the fetal vein.

Each tracer mixture contained about 1 $\mu$Ci of L-[1-$^{14}$C]glucose, an extracellular reference tracer (Yudilevich *et al.* 1979) and about 5 $\mu$Ci of a tritiated test amino acid. The tracer mixture was made up to the required injection volume by the addition of the appropriate perfusate.

The labelled amino acids used were L-[3-$^{3}$H]alanine, 2-aminoisobutyric [methyl-$^{3}$H]acid, $\alpha$[1-$^{14}$C]methylaminoisobutyric acid, L-[4,5-$^{3}$H]leucine, L-[4-$^{3}$H]phenylalanine, L-[3,5-$^{3}$H]tyrosine, L-[4,5-$^{3}$H]lysine and [1,2-$^{3}$H]taurine, All isotopes were purchased from New England Nuclear Chemical, GmBH Or Amersham International Ltd.

*Preparation of samples for liquid scintillation counting*

The venous samples, aliquots of the injectate, $^{3}$H and $^{14}$C channel standards and background samples were prepared for liquid scintillation counting by the addition of 4 ml. ethanol and 10 ml. of toluene containing 4 g $l.^{-1}$ 2,5-diphenyloxazole (PPO) and 100 mg $l.^{-1}$ 1,4-bis[2-15-phenyl-oxazolyl)] benzene (POPOP) (Packard). In later experiments samples were collected in glass mini-vials and 2 ml. of Aqualuma (Luma Systems A.G.) was added. Samples and standards were counted concurrently and individual quench corrections were unnecessary. Data were analysed using a Basic Programme in an ICL Modular 1 digital computer.

*Analysis of paired-tracer dilution curves*

The analysis of these experiments was similar to that previously reported for glucose (Yudilevich *et al.* 1979), and amino acid (Eaton & Yudilevich, 1981) transport studies. The activity of the test amino acid and extracellular tracer recovered in the venous samples was expressed as a percentage of the injected dose. Transport of the labelled amino acid into and out of the trophoblast could be assessed from the difference in their concentration–time profiles. The tissue uptake was calculated for each successive sample using the relationship:

$$\text{Uptake} = \left(1 - \frac{[[^3\text{H}]\text{amino acid}]}{[\text{L-}[^{14}\text{C}]\text{glucose}]}\right). \quad (1)$$

The uptake curves usually had an initial plateau value for about 20–40 sec after which tracer efflux from the tissue resulted in a progressive fall-off in the uptake curve.

The unidirectional influx, $v$, into the trophoblast was calculated from the average of the maximal uptake value ($U_{max}$) using the following relationship (Pardridge, Connor & Crawford, 1975; Bustamante, Mann & Yudilevich, 1981):

$$v = -F \cdot \ln(1 - U_{max}) \cdot C, \quad (2)$$

where $F$ is the perfusate flow per gram wet weight of placenta and $C$ is the concentration of the unlabelled amino acid in the perfusate.

*Chromatographic analysis of* L-[3-$^3$*H*]*alanine*

Experiments were performed in dually perfused placentae by collecting both venous outflows following a bolus injection of only L-[3-$^3$H] alanine into the maternal circulation. A Thin-Layer Berthold LB 2760 Chromatographic Scanner was used to analyse the cellulose thin layer chromatography plates (Merck 5577, BDH Chemicals). Similar experiments for L-[4-$^3$H]phenylalanine have previously been published as well as details of the analytical procedures (Eaton & Yudilevich, 1981).

In these experiments the presence of metabolites in the maternal venous effluent or in the fetal circulation following the significant transplacental transfer of labelled alanine was not detectable.

## RESULTS

### *Unidirectional uptake*

Results of the unidirectional tracer uptake of six naturally occurring and two synthetic amino acids at the fetal interface of the singly-perfused placenta are summarized in Table 1. In order to maximize the sensitivity of these measurements placentae were perfused with an amino acid-free Ringer solution and injectates

TABLE 1. Unidirectional amino acid uptake at the fetal interface of the syncytiotrophoblast

| Amino acid | Injectate concn. ($\mu$M) | $U_{max}$ % |
|---|---|---|
| L-[3-$^3$H]alanine | 0·4 | 76±1 (31) |
| 2-aminoisobutyric [methyl-$^3$H] acid | 2·1 | 18±5 (4) |
| $\alpha$[1-$^{14}$C]methylaminoisobutyric acid | 150 | 2±2 (6) |
| L-[4,5-$^3$H]leucine | 7·6 | 75±7 (6) |
| L-[4-$^3$H]phenylalanine | 2·2 | 90±3 (16) |
| L-[3,5-$^3$H]tyrosine | 7·6 | 82±1 (3) |
| L-[4,5-$^3$H]lysine | 0·4 | 65±2 (3) |
| [1,2-$^3$H]taurine | 3·8 | 8±4 (3) |

The fetal circulation was perfused with amino acid-free Krebs–Ringer solution while the maternal blood supply remained intact. The approximate amino acid concentrations in the isotope bolus injectates are listed. Values are given as mean±S.E. ($n$ = number of measurements).

contained the highest possible specific activity. The naturally occurring short-chain neutral amino acid L-alanine had four times the uptake observed for the non-metabolized analogue AIB. Methyl-AIB was not taken up even under these optimal conditions implying the absence of system A through which *N*-methyl amino acids are exclusively transported (Kilberg, Handlogten & Christensen, 1981). The three long-chain neutral L-amino acids (phenylalanine, leucine and tyrosine) tested had uptakes ranging between 75–90%. The basic amino acid L-lysine also exhibited a substantial uptake whereas the uptake of the $\beta$-amino acid taurine was negligible.

TABLE 2. Inhibition of tracer amino acid uptake at the fetal interface of the syncytiotrophoblast

| Inhibitor amino acid | % Inhibition of uptake L-[3-$^3$H]alanine | L-[4-$^3$H]phenylalanine |
|---|---|---|
| L-alanine | 76±7 | 19±3 |
| D-alanine | 8±2 | — |
| D,L-methylalanine | 2±1 | −1±1 |
| L-serine | 74±5 | 25±3 |
| L-aminobutyric acid | 70±8 | 29±3 |
| L-norvaline | 72±5 | — |
| L-cysteine | 76±4 | 22±6 |
| L-methionine | 55±10 | 28±5 |
| L-leucine | 67±6 | 43±8 |
| L-phenylalanine | 56±6 | 40±2 |
| D-phenylalanine | — | 11±1 |
| glycine | 37±3 | 10±4 |
| L-proline | 30±7 | 2±1 |
| L-diaminobutyric acid | 33±8 | 6±3 |
| L-lysine | 12±5 | 0±1 |
| L-arginine | 30±6 | 3±1 |

Unlabelled competitor amino acids were present in the injectate at a concentration of 100 mM. Values are mean±S.E. from four placentae.

In the dually perfused placenta uptake at the maternal interface was only measured for alanine, phenylalanine and lysine. Maximal uptake was determined at a given perfusate concentration of the unlabelled L-amino acid. The $U_{max}$ values obtained were: alanine (0·15 mM), 85%±3 ($n$ = 6); phenylalanine (0·15 mM), 81±4 ($n$ = 6) and lysine (0·38 mM), 75%±6 ($n$ = 3).

*Inhibition of uptake*

In order to assess cross-inhibition by a wide range of amino acids eight *in situ* placentae were perfused on the fetal side with an amino acid-free Ringer solution. Inhibition of L-[$^3$H]alanine or L-[$^3$H]phenylalanine uptake was determined by comparing $U_{max}$ values obtained in the presence or absence of different unlabelled competitor amino acids. The control uptake was measured using injectates containing 100 mM-mannitol. The unlabelled amino acid in the injectate (100 mM) would be diluted by the inflowing perfusate in a similar manner to the extracellular reference tracer. As the maximal L-[$^{14}$C]glucose concentration recovered in the venous effluent ranged between 4–6% of the injected dose, the concentration of the competitor at

the exchange site would approximate to 4–6 mM. Table 2 summarizes the percentage inhibition of the control tracer uptake which was obtained at micromolar injectate concentrations (Table 1). L-alanine uptake was only negligibly inhibited by D-alanine,

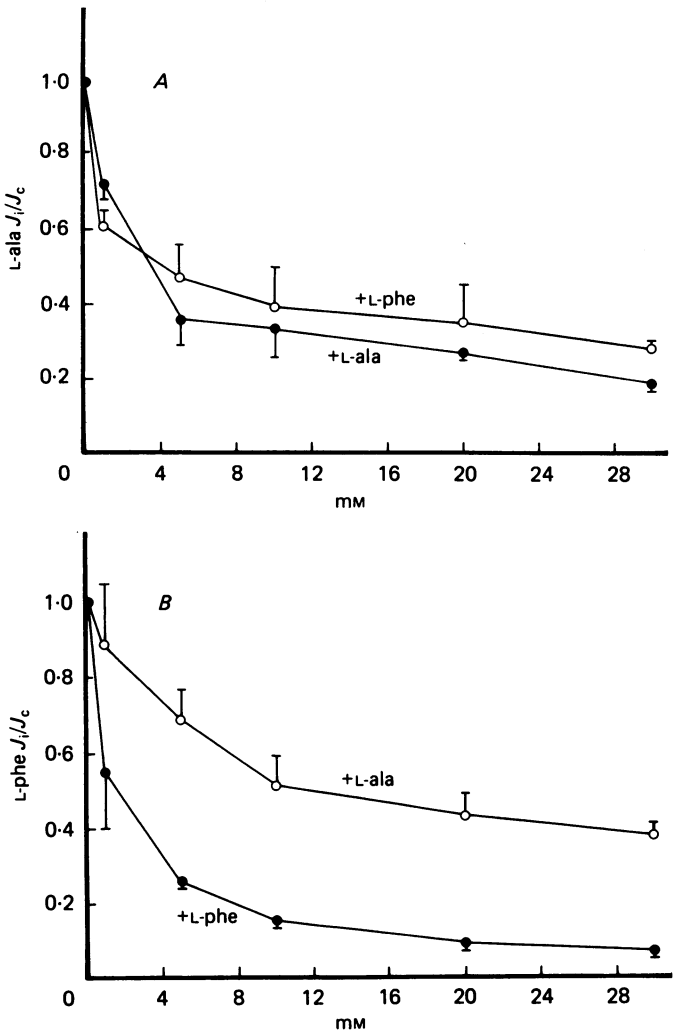


Fig. 1. Inhibition of alanine and phenylalanine influx across the maternal surface of the syncytiotrophoblast. *A*, inhibition of the influx of 0·15 mM-L-($^3$H)alanine by increasing concentrations of either unlabelled L-alanine or L-phenylalanine. The ordinate denotes the ratio of the influx measured in the presence of the inhibitor ($J_i$) to the control influx ($J_c$). *B*, equivalent data for the inhibition of the influx of 0·15 mM-L-[$^3$H]phenylalanine. Values are given as mean ± S.E. for three dually perfused placentae.

whereas the L-enantiomer and the other short-chain neutral amino acids (serine, aminobutyric, norvaline and cysteine) all caused over 70% inhibition. Methylated alanine had no inhibitory effect. Similarly large inhibitions were obtained with the long-chain neutral amino acids, methionine, leucine and phenylalanine. A comparable,

but less effective inhibition was observed with glycine and proline, and with the basic amino acids, diaminobutyric, lysine and arginine.

Phenylalanine uptake was less stereospecific with the D-isomer causing a small inhibition of 11% whereas the L-isomer inhibited by 40%. Inhibition was also observed with methionine and leucine. The short-chain neutral amino acids caused significant inhibition although they were generally less effective than the long-chain neutrals. The synthetic amino acid methylalanine was again ineffective in inhibiting uptake. Glycine caused a 10% inhibition, while proline and the basic amino acids had no effect. Generally, self- and cross-inhibition were smaller than those measured for alanine.

Fig. 1 shows results from six dually perfused placentae where influx of L-[$^3$H]alanine and L-[$^3$H]phenylalanine was characterized at the maternal interface. Inhibition of amino acid influx is expressed as the ratio of the influx measured either in the absence ($J_c$) or presence ($J_i$) of a competitor amino acid. The influx of alanine could be inhibited to a similar degree by either alanine or phenylalanine, whereas phenylalanine influx was more strongly self-inhibited than cross-inhibited by alanine. Furthermore, at equimolar concentrations self-inhibition of alanine influx was less effective than self-inhibition of phenylalanine influx.

*Transport kinetics for alanine and phenylalanine*

Influx kinetics on the fetal side were measured in the *in situ* placenta perfused successively with different solutions containing the unlabelled amino acid at concentrations ranging between 0·5–24 mM. Each measurement was made after 4 min of perfusion with a given perfusate. Michaelis–Menten parameters were estimated by fitting a single rectangular hyperbola weighted for the standard error at each mean (Cleland, 1967) to the data from three placentae (Fig. 2). The values for alanine were $K_m = 8{\cdot}4 \pm 1{\cdot}4$ mM and $V_{max} = 4{\cdot}4 \pm 0{\cdot}4$ $\mu$mol min$^{-1}$ g$^{-1}$ and those for phenylalanine were $K_m = 11{\cdot}9 \pm 1{\cdot}9$ mM and $V_{max} = 9{\cdot}5 \pm 0{\cdot}9$ $\mu$mol min$^{-1}$ g$^{-1}$.

Kinetics on the maternal side were measured in the isolated placenta in which both circulations were perfused with solutions containing a known concentration of a given amino acid. In each placenta only one labelled amino acid was tested and influx was measured at six different concentrations (0·15–30·15 mM) of either unlabelled alanine or phenylalanine. As on the fetal side influx was measured after 4 min of perfusion with a given solution. Control experiments with perfusates containing unlabelled mannitol (30 mM) excluded an osmotic effect on influx measurements. The kinetics constants estimated from data in Fig. 3 for alanine were $K_m = 10{\cdot}3 \pm 2{\cdot}7$ mM and $V_{max} = 14{\cdot}3 \pm 1{\cdot}8$ $\mu$mol min$^{-1}$ g$^{-1}$ and those for phenylalanine were $K_m = 3{\cdot}1 \pm 0{\cdot}8$ mM and $V_{max} = 3{\cdot}7 \pm 0{\cdot}3$ $\mu$mol min$^{-1}$ g$^{-1}$. These values as well as those obtained on the fetal side reflect the parameters for the over-all transport of alanine and phenylalanine rather than absolute constants for two separate carrier systems. No attempt was made to fit the data to a multi-carrier model.

*Sodium dependence of influx at maternal and fetal interfaces*

The effect of removing sodium from the perfusate on amino acid influx was investigated on both maternal and fetal sides of the isolated dually perfused placenta. The uptake of L-alanine, L-phenylalanine or L-lysine was measured in placentae

perfused with either a normal Ringer solution (143 mM-sodium) or a sodium-free perfusate. Both control and sodium-free perfusates contained a known concentration of a given amino acid (0·15–0·5 mM) and adenosine (1 mM). The presence of adenosine prevented vasoconstriction and changes in perfusion pressure which otherwise occurred in the absence of sodium. Table 3 illustrates that on the fetal side about

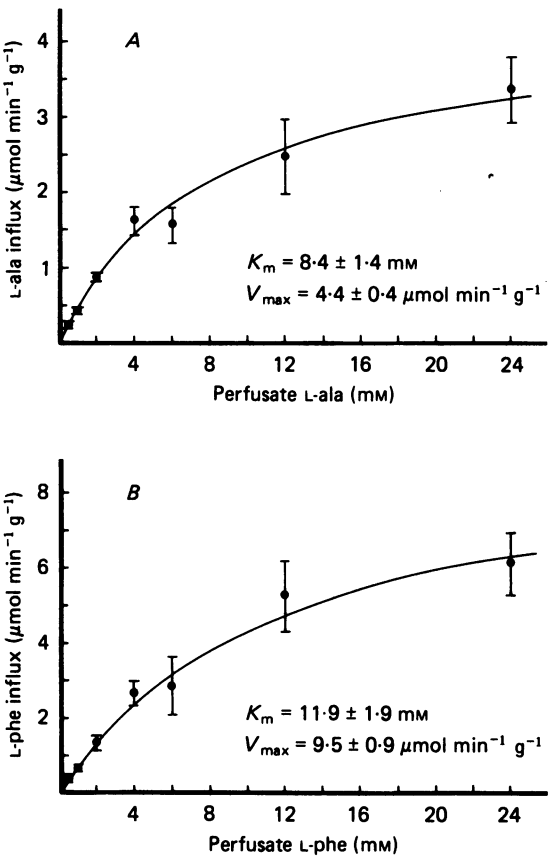


Fig. 2. Kinetics of L-alanine (*A*) and L-phenylalanine (*B*) influx across the fetal facing membrane of the syncytiotrophoblast in the *in situ* placenta. The alanine data are from four placentae and the phenylalanine are from three placentae. A single weighted hyperbola was fitted to each set of data and individual points are the mean ± S.E.

one half of the influx for all three amino acids was abolished in the absence of sodium. On the maternal side L-alanine influx was inhibited by 73% while L-phenylalanine influx was inhibited by only 38%. L-lysine influx was also significantly reduced. Reversibility of these effects was observed upon returning to control perfusates.

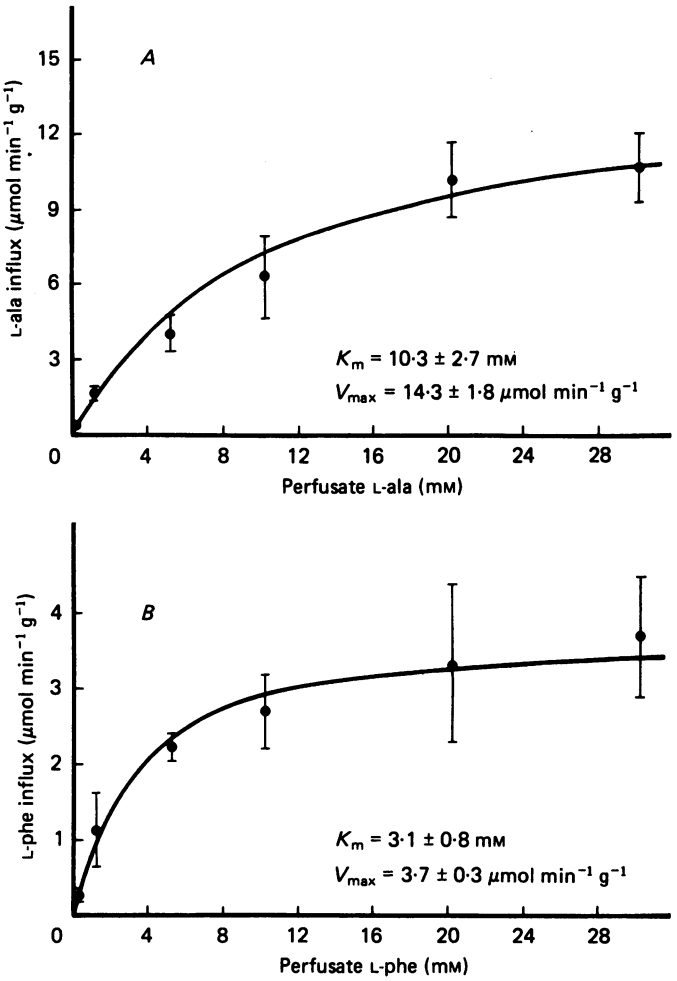


Fig. 3. Unidirectional influx kinetics for L-alanine (*A*) and L-phenylalanine (*B*) across the maternal interface of the syncytiotrophoblast in dually perfused placentae. The data in each panel were obtained from three placentae. Remaining details are given in the text and in the legend to Fig. 2.

TABLE 3. Sodium dependence of amino acid influx across maternal and fetal surfaces of the syncytiotrophoblast

| | % Reduction in influx | |
|---|---|---|
| | Maternal side | Fetal side |
| L-alanine | −73·5 ± 6·0 (3) | −43·1 ± 10·7 (3) |
| L-phenylalanine | −37·8 ± 5·6 (4) | −55·5 ± 6·3 (4) |
| L-lysine | −16.6, −29·9 | −40·5 ± 1·2 (3) |

Both maternal and fetal circulations of an isolated placenta were initially perfused with a normal Krebs–Ringer solution and then with one in which Na was replaced with buffered Trizma hydrochloride. The reversibility of this effect was tested by re-perfusing each placenta with control perfusate. Unidirectional influx was measured at a constant unlabelled amino acid concentration in the perfusate: L-[3-$^3$H]ala and L-[4-$^3$H]phe (0·15 mM), L-[4,5-$^3$H]lys (0·38–0·5 mM) and the reduction in influx expressed as mean ± S.E. ($n$ = number of placentae).

## DISCUSSION

The guinea-pig and human placenta are haemomonochorial and both surfaces of the syncytiotrophoblast are exposed to a different circulation. The role of this epithelium in amino acid transport has been the subject of numerous studies since the original observation of Christensen & Streicher (1948) that fetal plasma amino acid concentrations are considerably elevated compared to maternal plasma levels. Analyses of placental tissue (Hill & Young, 1973; Phillips, Holzman, Teng & Battaglia, 1978) revealed intracellular amino acid concentrations far in excess of even fetal plasma levels. These studies led to the hypothesis of active accumulation of amino acids at the maternal surface, producing the high tissue concentrations, followed by passive movement down the concentration gradient into the fetal circulation (Dancis, Money, Springer & Levitz, 1968; Reynolds & Young, 1971). Several other authors have supported this model (van Dijk & van Kreel, 1978; Schneider, Möhlen & Dancis, 1979). An alternative hypothesis has been proposed by Eaton & Yudilevich (1981) in which both sides of the trophoblast possess transport systems capable of actively producing concentration gradients between the circulation and the placental tissue.

In the dually perfused guinea-pig placenta Eaton & Yudilevich (1981) observed that at physiological amino acid concentrations the uptake into the trophoblast of a wide range of neutral and basic amino acids was similar when measured from either the maternal or fetal circulation whereas the backflux and transplacental transfer were asymmetric. Backflux was less pronounced at the maternal side than at the fetal side and this correlated with a transplacental transfer of a higher proportion of the injected radioactive amino acid in the maternal to fetal direction. These results implied that the concentration gradient favouring the fetal side was related to the asymmetric amino acid efflux from the placental tissue rather than asymmetry in the influx.

We have not attempted to characterize fully all the transport systems available to neutral amino acids. The cross-inhibition data on the maternal side (Fig. 1) and also on the fetal side (Table 2) suggest that the uptake of the two representative amino acids, alanine and phenylalanine, is mediated by more than one system. On the maternal side measurements were made using competing amino acids, present in the perfusate, up to a concentration of 30 mM. In all four cases the curves obtained were continuing to fall and presumably the influx of the test amino acid would be more completely inhibited at higher concentrations (Fig. 1).

On the fetal side the competitor amino acids (100 mM) were present only in the injectates and dilution by the perfusate reduced this concentration to an estimated effective concentration of about 4–6 mM. Under these conditions the self-inhibition of alanine uptake (76%) was greater than that observed for phenylalanine (40%). It is important to emphasize that the remaining fraction of the uptake cannot be ascribed to simple diffusion since the measurement is already corrected for diffusion; the labelled amino acid concentration profile is compared with that of a reference molecule of similar free-diffusibility.

On the maternal side the kinetic data for alanine and phenylalanine obtained in

the absence of any other amino acid indicated an affinity constant for alanine influx (10·3 mM) far greater than that for phenylalanine (3·1 mM). The number of transport sites available for alanine far exceeds those for phenylalanine as indicated by the $V_{max}$ measurements. Based on these findings phenylalanine would be expected to be a better inhibitor of alanine influx than alanine itself. Since this was not the case (see Fig. 1) these data are compatible with the presence of more than one transport system.

On the fetal side the $K_m$ for alanine (8·3 mM) was very similar to that on the maternal side. However, the value for phenylalanine (11·9 mM) was much higher than that on the maternal side, suggesting asymmetry of the influx mechanisms for hydrophobic amino acids at the two interfaces. These differences were not detected at the micromolar amino acid concentrations present in the TC199 tissue culture medium used as the perfusate in the previous studies (Eaton & Yudilevich, 1981).

An asymmetry in the sodium-dependency of amino acid transport was also observed in the present study (Table 3). The inhibition of influx indicated that an important fraction of amino acid uptake at both surfaces of the trophoblast was sodium-dependent. On the maternal side the relative sodium-dependency (alanine > phenylalanine > lysine) was similar to that reported in other tissues, such as the intestine (Sepúlveda & Smith, 1978). In contrast, on the fetal side measurements revealed that about half of the influx of all three amino acids was inhibited by the removal of sodium. These findings may be compared with those of Miller & Berndt (1974) in human placental slices in which the uptake of AIB was only partially inhibited in a sodium-free medium or by ouabain and DNP. The differences in the kinetic constants and the sodium-dependency on the two sides of the syncytiotrophoblast can be explained by differences in the proportion of the various transport sites at the two interfaces.

We further characterized the specificity of neutral amino acid transport systems by investigating the uptake and cross-reactivity of a range of amino acids at the fetal interface. A significant uptake of AIB was observed; however there was no uptake of methyl-AIB (Table 1), a substrate specific for the A-system (Christensen, 1979; Kilberg *et al.* 1981). The lack of an inhibitory effect of methylalanine on the uptake of either alanine or phenylalanine (Table 2) also lends support to the absence of an A-type system. Kilberg, Christensen & Handlogten (1979) described cysteine as a model substrate for characterizing the ASC system in rat hepatocytes. The inhibition data in Table 2 show that cysteine and other short-chain neutral amino acids inhibited the uptake of labelled alanine by up to 76%, and this suggests that the guinea-pig placenta possesses an ASC-type carrier. Dancis *et al.* (1968) observed that, although AIB was accumulated by both the human and guinea-pig placenta, insulin stimulated transport only in human placental tissue. Insulin stimulation of amino acid uptake has been shown to be restricted to an effect on the A-system (see review by Guidotti, Borghetti & Gazzola, 1978). Pre-incubation of human placental villous tissue in amino acid free medium was reported to markedly enhance uptake of AIB (Longo, Yen & Gusseck, 1973; Smith *et al.* 1973), and this effect has been proposed to be the result of an increase in the activity of the A-system (see review, Smith, 1981). It would therefore appear that a species difference exists between the human and

guinea-pig placenta since the guinea-pig may only have an ASC-type carrier whereas the human has both ASC- and A-type systems.

The results also suggest the presence of a second system for long-chain neutral amino acids, resembling Christensen's L-type carrier, on both the maternal and fetal sides of the trophoblast. This system exhibited a lower stereospecificity than that observed for alanine and a cross-reactivity with the short-chain neutral amino acids.

The presence of a separate transport system for basic amino acids is not clearly implied by the inhibition experiments summarized in Table 2. Although alanine can be transported by a basic amino acid transport system, as in the intestine (Paterson, Sepúlveda & Smith, 1981), there are examples of diamino acids being transported by neutral transport systems (Christensen, 1979). In our experiments alanine uptake was significantly inhibited by the basic amino acids whereas phenylalanine uptake was unaffected. It is interesting that in similar experiments in the salivary gland, alanine and phenylalanine uptake was not inhibited by either lysine or arginine (Bustamante *et al.* 1981).

Glycine and proline, which in some membranes appear to have a specific carrier, were found in the present study to interact with the ASC-system and not the L-system. The uptake of labelled glycine and proline has previously been reported to be similar on maternal and fetal interfaces (Eaton & Yudilevich, 1981). In preliminary experiments (unpublished results) glycine was found to be an effective inhibitor of labelled AIB uptake. These findings could reflect the presence of an imino-glycine transport system.

In conclusion, the guinea-pig placenta appears to possess at least two transport systems with overlapping specificity for neutral amino acids. These carriers, which resemble the ASC and L systems, may be present at both the maternal and fetal interfaces. Hence the kinetic constants measured here are the net result of uptake by several systems. The difference in influx kinetics and the sodium dependency on the two sides do, however, suggest differences in carrier distributions. This together with the preferential efflux of amino acids into the fetal circulation (Eaton & Yudilevich, 1981) could result in net maternal to fetal transfer while allowing for the regulation of fetal plasma levels by the placental tissue (Hayashi, Sanada, Sagawa, Yamada & Kido, 1978).

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