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Lysine and alanine transport in the perfused guinea-pig placenta

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The characteristics of L-lysine transport were investigated at brush-border (maternal) and basal (fetal) sides of the syncytiotrophoblast in the term guinea-pig placenta artificially perfused either through the umbilical vessels in situ or through both circulations simultaneously. Cellular uptake, efflux and transplacental transfer were determined using a single-circulation paired-tracer dilution technique. Unidirectional L-[^{3}H]lysine uptake (%) (perfusate lysine 50 μM) was high on maternal ($M = 87 \pm 1$) and fetal ($F = 73 \pm 2$) sides. L-[^{3}H]Lysine efflux back into the ipsilateral circulation was asymmetrical (F/M ratio = 2.3) and transplacental flux occurred in favour of the fetal circulation. Unidirectional lysine influx kinetics (0.05–8.00 mM) gave K_m values of 1.75 ± 0.70 mM and 0.90 ± 0.25 mM at maternal and fetal sides, respectively; corresponding V_{max} values were 1.95 ± 0.38 and 0.87 ± 0.10 μmol · min^{-1} · g^{-1}. At both sides, lysine influx (50 μM) could be inhibited (about 60–80%) by 4 mM L-lysine and L-ornithine and less effectively (about 10–40%) by L-citrulline, L-arginine, D-lysine and L-histidine. At the basal side: (i) lysine influx kinetics were greatly modified in the presence of 10 mM L-alanine ($K_m = 6.25 \pm 3.27$ mM; $V_{max} = 2.62 \pm 0.94$ μmol · min^{-1} · g^{-1}), but unchanged by equimolar L-phenylalanine or L-tryptophan; (ii) in the converse experiments, lysine (10 mM) did not affect the kinetic characteristics for either L-alanine or L-phenylalanine; (iii) L-lysine and L-alanine influx kinetics were not dependent on the sodium gradient; (iv) the inhibition of L-[^{3}H]lysine uptake by 4 mM L-homoserine was partially (60%) Na^+-dependent. At the maternal side the kinetic characteristics for alanine influx were highly Na^+-dependent, while lysine influx was partially Na^+-dependent only at low concentrations (0.05–0.5 mM). Bilateral perfusion with 2,4-dinitrophenol (1 mM) reduced L-[^{3}H]lysine uptake into the trophoblast and abolished transplacental transfer. It is suggested that lysine transport in the guinea-pig placenta is mediated by a specific transport system (y^+) for cationic amino-acids. The asymmetry in the degree of sodium-dependency at both trophoblast membranes may in part explain the maternal-to-foetal polarity of placental amino-acid transfer in vivo.

Introduction

Fetal acquisition of amino acids, required both for tissue protein formation and as a major metabolic fuel source (see Ref. 1), is achieved through an asymmetric bidirectional transfer with net movement occurring in favour of the fetal circulation [2,3]. The haemomonochorial placenta of human and guinea-pig has a single syncytial trophoblast separating maternal blood from the fetal placental microcirculation, such that transplacental nutrient movement involves transfer across two trophoblastic membranes. Much of the research both in vitro and in vivo has been directed towards understanding uptake of extracellular amino acids across the maternal-facing (microvillous) membrane with little consideration of the functional characteristics of the basal (fetal) side of the trophoblast (reviewed in Refs. 3 and 4).

Three transport systems for neutral amino acids have been demonstrated in human placental villous tissue fragments [5], which correspond approximately to Christensen's A (Na^+-dependent), ASC (Na^+-dependent) and L (Na^+-independent) systems (see Ref. 6). Competitive inhibition analysis has also shown that ASC- and L-type transporters (but not the A system) are expressed at maternal and fetal interfaces of the perfused guinea-pig placenta [7] and there have been several determinations of the kinetic characteristics for neutral substrates, both in guinea-pig [3,7] and human placental preparations [8–10]. Furthermore, recent evidence from studies in human placental microvillous membrane vesicles indicates that amino-acid transport

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at this membrane may be mediated by at least five systems, three of which are Na^+-dependent and two Na^+-independent [10]. However, surprisingly little is known about the cellular mechanisms involved in the placental transport of cationic amino acids, and, to date, no kinetic characterisation has been attempted for this group of amino acids either in membrane vesicles or in the perfused organ (see Refs. 3 and 4). In several eucaryotic cells, transport of these substrates occurs largely through a specific transport system denominated y^+ (Refs. 11 and 12 and reviewed in Ref. 13).

The paired-tracer dilution technique applied to the intact perfused placenta has allowed the separate characterisation of transport phenomena at maternal- and fetal-facing surfaces of the trophoblast (see Ref. 3). In the present study, we have utilised this method to examine (i) the kinetics, specificity and Na^+-dependency of L-lysine transport and (ii) the Na^+-dependency of L-alanine transport in the artificially perfused guinea-pig placenta. Our results are consistent with the presence of a previously undescribed y^+-like cationic amino-acid transport system in placenta. Parts of this work have been published in abstract form [14–16].

Methods

Perfused placental preparations

Experiments were performed in either an isolated, dually perfused preparation or in a singly perfused (fetal side only) placenta in situ. The surgical procedures adopted for both these preparations have been reported [17,18]. Briefly, white Dunkin-Hartley guinea-pig dams of approx. 60 days gestation were initially tranquilized with an intraperitoneal injection of 10 mg diazepam (Valium; Roche) and subsequently anaesthetized with 20–25 $mg \cdot kg^{-1}$ sodium pentobarbitone (Nembutal; Abbott Laboratories) introduced via a permanent forelimb vein cannula. Placental isolation was achieved using a pair of annular perspex clamps, uterine and umbilical vessels were cannulated and both maternal and fetal circulations were artificially perfused using an open-circuit system. For the singly perfused placenta, a fetus was exteriorised via a small uterine incision, the umbilical vessels were cannulated and the maternal circulation remained intact. In both placental preparations, the tissue was perfused at constant flow (3 $ml \cdot min^{-1}$) using peristaltic pumps (Pharmacia, P3) and effluents from cannulae inserted into the uterine and/or umbilical vein were collected for tracer or biochemical analyses. Mean perfusion pressures were stable throughout the experimental period and always less than 50 mmHg on the two sides of the dually perfused placenta.

Placental perfusates

A modified Krebs-Ringer solution of the following composition (mM) was employed: 120 NaCl/5.3 KCl/1.2 $MgSO_4$ 0.5 KH_2PO_4/2.5 $CaCl_2$/25 $NaHCO_3$/5.5 D-glucose. Dextran (40000) and bovine serum albumin (Cohn Fraction V) were added to give final concentrations of 40 and 1 $g \cdot l^{-1}$, respectively. Control perfusates always contained the appropriate unlabelled L-amino acid (either lysine, alanine or phenylalanine) at a concentration of 50 μM.

In experiments designed to test the sodium-dependency of amino-acid transport, the NaCl and $NaHCO_3$ in the medium were isoosmotically replaced with Tris-HCl buffer; these perfusates, and the relevant controls, also contained 0.1 mM adenosine to counteract the vasoconstriction induced under sodium-free conditions [7,18]. All solutions were prepared on the day of the experiment and gassed with 95% O_2/5% CO_2 to a pH value of 7.35–7.40. Perfusates could be interchanged rapidly without flow interruption by means of a two-way tap situated prior to the injection site.

Paired-tracer experiments

Rapid unidirectional amino-acid uptake at maternal and/or fetal interfaces was measured by directly comparing uterine or umbilical venous tracer concentration profiles [3,10–20] following a maternal or fetal arterial injection of a perfusate solution (100 μl in about 2 s) containing 7 μCi of an L-[^{3}H]amino acid and 2.1 μCi of L-[^{14}C]glucose (extracellular marker). The injectate tracer mixture was made up to the required volume with the same solution perfusing the placenta. The venous effluent from the injection side (ipsilateral) was sequentially sampled: 15 or 30 samples (200 or 100 μl, respectively) over a 70–80 s period followed by an additional 4 min (12 ml) sample to maximise tracer recoveries and to assess tracer efflux from the trophoblast. Up to 12 such injections could be made in an singly perfused placenta and a maximum of 14 in the dually perfused placenta. Transplacental transfer (dually perfused placenta only) was assessed simultaneously by collecting a single 6 min (18 ml) cumulative sample from the contralateral side venous outflow. Background activity was corrected for by collection of venous samples prior to bolus injection. (a) The time-course of L-[^{3}H]amino-acid uptake was determined by quantifying the uptake (U) in each effluent sample using the expression:

$$U = 1 - (\text{L-[}^3\text{H]amino acid})/(\text{L-[}^{14}\text{C]glucose})$$

A maximal uptake value, U_{max}, could be calculated by averaging the plateau values (Fig. 1). (b) Unidirectional influx, v, was estimated from the fractional maximal uptake U_{max}, the perfusion rate F ($ml \cdot min^{-1} \cdot g^{-1}$ wet weight) and the concentration (mM) of the unlabelled L-amino acid in the arterial perfusate (C_a):

$$v = -F \cdot \ln(1 - U_{max}) \cdot C_a$$

(c) L-[^{3}H]amino-acid efflux (backflux, b%) from the

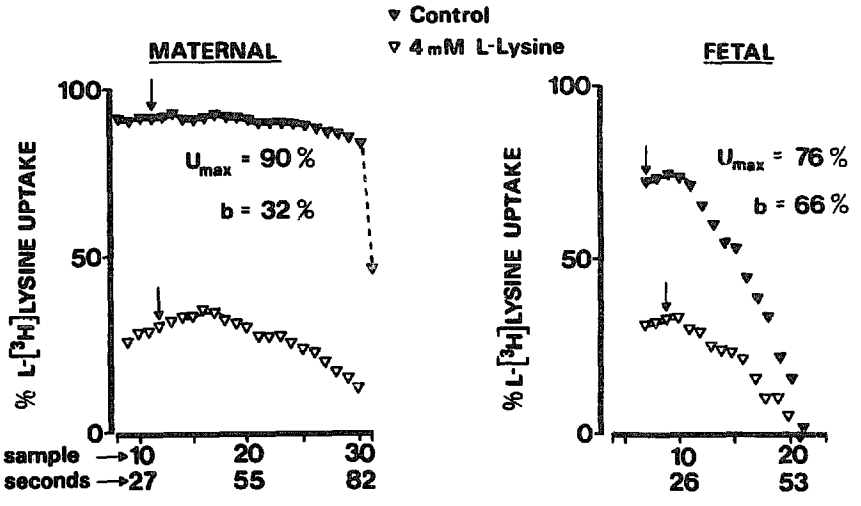

Fig. 1. Unidirectional uptake of L-[^{3}H]lysine and its inhibition by unlabelled L-lysine in the isolated dually perfused guinea-pig placenta. L-[^{3}H]Lysine uptake, U (see Methods), was determined successively at maternal and fetal sides under control conditions (▽; 50 μM perfusate L-lysine) and following a 4–5 min pre-perfusion period with solutions containing 4 mM unlabelled L-lysine (▽). Uptake is plotted against sample number and time in s after tracer injection. The maximal unidirectional uptake (U_{max}) was estimated from the joined points. The arrow represents the peak activity of the venous dilution curves (not shown). U_{max} and backflux, b, values in the presence of 4 mM L-lysine were: maternal side, 32 and 54%; fetal side, 36 and 85%. Data are taken from a representative experiment.

trophoblast back into the injection side circulation was estimated from the expression

$$b = (1 - (U_T/U_{max})) \times 100$$

where the overall amino-acid uptake, U_T, was calculated as in (a) but the tracer recoveries were integrated from the initial maximal value sample and including the final 4 min sample. (d) Transplacental transfer of both tracers (% dose) was measured from the single 6 min sample collected from the contralateral outflow. The highly variable transplacental 'leakage' [3,17] was indicated by the recovery of L-[^{14}C]glucose. Transplacental transfer was corrected for this non-specific pathway so that the L-[^{3}H]amino acid recovered in the contralateral circulation in excess of L-[^{14}C]glucose served as an index of specific amino-acid transfer. (e) Placental retention (R) of labelled amino acid was estimated from the total recoveries of the two tracers in both circulations over the entire 5–6 min sampling period

$$R = (\text{total L-[}^{14}\text{C]glucose}) - (\text{total L-[}^{3}\text{H]amino acid})$$

Sample processing

The venous outflow samples, diluted aliquots of the injectate, pure ^{3}H and ^{14}C isotope standards and background samples (perfusate only) were processed for liquid scintillation counting by the addition of 2 ml Readysolve H.P. scintillant (Beckman). Samples were counted concurrently in a Beckman 7500 beta counter and individual quench corrections were unnecessary. Data were analysed using a Basic program written for a Commodore CBM microcomputer.

Experimental design

Placentae were perfused successively with different concentrations of the appropriate amino acid and L-[^{3}H]amino-acid uptake was measured following a 4–5 min pre-equilibration period. Michaelis-Menten constants could then be estimated by fitting a single rectangular hyperbola, weighted for the reciprocal of the standard error [21] to the pooled influx data from several similar experiments [7], (see Ref. 3). The range of perfusate concentrations varied according to the amino acid under study (see Results).

Similar kinetic experiments employing sodium-free perfusates were performed in the singly perfused placenta (fetal side) and in the dually perfused placenta (maternal side). In the singly perfused placenta, an initial control injection was made when the perfusate contained 145 mM sodium. Thereafter, arterial perfusate was continuously sodium-free. Flow rates and perfusion pressures remained stable under these conditions. In the dually perfused placenta, preliminary experiments showed a tendency for the maternal-side pressure to increase in the absence of sodium; this was subsequently avoided by performing alternate control (145 mM sodium) and sodium-free injections which eliminated pressure changes in the maternal circuit. In all preparations, venous effluent concentrations of Na^+ and K^+ were measured periodically throughout the perfusion using a Type 343 Flame Photometer (Instrumentation Laboratory, U.K.). In singly perfused placentas the umbilical venous concentrations of Na^+ and K^+ were 9.4 ± 0.6 mM and 5.1 ± 0.2 mM, respectively (mean ± S.E. of 71 determinations in nine placentae). Concentrations (mM) in effluents from

Tris-perfused dually perfused placentas were: maternal, $Na^+ = 8.2 \pm 1.2$; $K^+ = 4.9 \pm 0.5$. Fetal, $Na^+ = 4.2 \pm 1.4$; $K^+ = 4.8 \pm 0.6$ (mean ± S.E. of 36 determinations in eight placentae).

Except where indicated, successive tracer injections were performed following a 4–5 min pre-equilibration period with a given perfusate.

Chromatographic analysis of venous outflow radioactivity

A single bolus injection of L-[^{3}H]lysine (40 μCi in 100 μl) was made into either the maternal circulation of the dually perfused placenta ($n = 2$) or the fetal circulation of the singly perfused placenta ($n = 4$). In the dually perfused placenta transplacental activity could also be analysed. Maternal and/or fetal outflows were immediately collected in four pooled samples over a 5–6 min period: 0–30, 30–60, 60–90 and 90–330 s. Aliquots of each sample (1.0 or 0.5 ml) were precipitated with 4.0 ml of absolute alcohol, centrifuged (9000 × *g* for 10 min) and the precipitate discarded. The supernatant was evaporated to dryness under a stream of nitrogen and the resultant material resuspended in 50 μl 50% ethanol. Samples (40 μl) were then applied to 0.1 mm cellulose-coated plates (Merck 5577, B.D.H. Chemicals Ltd., Poole, U.K.) and eluted using a mixture of *n*-butanol/acetic acid/water (12 : 3 : 5). The distribution of [^{3}H] activity on the plate was detected using a chromatographic scanner (Berthold LB 2760) and correlated with the R_f value of an L-[^{3}H]lysine standard. R_f values (mean ± S.D., n = number of samples) were: L-[^{3}H]lysine standard = 0.26 ± 0.05 ($n = 8$); injection side samples = 0.28 ± 0.09 ($n = 24$); contralateral side samples = 0.26 ± 0.06 ($n = 8$).

Materials

The radioactive molecules L-[1-^{14}C]glucose (47.0 mCi/mmol), L-[4,5(n)-^{3}H]lysine (90.8 Ci/mmol), L-[3-^{3}H]alanine (72.4 Ci/mmol) and L-[4-^{3}H]phenylalanine (26.0 Ci/mmol) were purchased from New England Nuclear, Dreieich, F.R.G. All other chemicals were obtained from Sigma Chemical Co., Poole, U.K.

Statistics

All data are expressed as mean ± S.E. Differences were assessed by Student's paired or unpaired *t*-test as appropriate and were considered significant when $P < 0.05$.

Results

L-[^{3}H]lysine uptake, backflux and transplacental flux

Fig. 1 illustrates representative L-[^{3}H]lysine uptake vs. time profiles obtained during a single transit through the maternal or fetal circulation of an isolated placenta dually perfused with a control medium (50 μM L-lysine). At both blood-tissue interfaces a maximal uptake plateau (U_{max}) was evident and was followed by rapid tracer backflux, reflected in a decreasing apparent uptake. Although the U_{max} appeared similar at the two sides, tracer backflux exhibited a marked asymmetry, being greater into the fetal (55%) than in to the maternal circulation (22%). The combined control data from a total of 19 isolated preparations (Table I) indicated that unidirectional L-[^{3}H]lysine uptake and influx (see Methods) were significantly greater at the maternal surface, while L-[^{3}H]lysine efflux back into the injection side circulation was generally 2-fold higher at the fetal

TABLE I

Paired-tracer data for L-[^{3}H]lysine in the dually perfused and in situ perfused guinea-pig placenta

L-Lysine transport was assessed following arterial bolus injection of L-[^{3}H]lysine and L-[^{14}C]glucose into either the maternal or fetal circuit of dually perfused placenta, or the fetal inflow of singly perfused placenta. Measurements were made during perfusion with solutions containing 50 μM unlabelled L-lysine. The value given for the backflux ratio is the mean of the F/M ratio from 16 individual paired-tracer injections. Transplacental transfer could not be measured directly in the in situ preparation. Data are from a total of 19 dually perfused and 14 in situ perfused placentae and values are mean ± S.E. (number of injections in parentheses). M, maternal; F, fetal; n.s., not significant ($P > 0.05$).

	Dually perfused placenta			In situ perfused placenta	
	maternal	fetal	P *	fetal	P **
U_{max} (%)	87 ±1 (28)	73 ±2 (28)	< 0.001	73 ±2 (34)	n.s.
Unidirectional influx (μmol·min^{-1}·g^{-1})	0.078 ± 0.006	0.048 ± 0.004	< 0.001	0.050 ± 0.004 (34)	n.s.
Backflux (%)	26 ±4 (28)	53 ±3 (28)	0.001	47 ±2 (34)	n.s.
Backflux ratio (*F/M*)	2.3 ±0.4				
Contralateral side excess L-[^{3}H]lysine (% dose)	31 ±4 (28)	5 ±2 (28)	0.001		
Tissue retention (% dose)	16 ±2 (28)	24 ±2 (28)	< 0.05		

* Paired *t*-test, maternal versus fetal in dually perfused placenta.

** Unpaired *t*-test, singly perfused placenta versus fetal side of dually perfused placenta.

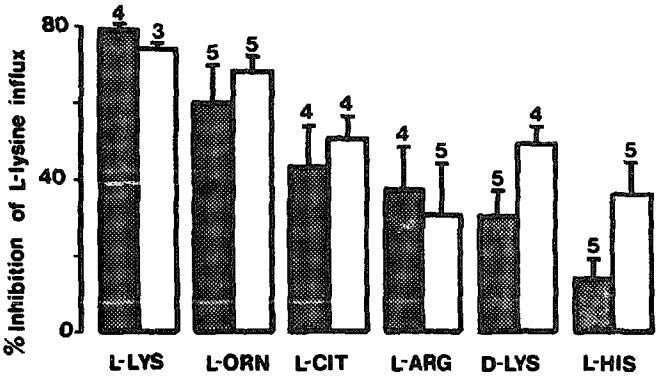

Fig. 2. Inhibitory effect of various unlabelled amino acids on unidirectional L-lysine influx at maternal and fetal sides. Paired-tracer injections were made into either the maternal (stippled columns) or fetal (open columns) arterial inflow of the dually perfused placenta. Placentae were pre-perfused (both sides) for 4–5 min with a given amino acid before measurement of L-[^{3}H]lysine uptake. Inhibition (%) of influx was calculated from: $(J_c - J_i/J_c) \times 100$, where J_i is the influx measured in the presence of amino acid (4 mM) and J_c the control influx (50 μM L-lysine) determined in the same placenta. Values are given as mean ± S.E., n = number of placentae. Data are from a total of eight preparations.

side. Specific transplacental L-[^{3}H]lysine transfer (in excess of the extracellular tracer) occurred preferentially in the maternal-to-fetal direction. Also shown in Table I are the corresponding data obtained at the fetal side of the in situ placenta under similar perfusion conditions; these parameters were not different from those measured at the fetal side of the isolated preparation.

Inhibition of L-lysine influx by cationic substrates

L-Lysine influx was determined under control conditions (50 μM L-lysine) and when the perfusate contained an additional amino acid at 4 mM (Fig. 2). L-Lysine itself (see also Fig. 1), L-ornithine and L-arginine inhibited lysine influx by approx. 80, 60 and 40%, respectively. L-Citrulline induced a similar reduction in influx to L-arginine, whereas L-histidine was less effective. The lysine transport system would appear not to be entirely stereospecific, since the D-enantiomer of lysine reduced influx by between 30 and 50% (Fig. 2). The inhibitory effect for all molecules tested was generally similar at maternal and fetal poles of the trophoblast.

Kinetics of L-lysine influx and the effect of sodium-free perfusates

L-[^{3}H]Lysine uptake, determined over a range of perfusate concentrations (50 μM to 8 mM), was reduced in a concentration-dependent manner (see insets to Fig. 3). These data allowed measurement of the unidirectional influx (see Methods) across either placental interface (Fig. 3). It can be seen that the estimated K_m values under control conditions were not significantly different at maternal and fetal sides, while the maximal transport capacity, V_{max}, was greater at the maternal surface. Removal of extracellular sodium had no effect on L-[^{3}H]lysine uptake at the fetal side and the kinetic parameters were similar to the control estimates (Fig. 3B). On the maternal surface, although the overall kinetic constants appeared similar to controls (Fig. 3A), an inhibition of uptake occurred in the absence of extracellular Na^+, which was evident primarily over the lower end of the concentration range (50 μM to 0.5 mM). At 50 μM, L-lysine influx was inhibited by approx. 64% (control = 0.072 ± 0.004; Na^+-free = 0.026 ± 0.002 μmol · min^{-1} · g^{-1}).

Effect of sodium-free perfusates on L-alanine influx kinetics

L-[^{3}H]Alanine uptake was determined in the absence of sodium over concentration ranges of 50 μM to 30 mM (maternal side) and 50 μM to 24 mM (fetal side, in situ preparation) (Fig. 4); the results were compared directly with control data previously obtained in this laboratory [7]. At the fetal side, the K_m value was similar in the presence and absence of sodium, while the V_{max} was elevated under sodium-free conditions. In contrast, removing sodium from the maternal perfusate reduced alanine influx throughout the entire concentration range (Fig. 4A); the estimated V_{max} was lowered by over 50% and a marked reduction in the apparent affinity was observed. Influx values at 50 μM L-alanine in the presence and absence of sodium were 0.042 ± 0.003 and 0.008 ± 0.002 μmol · min^{-1} · g^{-1}, respectively.

TABLE II

Kinetic constants for L-lysine, L-alanine and L-phenylalanine at the fetal side of the in situ perfused placenta

Unidirectional influx kinetics for L-lysine, L-alanine and L-phenylalanine were measured under control conditions and during continuous perfusion with an additional unlabelled amino acid (10 mM). The control kinetic parameters given for lysine and alanine are those derived from the data presented in Fig. 3B and Fig. 4B, respectively. Values are given as mean ± S.E., n = number of in situ perfused placentae. Results are from a total of 33 preparations.

	n	Apparent K_m (mM)	V_{max} (μmol · min^{-1} · g^{-1})
L-Lysine			
Control	5	0.90 ± 0.25	0.87 ± 0.10
Plus 10 mM L-alanine	4	6.25 ± 3.27	2.62 ± 0.94
Plus 10 mM L-phenylalanine	5	1.51 ± 0.16	0.82 ± 0.04
Plus 10 mM L-tryptophan	4	1.12 ± 0.33	0.81 ± 0.10
L-Alanine			
Control *	4	8.42 ± 1.41	4.40 ± 0.45
Plus 10 mM L-lysine	4	5.41 ± 1.63	4.04 ± 0.52
L-Phenylalanine			
Control *	3	11.98 ± 1.92	9.50 ± 0.94
Plus 10 mM L-lysine	4	14.81 ± 2.97	12.50 ± 1.56

* Data taken from Eaton et al. [7].

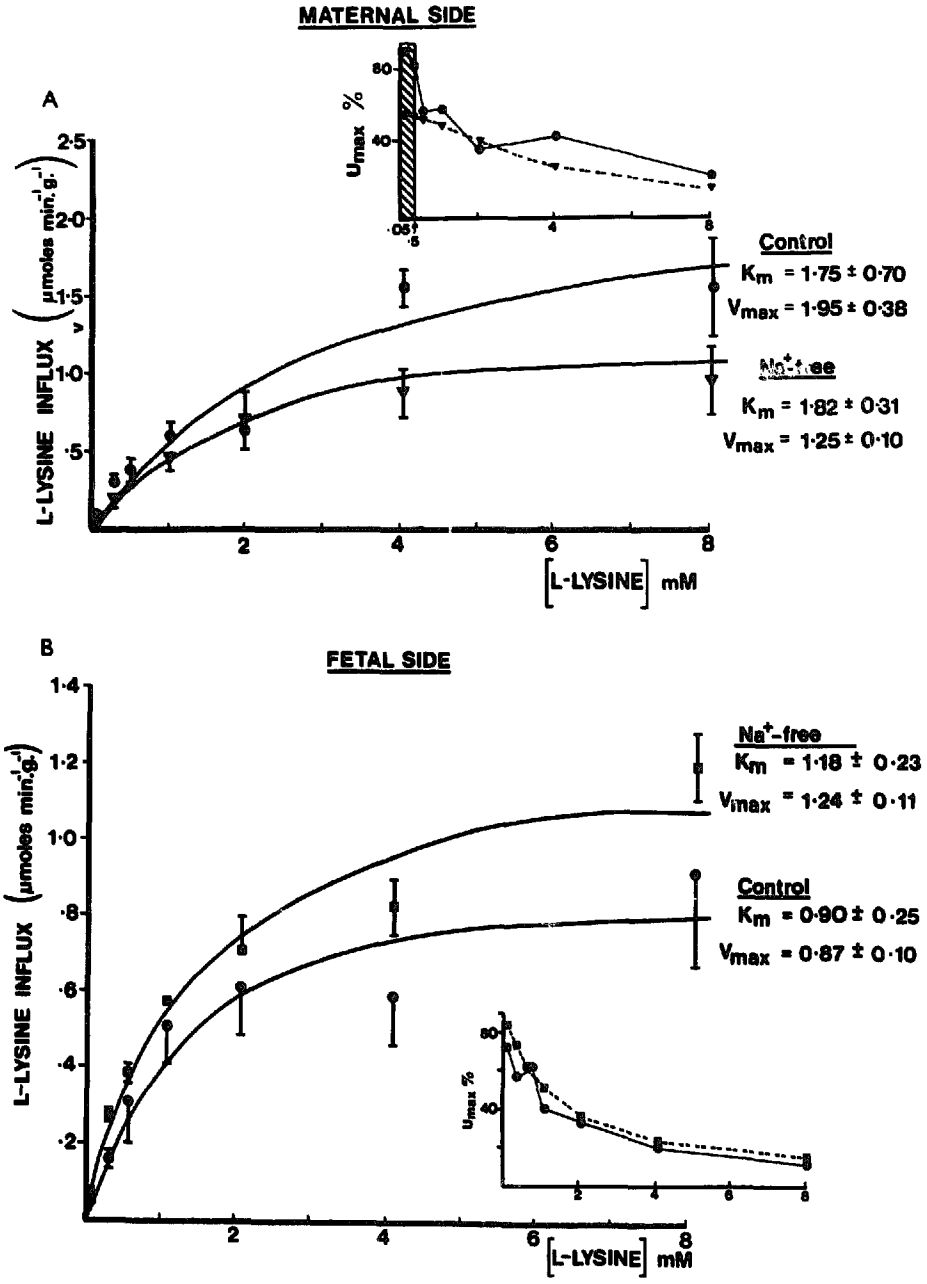

Fig. 3. L-Lysine influx kinetics at maternal and fetal surfaces of the trophoblast. Unidirectional L-lysine influx was measured successively at seven different perfusate concentrations of unlabelled L-lysine (0.05–8 mM). Experiments were conducted at the maternal side of the dually perfused placenta (A) and at the fetal side of the in situ perfused placenta (B) as described in Methods. Saturation kinetics were determined in the presence of sodium (143 mM; ●) and when the sodium in the perfusion fluid was replaced with Tris (▼, ■). The curves are rectangular hyperbolas derived from a direct fit of the mean influx value at each lysine concentration and weighted for the reciprocal of their respective standard error. Individual points are the mean ± S.E., $n = 3$–5 placentae in each case. The inset to each panel illustrates the concentration-dependence of the maximal L-[^{3}H]lysine uptake (mean values given) under both experimental conditions. The hatched area in the inset to panel A denotes the perfusate L-lysine concentration over which transport appeared to be Na^+-dependent. K_m is given in mM and V_{max} in μmol $min^{-1} \cdot g^{-1}$.

Sodium-dependent inhibition of L-lysine uptake by L-homoserine

When in situ placentae were perfused with solutions containing the neutral amino acid L-homoserine (4 mM), L-[^{3}H]lysine uptake (50 μM) was reduced by 48 ± 5% ($n = 4$ placentae). When the sodium in the perfusion fluid was replaced with Tris, the inhibition induced by L-homoserine was significantly reduced (22 ± 4%; $P <$ 0.01, paired t-test).

Effect of L-alanine, L-phenylalanine and L-tryptophan on L-lysine influx kinetics

The kinetic characteristics of L-lysine influx were examined at the fetal side (in situ placentae) in the

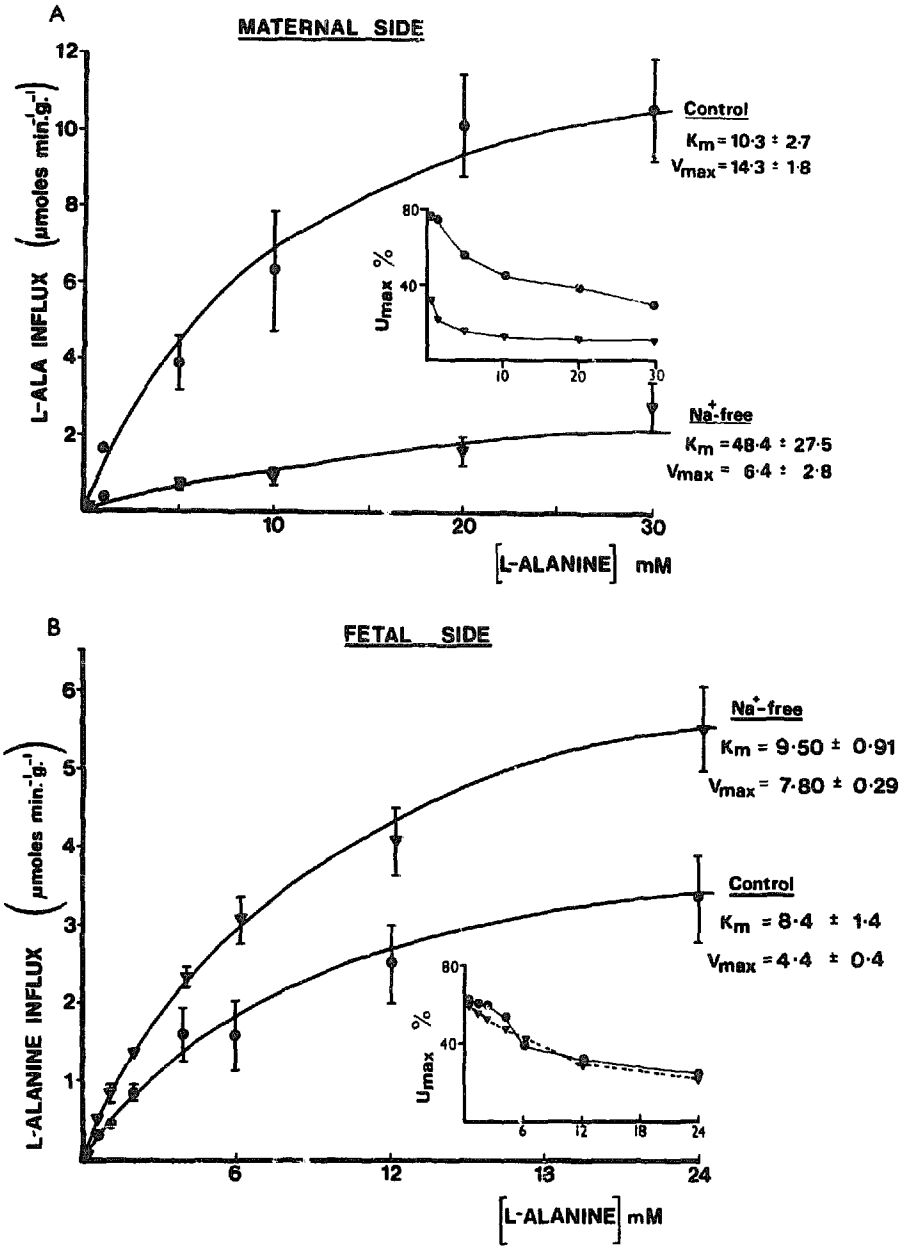

Fig. 4. Effect of extracellular sodium depletion on the kinetics of L-alanine influx. Influx was measured at maternal- (A; dually perfused) and fetal- (B; singly perfused) facing membranes of the trophoblast at various unlabelled L-alanine concentrations. Individual points represent the mean ± S.E., $n = 3$–5 placentae. Insets show the mean maximal L-[^{3}H]alanine uptake in the presence or absence of perfusate sodium (error bars not shown). Control data in each panel are taken from Ref. 7. See text and legend to Fig. 3 for further details. K_m is given in mM and V_{max} in μmol $min^{-1} \cdot g^{-1}$.

continuous presence of unlabelled L-alanine (10 mM). L-[^{3}H]Lysine uptake was inhibited (from 73 ± 2 to 35 ± 4% at 50 μM, $n = 4$ placentae) and marked increases in both the K_m and V_{max} for influx were observed (Table II). Conversely, in the presence of the large, branched-chain neutral amino acids, L-phenylalanine and L-tryptophan (10 mM), the kinetic constants for L-lysine were only marginally affected (Table II).

Effect of L-lysine on influx kinetics for L-alanine and L-phenylalanine

In further experiments, L-[^{3}H]phenylalanine and L-[^{3}H]alanine uptake was assessed in the presence of 10 mM L-lysine over unlabelled perfusate concentrations ranging from 50 μM to 24 mM. The estimated kinetic parameters under these experimental conditions were similar to those previously determined in the absence of

L-lysine [7]. For comparison, the latter values [7] together with those obtained in the present study are given in Table II.

The effect of 2,4-dinitrophenol on L-lysine transport at maternal and fetal sides

The effect of dinitrophenol on L-[^{3}H]lysine transport was evaluated by including the inhibitor (1 mM) in the perfusion medium and determining lysine uptake successively at maternal and fetal interfaces ($n = 5$ dually perfused placentas) throughout a continuous 60 min perfusion period. At the maternal side, uptake prior to addition of dinitrophenol was 86 ± 1% and was reduced to 49 ± 16% ($P < 0.05$) after 10 min dinitrophenol perfusion. A similar degree of inhibition was seen at the fetal side, but only after 40 min exposure to dinitrophenol (72 ± 1% reduced to 48 ± 10%). A concomitant 2-fold stimulation in L-[^{3}H]lysine efflux back into the maternal circulation was evident (control 26 ± 4%, plus dinitrophenol 55 ± 15%), whereas a much smaller change in efflux was observed at the fetal side (control 58 ± 3%, plus dinitrophenol 70 ± 3%). Transplacental L-[^{3}H]lysine transfer towards the fetus (37 ± 6% dose prior to dinitrophenol exposure) showed a time-dependent reduction and was practically abolished (4 ± 4%) after 20 min perfusion with dinitrophenol.

Discussion

Our findings have demonstrated the presence of saturable transport systems for lysine at both surfaces of the trophoblast with efflux occurring in favour of the fetal circulation (Table I). Furthermore, the higher maximal flux at the maternal side, compared to the fetal surface, suggests that a greater number of lysine transport sites are available at the microvillous border. These results are consistent with the asymmetric transport reported for a number of other naturally occurring amino acids [2].

Several features of L-[^{3}H]lysine uptake by the placenta support the presence of a distinct cationic-preferring agency resembling system y^+, originally described in Ehrlich ascites cells [24] and subsequently characterised in human fibroblasts [12], hepatoma cells [11], murine P388 leukaemia cells [25], the basolateral membranes of renal epithelial cell lines [26] and in the in situ perfused salivary gland [27].

Transmembrane transport of cationic amino acids via system y^+ typically occurs with high affinity and the K_m values in isolated cell preparations range between 0.03 in cultured human fibroblasts [12] and 0.94 mM in rat hepatocytes [28] (see Ref. 13 for review). In the present study, the estimated K_m for undirectional lysine influx was several-fold lower than values previously reported for L-alanine and L-phenylalanine [7], a difference which might suggest that placental lysine transport is mediated by a system distinct from those serving neutral amino acids.

Investigation of the selectivity of the lysine transport system at both placental interfaces showed that uptake was inhibited by other basic amino acids (L-ornithine, L-arginine and D-lysine), by L-citrulline, another intermediate of the urea cycle, and by L-histidine. As in the classical y^+ system, preference for the L-configuration was evident, although there was no absolute stereospecificity. The less potent inhibition induced by L-histidine may be related to the small cationic fraction (about 0.1) existing at physiological pH values [27]. Unidirectional uptake values previously determined for L-[^{3}H]arginine and L-[^{3}H]histidine [2] are considerably lower than those reported here for L-[^{3}H]lysine and correlate well with the extent of inhibition observed with the unlabelled amino acids (Fig. 2). In general, system y^+ has a high affinity for ornithine [11], while citrulline appeared to be a very weak inhibitor of lysine uptake in the reticulocyte [29]. In renal [30–33] and intestinal [34] microvillous membranes, lysine, arginine and ornithine also share a common transport system.

Our characterisation of neutral–cationic interactions in placenta showed that alanine markedly reduced lysine influx, suggesting that lysine transport is mediated by a y^+ system which may also transport alanine but has minimal interaction with the typical L-system amino acids phenylalanine and tryptophan. The reciprocal experiments demonstrated that unlabelled lysine was ineffective as an inhibitor of both L-[^{3}H]alanine and L-[^{3}H]phenylalanine uptake, providing further evidence for lysine influx through a separate system. Cross-reactivity between cationic acid and neutral amino-acid transport has also been reported in intestine [35,36]. In human placenta, lysine did not affect the uptake of either aminoisobutyric acid or glycine (see Ref. 3), nor did it reduce valine uptake into rat placental villous fragments [37]. However, in a recent detailed report on human placental microvillous membrane vesicles [10], lysine (30 mM) inhibited neutral amino-acid uptake only by about 5% in the presence of a sodium-gradient, whereas the Na^+-independent transport of proline, methionine, leucine, glutamate, glycine and cysteine was inhibited by 95%. In addition, all these substrates (30 mM) caused a greater than 80% inhibition of Na^+-independent L-[^{14}C]lysine uptake. Hence, in contrast to the present results, lysine uptake across the human placental brush border appears to be mediated by a transport system with very broad specificity and not by system y^+ [10].

Studies in other tissues have demonstrated Na^+-dependent inhibition of lysine uptake by hydroxyamino acids, and by glutamine which has some structural resemblance to citrulline [11,26]. The partial Na^+-dependent inhibition of lysine uptake by L-homoserine in the present study may also be explained by assuming

that the sodium ion occupies a position at the transport site that can otherwise be taken by the distal cationic group of a basic amino acid [11,26,38]. Whether the inhibition of lysine uptake by alanine (Table II) and citrulline (Fig. 2) is Na^+-dependent cannot be determined from the available data, since experiments were performed in the presence of sodium.

At the brush border, unidirectional L-alanine influx was markedly reduced in the absence of a sodium gradient, suggesting Na^+-coupled transport at the maternal-facing membrane (Fig. 4). Since system A (Na^+-dependent) does not appear to be expressed in the guinea-pig placenta [7], this uptake is likely to be confined to an ASC-type system, the residual influx reflecting transport largely though Na^+-independent system L [7]. In comparable studies in the dually perfused guinea pig placenta, L-[^{3}H]alanine uptake at the maternal side was reduced from 78 to 24% after low-sodium perfusion [39].

In its usual form, system y^+ is not Na^+-coupled [24] (see 13). In our experiments the affinity constant determined for L-lysine was similar in the absence or presence of sodium with some reduction in the maximal transport rate. However, at low lysine concentrations (0.05–0.5 mM), 60% of the influx appeared to be via a Na^+-dependent pathway (Fig. 3A). From these findings it would seem that the microvillous trophoblast membrane resembles the brush-border surfaces of renal [31] and intestinal [40] epithelia, in which there is clear evidence for coexistence of Na^+-dependent and Na^+-independent lysine uptake.

The influx of alanine and lysine at the fetal side appeared to be mediated exclusively by Na^+-independent pathways, supporting the presence of system y^+ in the basal membrane. With respect to alanine, previous studies have shown uptake of this amino acid at the fetal membrane to be mediated by both ASC- and L-type transport systems [7]. It remains to be determined whether the Na^+-independent nature of its transport reflects the presence of the Na^+-independent variant (asc) of system ASC [41,42] These findings in the in situ perfused placenta are in conflict with those obtained in the dually perfused preparation. In an earlier study from this laboratory, Na^+-dependent influx accounted for approx. 40% of the total alanine and lysine influx at the fetal side of the dually perfused placenta [7]. These results were recently confirmed in the same preparation where the absence of the sodium gradient diminished alanine influx by 50% [39]. Nevertheless, there was still a marked asymmetry between the degree of sodium-dependency (maternal greater than fetal) [39]. As yet, there have been no studies on membrane vesicles prepared from the basal side of the trophoblast, which could clarify the nature of sodium coupling to alanine and lysine uptake at this membrane.

Uncouplers and inhibitors of the electron-transport system, including 2,4-dinitrophenol and cyanide, have been reported to effect influx and efflux of neutral amino acids in placental tissue (reviewed in Ref. 3). In our study, dinitrophenol did not completely suppress lysine influx into the trophoblast but was very effective in abolishing transplacental flux. The relatively lower sensitivity of lysine influx at the fetal membrane may reflect the Na^+-independent uptake at this surface. These results are in keeping with a recent report in human placental brush-border vesicles, in which preincubation with 1 mM ATP enhanced Na^+-dependent, but not Na^+-independent, aminoisobutyrate uptake [43].

In conclusion, the present study has provided evidence for the presence of a y^+-like amino-acid transport system mediating the uptake of cationic substrates across the basal membrane of the guinea-pig placenta. At the maternal-side, lysine transport resembles that found in other brush-border membranes and whether this occurs through a specific cationic system(s) requires further investigation. However, the functional polarity of amino-acid transfer could be explained by the marked differences in the relative distribution of sodium-dependent transporters between the two syncytiotrophoblast membranes such that the most strongly concentrated uptake occurs at the maternal-facing surface [44].

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