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AMINO ACID CARRIERS AT MATERNAL AND FETAL SURFACES OF THE PLACENTA BY SINGLE CIRCULATION PAIRED-TRACER DILUTION
KINETICS OF PHENYLALANINE TRANSPORT

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Summary

The unidirectional uptake into the trophoblast of L-leucine, L-isoleucine, L-phenylalanine, L-tryptophan, L-methionine and L-tyrosine from either the maternal or fetal circulations of an isolated dually-perfused guinea-pig placenta was studied using a single circulation paired-tracer dilution technique. Significant and equal uptakes were found on both sides. L-Phenylalanine uptake kinetics on the fetal side indicated an apparent K_m of 17.0 mM and a V of 8.2 μmol/min per g.

The passage of substances from the maternal blood into the fetal circulation implies movement across the placental barrier, which, in guinea-pig and man is a single layered syncytiotrophoblast [1]. Christensen and Streicher in 1948 made the original observation that amino acid transport in the placenta involves an active transport process [2]. Hill and Young [3] suggested that this occurs via an accumulative mechanism at the maternal side followed by transfer into the fetal circulation by diffusion down the concentration gradient.

Characterisation of the amino acid transport systems has mainly been made in vitro in placental slices and vesicles [4—8]. Generally in vivo methods are limited since they are based on overall transplacental transfer e.g. Ref. 9, see Ref. 10 for review) and do not allow separate characterisation of the phenomena at the two blood-tissue interfaces. Furthermore, some molecules may be taken up from either the maternal or fetal circulations and not be transferred to the other side but enter metabolic cycles within the tissue,

TABLE I

MAXIMAL UPTAKE FOR A GROUP OF AMINO ACIDS SHARING THE L-SYSTEM CARRIER [17]

The concentrations given are those of the Difco TC199 tissue culture perfusate. The tissue concentrations were taken from Hill and Young [3]. Percentage uptakes are given as mean ± S.E. (n = number of observations).

Amino acid	Perfusate concn. (mM)	Tissue concn. (mmol/kg)	Maximal uptake (%)	
			Maternal side	Fetal side
L-[4,5-^{3}H] Leucine	0.46	0.63	45 ± 8(3)	32 ± 7(3)
L-[4,5-^{3}H] Isoleucine	0.15	0.30	55 ± 8(3)	44 ± 8(3)
L-[4-^{3}H] Phenylalanine	0.15	0.71	58 ± 3(13)	58 ± 5(15)
L-[5(n)-^{3}H] Tryptophan	0.05	—	47 ± 7(8)	45 ± 5(10)
L-[2(n)-^{3}H] Methionine	0.10	0.12	39 ± 6(3)	35 ± 2(3)
L-[3,5-^{3}H] Tyrosine	0.22	0.33	37 ± 14(3)	48 ± 7(5)

since the metabolic rate of the placenta is comparable to that of liver, kidney, lung or certain endocrine glands [11].

Recently, a method based on the single circulatory passage of a pair of labelled molecules has been introduced by Yudilevich et al. to separately characterise sugar transport at the maternal and fetal surfaces of the trophoblast, in the guinea-pig placenta, independent of cellular metabolism or transplacental transfer [12]. This method, which employs an extracellular reference tracer, is extended here to the study of amino acid transport and the measurement of kinetic constants.

A dually-perfused isolated placenta obtained from a pentobarbitone anaesthetised guinea-pig was used [12, 13]. The umbilical and maternal side vessels were perfused at identical flow rates (about 3 ml/min) with TC 199 tissue culture medium (Difco Ltd.) to which 40 g/l dextran and 1 g/l albumin were added. This perfusate contained various amino acids some of which are shown in Table I. In another series of experiments the fetus was removed, the umbilical vessels were cannulated and an artificial fetal circulation was established using a Ringer perfusate, while the maternal circulation remained intact [14, 15].

A mixture containing 7 μC of a tritiated amino acid and 1.4 μC of a ^{14}C-labelled extracellular marker was injected close-arterially (100 μl in 1—2 seconds) and 20—30 consecutive venous samples were immediately collected from the same circulation and prepared for liquid scintillation counting. All labelled substances were obtained from the Radiochemical Centre, Amersham, U.K.

Fig. 1 illustrates typical paired-tracer dilution curves obtained in the dually-perfused isolated placenta where successive maternal and fetal injections of a mixture containing L-[^{3}H] phenylalanine and L-[^{14}C] glucose were performed. It can be seen that the initial concentrations of L-[^{3}H]-phenylalanine were less than that of the L-[^{14}C] glucose in both the runs illustrated in Fig. 1. L-Glucose 'uptake' has been used as a measure of non-specific diffusion in adipocytes [16] and it appeared to be suitable as an extracellular marker in the placenta during a single circulation [12]. Therefore the amino acid uptake into the trophoblast, U, was calculated from:

$$U = (1 - [\text{L-}[^3\text{H}]\text{phenylalanine}]/[\text{L-}[^{14}\text{C}]\text{glucose}]) \times 100.$$

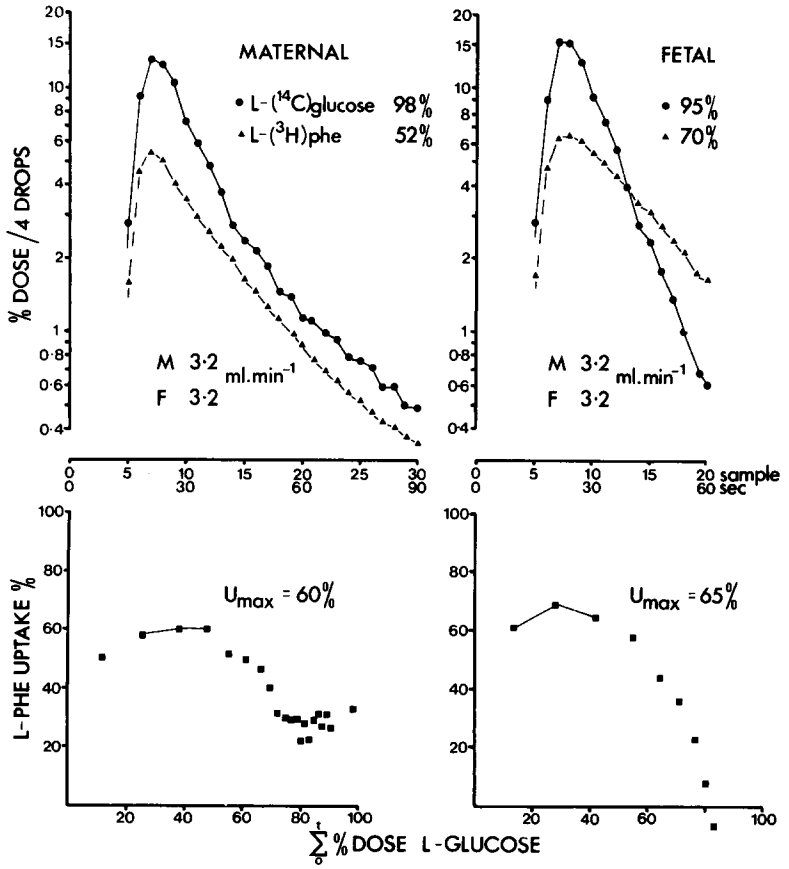

Fig. 1. Measurement of the maximal uptake (U_{max}) of a labelled amino acid from paired-tracer single circulation dilution profiles. Upper panel: The normalised concentrations (expressed as a percentage of the injected dose) of the two tracers in the venous samples are plotted against the sample number and the accumulated time after the injection. The total tracer recovery over a 5 min period is shown. The maternal (M) and fetal (F) perfusate outflows are shown and they were equal to the pump inflow rates into the two circulations of the isolated placenta. The outflow stability was used as an index of the suitability of the preparation. Lower panel: The L-[^{3}H]phenylalanine cellular uptake in each sample is plotted against the accumulated reference tracer recovery as a means of weighting the measurements. U_{max} was estimated as the mean of the joined points, and maximal uptake occurs within 30 s in both circulations.

The time course of the uptake (Fig. 1) showed an initial maximal value, U_{max}, followed by a rapid fall-off due to tracer backflux into the circulation [12]. The faster backflux on the fetal side compared with that on the maternal side was generally found for phenylalanine as well as for other amino acids (unpublished observations). The maximal uptake of L-[^{3}H] phenylalanine was similar from both the maternal and fetal circulations in all 10 isolated placentae (Table I): maternal side U_{max} was 58 ± 3(S.E.) and fetal side U_{max} was 58 ± 5 (S.E.).

We also investigated the placental uptake of five other labelled neutral amino acids selected from the group identified by Christensen as sharing a common transport system, the L-system [17]. It can be seen in Table I, that all these amino acids had approximately equal maximal uptakes from both circulations suggesting symmetry of the transport systems on the maternal

and fetal faces of the trophoblast. This symmetry is similar to that found for various labelled sugars in the same preparation [12].

The unidirectional amino acid influx, v, can be calculated from the product of U_{max}, the perfusate flow and the perfusate amino acid concentration [12, 18, 19]. The measurement of v, at constant perfusion flow, and at various perfusate amino acid concentrations, was used for the estimation of the transport kinetic constants. This type of experiment was performed with L-phenylalanine, as a typical L-system amino acid. The in situ placenta was considered to be more suitable than the isolated dually-perfused placenta for these studies. Solutions containing phenylalanine concentrations ranging between 0.15 and 25 mM, were successively perfused, in random order, through the fetal circulation and paired-tracer dilution runs were performed after three minutes perfusion with a given solution.

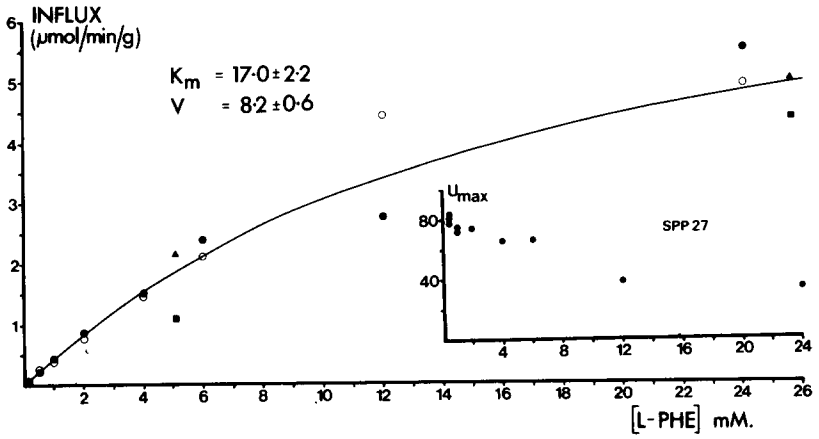

Fig. 2. L-Phenylalanine uptake kinetics on the fetal side. The results of four placentae were pooled to estimate Michaelis-Menten constants. The inset illustrates the effect of increasing phenylalanine perfusate concentrations on the uptake of L-[^{3}H]phenylalanine in one of these experiments.

Fig. 2 represents the results obtained from four placentae. The data have been fitted to a single hyperbola (Michaelis-Menten analysis) as the simplest and commonest solution, though it is realised that other, more complicated, models could fit the experimental points [20]. The use of reference and test tracers of similar size and free-diffusibilities provides an estimate of cellular uptake corrected for free diffusion.

The apparent K_m of 17 mM can be compared with that obtained in the rat heart sarcolemma, 3.7 mM [21] and in the rat brain, 0.12 mM [22]. Both these measurements were also obtained from blood-tissue tracer uptake in a single circulation. It is interesting that in their kinetic studies in the heart Baños et al. [21] were obliged to raise plasma amino acid concentrations to comparably high levels to obtain saturation: 10.2 mM for phenylalanine and up to 78 mM for the other amino acids they examined. Our finding of a very large V (8.2 μmol/min per g) suggests a population of transport sites 10 times that of the rat heart sarcolemma and 200 times that of the rat brain.

The in vitro studies of Enders et al. [7] with human placenta have shown the presence of at least three distinct carrier systems for neutral amino acids

corresponding to Christensen's A, L and ASC systems. However, kinetic constants have only been determined for a non-metabolisable amino acid representing the A system, in human placenta tissue [5, 8] and in perfused guinea-pig placenta [23]. The present results suggest that kinetic parameters could be measured in vivo for any metabolizable amino acid and therefore studies on the regulations of their transport systems appear possible.

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