Carrier-mediated L-lactate transport in brush-border membrane vesicles from rat placenta during late gestation

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The mechanism for L-lactate transport across microvillous membrane vesicles prepared from rat placenta was examined. Uptake of L-lactate into these vesicles was mainly the result of transport into the intravesicular (osmotically active) space. The initial rate of L-lactate uptake was not affected by the presence of an inward gradient of either Na⁺ or K⁺. In the presence of an inward-directed proton gradient, L-lactate uptake was markedly stimulated, accumulating at concentrations 6–7-fold higher than the equilibrium. Lower transmembrane pH gradients were associated with slower initial uptakes and smaller overshoots. L-Lactate uptake determined under an inside-directed pH gradient was strongly inhibited by *p*-chloromercuriphenylsulphonic acid, a protein-thiol oxidizing agent. L-Lactate uptake was: (1) saturable as a function of the concentration of L-lactate, (2) inhibited by monocarboxylic acids such as pyruvate, D-lactate, β -hydroxybutyrate and α -cyano-4-hydroxycinnamic acid, and (3) temperature-dependent. When present inside the vesicles, L-lactate, pyruvate and β -hydroxybutyrate caused *trans*-stimulation of L-lactate uptake both in the presence and in the absence of an inside-directed pH gradient, indicating that L-lactate transport is a reversible process that can be shared by other monocarboxylic acids. There were no significant changes in maximal initial rate or in the kinetic parameters of L-lactate transport during the last 3 days of gestation.

INTRODUCTION

The role played by L-lactate as an important metabolic fuel for the fetus and newborn is supported by a considerable body of evidence. Thus lactate is utilized by fetal tissues as a source of energy and carbon skeletons (see Medina *et al.*, 1990). Actually, lactate is actively utilized by perinatal brain in the rat (Itoh & Quastel, 1970; Shambaugh *et al.*, 1977; Arizmendi & Medina, 1983; Fernández & Medina, 1986), dog (Hellman *et al.*, 1982) and glucose-6-phosphatase-deficient human babies (Fernández *et al.*, 1984). Lactate accumulates in maternal blood during late gestation (Valcarce *et al.*, 1985), a fact that suggests that maternal lactate may be available for fetal growth. Because the blood–brain barrier is not yet present at this stage of development, the placenta probably controls the passage of lactate to the fetal brain. Considerable evidence supporting lactate transport through placenta has been reported (see Shennan & Boyd, 1987). Moreover, the occurrence of a carrier-mediated transport system for L-lactate has been characterized in brush-border membrane of human placenta (Balkovetz *et al.*, 1988). This system is driven by a proton gradient (Balkovetz *et al.*, 1988), as also reported in other tissues (see Edlund & Halestrap, 1988).

The aim of the present work was to investigate the possible occurrence of a carrier-mediated L-lactate transport system in microvillous membrane of syncytiotrophoblasts from rat placenta. We also studied the possible changes in the affinity and capacity of the lactate carrier that may occur during the last 3 days of gestation.

EXPERIMENTAL

Materials

L-[U-¹⁴C]Lactic acid was purchased from New England Nuclear (Itisa, Madrid, Spain). L-Lactic acid was purchased from

Serva (Heidelberg, Germany). D-Lactic acid, pyruvate, α -cyano-4-hydroxycinnamic acid, valinomycin, phenylmethanesulphonyl fluoride and BSA (Fraction V) were purchased from Sigma (St. Louis, MO, U.S.A.). All other reagents were of analytical grade.

Preparation of microvillous membranes

Microvillous membranes from rat placenta were prepared by a modification of the method of Smith *et al.* (1977). Albino Wistar rats, of known gestational age (19.5, 20.5 and 21.5 days) fed on stock laboratory diet (carbohydrate 58.7%, protein 17.0%, fat 3.0% and added salts and vitamins) were used for the experiments. Females (mean weight 250 g) were caged with males overnight, and conception was confirmed the following morning by the presence of spermatozoa in vaginal smears. Pregnant rats were killed by cervical dislocation, the fetal–placental units were removed and the fetuses weighed as a further measure of gestational age. Each placenta was perfused with cold low-Ca²⁺ Krebs–Henseleit buffer (pH 7.4) (García-Marín *et al.*, 1985) until the placenta appeared free of blood. All subsequent steps were then carried out at 4 °C. The maternal layer of each placenta was peeled off and the remaining labyrinthine tissue was washed once in cold Krebs–Henseleit buffer (pH 7.4). The placentas were rinsed three times with 100 mM-CaCl₂, weighed and used immediately. The tissue was minced in buffer A (500 mM-sorbitol, 1 mM-EGTA, 0.1 mM-phenylmethanesulphonyl fluoride, 20 mM-Tris/Mes, pH 7.4) and homogenized by applying 10 up–down strokes of a motor-driven Teflon/glass homogenizer (S-778; B. Braun, Melsungen, Germany). The homogenate was then made up to 10 times the tissue weight with additional buffer A. MgCl₂ was added to a final concentration of 10 mM and, after standing for 30 min, the homogenate was centrifuged at 800 g for 10 min in a Beckman L8-M centrifuge. This yielded a pellet (P1) and a supernatant (S1). P1 was washed with 5 times the weight of the placenta with buffer A and homogenized with 8 up–down

Abbreviations used: BBMVs, brush-border membrane vesicles; *p*-CMBS, *p*-chloromercuriphenylsulphonic acid.

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strokes of the motor-driven Teflon homogenizer. After addition of MgCl_2 to a final concentration of 10 mM, the suspension was left for 15 min. The washed P1 was centrifuged at 800 *g* for 10 min, resulting in another pellet, which was discarded, and a supernatant (S1'). Supernatants S1 and S1' were pooled together and centrifuged at 10400 *g* for 20 min. The resulting pellet (P2) was discarded, and the supernatant (S2) was centrifuged at 110000 *g* for 45 min in a Beckman L8-M ultracentrifuge. The final supernatant (S3) was discarded, and the cream-coloured pellet (P3) was resuspended in a small volume of buffer B (500 mM-sorbitol, 20 mM-Tris/Mes, pH 7.4) and homogenized 6 times through a 25-gauge hypodermic needle.

Brush-border membrane vesicles (BBMV) from human term placenta, kindly supplied by the Gynaecology and Obstetrics Unit of the 'Virgen de la Vega' Hospital, Salamanca, Spain, were prepared by the method of Boyd & Lund (1981) and resuspended in the same buffer used for BBMV from rat placenta. The BBMV preparation from human placenta showed an alkaline phosphatase enrichment of 26-fold compared with the washed placental tissue homogenate.

Both human and rat membrane vesicles were adjusted to about 15 mg of protein/ml and stored in liquid nitrogen until use. In order to obtain vesicles preloaded with certain ions or substrates, two distinct procedures were used, with similar results, namely either entrapment during vesicle formation or application of two cycles of freezing and thawing in the presence of the desired solution.

Enzyme assays

The microvillous-membrane vesicle preparation was tested for contamination by other cellular materials by analysing the activity of the following marker enzymes: alkaline phosphatase (EC 3.1.3.1) (Bretaudiere & Spilman, 1984), 5'-nucleotidase (EC 3.1.3.5) (Bertrand & Buret, 1982), γ -glutamyl transpeptidase (EC 2.3.2.2) (Persijn, 1976), Na^+/K^+ -ATPase (EC 3.6.1.37) (Scharschmidt *et al.*, 1979), L-(+)-tartrate-sensitive acid phosphatase (EC 3.1.3.2) (Moss, 1984), glucose-6-phosphatase (EC 3.1.3.9) (Baginski *et al.*, 1974) and succinate dehydrogenase (EC 1.3.99.1) (Pennington, 1961). Na^+/K^+ -ATPase activity was determined on the day on which the membrane fractions were prepared. All other determinations were carried out after storage at -80°C . Protein concentration was measured by a modification of the Lowry procedure (Markwell *et al.*, 1978), with BSA as standard.

Uptake assays

L-Lactate uptake by microvillous-membrane vesicles was determined by a rapid-filtration technique as described by Hopfer *et al.* (1973). For most experiments incubations at 37°C were started by mixing 10 μl of the vesicle preparation in buffer B with 90 μl of buffer B with ^{14}C -labelled lactate solution adjusted to either pH 5.4 or 7.4. All experiments were performed with the same inward-directed net osmotic gradient (500 mM/600 mM in/out). Incubations were stopped with 4 ml of ice-cold stop solution containing 20 mM-Tris/Mes buffer, 350 mM-KCl and 25 mM- MgSO_4 . The diluted samples were rapidly filtered under negative pressure through wetted cellulose/nitrate filters (0.65 μm pore; Millipore Ibérica, Madrid, Spain), which were then rinsed with 4×4 ml of stop solution. The entire procedure lasted less than 15 s. Appropriate blanks were performed to correct for non-specific marker retention on the filters. After dissolution of the filters in 4 ml of scintillation fluid (Ready Safe, Beckman), radioactivity was measured in a scintillation spectrophotometer. Uptake buffers were prepared at different pH values by adding Tris or Mes but keeping the sum $[\text{Tris}] + [\text{Mes}]$ equal to 20 mM. The exact composition of the uptake buffers and preloading

buffers is specified in the legends to Figures and Tables. Initial uptake rates (1.7 s) were determined with a Short-Time Incubation apparatus (Innovativ Labor A.G., Zürich, Switzerland).

Fitting of transport results to the Michaelis-Menten equation

Absolute velocity data as a function of the substrate concentration were fitted by iteration to an equation involving the sum of one saturable and one non-saturable component (van Melle & Robinson, 1981; Robinson & van Melle, 1983).

Expression of results

Results are expressed as either absolute uptakes (nmol/mg of protein), absolute velocities (nmol/s per mg of protein) or relative velocities (nl/s per mg of protein) (Brot-Laroche *et al.*, 1986). The data are presented as the means ($\pm$ S.D. or S.E.M.) from several experiments performed with at least two different membrane preparations. Occasionally, representative results obtained with a single membrane preparation are presented. The uptake experiments were routinely done in triplicate.

Statistical analysis

Statistical analyses were carried out with Student's *t* test. Saturation curves were solved by applying non-linear regression analysis that includes an *F* test (van Melle & Robinson, 1981). All the calculations were performed with an Apple-Macintosh SE microcomputer.

RESULTS AND DISCUSSION

Purity of microvillous-membrane preparations

The purity of the brush-border membrane preparation was verified by assaying for alkaline phosphatase, 5'-nucleotidase and γ -glutamyl transpeptidase, enzymes commonly used as markers for brush-border membranes in placenta; enrichment over the activities found in the homogenate was 5–6-fold (Table 1). Succinate dehydrogenase activity in the microvillous-membrane preparations was not detectable, indicating the absence of mitochondrial contamination in the preparations. The specific activity of glucose-6-phosphatase (a marker for endoplasmic reticulum) increased 1.6-fold. The specific activity of Na^+/K^+ -ATPase (a basal membrane marker; Boyd *et al.*, 1979) increased 1.5-fold (results not shown), whereas the specific activity of L-(+)-tartrate-sensitive acid phosphatase was unchanged. This indicates that our microvillous-membrane vesicles were contaminated with endoplasmic reticulum and with basolateral membranes and, to a lesser extent, with lysosomal material. The recovery obtained by our procedure was 1.69 ± 0.12 (mean $\pm$ S.E.M., $n = 6$) mg of membrane protein/g of tissue. The efficiency of Mg^{2+} treatment was determined on the basis of the enrichment in alkaline phosphatase in our BBMV, which showed a 5-fold higher purification than in BBMV prepared in a similar manner but without Mg^{2+} treatment (McArdle *et al.*, 1984).

Evaluation of non-specific binding of L-lactate to the BBMV from rat placenta

To differentiate between the binding of L-lactate to membrane surfaces and transport into the intravesicular compartment, we examined the initial uptake rate (at 1.7 s) as a function of L-lactate concentration by using BBMVs disrupted with 0.5% (v/v) Triton X-100. Under these conditions L-lactate uptake was negligible at all the concentrations studied, and did not differ statistically from the amount of labelled L-lactate non-specifically bound to the filters (blank). Similar results have been obtained in experiments in which osmolarity of the incubation medium was modified (results not shown). We conclude that all L-lactate

Table 1. Purification of brush-border plasma membrane from rat placenta

The specific activity of each marker enzyme was determined in each fraction, and is expressed in units/mg of protein. Recoveries (%) are means of the recoveries from each experiment. N.D. indicates lowest detectable level. Values are means $\pm$ S.E.M., with numbers of determinations in parentheses.

Fraction	Alkaline phosphatase	5'-Nucleotidase	γ -Glutamyl transpeptidase ($\times 10^{-3}$)	Glucose-6-phosphatase	Acid phosphatase	Succinate dehydrogenase ($\times 10^{-3}$)
H	0.73 $\pm$ 0.014 (9)	0.05 $\pm$ 0.002 (9)	5.50 $\pm$ 0.250 (8)	0.08 $\pm$ 0.004 (6)	0.03 $\pm$ 0.001 (5)	9.10 $\pm$ 0.930 (6)
%	100	100	100	100	100	100
S1	0.32 $\pm$ 0.016 (6)	0.04 $\pm$ 0.002 (8)	3.70 $\pm$ 0.300 (8)	0.06 $\pm$ 0.008 (4)	0.02 $\pm$ 0.002 (4)	4.20 $\pm$ 0.440 (6)
Enrichment	0.44	0.80	0.67	0.75	0.66	0.46
P1	0.32 $\pm$ 0.038 (5)	0.03 $\pm$ 0.001 (9)	5.10 $\pm$ 0.410 (4)	0.07 $\pm$ 0.013 (4)	0.03 $\pm$ 0.002 (4)	10.60 $\pm$ 0.470 (6)
Enrichment	0.44	0.60	0.92	0.87	1.00	1.20
%	40.00 $\pm$ 2.16	56.00 $\pm$ 4.64	32.00 $\pm$ 2.66	65.00 $\pm$ 1.20	35.00 $\pm$ 1.50	53.00 $\pm$ 2.40
S2	0.33 $\pm$ 0.036 (7)	0.04 $\pm$ 0.002 (9)	3.80 $\pm$ 0.160 (4)	0.03 $\pm$ 0.007 (4)	0.01 $\pm$ 0.001 (4)	N.D.
Enrichment	0.45	0.80	0.69	0.37	0.33	—
P2	0.42 $\pm$ 0.038 (6)	0.05 $\pm$ 0.003 (8)	11.90 $\pm$ 0.010 (5)	0.12 $\pm$ 0.010 (4)	0.03 $\pm$ 0.002 (5)	13.90 $\pm$ 0.660 (6)
Enrichment	0.57	1.00	2.16	1.50	1.00	1.50
%	28.00 $\pm$ 1.32	31.00 $\pm$ 2.87	27.00 $\pm$ 1.10	30.00 $\pm$ 2.21	39.00 $\pm$ 2.25	29.00 $\pm$ 1.79
S3	0.05 $\pm$ 0.003 (4)	0.01 $\pm$ 0.001 (7)	N.D.	N.D.	0.01 $\pm$ 0.001 (4)	N.D.
Enrichment	0.07	0.20	—	—	0.33	—
P3	4.69 $\pm$ 0.150 (7)	0.24 $\pm$ 0.009 (8)	26.70 $\pm$ 0.020 (7)	0.13 $\pm$ 0.010 (4)	0.03 $\pm$ 0.001 (5)	N.D.
Enrichment	6.40	4.80	4.85	1.60	1.00	—
%	25.00 $\pm$ 1.78	3.30 $\pm$ 0.20	21.00 $\pm$ 0.70	—	20.00 $\pm$ 0.90	6.00 $\pm$ 0.35
Total recovery	99	100	89	95	99	88

Fig. 1. Effect of *cis* Na⁺ or K⁺ and of increasing inward-directed proton gradients on L-lactate transport in BBMVs from rat placenta

BBMV were preloaded with a buffer containing 500 mM-sorbitol and 20 mM-Tris/Mes (pH 7.4). Incubation was performed at 37 °C in buffers containing 1 mM-L-[¹⁴C]lactate, 599 mM-sorbitol and 20 mM-Tris/Mes, or 1 mM-L-[¹⁴C]lactate, 399 mM-sorbitol and 100 mM-NaCl or -KCl and 20 mM-Tris/Mes adjusted to pH 5.4 (upper curve) or pH 7.4 (lower curve). In the presence of an inward-directed proton gradient, statistically identical results (pooled under the $\square$ symbol, upper curve) were obtained in the presence of either *cis* Na⁺ or K⁺ and in the presence of an outwardly directed K⁺ gradient ([K⁺]_{in}: [K⁺]_{out}, 100 mM: 10 mM) independently of the presence or absence of valinomycin. Statistically identical results (pooled under the $\blacksquare$ symbol, lower curve) were obtained in the absence of a proton gradient. Each data point represents the mean $\pm$ S.E.M. of four experiments.

taken up under standard conditions represents entrapment into the osmotically active intravesicular space. Storelli *et al.* (1980) reported a slight degree of L-lactate binding in BBMVs from rat enterocytes prepared by the Ca²⁺-precipitation method. However, this result may be due to a limited shrinking capacity of membrane vesicles isolated by this method rather than the

binding of L-lactate to the membranes. Nevertheless, this phenomenon was not observed in our BBMVs from rat placenta prepared by the Mg²⁺-precipitation method.

Effect of univalent cations on L-lactate uptake

The effect of inside-positive diffusion potentials generated by cations was examined by studying the effect of a 100 mM-Na⁺ or -K⁺ gradient (in/out = 0/100 mM in both cases) on the uptake of 1 mM-L-lactate, both with and without a pH gradient. No significant increase in the initial uptake rates was observed in either case (Fig. 1). These results lead us to conclude that, in agreement with those described in BBMVs from human placenta (Balkovetz *et al.*, 1988), L-lactate transport is not coupled to either a Na⁺ or a K⁺ gradient. In contrast, an inward-directed proton gradient can provide the driving force for L-lactate uphill transport across the brush-border membrane of trophoblast rat placenta (see below).

Effect of extravesicular pH on L-lactate uptake

We examined the effect of changing the pH value of the incubation buffer on the transport of 1 mM-L-lactate into BBMVs from rat placenta. The extravesicular pH was varied from 5.2 to 8.2 and the intravesicular pH was fixed at 7.4. The results (Fig. 2) show that the maximum uptake rate was observed at pH 5.2, and was about 20-fold higher than at pH 8, suggesting that the proton gradient is responsible for the stimulation of L-lactate transport, as also observed by Tiruppathi *et al.* (1988) and by Balkovetz *et al.* (1988) in other preparations.

Effect of an inward-directed H⁺ gradient on L-lactate uptake

The transport of L-lactate in BBMVs from rat placenta was examined in the presence and absence of an inward-directed pH gradient. The results (Fig. 1) show that in the presence of an inward-directed proton gradient (pH_{in} = 7.4 and pH_{out} = 5.4) 1 mM-L-lactate uptake was rapid, and a transient accumulation 'overshoot' was observed (5–6-fold higher at 30 s than that at

Fig. 2. Effect of extravesicular pH on L-lactate transport in BBMVs from rat placenta

BBMV were preloaded with a buffer containing 500 mM-sorbitol and 20 mM-Tris/Mes (pH 7.4). Incubation was performed for 10 s at 37 °C in a buffer containing 1 mM-L-[¹⁴C]lactate, 599 mM-sorbitol and 20 mM-Tris/Mes adjusted to the different pH. Each data point represents the mean $\pm$ S.E.M. of four to five experiments.

equilibrium). When the pH gradient was decreased (e.g. by making $\text{pH}_{\text{in}} = 7.4$ and $\text{pH}_{\text{out}} = 6.6$) both the initial uptake rate and the size of L-lactate 'overshoot' decreased proportionally (results not shown). By contrast, in the absence of a pH gradient the initial uptake rate was strongly diminished and no 'overshoot' was observed (Fig. 1, lower curve). The effect of a diffusion potential presumably generated by proton movements across the membrane was evaluated by studying the time course of L-lactate uptake in the presence of a K^+ gradient ($[\text{K}^+]_{\text{in}} : [\text{K}^+]_{\text{out}} = 100 : 10 \text{ mM}$) both with and without a pH gradient. Our results show that, in the presence or the absence of a pH gradient and an outwardly directed K^+ gradient, valinomycin does not affect L-lactate uptake significantly (Fig. 1), as would be expected if the overshoot supported by the pH gradient mainly involves an electroneutral mechanism. These results point to the absence of an electrogenic L-lactate uniport activity, as has been concluded for sialic acid (Mancini *et al.*, 1989) and Cl^- -coupled proton transport (Vasseur *et al.*, 1989). However, our results contrast with those obtained by Balkovetz *et al.* (1988) in BBMVs from human placenta, in which an inside-negative membrane potential inhibited L-lactate uptake.

Effect of intravesicular pH on L-lactate uptake

In order to elucidate further the role of protons on L-lactate transport in BBMVs from rat placenta, we tested the effect of variations in pH in the *trans*-side of the membrane on L-lactate uptake. In Table 2 a decrease is seen in L-lactate uptake with the decrease in internal pH, indicating that protons on the *trans*-side of the membrane are inhibitory and/or that the transporter is sensitive to the pH gradient, as has been suggested by Trosper & Philipson (1987) and by Roth & Brooks (1990) for L-lactate uptake in sarcolemmal vesicles.

Temperature-dependence of L-lactate uptake

L-Lactate uptake increased 4-fold when temperature rose from 25° to 37 °C (results not shown), suggesting a strong temperature-dependence of L-lactate transport across BBMVs from rat placenta. A roughly similar temperature-dependent increase in L-lactate uptake has been described by Trosper & Philipson (1987) in cardiac sarcolemmal vesicles. These facts support the idea of the existence of a carrier-mediated pathway for L-lactate uptake in BBMVs from rat placenta. Additionally, the Arrhenius plot for the accumulation of L-lactate in the 10–40 °C range showed a

Table 2. Effect of intravesicular pH on L-lactate uptake in BBMVs from rat placenta

BBMV were preloaded with a buffer containing 500 mM-sorbitol and 20 mM-Tris/Mes adjusted to the pH indicated. Incubations were performed at 37 °C for 10 s in a buffer containing 1 mM-L-[¹⁴C]lactate, 599 mM-sorbitol and 20 mM-Tris/Mes adjusted to pH 7.4. L-Lactate uptakes are expressed in nmol/10 s per mg of protein and are means $\pm$ S.E.M. of at least three experiments.

Intravesicular pH	L-Lactate uptake
8.8	0.590 $\pm$ 0.025
7.4	0.280 $\pm$ 0.012
6.7	0.080 $\pm$ 0.001
6.0	0.042 $\pm$ 0.001

Table 3. Initial rates of L-lactate uptake in BBMVs from rat placenta

BBMV were preloaded with a buffer containing 500 mM-sorbitol and 20 mM-Tris/Mes adjusted to pH 7.4. Incubations were performed at 37 °C in buffers containing 1 or 100 mM-L-[¹⁴C]lactate, enough sorbitol to maintain the osmolality and 20 mM-Tris/Mes adjusted to pH 5.4. L-Lactate uptakes are expressed in nmol/s per mg of protein and are means $\pm$ S.E.M. of at least three experiments.

Incubation time (s)	L-Lactate uptake	
	1 mM-L-Lactate	100 mM-L-Lactate
1.2	0.98 $\pm$ 0.063	34.1 $\pm$ 1.023
1.7	1.14 $\pm$ 0.075	33.8 $\pm$ 1.130
2.7	1.13 $\pm$ 0.043	24.1 $\pm$ 1.220
5.7	0.93 $\pm$ 0.040	14.5 $\pm$ 0.790

break point at 25 °C (results not shown). These results correlate well with the thermotropic behaviour of the L-lactate transport system. According to some authors, the lipid environment is one of the main factors affecting carrier-mediated uptake of substrates across the membranes (Basu *et al.*, 1986).

Effect of *p*-CMBS on L-lactate uptake

p-CMBS is a fairly specific reagent that oxidizes the thiol groups of proteins, and it therefore inhibits carrier-mediated uptake of L-lactate in cardiac sarcolemmal vesicles (Trosper & Philipson, 1987). In order to understand better the mechanism of action of *p*-CMBS, we carried out experiments at constant inhibitor concentration (0.55 mM), with and without preincubation. After 45 min at 25 °C there was a 45 % inhibition of L-lactate uptake, whereas the inhibition was nil in the absence of preincubation (4.50 $\pm$ 0.38 versus 4.38 $\pm$ 0.20 nmol/10 s per mg of protein). This time-dependent action of *p*-CMBS is compatible with an effect of this agent on thiol groups that are partially hidden, i.e. not really accessible at the surface of the vesicles.

Concentration-dependence of L-lactate uptake

The transport of L-lactate in BBMVs from rat placenta was examined as a function of increasing substrate concentrations in the incubation medium in the presence of an inwardly directed pH gradient ($\text{pH}_{\text{in}}/\text{pH}_{\text{out}}$ 7.4/5.4) at 37 °C. The incubation time for these experiments was fixed at 1.7 s in order to estimate initial rates even at concentrations of L-lactate as high as 100 mM (Table 3). Fig. 3 shows that L-lactate uptake is a saturable function of its own concentration.

Fig. 3. Transport of L-lactate as a function of concentration of L-lactate in BBMV from rat placenta

BBMV were preloaded with a buffer containing 500 mM-sorbitol and 20 mM-Tris/Mes (pH 7.4). Incubation was performed at 37 °C for 1.7 s in buffers containing increasing concentrations of L-[¹⁴C]lactate, 20 mM-Tris/Mes adjusted at pH 5.4 and enough sorbitol to maintain the osmolality. Inset: Eadie-Hofstee plot for the data. Results are means $\pm$ S.E.M. of three to four experiments.

As indicated by Poole *et al.* (1989), most kinetic work on L-lactate transport has so far failed to define the boundaries separating simple diffusion from carrier-mediated transport of this substrate. In order to make this distinction, L-lactate saturation curves were subjected to a non-linear-regression analysis according to an equation involving one saturable and one non-saturable (diffusional) component (van Melle & Robinson, 1981):

$$v = (V_{\max,1} \cdot [S]/K_{m1} + [S]) + K_d \cdot [S]$$

where K_d is the permeability component, i.e. the kinetic constant obtained by non-linear regression of the absolute velocity data. To assess K_d with a certain degree of accuracy, our iteration procedure would require the use of substrate concentrations above the saturation point of the system (Brot-Laroche *et al.*, 1986). The best fit of our results yielded evidence for the existence of a single saturable process ($V_{\max}$, 57 nmol/s per mg of protein, apparent K_m 59 mM) but indicated the diffusion constant, K_d , to be indistinguishable from zero. These results suggest that L-lactate uptake across BBMV from rat placenta is the result of a carrier-mediated process. Linearization of the data by the Eadie-Hofstee transformation (Fig. 3, inset) gave the same values for kinetic parameters, and also showed the occurrence of a single transport system with very low affinity for L-lactate in rat placenta. In order to understand the physiological meaning of this kind of L-lactate transport system with such a low affinity, we carried out the experiment on concentration-dependence of L-lactate uptake in BBMV from human placenta under the same experimental conditions. Non-linear-regression analysis of the data (van Melle & Robinson, 1981) gave the following results: K_d = 0 nl/s, $V_{\max}$ = 51.2 nmol/s per mg of protein, and K_m = 46.5 mM. These results are very similar to those found in rat placenta, suggesting that L-lactate crosses the brush-border trophoblast membrane from both rat and human placenta through a carrier-mediated system with a high capacity and very low affinity.

However, the apparent K_m values for L-lactate in BBMV from rat and human placenta were much higher than the reported concentrations of L-lactate in human (1 mM; Balkovetz *et al.*, 1988) and rat plasma at term (5 mM; Juanes *et al.*, 1986). Nevertheless, the existence of a specific transport system for L-

Table 4. *trans*-Stimulation of L-lactate uptake by preloading BBMV with unlabelled L-lactate, pyruvate or β -hydroxybutyrate

BBMV were preloaded both with and without (control) 10 mM unlabelled L-lactate, pyruvate or β -hydroxybutyrate at pH 7.4. Uptake rates of L-lactate (1 mM) were measured at 37 °C for 10 s at the pH indicated. L-Lactate uptakes are expressed in nmol/10 s per mg of protein and are means $\pm$ S.E.M. of at least four experiments. Percentages were calculated by taking each corresponding control as 100 %.

	pH 5.4	pH 7.4
Control	5.14 $\pm$ 0.36	0.37 $\pm$ 0.02
10 mM-lactate	10.7 $\pm$ 0.60 (208 %)	0.89 $\pm$ 0.05 (238 %)
10 mM-pyruvate	10.9 $\pm$ 0.22 (213 %)	1.54 $\pm$ 0.06 (409 %)
10 mM- β -hydroxybutyrate	7.17 $\pm$ 0.21 (140 %)	0.75 $\pm$ 0.03 (200 %)

lactate in the apical membrane of trophoblasts would explain the transfer of such an important metabolite for the conceptus growth, because the low capacity of the passive-diffusion mechanism for L-lactate observed in our experiments could not account for a net passage of lactate through the microvillous membrane.

It should be mentioned that the kinetic parameters of the L-lactate transport system described by Balkovetz *et al.* (1988) in BBMV from human placenta (K_m = 4 mM and $V_{\max}$ = 1.8 nmol/s per mg of protein) differ considerably from those found by us in BBMV from rat and human placenta. These discrepancies about the values of the kinetic parameters are easily explained by the fact that our results were obtained by working at the true initial rate (at 1.7 s; see Table 3), whereas Balkovetz *et al.* (1988) used longer incubation times (30 s).

trans-Stimulation of L-lactate uptake by several monocarboxylic acids

In order to demonstrate the symmetrical function and the specificity towards L-lactate itself and other monocarboxylic acids, we examined the transport of 1 mM-L-lactate in BBMV from rat placenta preloaded with 10 mM-L-lactate, -pyruvate and - β -hydroxybutyrate at 37 °C for 10 s in both the presence and absence of an inward-directed proton gradient. As shown in Table 4, all the monocarboxylic acids preloaded inside the vesicles stimulated L-lactate uptake under conditions both with and without a pH gradient. This phenomenon of countertransport suggests that under appropriate biochemical conditions L-lactate and the other monocarboxylic acids tested can be transported in both directions by the same carrier. A similar interpretation has been offered for countertransport of acidic monosaccharides in lysosomal membranes (Mancini *et al.*, 1989). Since L-lactate transfer should occur either from maternal to fetal blood or vice versa, it is likely that the reversibility of the carrier would represent a physiological mechanism for the transfer of L-lactate between both compartments. Thus at the end of gestation the L-lactate produced by the fetus must then be transported to the maternal circulation (Kraan & Dias, 1975).

Inhibitory effect of monocarboxylic acids on L-lactate uptake

In order to elucidate whether monocarboxylic acids share a common carrier in BBMV from rat placenta, as has been suggested for human placenta (Balkovetz *et al.*, 1988) and cardiac myocytes (Poole *et al.*, 1989), we have studied the *cis*-inhibitory effect of selected monocarboxylic acids such as L-lactate, D-lactate, pyruvate, α -cyano-4-hydroxycinnamate and β -hydroxybutyrate at concentrations of 5 and 25 mM on the transport of 1 mM-L-lactate in the presence of an inwardly directed proton gradient. The inhibitory effect of all the

Table 5. Inhibitory effect of monocarboxylates on L-lactate uptake by BBMV from rat placenta

Membrane vesicles were preloaded with 500 mM-sorbitol and 20 mM-Tris/Mes buffer (pH 7.4). Uptake of 1 mM-L-lactate was measured at 37 °C for 10 s by incubating the membrane vesicles in 20 mM-Tris/Mes buffer (pH 5.4) containing 5 or 25 mM of inhibitors. Enough sorbitol was added to maintain the osmolarity. Each value represents the mean $\pm$ S.E.M. of three to five experiments.

Inhibitor	Relative uptake (% of control)
None	100
L-Lactate	
5 mM	79.0 $\pm$ 5.7
25 mM	42.0 $\pm$ 2.0
D-Lactate	
5 mM	71.0 $\pm$ 3.0
25 mM	29.0 $\pm$ 2.0
Pyruvate	
5 mM	75.0 $\pm$ 5.0
25 mM	25.0 $\pm$ 1.5
α -Cyano-4-hydroxycinnamate	
5 mM	96.0 $\pm$ 4.4
25 mM	58.0 $\pm$ 2.5
β -Hydroxybutyrate	
5 mM	66.0 $\pm$ 1.5
25 mM	34.0 $\pm$ 0.2

Table 6. Kinetic parameters for L-lactate uptake in microvillous membrane vesicles from rat placenta during the last 3 days of gestation

BBMV from rat placenta prepared at 19.5, 20.5 and 21.5 days of gestation were preloaded with a buffer containing 500 mM-sorbitol and 20 mM-Tris/Mes (pH 7.4). Incubations were performed at 37 °C for 1.7 s in buffers adjusted at pH 5.4 containing 20 mM-Tris/Mes, increasing concentrations of L-lactate and enough sorbitol to maintain the osmolarity. Uptake-rate data were fitted by non-linear regression analysis to an equation involving one saturable component and one non-saturable component. The *F* test includes the degree of freedom (df) in parentheses.

Days of gestation	$V_{\max}$ (nmol/s per mg)	K_m (mM)	K_d (nl/s per mg)	<i>F</i> (df)
19.5	50.7	57.4	0	0.7 (5,12)
20.5	79.8	68.5	0	1.6 (5,14)
21.5	57.2	58.5	0	1.0 (4,34)

monocarboxylic acids tested (Table 5) suggests that they share a common carrier in BBMV from rat placenta.

Development of the L-lactate transport system during the last 3 days of gestation in the rat

Owing to the presumed changes in the rates of lactate transport during fetal development, we studied the maximal initial rates and the kinetic parameters of the L-lactate transport system in BBMV of rat placenta of 19.5, 20.5 and 21.5 days of gestation. The time course of L-lactate transport was estimated in the presence of an inward-directed proton gradient. No statistically significant differences were found in the initial L-lactate uptake rates observed in BBMV from rat placenta at 19.5, 20.5 or 21.5 days of gestation (results not shown). In addition, the kinetic parameters of L-lactate transport during the last 3 days of gestation were estimated by measuring L-lactate transport as a

function of increasing substrate concentrations in the incubation medium in the presence of an inwardly directed proton gradient (pH_{in}/pH_{out} 7.4/5.4) at 37 °C and 1.7 s of incubation time. The kinetic parameters of L-lactate transport in BBMV from 19.5 and 21.5 days gestation were not statistically different (Table 6). Thus at 20.5 days of gestation $V_{\max}$ and apparent K_m for L-lactate increased as compared with 19.5 or 21.5 days gestation, although the difference was not statistically significant. These results virtually rule out the existence of modifications in rat placental L-lactate transport properties during late gestation.

In conclusion, our results suggest that passive diffusion of L-lactate is virtually nil in both rat and human placenta. This result is easy to understand if we take into consideration the *pK* of lactic acid. In the pH range used in our experiments, the lactate ion will be the only quantitatively significant species present in solution. Because of its lipophobicity, it can therefore be expected that the overall rate of diffusion of the lactic acid/lactate pair across the membrane would be nil. However, L-lactate is transported by a saturable low-affinity high-capacity carrier driven by an inside-positive proton gradient. This system is reversible and may be shared by other monocarboxylic acids. The affinity and the capacity of the carrier do not change during the last 3 days of gestation, suggesting that the putative vectorial changes in L-lactate transport observed during gestation are not achieved by modifications of the intrinsic properties of the L-lactate carrier.

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