Facilitated Transport of Lactate by Rat Jejunal Enterocyte

M.N. Orsenigo[1], M. Tosco[1], U. Laforenza[2], A. Faelli[1]
[1]Dipartimento di Fisiologia e Biochimica Generali, Università di Milano, via Celoria 26, I-20133 Milano, Italy
[2]Istituto di Fisiologia Umana, Università di Pavia, Via Forlanini 6, I-27100 Pavia, Italy

Abstract. L-lactate transport mechanism across rat jejunal enterocyte was investigated using isolated membrane vesicles. In basolateral membrane vesicles L-lactate uptake is stimulated by an inwardly directed H^+ gradient; the effect of the pH difference is drastically reduced by FCCP, pCMBS and phloretin, while furosemide is ineffective. The pH gradient effect is strongly temperature dependent. The initial rate of the proton gradient-induced lactate uptake is saturable with respect to external lactate with a K_m of 39.2 ± 4.8 mM and a J_{max} of 8.9 ± 0.7 nmoles mg protein^{-1} sec^{-1}. A very small conductive pathway for L-lactate is present in basolateral membranes. In brush border membrane vesicles both Na^+ and H^+ gradients exert a small stimulatory effect on lactate uptake. We conclude that rat jejunal basolateral membrane contains a H^+-lactate cotransporter, whereas in the apical membrane both H^+-lactate and Na^+-lactate cotransporters are present, even if they exhibit a low transport rate.

Key words: Rat jejunum — Basolateral membrane vesicles — Brush border membrane vesicles — Proton-lactate cotransport — Na-lactate cotransport

Introduction

Transport systems for monocarboxylic acids have been identified and characterized in plasma membranes from several tissues (Poole & Halestrap, 1993). In the most thoroughly investigated cells, erythrocytes, three pathways for lactate transport have been defined: first, carrier-mediated transport by a proton-coupled monocarboxylate transporter (MCT); second, exchange with inorganic anions mediated by the band 3 protein; third, passive diffusion across the lipid bilayer. Under physiological conditions, the MCT is believed to mediate 90% of lactate transport (Deuticke et al., 1982). MCTs have been described functionally in multiple cell types and tissues (Poole & Halestrap, 1993); two of these transporter isoforms have now been cloned and sequenced: MCT1 from Chinese hamster ovary cells (Garcia et al., 1994; Tamai et al., 1995) and MCT2 from Syrian hamster liver (Garcia et al., 1995). The two isoforms differ in substrate and inhibitor specificities and in tissue distribution (Poole & Halestrap, 1993).

The majority of mammalian cells require lactic acid to be transported across the plasma membrane. Although some studies have been performed in proximal small intestine, the mechanisms of lactate movement across the enterocyte are still doubtful. Evidence was given that in intestinal brush border membrane vesicles the transport of lactate occurs via a lactate-sodium cotransport mechanism (Hildmann et al., 1980; Storelli et al., 1980); however a lactate-proton symport was also evidenced in apical membranes from proximal small intestine (Tiruppathi et al., 1988). A Na-independent carrier-mediated transport system was suggested to occur in basolateral membranes (Storelli et al., 1980; Cheeseman, Shariff & O'Neill, 1994). Besides lactate absorption, it is known that the jejunal enterocyte itself produces large quantities of lactic acid (HL), despite an adequate oxygen supply, and that L-lactate is preferentially released in the serosal side (Lamers & Hulsmann, 1972; Hanson & Parson, 1976). Since lactic acid (pK = 3.86) is mostly dissociated at physiological pH, its diffusion in the undissociated form would be quantitatively negligible if it occurred. The lactate ion could not readily cross the lipophilic membrane and it would require specialized transport mechanisms. Anion exchangers have been found to carry lactate in red blood cells and other cell types (Deuticke, Rickert & Beyer, 1978; Poole & Halestrap, 1993; Kenyon et al., 1994). Although evidence

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Correspondence to: M.N. Orsenigo

for the existence of a basolateral Cl^-/HCO_3^- exchanger in jejunal enterocyte has previously been published (Orsenigo et al., 1991; Orsenigo et al., 1992; Orsenigo, Tosco & Faelli, 1994), anion antiport would not help mediate basolateral lactate transport, as it does not exchange lactate for either Cl^- (Orsenigo et al., 1991) or HCO_3^- (Orsenigo et al., 1992). Since the glycolytic metabolism of jejunal enterocyte leads to the concomitant production of lactate and H^+, it is reasonable to hypothesize that H^+-lactate cotransport is present in basolateral membranes.

Aim of this work is to get more insight on lactate transport mechanisms across rat jejunal enterocyte using isolated membrane vesicles and to investigate whether the lactate transporters share any properties with the known MCTs.

Materials and Methods

BASOLATERAL MEMBRANE ISOLATION

Two male albino rats (Wistar strain, Charles River Italiana) weighing 250–300 g (about two months age), fed a rodent laboratory chow and tap water, were used for each experiment. Jejunal enterocytes were collected by scraping off the mucosal layer and diluted in 250 mM sucrose, 1 mM dithiothreitol, 0.2 mM phenylmethanesulphonilfluoride (PMSF), 0.01% (v/v) ethanol, 20 mM Hepes/Tris buffer, pH 7.5. Basolateral plasma membranes were isolated and purified as described previously (Orsenigo et al., 1985). Briefly, basolateral membranes collected by self-orienting Percoll-gradient centrifugation (Kontron, Centrikon mod. T 2070 ultracentrifuge; Haake-Buchler, Auto Densi-Flow IIC apparatus), were suspended in the appropriate buffer (*see* single experiment). Seven mM $MgCl_2$, which aggregates preferentially all membranes except brush border, was added. Collected pellets (basolateral membrane fraction) were washed and used for analysis and for L-[^{14}C]lactate uptake experiments by a rapid microfiltration technique. To control the purity of the basolateral membrane fraction, as a rule total protein, γ-glutamyltransferase (γ-GT, a marker enzyme for brush border membrane), cytochrome c oxidase (a marker enzyme for mitochondria) and (Na, K)-ATPase (a marker enzyme for basolateral membranes) were determined as published (Orsenigo et al., 1985).

Brush Border Membrane Isolation

Brush border membrane vesicles were isolated from rat jejunum enterocytes by the Ca^{2+} (Mg^{2+}) precipitation method first described by Schmitz et al. (1973). Mucosal scrapings, homogenized in hypotonic solution (50 mM sorbitol and 2 mM Tris Cl, pH 7.1) and incubated with 10 mM $MgCl_2$ for 15 min at 0°C, were centrifuged at $3,000 \times g$ for 15 min. The supernatant was centrifuged at $27,000 \times g$ for 30 min. The pellet was resuspended in the appropriate buffer and centrifuged at $43,000 \times g$ for 20 min. The final pellet was resuspended again in the above solution.

UPTAKE EXPERIMENTS

Transport of either 0.5 mM L-[^{14}C]lactate or 0.5 mM D-[^{14}C]glucose into vesicles was measured at 37°C or 28°C by a rapid microfiltration technique. A volume of membrane suspension (2–4 mg protein/ml) equilibrated with 0.2 mM EDTA, was mixed with the proper incubation

solution (zero time). The composition of the resuspension buffers and incubation media are given in the legends of the figures. Samples were removed at selected times and diluted with 0.8 ml of ice-cold reaction stopping solution (122 mM NaCl, 0.2 mM PMSF, 0.01% (v/v) ethanol, 20 mM Hepes/Tris buffer pH 7.5), filtered on wetted cellulose nitrate filters (0.45 μm pore size) and immediately rinsed with 5 ml of the stop solution. The radioactivity of the filters was counted by liquid scintillation spectrometry (Tri-Carb, Packard, mod. 4000).

In a second kind of experiments L-[^{14}C]lactate at different concentrations was present in the incubation fluid; 10 μl of membrane suspension were mixed for a predetermined time interval with 40 μl of incubating solution (composition in figure legends), using a homemade rapid-exposure uptake apparatus. All reaction mixture was collected on presoaked cellulose nitrate filters and treated as described above. All experiments were performed in voltage-clamp conditions. The solutions used were pre-filtered through 0.22 μm pore size filters. Individual uptake experiments in triplicate, representative of more than three repetitions with qualitatively identical results, are presented throughout the paper.

REAGENTS

L-[^{14}C]lactate (7.4 MBq/ml) and D-[^{14}C]glucose (7.4 MBq/ml) were obtained from Amersham, UK; all other reagents were from Sigma Chemical (St. Louis, MO).

Results

From the (Na,K)-ATPase, γ-GT and cytochrome c oxidase determinations, we calculated that the basolateral membranes were enriched about 12 times over the initial homogenate, while brush border and mitochondria were reduced to about a half or less, according to results previously published (Orsenigo et al., 1985). In the brush border membrane fraction the γ-GT enrichment factor was 13.8 ± 1.8 ($n = 8$), while the enrichment of (Na,K)-ATPase was 0.58 ± 0.04. Firstly, we demonstrated that 0.5 mM L-lactate uptake at 60 min is inversely proportional to the osmolarity of the incubation medium (Fig. 1); evidence is also given that no binding on vesicles surface occurs at pH 8.0, while at pH 6.0 L-lactate binds to a certain extent on the membranes. However, binding is always small compared with transport. Since adsorption on outer vesicle surface depends on external pH, this was kept constant in all conditions, the control included.

If a proton-lactate symport works in the basolateral membrane, then transport of L-lactate would be expected to be pH-dependent. Figure 2 depicts the effect of a pH gradient on L-lactate uptake. An inwardly directed proton gradient enhances lactate uptake over the equilibrium value; there is no stimulation in the absence of ΔpH. The H^+ gradient-dependent stimulation of lactate uptake is not due to the effect of a H^+ diffusion potential, since all experiments were performed in voltage-clamp conditions. Uptake values obtained after 10 and 25 sec incubation (*not reported*) are significantly lower than the 15-sec value, that is depicted in Fig. 2. Since L-lactate
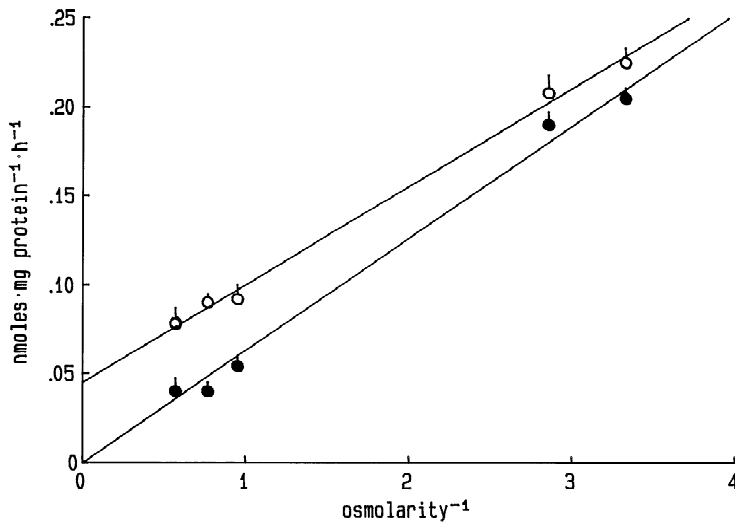
Fig. 1. Effect of extravesicular osmolarity on 0.5 mM L-lactate uptake into basolateral membrane vesicles. 150 μl basolateral membrane vesicles (0.5–1 mg protein/ml), pre-equilibrated with 25 μM valinomycin, were incubated for 60 min at 37°C with 300 μl of incubation medium. Vesicles were obtained in 53 mM sucrose and either 100 mM Hepes/Tris buffer at pH 8.0 (filled circles) or 100 mM Mes/Tris buffer at pH 6.0 (open circles). The incubation medium contained either 100 mM Hepes/Tris buffer at pH 8.0 (filled circles) or 100 mM Mes/Tris buffer at pH 6.0 (open circles), 0.5 mM L-[^{14}C]lactate and 53, 103, 803, 1053 or 1503 mM sucrose. All solutions contained 80 mM KCl, 0.2 mM PMSF and 0.01% (v/v) ethanol. In both experiments, after 60-min incubation, 100 μl samples were processed as described. Ordinate: lactate uptake, mean values ± SE (= vertical bars). Abscissa: 1/osmolarity.

uptake peaks at 15 sec, this time point was chosen in all experiments. To give more evidence that the pH stimulation of lactate uptake is due to H$^+$-lactate cotransport, the effect of the protonophore p-trifluoromethoxyphenyl-hydrazone (FCCP) on proton gradient-dependent lactate uptake was tested; as expected, FCCP (50 μM), by dissipating the pH gradient across membrane vesicles, yields a drastic reduction of lactate uptake. Therefore it seems that the transport of L-lactate is coupled to the cotransport of an H$^+$ ion (or the countertransport of OH$^-$ ion). This hypothesis is also supported by the drastic inhibition exerted by 1 mM p-chloromercuribenzenesulphonate (pCMBS), a protein-thiol oxidizing reagent which is known to block proton-linked monocarboxylate transport (Alonso De La Torre et al., 1991; Poole & Halestrap, 1993); in contrast 1 mM furosemide is ineffective, as reported in other substrates (Balkovetz et al., 1988; Roth & Brooks, 1990).

A recent work failed to demonstrate an H$^+$-lactate symport in basolateral membranes from rat jejunum (Cheeseman et al., 1994). Since the experimental protocol reported in that study was undertaken in the presence of 120/150 mM KSCN on both sides of BLM, we tested the effect of KSCN on the proton-driven lactate uptake. The presence of 80 mM KSCN instead of 80 mM KCl causes a drastic reduction of lactate uptake, suggesting an inhibitory effect of SCN$^-$ on H$^+$-lactate cotransport (unpublished). Actually, a rough calculation suggests that, in the presence of SCN$^-$, lactate flux is comparable to that reported in the cited study. We do not know the
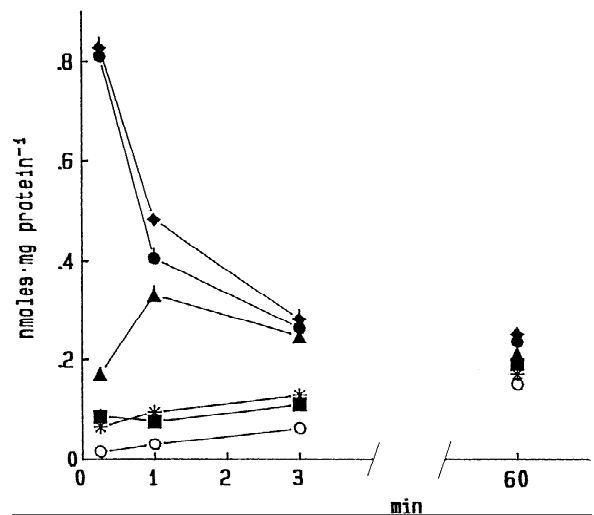
Fig. 2. 0.5 mM L-lactate uptake into basolateral membrane vesicles under different experimental conditions. 40 μl basolateral membrane vesicles obtained either in 100 mM Mes/Tris buffer pH 6.0 (open circles), or in 100 mM Hepes/Tris buffer pH 8.0 (dark circles, diamonds, triangles), added with either 50 μM FCCP (squares), or 1 mM pCMBS (stars), or 2 mM phloretin (triangles) were incubated at 37°C in 360 μl of either 100 mM Mes/Tris buffer pH 6.0 (circles, stars and triangles), or in the same solution added with 50 μM FCCP (squares), or 1 mM furosemide (diamonds). 0.5 mM L-[^{14}C]lactate was in the incubating solutions. All solutions contained 80 mM KCl, 0.2 mM PMSF and 0.01% (v/v) ethanol (0.11% in the presence of FCCP). Vesicles were preincubated with 25 μM valinomycin. Ordinate: lactate uptake, mean values ± SE (= vertical bars, absent if less than symbol height). Abscissa: incubation time.
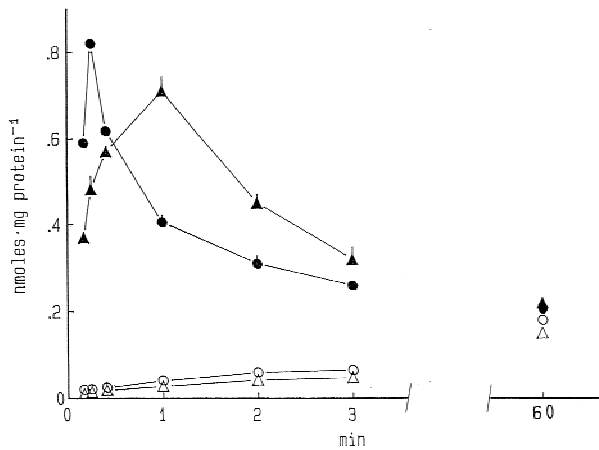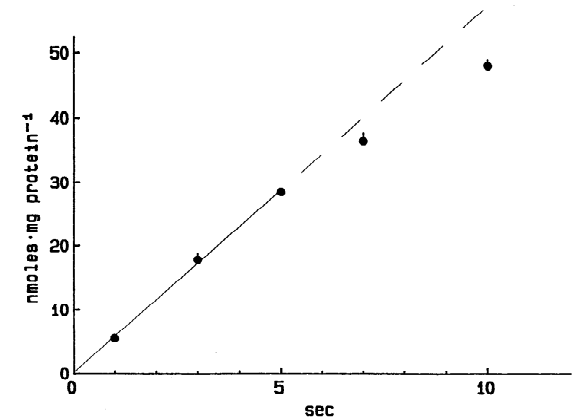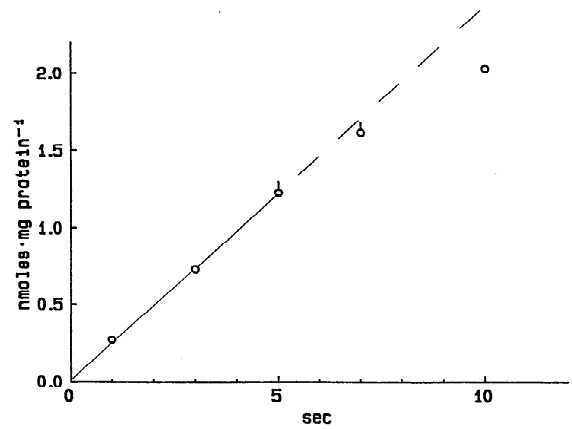
Fig. 3. 0.5 mM L-lactate uptake into basolateral membrane vesicles at two temperatures both in the presence and in the absence of a pH gradient. 40 μl basolateral membrane vesicles obtained either in 100 mM Mes/Tris buffer pH 6.0 (open circles and triangles) or in 100 mM Hepes/Tris buffer pH 8.0 (filled circles and triangles) were incubated in 360 μl of 100 mM Mes/Tris buffer pH 6.0 both at 28°C (triangles) and 37°C (circles). 0.5 mM L-[¹⁴C]lactate was in the incubating solutions. All solutions contained 80 mM KCl, 0.2 mM PMSF and 0.01% (v/v) ethanol. Vesicles were preincubated with 25 μM valinomycin. Ordinate: lactate uptake, mean values ± SE (= vertical bars, absent if less than symbol height). Abscissa: incubation time.

cause for the inhibition; however a 30% decrease is already evident with 5 mM extravesicular SCN⁻ (*see* Table 2). The effect of SCN⁻ could be due, like that of FCCP, to a proton carrier function of SCN⁻, dissipating pH gradients.

In Fig. 3 we present data illustrating the effect of temperature on the rate of transport of 0.5 mM L-lactate into basolateral membrane vesicles. As in other membranes (Alonso De La Torre et al., 1991), the initial rate of lactate transport is strongly temperature dependent, since the 15-sec value at 25°C is about half the one observed at 37°C.

In the presence of an inwardly directed pH gradient, the uptake of both 2 mM and 50 mM lactate was determined as a function of short incubation times, resulting in linear response for at least 4 sec (Fig. 4). Thus, a 4-sec uptake can be reasonably considered as an estimate of the initial uptake rate. Figure 5 shows the results of an experiment in which the transport of lactate was examined as a function of increasing substrate concentrations in the incubation medium in the presence of an inwardly directed proton gradient. The Michaelian hyperbolic relationship obtained was linearized by Eadie-Hofstee transformation of the data, showing a good correlation coefficient (inset of Fig. 5). Calculated kinetic parameters were: $K_m = 39.2 \pm 4.8$ mM and $J_{max} = 8.9 \pm 0.7$ nmoles mg protein⁻¹ sec⁻¹. Since kinetic parameters of proton-lactate symport are known to depend on the pHs on both sides of the membrane, K_m and J_{max} were also
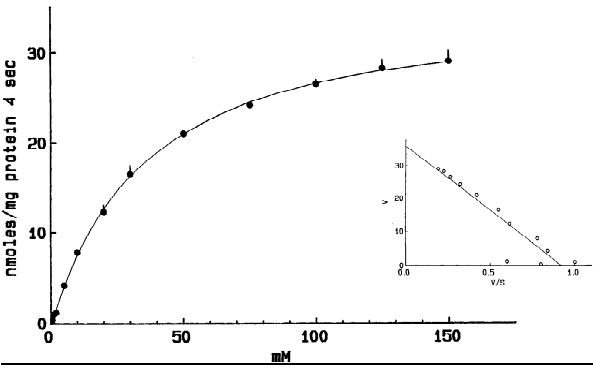

Fig. 4. L-lactate uptake into basolateral membrane vesicles. 10 μl vesicles obtained in 125 mM mannitol and 100 mM Hepes/Tris buffer pH 8.0 were incubated in 40 μl of 100 mM Mes/Tris buffer pH 6.0 and 1 mM amiloride added with either 125 mM mannitol and 2 mM L-lactate (upper panel) or 50 mM lactate (lower panel). All solutions contained 80 mM KCl, 0.2 mM PMSF and 0.01% (v/v) ethanol. Vesicles were preincubated with 25 μM valinomycin and incubated for 1-3-5-7-10 sec. Ordinate: lactate uptake, mean values ± SE (= vertical bars, absent if less than symbol height). Abscissa: incubation time.

determined as a function of both cis- and trans-pH. As shown in Table 1, the K_m value increased and the J_{max} decreased as the pH was raised in the incubating solution; on the contrary, intravesicular pH variations had no effect (unpublished data).

In Fig. 6 the effect of both positive and negative superimposed membrane potentials on 0.5 mM lactate uptake is presented: since K gradients plus valinomycin affect only to a small extent lactate movement, data suggest the existence of a poorly conductive pathway for lactate.

Table 2 describes the effect of different compounds on the initial rate of proton gradient-dependent lactate uptake. The extent of inhibition is high for L-lactate, D-lactate and pyruvate, whereas varying degrees of inhi-

Fig. 5. Effect of increasing concentration of L-lactate on the initial rate of lactate uptake (4-sec incubation) into basolateral membrane vesicles. 10 μl basolateral membrane vesicles obtained in 336 mM mannitol and 100 mM Hepes/Tris buffer pH 8.0 were incubated in 1 mM amiloride, 100 mM Mes/Tris buffer pH 6.0 and various concentrations of L-lactate from 0.5 to 150 mM. Iso-osmolarity was maintained with mannitol. All solutions contained 80 mM KCl, 0.2 mM PMSF and 0.01% (v/v) ethanol. Vesicles were preincubated with 25 μM valinomycin. Ordinate: lactate uptake, mean values ± SE (= vertical bars, absent if less than symbol height). Abscissa: L-lactate concentration (mM). Inset: Eadie-Hofstee plot of the same data.

Table 1. Effect of cis-pH on kinetic parameters for lactate transport during initial influx

| pH	K_M	J_{max}
6.0	39.2 ± 4.8	8.9 ± 0.7
6.5	40.4 ± 4.3	6.3 ± 0.8
7.0	42.3 ± 3.9	4.3 ± 0.3
7.5	55.5 ± 5.6	1.7 ± 0.2

Experimental conditions were the same as in Fig. 5. Intravesicular pH was kept constant at 8.0, whereas external pH was varied. Units: K_M = mM; J_{max} = nmoles mg protein^{-1} s^{-1}. Each value represents the mean ± SE of 4 experiments.

bition were obtained with other substances, known to be specific or nonspecific inhibitors of H$^+$-lactate cotransport in other systems.

To determine the mechanism of lactate transport across the apical membrane of rat jejunal enterocyte, we performed uptake experiments using brush border membrane vesicles. Figure 7 depicts the effect of inwardly directed gradients of both Na$^+$ and H$^+$ on lactate uptake; to test whether the functional integrity of membranes was preserved, Na$^+$-dependent D-glucose uptake was also measured and reported in the same figure. Both Na$^+$ and H$^+$ gradients seem to exert a stimulatory effect on lactate uptake, even if to a small extent.

Discussion

The main purpose of this research was to investigate the mechanism of lactate transport across the basolateral
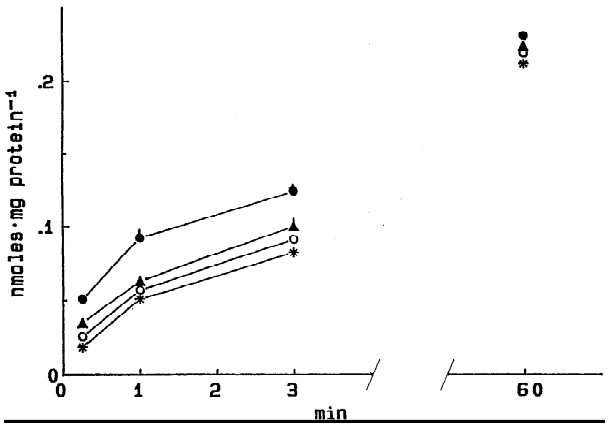
Fig. 6. Effect of membrane potential on 0.5 mM L-lactate uptake into basolateral membrane vesicles. 40 μl basolateral membrane vesicles obtained in 1 mM K$_2$SO$_4$ and 220 mM sorbitol were incubated in 360 μl of either 100 mM K$_2$SO$_4$ (dark circles), or 10 mM K$_2$SO$_4$ and 200 mM sorbitol (triangles), or 1 mM K$_2$SO$_4$ and 220 mM sorbitol (open circles), or 0.1 mM K$_2$SO$_4$ and 220 mM sorbitol (stars). 0.5 mM L-[^{14}C]lactate was in the incubating solutions. All solutions contained 100 mM Hepes/Tris buffer pH 7.0, 0.2 mM PMSF and 0.01% (v/v) ethanol. Vesicles were preincubated with 25 μM valinomycin. Ordinate: lactate uptake, mean values ± SE (= vertical bars, absent if less than symbol height). Abscissa: incubation time.

Table 2. Inhibitory effect of various compounds on L-lactate uptake into basolateral membrane vesicles

Tested Compounds	L-Lactate uptake	
	nmol/mg protein · 15 Sec	% Inhibition
None	0.81 ± 0.08	0%
L-lactate 5 mM$_{Out}$	0.21 ± 0.06	74%
D-Lactate 5 mM$_{Out}$	0.32 ± 0.06	60%
Quercetin 5 mM$_{In}$	0.43 ± 0.07	47%
Pyruvate 5 mM$_{Out}$	0.54 ± 0.09	41%
DIDS 1 mM$_{Out}$	0.52 ± 0.05	30%
NaSCN 5 mM$_{Out}$	0.57 ± 0.05	30%
CnCN* 5 mM$_{In}$	0.59 ± 0.08	27%
IBMX** 5 mM$_{In}$	0.69 ± 0.05	15%

Vesicles obtained in 100 mM Hepes/Tris buffer pH 8.0 were incubated for 15 sec in 100 mM Mes/Tris buffer pH 6.0. Tested compounds were added either in the resuspending solution or in the incubation medium, as indicated. 0.5 mM L-(^{14}C) lactate was in the incubating solution. All solutions contained 80 mM KCl, 0.2 mM PMSF, 0.01% (v/v) ethanol. Vesicles were preincubated with 25 μM valinomycin. Each value represents the mean ± SE of three to six experiments.
*α-cyano-4-hydroxycinnamic acid
**3-isobutyl-1-methylxanthine

membrane of rat jejunal enterocyte. In a previous work (Orsenigo et al., 1994), we gave evidence that basolateral membrane vesicles are about 90% right side out-oriented. The purity of the basolateral membrane fraction is an absolute requirement since an H$^+$-driven com-
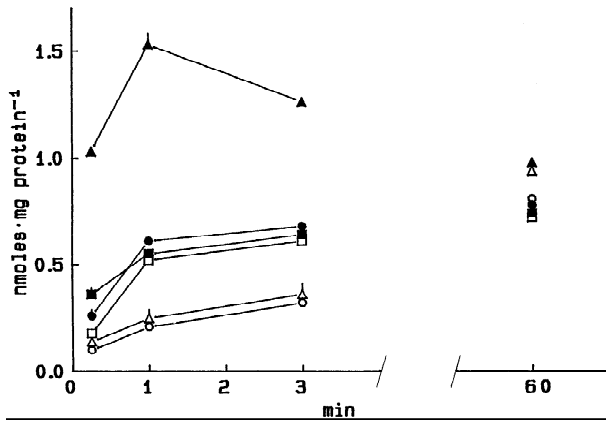
Fig. 7. L-lactate and D-glucose uptake into brush border membrane vesicles. 40 μl brush border membrane vesicles obtained in 93 mM sorbitol and 100 mM Hepes/Tris buffer pH 8.0 in the presence (open squares) or in the absence (all other symbols) of 2 mM phloretin, were incubated in 360 μl of either 0.5 mM L-[^{14}C]lactate, 93 mM sorbitol and 100 mM Mes/Tris buffer pH 6.0 (open and filled squares), or 0.5 mM L-[^{14}C]lactate, 50 mM NaCl, 1 mM amiloride and 100 mM Hepes/Tris buffer pH 8.0 (filled circles), or 0.5 mM L-[^{14}C]lactate, 93 mM sorbitol and 100 mM Hepes/Tris buffer pH 8.0 (open circles), or 0.5 mM D-[^{14}C]glucose, 50 mM NaCl, 0.1 mM amiloride and 100 mM Hepes/Tris buffer pH 8.0 (filled triangles), or D-[^{14}C]glucose, 93 mM sorbitol and 100 mM Hepes/Tris buffer pH 8.0 (open triangles). All solutions contained 80 mM KCl, 0.2 mM PMSF and 0.01% (v/v) ethanol. Vesicles were preincubated with 25 μM valinomycin. Ordinate: L-lactate or D-glucose uptake, mean values ± SE (= vertical bars, absent if less than symbol height). Abscissa: incubation time.

ponent for lactate transport has been evidenced in the brush-border of rabbit intestine (Tiruppathi et al., 1988) and in mitochondrial membranes (Poole & Halestrap, 1993). We can exclude any contribution of brush-border and mitochondria transport systems in our results, since the marker enzyme analysis shows that the basolateral membrane preparation is very well purified; moreover, in a previous work (Orsenigo et al., 1991) our basolateral membrane vesicles were tested for their ability to accumulate D-glucose in the presence of an inward Na$^+$ gradient. The lack of effect is in agreement with the absence of brush-border contamination. A proton-lactate cotransport is also present in sarcolemmal membranes from smooth muscle underlying the mucosa (Poole & Halestrap, 1993; McDermott & Bonen, 1993), but in our separation procedure only mucosal layer is scraped off and processed; therefore a contamination of the basolateral membrane fraction by sarcolemmal membranes is excluded.

In a previous work, carried out using a jejunal tract incubated ''in vitro'' (Faelli et al., 1980), pH and pCO$_2$ measurements suggested the simultaneous movement of lactate ion and H$^+$ from the cell into the serosal compartment: thus diffusion of undissociated lactic acid was hypothesized. However, HL diffusion is unlikely to be the main mechanism for lactate movement; moreover

evidence was given that lactate transport shows stereospecificity, as L-lactate uptake proceeds faster than the uptake of its stereoisomer D-lactate (Storelli et al., 1980). Thus it appeared possible that lactate ion may undergo cotransport with H$^+$ across the basolateral cell membrane, as it happens at the basolateral pole of cecum, stomach and kidney (Garcia et al., 1995; Siebens & Boron, 1987).

Results presented in Fig. 2 show the actual presence of a carrier system capable of transporting lactate coupled to the symport of an H$^+$ ion. Experimental limitations do not permit us to distinguish whether L-lactate is physically cotransported with H$^+$ or reflects the countertransport of negative ions, such as OH$^-$. The effects of all substances tested in Fig. 2 support the presence of a pH-dependent lactate transport according to data reported in the literature (Roth & Brooks, 1990; Alonso De La Torre et al., 1991); a characteristic behavior is exhibited by phloretin, a well-known reversible inhibitor of H$^+$-lactate symport (Poole & Halestrap, 1993), whose effect is evident only at 15-sec incubation. A possible explanation could lie in its insolubility in acid solutions: as gradually intravesicular pH acidifies, phloretin precipitates removing its reversible inhibitory effect. The same behavior was exhibited by quercetin, whose action is reported in Table 2.

As shown in Fig. 3, as in other membranes (Alonso De La Torre et al., 1991) pH gradient-dependent lactate uptake is strongly affected by temperature, giving further evidence for the involvement of a carrier-mediated transport system. Since the initial rate of transport is higher at 37°C, we chose this temperature for the subsequent work.

Figure 5 shows that L-lactate transport driven by H$^+$ gradients is saturable. Saturation curves were subjected to a nonlinear regression analysis, which yielded evidence that the diffusional component of lactate transport was indistinguishable from zero. Linearization of the data by Eadie-Hofstee transformation give kinetic parameters that suggest the occurrence of a transport system with low affinity and high capacity for L-lactate. As a matter of fact, kinetic parameters of the lactate transport systems described in the literature are either smaller (Tiruppathi et al., 1988; Poole et al., 1989; McDermott & Bonen, 1993; Rosenberg, Fadilt & Schuster, 1993; McDermott & Bonen, 1994; Jouel et al., 1994) or similar (Roth & Brooks, 1990; Alonson De La Torre, 1991) to those found in the present research. The low affinity, high capacity system is fit for the transport of the quite large amounts of lactate that are produced by the intestine.

It is known that kinetic parameters of H$^+$-monocarboxylate cotransporters are crucially pH-dependent and it has been shown that kinetic analysis of initial transport can be used to discriminate between the

possible reaction types of the symport carrier (Deuticke, 1982; De Bruijne, Vreeburg & Van Steveninck, 1983). Data in Table 1 show that the affinity for lactate changes with cis- (extravesicular) pH, with the apparent K_m increasing from 39.2 mM at pH 6.0 to 55.5 mM at pH 7.5; also the J_{max} value is affected, decreasing from 8.9 nmoles mg protein^{-1} sec^{-1} at pH 6.0 to 1.7 nmoles mg protein^{-1} sec^{-1} at pH 7.5. On the contrary, kinetic parameters are insensitive to variations of the trans- (intravesicular) pH. According to theoretical models (De Bruijne et al., 1983), these results would exclude lactate-OH$^-$ antiport, as well as lactate-proton symport system with ordered binding, proton binding first. This seems to differentiate jejunal proton-lactate cotransport from the monocarboxylate transporter of the erythrocytes (Deuticke, 1982; De Bruijne et al., 1983; Poole & Halestrap, 1993). In jejunal basolateral membranes, lactate uptake could theoretically be accounted for by a lactate-proton symport system with either ordered binding, lactate first, or random binding to a nonmobile carrier. However, a more detailed kinetic analysis will be necessary to better focus this matter.

The lack of a conductive pathway for lactate ion in jejunal basolateral membranes, suggested by the saturation curve reported in Fig. 5, is confirmed by the experiment depicted in Fig. 6. Electrical potential differences were superimposed across membrane vesicles by K gradients plus valinomycin; their calculated values were −44 mV, +0.59 mV and +120 mV. As it is apparent from the figure, only the highest membrane potential affects lactate uptake to a certain extent; however this value is unlikely in physiological conditions. Moreover, we cannot exclude that this small conductance might be a consequence of the separating procedure.

The inhibitory effect of various compounds on L-lactate uptake is illustrated in Table 2. Quercetin, α-cyano-4-hydroxycinnamate (CnCN) and 3-isobutyl-1-methylxanthine (IBMX) improve their solubility if dissolved in an alkaline solution. Therefore they were added in the vesicle resuspending solution, that was at pH 8. The inhibitors are probably not readily permeable, so they are likely to act on the external surface of the membrane.

As illustrated in Table 2, the cis-inhibitory effect of selected monocarboxylic acids such as L-lactate, D-lactate and pyruvate suggests that they share a common carrier with rather broad specificity. It is noteworthy that CnCN and IBMX exert a small inhibitory effect besides the high concentration used; however low degrees of inhibition have been reported in various substrates (Storelli et al., 1980; Quintana et al., 1988; Alonso De La Torre et al., 1991; McDermott & Bonen, 1993; Rosenberg et al., 1993; Wilbrand & Jouel, 1994).

To sum up, the present study suggests that, besides undissociated lactic acid diffusion, a H$^+$-lactate symport

could drive across jejunal basolateral membrane L-lactate both absorbed from the lumen and produced by glucose anaerobic metabolism in the enterocyte. The net movement of the anion across the basolateral cell membrane could be favored by the activity of the Cl$^-$/HCO$_3^-$ exchange previously evidenced (Orsenigo et al., 1991; Orsenigo et al., 1992; Orsenigo et al., 1994), which, by extruding a base, would stimulate the H$^+$-coupled exit of lactate.

All the features evidenced for the jejunal basolateral proton-lactate symport agree with the ones reported for the monocarboxylate transporter (MCT) family. Up to now two MCT isoforms have been cloned and sequenced (Garcia et al., 1994; Tamai et al., 1995; Garcia et al., 1995; Jackson & Halestrap, 1996). The two isoforms, designated MCT1 and MCT2, show a strikingly different tissue distribution: MCT1, but not MCT2, was found on the basolateral surfaces of epithelial cells in the cecum, in the proximal tubules, in the stomach (but not in the oxyntic cells), whereas MCT2 was detected on the sinusoidal surfaces of hepatocytes, on the basolateral membrane of epithelial cells in the connecting duct and in the oxyntic cells. The most striking difference between MCT1 and MCT2 is the differential sensitivity to the organomercurial thiol reagent pCMBS (Garcia et al., 1994; Garcia et al., 1995; Jackson & Halestrap, 1996): whereas MCT1 is sensitive to this agent, MCT2 is resistant. Since we have evidenced a complete inhibitory effect of pCMBS on lactate uptake (Fig. 2), we could argue that MCT1 isoform might be expressed in rat jejunal basolateral membranes. It will be of interest in the future to get more insight on this hypothesis.

To get a complete picture of lactate movements in the physiology of rat jejunal enterocyte, a reexamination of its transport mechanism across the brush border was needed: as a matter of fact, a lactate-sodium cotransport was suggested in rat intestine (Hildmann et al., 1980; Storelli et al., 1980), but a lactate-proton symport was also evidenced in rabbit (Tiruppathi et al., 1988). Data of Fig. 7 indicate that both proton and Na$^+$ gradients stimulate lactate uptake. The pH gradient effect, inhibited by phloretin, is much lower in the brush border vesicles than in the basolateral ones, suggesting the possibility that H$^+$-stimulated lactate transport is actually occurring via the basolateral membranes contaminating the brush border membrane fraction. To check this possibility, the membrane preparation was washed three times by the Mg^{2+} precipitation technique, but uptake results did not change. Thus both H$^+$ linked and Na$^+$-linked symport mechanisms seem to occur simultaneously in the apical membrane, as seen both in human and in rabbit jejunal brush border membrane vesicles (Friedrich, Murer & Berger, 1991; Friedrich et al., 1992), even if both exhibit a low transport activity.

The results of this study suggest that cellular mecha-

nisms of secondary active transport facilitate diffusion of lactate through rat jejunal enterocyte. Lactate is first absorbed by apical H^+-lactate and Na^+-lactate cotransports and then, together with lactate produced by cellular glycolytic activity, it efficiently crosses the basolateral membrane by the proton-lactate symport.

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