A proton gradient, not a sodium gradient, is the driving force for active transport of lactate in rabbit intestinal brush-border membrane vesicles

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An inward-directed H^+ gradient markedly stimulated lactate uptake in rabbit intestinal brush-border membrane vesicles, and uphill transport against a concentration gradient could be demonstrated under these conditions. Uptake of lactate was many-fold greater in the presence of a H^+ gradient than in the presence of a Na^+ gradient. Moreover, there was no evidence for uphill transport of lactate in the presence of a Na^+ gradient. The H^+ -gradient-dependent stimulation of lactate uptake was not due to the effect of a H^+ -diffusion potential. The uptake process in the presence of a H^+ gradient was saturable [K_t (concn. giving half-maximal transport) for lactate 12.7 ± 4.5 mM] and was inhibited by many monocarboxylates. It is concluded that a H^+ gradient, not a Na^+ gradient, is the driving force for active transport of lactate in rabbit intestinal brush-border membrane vesicles.

INTRODUCTION

The role of a protonmotive force as the source of energy for active transport of organic solutes in mammalian systems has become increasingly evident in recent years. Intestinal and renal transport of small peptides (Ganapathy & Leibach, 1985, 1986; Ganapathy *et al.*, 1987a,b; Hoshi, 1985) and intestinal transport of folate (Said *et al.*, 1987; Schron *et al.*, 1985) have been shown to be energized by an inward-directed H^+ gradient, and there is no direct participation of a Na^+ gradient in these transport processes. Moreover, many of the Na^+ -gradient-driven transport systems have also been shown to be energized and/or stimulated by a H^+ gradient in the presence, as well as in the absence, of Na^+ (Eveloff, 1987; Hoshi *et al.*, 1986; Jorgensen & Sheikh, 1987; Nelson *et al.*, 1983; Rajendran *et al.*, 1987; Roigaard-Petersen *et al.*, 1987; Vorum *et al.*, 1988). There have been two studies on lactate transport in purified intestinal brush-border membrane vesicles, and both studies have demonstrated that the transport of lactate across this membrane occurs via a lactate- Na^+ co-transport mechanism (Hildmann *et al.*, 1980; Storelli *et al.*, 1980). However, the role of a H^+ gradient, if any, in lactate transport was not investigated in these studies. The present study was undertaken to compare and contrast the effects of a Na^+ gradient and a H^+ gradient on the uptake of lactate in brush-border membrane vesicles isolated from the proximal intestine of the rabbit. The results demonstrate that an inward-directed H^+ gradient, not an inward-directed Na^+ gradient, is the driving force for uphill transport of lactate across this membrane.

EXPERIMENTAL

Materials

L-[U- ^{14}C]Lactate (sp. radioactivity 177.3 mCi/mmol) and D-[1- 3H (n)]glucose (sp. radioactivity 15.5 Ci/mmol) were obtained from Dupont-NEN, Boston, MA, U.S.A. L-Lactic acid, pyruvate, propionate and α -hydroxy-

butyrate were from Sigma, St. Louis, MO, U.S.A. Carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) was purchased from Aldrich, Milwaukee, WI, U.S.A. All other chemicals were of the highest analytical grade available.

Methods

Brush-border membrane vesicles from rabbit proximal small intestine were prepared by Mg^{2+} precipitation in the presence of EGTA as described previously (Tiruppathi *et al.*, 1988). Preparation of membrane vesicles by the Ca^{2+} -precipitation technique was carried out as described by Kessler *et al.* (1978). The composition of the preloading buffer in which purified brush-border membrane vesicles were suspended varied depending on the nature of the individual experiment, and is described in the legends to Figures and Tables. In most experiments, the preloading buffer consisted of 20 mM-Hepes, 10 mM-Tris and 280 mM-mannitol, pH 7.5. Uptake of lactate was usually measured in a 20 mM-Mes/Tris buffer, pH 5.0, containing 280 mM-mannitol. The membrane suspension was adjusted to a protein concentration of 10 mg/ml and stored in liquid N_2 until use.

Uptake experiments were done as described by Ganapathy *et al.* (1981), by a rapid-filtration technique. Millipore filters (DAWP type; 0.65 μm pore size) were employed for this purpose. Uptake measurements were routinely made in duplicate or triplicate, and the variation among replicate values was less than $\pm 10\%$ of the mean value.

Statistical analysis was done by Student's *t* test, and a *P* value less than 0.05 was considered significant. The results are usually given as means $\pm$ s.d.

RESULTS AND DISCUSSION

Effect of extravesicular pH on lactate uptake

We investigated the effect of extravesicular pH on lactate uptake in intestinal brush-border membrane vesicles, by using a Na^+ -free medium. Membrane vesicles

Abbreviation used: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

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Fig. 1. Effect of extravesicular pH on lactate uptake

Brush-border membrane vesicles were preloaded with 20 mM-Hepes/Tris buffer, pH 7.5, containing 280 mM-mannitol. Uptake buffers with different pH values were prepared by varying the concentrations of Hepes, Mes and Tris, but keeping the sum [Hepes]+[Mes]+[Tris] equal to 40 mM. In addition, all uptake buffers contained 280 mM-mannitol. The indicated extravesicular pH values represent the final pH values after mixing the membrane vesicles with uptake buffer. Uptake rates of lactate ($5 \mu\text{M}$) were measured with 1 min incubations.

were preloaded with 20 mM-Hepes/Tris buffer, pH 7.5, containing 280 mM-mannitol. Uptake rates of lactate ($5 \mu\text{M}$) were measured with 1 min incubations with uptake buffers of different pH. The results illustrated in Fig. 1 show that lactate uptake was markedly stimulated by decreasing extravesicular pH. The uptake rate was 25 times greater at an extravesicular pH of 5.6 than at pH 8.0.

Effects of a H^+ gradient and a Na^+ gradient on lactate uptake

Stimulation of lactate uptake observed when the extravesicular pH was lowered may be due either to the direct effect of the external pH itself or to the effect of the inward-directed H^+ gradient which exists across the brush-border membrane under these conditions. To differentiate between these two effects, uptake of lactate measured in the presence of an inward-directed H^+ gradient ($\text{pH}_i = 7.5$; $\text{pH}_o = 5.5$) was compared with the uptake measured in the absence of a H^+ gradient ($\text{pH}_i = \text{pH}_o = 5.5$). The extravesicular pH in both cases remained the same, and these experiments were conducted in Na^+ -free media. The results are given in Fig. 2.

Fig. 2. Effects of a H^+ gradient and a Na^+ gradient on lactate uptake

Brush-border membrane vesicles were preloaded with either 20 mM-Hepes/Tris/280 mM-mannitol, pH 7.5, or 20 mM-Mes/Tris/280 mM-mannitol, pH 5.5. In one set of experiments, uptake was measured with either 20 mM-Mes/Tris/280 mM-mannitol, pH 5.0, or 20 mM-Mes/Tris/280 mM-mannitol, pH 5.5, as uptake buffers. In another set of experiments, uptake was measured with either 20 mM-Hepes/Tris/140 mM-NaCl, pH 7.5, or 20 mM-Hepes/Tris/140 mM-KCl, pH 7.5, as uptake buffers. Final concentration of lactate was $5 \mu\text{M}$. ●, $\text{pH}_i = 7.5$; $\text{pH}_o = 5.5$. ○, $\text{pH}_i = \text{pH}_o = 5.5$. ■, $[\text{Na}^+]_i = 0$; $[\text{Na}^+]_o = 112 \text{ mM}$. □, $[\text{K}^+]_i = 0$; $[\text{K}^+]_o = 112 \text{ mM}$.

was accelerated many-fold in the presence of an inward-directed H^+ gradient, and the time course of uptake exhibited an overshoot phenomenon, indicating transient accumulation of lactate inside the vesicles against a concentration gradient. The initial rates of uptake measured with 30 s incubations were 8–10-fold greater in the presence of a H^+ gradient than in its absence, and the intravesicular concentration of lactate at the peak of the overshoot (2.5 min) was 9 times the equilibrium value (60 min). These data demonstrate that the stimulation of lactate uptake seen at low extravesicular pH was due to the inward-directed H^+ gradient rather than the extravesicular pH itself.

Fig. 2 also describes the effects of inward-directed Na^+ and K^+ gradients on lactate uptake in the absence of a H^+ gradient. The initial rates of lactate uptake were significantly greater (2–3-fold) in the presence of a Na^+ gradient than in the presence of a K^+ gradient, but the equilibrium value was the same in both cases. In spite of the apparent stimulation of lactate uptake by a Na^+ gradient, there was no evidence for the uphill transport

Fig. 3. Effects of an inside-positive membrane potential on H^+ -dependent lactate uptake and Na^+ -dependent glucose uptake

Brush-border membrane vesicles were preloaded with 20 mM-Hepes/Tris buffer, pH 7.5, containing 280 mM-mannitol. Lactate uptake ($5 \mu M$) was measured with the uptake buffer 20 mM-Mes/Tris/280 mM-mannitol, pH 5.0 (a). Glucose uptake ($10 \mu M$) was measured with the uptake buffer 20 mM-Mes/Tris/140 mM-NaCl, pH 5.0 (b). A stock solution of FCCP, made in ethanol, was added to the uptake buffer, and control experiments were done with an equal volume of ethanol alone added to the uptake buffer. Final concentrations of FCCP and ethanol were $20 \mu M$ and 0.2% respectively. Key: ●, control; ○, FCCP.

of lactate under these conditions. A more interesting and important observation is that the initial uptake rates were 5–8-fold greater in the presence of inward-directed H^+ gradient than in the presence of an inward-directed Na^+ gradient.

In these studies, which demonstrate the H^+ -gradient-driven uphill uptake of lactate in intestinal brush-border membrane vesicles, we have used a very low concentration of lactate ($5 \mu M$). On the other hand, previous investigators (Hildmann *et al.*, 1980; Storelli *et al.*, 1980), who have concluded that Na^+ -coupled lactate transport is the mechanism of lactate uptake in these vesicles, utilized a much higher concentration of lactate ($540 \mu M$) in their studies. In order to probe the possibility that the differences between the results of our studies and theirs may be due to the different concentrations of lactate used, we investigated the role of a H^+ gradient and a Na^+ gradient on the uptake of $500 \mu M$ -lactate in our vesicle preparations. Uptake of lactate in the presence of a Na^+ gradient ($[Na^+]_i = 0$; $[Na^+]_o = 80 \text{ mM}$) was significantly greater than uptake in the presence of a K^+ gradient ($[K^+]_i = 0$; $[K^+]_o = 80 \text{ mM}$) (0.14 ± 0.02 versus $0.05 \pm 0.01 \text{ nmol/45 s per mg of protein}$; $P < 0.001$). These results are similar to those reported by Hildmann *et al.* (1980) and by Storelli *et al.* (1980). However, uptake of lactate in the presence of a H^+ gradient ($pH_i = 7.5$; $pH_o = 5.5$) was many-fold greater than uptake in the presence of a Na^+ gradient (0.74 ± 0.04 versus $0.14 \pm 0.02 \text{ nmol/45 s per mg of protein}$). These results demonstrate that lactate uptake was stimulated to a greater extent by a H^+ gradient than by a Na^+ gradient, at both low and high concentrations of lactate.

Influence of a membrane potential (inside-positive) on the H^+ -gradient-dependent lactate uptake

There are two possibilities by which an inward-directed H^+ gradient may accelerate lactate uptake in these membrane vesicles. Firstly, if lactate uptake occurs via diffusion of lactate anions, a H^+ -gradient-induced inside-positive H^+ -diffusion potential would stimulate the uptake, and secondly, if lactate uptake occurs via a lactate- H^+ symport (or lactate- OH^- antiport) mechanism, an inward-directed H^+ gradient would provide the driving force for the process. To differentiate between these two possibilities, we studied the effect of an FCCP-induced, inside-positive, H^+ -diffusion potential on lactate uptake. The rationale for this experiment was that, if the first possibility were to be true, the H^+ -gradient-dependent lactate uptake would be stimulated further in the presence of FCCP. But the data in Fig. 3(a) show that FCCP had no influence on lactate uptake. It can be argued, however, that the absence of a FCCP effect may be due to the inability of the ionophore to generate an inside-positive H^+ -diffusion potential in these membrane vesicles. But this argument is untenable for the following reasons. Intestinal brush-border membrane vesicles have been shown to have a very low H^+ conductance (Murer *et al.*, 1975), and hence FCCP, a proton ionophore, is expected to increase the H^+ conductance and thus produce an inside-positive membrane potential. That FCCP did in fact generate an inside-positive H^+ -diffusion potential under the experimental conditions is evident from the FCCP-induced inhibition of the electrogenic glucose- Na^+ co-transport (Fig. 3b). Taken

collectively, these data demonstrate that either lactate- H^+ symport or lactate- OH^- antiport is responsible for the H^+ -gradient-dependent lactate uptake in intestinal brush-border membrane vesicles.

Effects of 4,4'-di-isothiocyanostilbene-2,2'-disulphonic acid (DIDS) and furosemide on lactate uptake

The effects of DIDS and furosemide, inhibitors of the anion-exchange process, on H^+ -gradient-dependent lactate uptake were then investigated. These compounds had no effect on lactate uptake (results not shown), indicating the non-involvement of DIDS- and furosemide-sensitive lactate- OH^- exchange in intestinal lactate transport.

Saturability of the intestinal lactate-transport system

The dependence of lactate uptake on lactate concentration was studied in the presence of an inward-directed H^+ gradient. The uptake rates were linear at least up to 45 s ($r = 0.96$), and therefore a 30 s incubation was used to determine the initial uptake rates for kinetic analysis. Fig. 4 shows that lactate transport in intestinal brush-border membrane vesicles is a saturable process. An Eadie-Hofstee plot of these data (Fig. 4, inset) gave an apparent K_t (concn. giving half-maximal transport) for lactate of 12.7 ± 4.5 mM and a V_{max} of 28.2 ± 8.0 nmol/30 s per mg of protein.

Inhibition of lactate uptake by monocarboxylates

Table 1 describes the effects of lactate, pyruvate, propionate and α -hydroxybutyrate on the H^+ -gradient-dependent uptake of radiolabelled lactate ($10 \mu M$). All these compounds, at a concentration of 20 mM, caused 40–60% inhibition, suggesting that these mono-

Fig. 4. Dependence of initial rates of lactate uptake on the concentration of lactate

Initial rates of lactate uptake were measured with 30 s incubations, in the presence of an inward-directed H^+ gradient ($pH_i = 7.5$; $pH_o = 5.5$). Lactate concentration range was 1–25 mM. Osmolality and pH of uptake buffers containing different concentrations of lactate were maintained constant by varying the concentrations of Tris and mannitol. Inset: Eadie-Hofstee plot; v , initial uptake rate (nmol/30 s per mg of protein); s , lactate concentration (mM).

Table 1. Inhibition of H^+ -gradient-dependent lactate uptake by various monocarboxylates

Brush-border membrane vesicles were preloaded with 20 mM-Hepes/Tris buffer, pH 7.5, containing 280 mM-mannitol. Initial uptake rates of radiolabelled lactate ($10 \mu M$) were measured with 30 s incubations in the presence of an inward-directed H^+ gradient ($pH_i = 7.5$; $pH_o = 5.8$). Final concentration of the monocarboxylates was 20 mM. When these inhibitors were present, osmolality and pH of the uptake medium were kept equal to those in control experiments by varying the concentrations of mannitol and Tris.

Monocarboxylate	Lactate uptake	
	(pmol/30 s per mg of protein)	(%)
Control	15.5 ± 0.4	100
Lactate	7.4 ± 0.3	48
Pyruvate	9.1 ± 0.1	59
Propionate	8.2 ± 0.3	53
α -Hydroxybutyrate	6.7 ± 0.3	43

Table 2. Effects of a H^+ gradient and a Na^+ gradient on lactate uptake in brush-border membrane vesicles prepared by a Ca^{2+} -precipitation technique

Uptake rates of lactate were measured with 45 s incubations. Final concentration of lactate was $540 \mu M$, of which the concentration of radiolabelled lactate was $10 \mu M$.

Incubation conditions	Lactate uptake	
	(nmol/45 s per mg of protein)	(%)
Effect of a H^+ gradient		
$pH_i = pH_o = 5.5$	0.23 ± 0.01	100
$pH_i = 7.5$; $pH_o = 5.5$	1.18 ± 0.04	513
Effect of a Na^+ gradient		
$[K^+]_i = 0$; $[K^+]_o = 100$ mM	0.11 ± 0.01	100
$[Na^+]_i = 0$; $[Na^+]_o = 100$ mM	0.26 ± 0.01	236

carboxylates may also be transported into the vesicles via the lactate-transport system.

Effects of a H^+ gradient and a Na^+ gradient on lactate uptake in brush-border membrane vesicles prepared by a Ca^{2+} -precipitation technique

All the experiments described so far in this paper were done with brush-border membrane vesicles isolated by a Mg^{2+} -precipitation technique. However, the previous studies by Hildmann *et al.* (1980) and by Storelli *et al.* (1980), describing Na^+ -lactate co-transport in small intestine, were done with brush-border membrane vesicles prepared by a Ca^{2+} -precipitation technique. Therefore we investigated the role of a H^+ gradient in lactate transport, using membrane vesicles which were isolated by a procedure similar to that described by those

investigators. Table 2 compares the effects of a H^+ gradient and a Na^+ gradient on lactate uptake in these membrane vesicles. As previously shown in the aforementioned studies, uptake of lactate was 2–3-fold greater in the presence of a Na^+ gradient than in the presence of a K^+ gradient. But a H^+ gradient markedly stimulated lactate uptake in these vesicles, as was the case in the vesicles isolated by a Mg^{2+} -precipitation technique. More importantly, lactate uptake in the presence of a H^+ gradient was 4–5-fold greater than in the presence of a Na^+ gradient.

These results demonstrate that an inward-directed H^+ gradient, not a Na^+ gradient, is the driving force for uphill transport of lactate in intestinal brush-border membrane vesicles. Since a Na^+/H^+ exchange system is present in rabbit small intestine (Tiruppathi *et al.*, 1988), it is very likely that the greater uptake of lactate in the presence of a Na^+ gradient than in the presence of a K^+ gradient is due to the Na^+ -gradient-induced generation of a H^+ gradient via the exchange system. Hildmann *et al.* (1980) have excluded this possibility, because they demonstrated that the presence of Na^+ enhances lactate uptake at a concentration of 0.3 mM even in the absence of a Na^+ gradient. However, when we measured lactate uptake in the presence of Na^+ , but in the absence of a gradient ($[Na^+]_i = [Na^+]_o = 100$ mM), the uptake was not significantly different from the uptake measured in the presence of K^+ ($[K^+]_i = [K^+]_o = 100$ mM). At a lactate concentration of 10 μ M, uptake was 2.1 ± 0.4 pmol/45 s per mg of protein in the presence of Na^+ and 2.2 ± 0.1 pmol/45 s per mg of protein in the presence of K^+ . Similar results were obtained at a lactate concentration of 0.5 mM (116.8 ± 10.0 pmol/45 s per mg of protein in the presence of Na^+ and 113.4 ± 0.7 pmol/45 s per mg of protein in the presence of K^+). The reasons for the discrepancies between our results and those of Hildmann *et al.* (1980) are not immediately apparent, but the present data strongly corroborate the conclusion that the stimulatory effect of a Na^+ gradient on lactate uptake observed in our studies and in the studies of Hildmann *et al.* (1980) may be an indirect effect of a Na^+/H^+ -exchanger-mediated, Na^+ -gradient-induced, H^+ gradient on the uptake of lactate.

The role of a H^+ gradient as the driving force for active transport of lactate in small intestine has physiological significance. In intestine, the pH in the close vicinity of the brush-border membrane is acidic compared with the pH of the bulk of the luminal solution, and this 'microclimate pH' has been determined to be in the range 5.5–6.0 (Lucas, 1984). But the intracellular pH of intestinal epithelial cells is approx. 7.0 (Kurtin & Charney, 1984). Therefore it is obvious that an inward-directed H^+ gradient exists across the small-intestinal

brush-border membrane *in vivo*. This H^+ gradient may provide the driving force for active absorption of lactate from the lumen as it does for active absorption of folate and small peptides.

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