Pflügers Arch (1982) 392:366–371

Pflügers Archiv
European Journal of Physiology

Asymmetry in the Transport of Lactate by Basolateral and Brush Border Membranes of Rat Kidney Cortex

M. Barac-Nieto[2,*], H. Murer[1], and R. Kinne[1,**]

[1] Max Planck Institut für Biophysik, D-6000 Frankfurt/Main, Federal Republic of Germany
[2] Physiology Section, University del Valle, Cali, Colombia

Abstract. The uptake of L(+)lactate into rat renal cortical brush border (BBV) and basolateral (BLV) membrane vesicles, isolated through differential centrifugation and free flow electrophoresis, were studied using a rapid filtration technique. In contrast to the lactate transport into the BBV, that into the BLV: 1) was found to proceed only towards equilibrium, 2) showed Na^+-independent coupling of the influx of L(+)lactate and the efflux of L(+) but not to the efflux of D(−)lactate, 3) was not inhibited by D(−)lactate, 2-thiolactate or 3-phenyl-lactate, but 4) was inhibited by 3-thiolactate and α-hydroxybutyrate and 5) was accelerated by changes in inwardly directed ionic gradients or by increases in cation conductance both of which led to increased intravesicular positivity. The latter changes had the opposite effect on the uptake of L(+)lactate by BBV. Thus, while the L(+)lactate transport system present in BBV showed the characteristics of Na-dependent electrogenic cotransport system, that in the BLV was consistent with a carrier mediated Na-independent, facilitated diffusion system.

Key words: Lactic acid – Anion – Basolateral – Brush border – Rat – Renal – Transport

Introduction

We have recently reported on the characteristics of the lactate-Na cotransport system present in the brush border membranes of rat kidney cortex [1]. In an attempt to understand more fully the handling of lactate by the renal proximal tubular cells we compare now the characteristics of the lactate transport across the basolateral plasma membrane with those of the brush border membranes. We present evidence for the existence of a specific transport system for the transfer of L(+)lactate across the basolateral membrane of the renal proximal cells. However and in contrast with the findings in the brush border membrane, the only force identified which was capable of driving this system was the electrochemical potential gradient for lactate across the membrane.

Send offprint requests to M. Barac-Nieto's present address
* Present address: University of Rochester, Physiology Department, Box 642, Rochester, New York 14642, USA
** Present address: Albert Einstein College of Medicine, Department of Physiology. 1300 Morris Park, Bronx, New York 10401

Methods

Brush border and basal-lateral membrane vesicles from rat renal cortical cells were isolated by differential centrifugation and free flow electrophoresis as described elsewhere [7]. Na + K ATPase specific activity (2.5 ± 0.29 U/mg, $n = 12$) was enriched by 22 ± 1.3 fold in the basal lateral membrane fraction while alkaline phosphatase (12.5 ± 1.1 U/mg, $n = 12$) was enriched by 12 ± 0.9 fold in the brush border membrane fraction with respect to the values in the initial renal cortex homogenate.

The rapid filtration technique used for the study of lactate uptake into the vesicles has been previously described [1]. Briefly, aliquots (20 µl) of a membrane suspension (5 mg of membrane protein/ml) in 0.05 M mannitol, 0.02 M HEPES-Tris, pH 7.4 (M-HT buffer), were rapidly mixed with 100 µl of incubation medium at 25° C. The incubation media contained 0.05 M mannitol, 0.02 M HEPES-Tris, pH 7.4, [1-^{14}C] L(+)lactate and different salts as indicated in each case; 20 µl samples of the incubate were taken at different time intervals. The sampled aliquots were delivered onto wetted callulose nitrate filters (0.6 µm pore diameter) under vacuum (710 torr below atmospheric), washed free of extravesicular radioactivity with 5 ml of cold "washing" buffer (0.05 M Na_2SO_4, 0.05 M mannitol, 0.02 M HEPES-Tris, pH 7.4) containing phloretin (0.1 mM) and mercuric acetate (5×10^{-5} M). The filters were counted for ^{14}C in Triton X100-Toluene fluor by liquid scintillation. The recovered radioactivity was corrected for radioactivity in the blanks. The blanks were incubation media (100 µl), to which M-HT buffer (20 µl) instead of vesicle suspension had been added. Aliquots (20 µl) of the blanks were filtered and the radioactivity retained on the filters after washing with cold "washing" buffer taken as the radioactivity in the blanks. Aliquots of the membrane suspension were assayed for protein [9] and the amount of membrane protein added onto the filters was calculated. The radioactivity retained by the filters was thus expressed for mg of membrane protein and then converted to µmoles of lactate retained in the vesicles per mg of membrane protein on the basis of the specific activity of the ^{14}C-lactate, determined in 5 µl aliquots of the incubation media [1]. Uptake of L(+)lactate into basolateral and brush border vesicles was the same after 30 or 60 min of incubation under the various conditions here reported (equilibrium). At equilibrium, the intravesicular distribution volumes for L(+)lactate in basolateral (2.1 ± 0.2 µl/mg protein) and brush border (3.2 ± 0.4 µl/mg protein) vesicles ($n = 12$) were not significantly different than those simultaneously determined for glucose. The similarities occurred both in the presence and in

0031-6768/82/0392/0366/$01.20

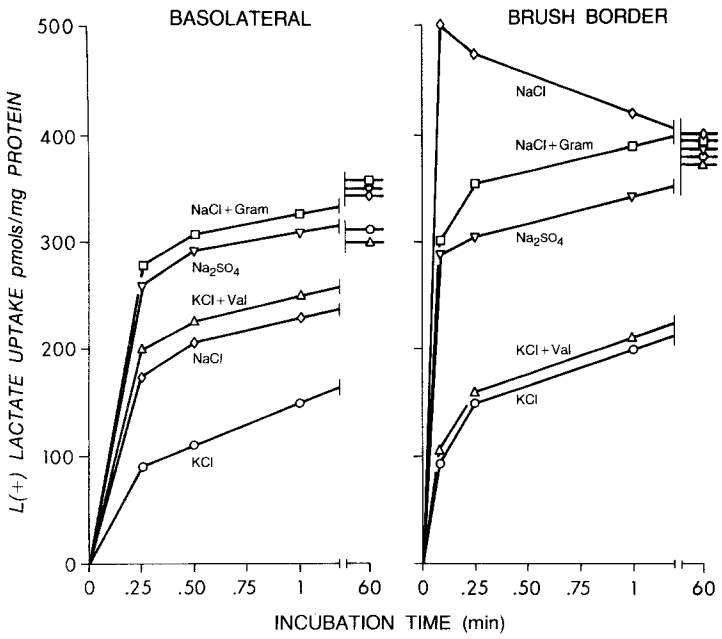

Fig. 1
Effects of ionophores and of different inwardly directed ionic gradients on the uptake of L(+)lactate into basolateral and brush border membrane vesicles. Uptake of 0.1 mM [1-^{14}C]-L(+)lactate into vesicles loaded with 0.05 M mannitol, 0.02 M HEPES-Tris, pH 7.4 buffer (M-HT buffer) and incubated in 100 mM NaCl, or 100 mM KCl or 50 mM Na_2SO_4 in M-HT buffer were measured. Samples were taken at the indicated times after the start of incubation. Valinomycin (5 μg/mg protein) or gramicidin-D (8 μg/mg protein) were added where indicated, as ethanolic solutions (final ethanol concentration 1%). Ethanol alone was added to the controls. The average of duplicate determinations in a single membrane preparation are shown. In two additional membrane preparations qualitatively similar results but with different absolute uptake values (0.5 to 2.5 fold range) were observed

the absence of inwardly directed salt (NaCl, KCl, choline chloride) gradients. Since glucose is known to enter an osmotically responsive intravesicular space [8] these observations indicate that no binding of L(+)lactate to the membrane vesicles occurs. Thus it was the uptake of lactate across the membrane of the vesicles which was studied. Means ± one standard error of the mean are presented. All experiments were the result of duplicate determinations in at least three different membrane vesicle preparations. A change was considered to be statistically significant when $P<0.05$ (Student t-test). Where data from a single duplicate experiment are presented, similar results were obtained in at least two additional membrane vesicle preparations.

Results

Uptake of L(+)lactate (0.1 mM) into baso-lateral membrane vesicles in the presence of an inwardly directed 100 mM NaCl gradient was more rapid than in the presence of a similar KCl gradient (Fig. 1). However in contrast to the L(+)lactate uptake into brush border membrane vesicles, the L(+)lactate uptake into BLV did not exceed the final equilibrium value: i.e. there was no concentrative uptake. Also, in contrast with the L(+)lactate uptake into brush border vesicles, uptake into baso-lateral vesicles in the presence of an inwardly directed NaCl gradient was increased by pretreatment of the membrane suspension with gramicidin-D (8 μg/mg protein) indicating that increases in the cation conductance and hence in the inside positive Na^+ diffusion potential led to increases in L(+)lactate uptake in basolateral but to decreased uptake in brush border membrane vesicles (Fig. 1). In the presence of an inwardly directed KCl gradient (100 mM) L(+)lactate (0.1 mM) uptake into basal lateral membrane vesicles was increased by pretreatment of the vesicles with valinomycin (5 μg/mg protein) while a similar pretreatment of the brush border membranes had little effect on the uptake of L(+)lactate (Fig. 1).

In addition, uptake of L(+)lactate into basal-lateral membrane vesicles was more rapid in the presence of an inwardly

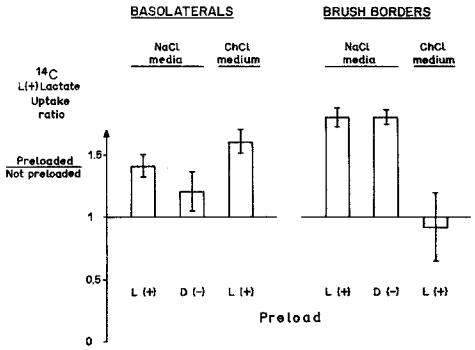

Fig. 2. Effects of lactate preloading on the uptake of L(+)lactate into basolateral and brush border membrane vesicles in the presence of NaCl, or choline chloride. Membrane vesicles loaded with 100 mM mannitol, 25 mM KCl and 20 mM HEPES-Tris were preincubated for 1 h at 25° C in 75 mM NaCl (or choline chloride) 25 mM KCl, 100 mM mannitol, 20 mM HEPES-Tris pH 7.4 with or without D(−) or L(+)lactate (1 mM) and incubated at 25° C in identical solutions but with ^{14}C-L(+)lactate. Uptake (at 5 s) of L(+)lactate was determined as explained in the text. The ratios of the uptake into vesicles preloaded with lactate with respect to those not preloaded, under otherwise identical incubation conditions, are illustrated for each condition used. Equilibrium (60 min) L(+)lactate uptake values in basolateral vesicles were 2.1 ± 0.1 pmoles/μg protein (NaCl) and 1.8 ± 0.1 pmoles/μg protein (choline chloride). Corresponding values in brush border membranes were 3.6 ± 0.2 and 3.2 ± 0.3 respectively. Lactate uptake at 5 s in vesicles not preloaded with lactate were 13 ± 2% (NaCl) and 13 ± 1% (choline chloride) of equilibrium values in basolateral membranes. Corresponding values in brush border membranes were 75 ± 3% (NaCl) and 55 ± 2% (choline chloride). The means and S.E. of duplicate determinations in three different membrane preparations are shown

directed 50 mM Na_2SO_4 gradient than in the presence of a similar 100 mM NaCl gradient while the opposite occurred with the brush border membrane vesicles (Fig. 1). Thus, uptake of L(+)lactate into basal-lateral membrane vesicles was accelerated by changes in ionic gradients and by changes in cationic conductance which led to increased intravesicular positivity while such conditions inhibited the sodium de-

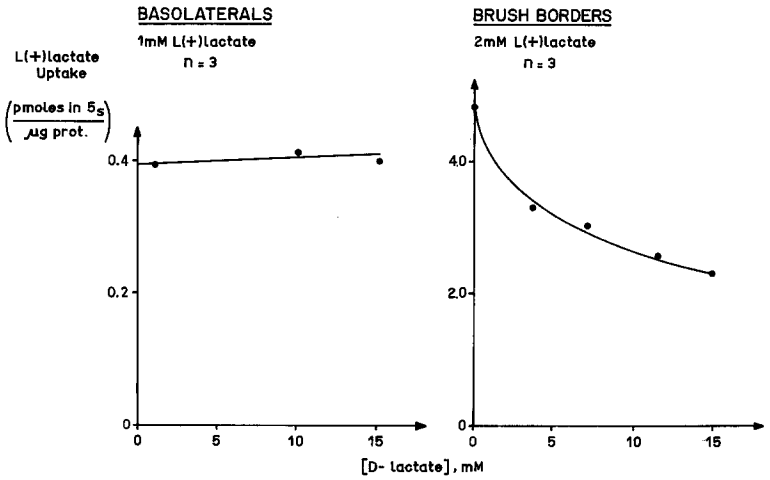

Fig. 3
Effects of D(−)lactate on the uptake of L(+)lactate in basolateral and brush border membrane vesicles of rat kidney cortex. Uptake at 5 s of [1-^{14}C] L(+)lactate incubated in the presence of 100 mM NaCl in M-HT buffer were determined as described in the text, at varying medium D(−)lactate concentrations and at the L(+)lactate concentrations indicated. The averages of duplicate determinations in a single membrane preparation are shown. Three additional experiments with different membrane preparations showed qualitatively similar results but with different solute uptake values (0.75 to 1.8 fold range)

pendent L(+)lactate uptake into brush border membrane vesicles.

Uptake of 1 mM 1-^{14}C-L(+)lactate into brush border membrane vesicles preloaded with 1 mM unlabelled L(+)lactate was faster in vesicles pre-equilibrated with 100 mM NaCl than in those pre-equilibrated with 100 mM choline chloride (Fig. 2). By contrast uptake of [1-^{14}C]-L(+)lactate into basolateral plasma membrane vesicles preloaded with 1 mM L(+)lactate was independent of the cation (Na^+ or choline) present, but always faster than into vesicles not preloaded with 1 mM L(+)lactate (Fig. 2). Thus, tracer exchange of L(+)lactate in basolateral membrane vesicles was shown to occur and was independent of Na^+ while this same process in brush border membranes only occurred in the presence of Na^+.

Also, the net uptake (at 5 s) of 1 mM ^{14}-L(+)lactate into basolateral membrane vesicles (not preloaded with lactate) was independent of the cation with which the vesicles had been preequilibrated (100 mM NaCl or choline chloride) and amounted to $13 \pm 1\%$ ($n = 6$) of the final lactate equilibrium uptake values (2.1 ± 0.1 pmoles/µg protein, $n = 3$ in NaCl; 1.8 ± 0.1 pmoles/µg protein, $n = 3$ in choline chloride). Thus neither the net uptake (at 5 s) nor the unidirectional ^{14}C-L(+)lactate influx into the basolateral membrane vesicles were altered by the presence of Na^+ instead of choline (equilibrated).

Taken together, the preceeding observations indicate that the lactate transport system present at the basolateral plasma membrane is carrier mediated since it exhibits coupling of tracer fluxes but is independent of Na^+ while lactate transport at the brush border membrane shows the characteristics of a Na^+-gradient driven electrogenic cotransport system.

Uptake of 1 mM [1-^{14}C]-L(+)lactate into basolateral membrane vesicles was not significantly altered by preloading of the vesicles with 1 mM unlabelled D(−)lactate (Fig. 2). The ^{14}C-L(+)lactate uptake ratio [preloaded with D(−)/not preloaded] was not significantly different from 1. By contrast uptake of [1-^{14}C]-L(+)lactate into brush border membrane vesicles was accelerated by such a preloading with D(−)lactate when Na^+ (100 mEq/l) was present in the system (Fig. 2).

In addition uptake of [1-^{14}C]-L(+)lactate (1 mM) into basolateral membrane vesicles in the presence of an inwardly directed 100 mM NaCl gradient was unaltered by the presence of either 5, 10 or 15 mM D(−)lactate in the incubation media while uptake of [1-^{14}C]-L(+)lactate into brush border vesicles was progressively inhibited by increasing concentrations of D(−)lactate (Fig. 3, Ref. [1]). Thus L(+)lactate uptake into basolateral membrane vesicles was independent of D(−)lactate (up to 15 mM) while that into brush border vesicles was inhibited by the presence of D(−)lactate on the cis-side or was stimulated by D(−)lactate at the trans-side, when Na^+ was present in the medium. Competition between L(+) and D(−)lactate in the brush border membranes could arise from interaction at a common transport site or from sharing of a common driving force: the electrochemical Na^+ gradient. However, the observed transstimulation of ^{14}C-L(+)lactate influx by D(−)lactate, in the absence of an electrochemical gradient for Na^+, indicates that they interact at a common transport site. In sum, the lactate transport system in the basolateral membrane seems to be stereospecific for the L(+)enantiomorph while that in the brush border membrane is shared by L(+) and D(−)lactate which compete effectively for the system.

Further characterization of the two systems revealed that while that in the basolateral membranes showed a linear dependency of L(+)lactate uptake (at 5 s) upon the medium L(+)lactate concentration (up to 15 mM), L(+)lactate uptake in the brush border membrane had a dual behavior: in the absence of sodium (100 mM choline chloride gradient) the uptake rates were similarly linearly related to the medium L(+)lactate concentration; in the presence of sodium the uptake was saturable (Fig. 4).

L(+)lactate uptake into both brush border and basolateral membrane vesicles (in the presence of a 100 mM NaCl gradient directed towards the inside of the vesicles) were inhibited by phloretin 0.5 mM, mercuric acetate (0.5 mM) and 4 acetamido 4′-isothiocyanostylbene 2 2′disulfonic acid (1 mM) although with differing potencies (Table 1).

Uptake of L(+)lactate (1 mM) into basolateral but not into brush border membrane vesicles – incubated with a 100 mM inwardly directed NaCl gradient – was inhibited by 20% by 5 mM 3-thiolacetate or by 5 mM DL-α-hydroxybutyrate (Table 1). Uptake of L(+)lactate (1 mM) into brush border but not into basolateral membrane vesicles – in the presence of a 100 mM inwardly directed NaCl gradient – was inhibited by about 50% by the presence of 10 mM D-lactate, 5 mM phenyllactate or 2 mM 2-thiolactate in the media (Table 1).

The uptake of D(−)[1-^{14}C]lactate (2 mM) across the brush border membrane vesicles in the presence of an inwardly

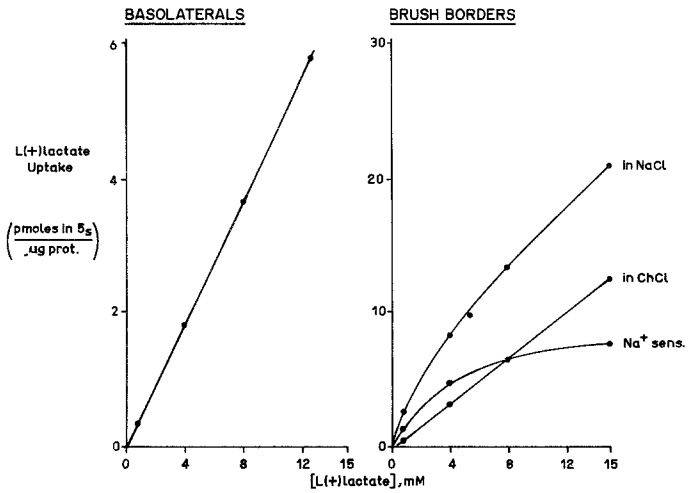

Fig. 4
Comparison of the concentration dependency of the uptakes of L(+)lactate into basolateral an brush border plasma membrane vesicles of rat kidney cortex. Uptake (5 s) of 1-^{14}C-L(+)lactate into the membrane vesicles was determined as explained in the text, at different medium L(+)lactate concentrations. Choline chloride (100 mM) or NaCl (100 mM) were present in the incubation media (M-HT buffer). The Na sensitive uptake represents the difference between uptake in NaCl versus uptake in choline chloride medium at each medium lactate concentration. The averages of triplicate determinations in a single membrane preparation are presented. In two additional membrane preparations qualitatively similar results were observed but absolute uptake values were different by 0.5 to 2 fold

Table 1. [1-^{14}C]Lactate uptake [D(−) or L(+)] into vesicles incubated in the presence of inwardly directed 100 mM NaCl gradients in M-HT buffer and the indicated inhibitor concentrations were compared to those in the absence of inhibitor. Average percentage inhibition observed in duplicate determinations with three different membrane preparations are presented. * $P < 0.05$ with respect to 0

Lactate isomer	mM	Inhibitor tested	mM	% Inhibition of uptake at 5 s with respect to paired controls (n = 3) without inhibitor	
				Basolateral	Brush borders
L(+)	2	Phloretin	0.5	50 ± 10*	60 ± 8*
D(−)	2	Phloretin	0.5	0 ± 6	48 ± 6*
L(+)	1	Hg Acetate	0.5	40 ± 8*	90 ± 10*
L(+)	1	SITS	1	61 ± 7*	40 ± 5*
L(+)	1	3-Thiolactate	5	23 ± 6*	3 ± 8
L(+)	1	α-OH butyrate	5	21 ± 7*	−2 ± 7
L(+)	1	D-Lactate	10	4 ± 8	50 ± 6*
L(+)	1	2-Thiolactate	2	5 ± 8	50 ± 7*
L(+)	1	3-Phenyllactate	5	2 ± 6	42 ± 5*
L(+)	1	Pyruvate	5	3 ± 5	4 ± 6
L(+)	1	Proprionate	5	3 ± 6	4 ± 5

directed 100 mM NaCl gradient, was inhibited (50%) by phloretin (0.5 mM) while that into basolateral membrane vesicles was not altered (Table 1).

Thus, the lactate transport systems in basolateral and brush border membrane vesicles showed different inhibitor specificities. In sum, the different Na dependency, stereospecificity and inhibitor specificity indicate that there is an asymmetry in the characteristics of the lactate transport systems at the two poles of the renal proximal cells: the luminal membrane has a Na-dependent electrogenic L(+)lactate cotransport system which can be shared by D(−)-lactate and is inhibited by 2-thiolacetate and 3-phenyllactate. At the antiluminal membrane there is a carrier mediated stereospecific L(+)lactate transport system which showed a different inhibitor specificity than that in the brush border membranes and which was driven by the electrochemical potential gradient for L(+)lactate.

Discussion

Coupling of Tracer Fluxes. The uptake of tracer L-lactate into basolateral membrane vesicles occurs more rapidly when it occurs in exchange for unlabelled L-lactate than when net uptake of L-lactate occurs. This is consistent with the coupling of influx of ^{14}C-L(+)lactate to the efflux of L(+)lactate and thus with participation of a system different from simple diffusion in the transfer of L-lactate across the basolateral plasma membranes.

However, a higher uptake (at 5 s) of ^{14}C-L(+)lactate in basolateral vesicles preloaded with L(+)lactate than in vesicles not preloaded could also result from minimization of the ^{14}C-L(+)lactate efflux in the preloaded vesicles due to dilution of the intravesicular ^{14}C-L(+)lactate by the unlabelled L(+)lactate. That such a dilution cannot account for our observations is indicated by the following estimates: at 5 s lactate uptake into basolateral vesicles equilibrated with NaCl or with choline chloride was 13% of the final equilibrium concentration (1 mM). Since fluxes were found to be proportional to the lactate concentration (Fig. 4), efflux of lactate at 5 s represented 13% of influx and net uptake was 100 − 13 = 87% of influx. Thus the ratio of influx (100%) to net uptake (87%) was 1.15. This ratio represents an overestimate since at times before 5 s, efflux was most surely less than 13% of influx. The observed ratio of unidirectional ^{14}C-L(+)lactate influx (into preloaded vesicles)/net uptake (into not preloaded vesicles) at 5 s were 1.40 and 1.62 in the presence of NaCl or choline chloride respectively, higher than the maximum estimate possibly due to differences in ^{14}C-L(+)lactate efflux. Thus, we view the higher uptake of ^{14}C-L(+)lactate into vesicles preloaded with L(+)lactate than in those not preloaded, as representing mostly coupling of the tracer fluxes of lactate across the basolateral membrane.

Inhibition. While phloretin inhibits the transfer of L-lactate (Table 1) and D-glucose [8] across the basolateral plasma membranes it does not alter the uptake of D-lactate (Table 1), thus indicating a specific inhibitory effect on the L-lactate and the D-glucose transport systems. These effects of phloretin contrast with those observed in the brush border membrane vesicles where both the uptake of D- and L-lactate where partially inhibited by phloretin (Table 1).

In contrast to the observations with BBV, in basolateral membrane vesicles trans-stimulation of L-lactate uptake

occurred specifically with L-lactate but not with D-lactate preloading. In addition D-lactate did not inhibit L-lactate uptake into basolateral plasma membrane vesicles. Thus, the transport system present in these BLV seems to be stereospecific for the L(+)enantiomorph in contrast to the transport systems present in the brush border membrane [1], and in red cells [4].

D(−)lactate or other monocarboxylates which may be contransported with Na^+ in the brush border vesicles, could inhibit L(+)lactate cotransport either by competing for the same transport path or by promoting the dissipation of the NaCl gradient, the major driving force for these systems. However, the observation that D(−)lactate transstimulates with L(+)lactate in the presence of Na^+ but in the absence of a Na^+ gradient, suggests that at least this monocarboxylate, shares the same transport system with L(+)lactate in the brush border membrane. In the basolateral membranes the uptake of L(+)lactate was only slightly inhibited by certain monocarboxylates (α-hydroxy-butyrate, 3-thiolacetate) but not at all by others [D(−)lactate, 2-thiolactate, 3-phenyllactate]. It is possible that this system has very specific steric requirements for transport or that while some monocarboxylates enter fast enough to alter (inside more negative) the electrical potential across the membrane and thus inhibit the entry of the lactate anion, others enter very slowly (D-lactate, 3-phenyllactate, 2-thiolactate) and thus do not alter this driving force for L(+)lactate entry. Our present data do not allow us to differentiate between those possibilities but do indicate that whatever the mechanism(s) by which these monocarboxylate exert their inhibitory effect on the basolateral membranes these are different from those observed on the uptake of L(+)lactate in the brush border membranes.

Saturability. The transfer of L-lactate across the basolateral plasma membrane vesicles could occur through a low affinity high capacity system since no evidence for saturation was obtained up to medium lactate concentrations of 12 mM. Unspecific effects of lactate on the glucose distribution space at high lactate concentrations (30 and 60 mM) precluded valid observations of lactate uptake at these levels. The studied concentration range covers howevers the levels of physiological interest at which the system does not show evidence of saturation whether in Na^+ or in choline containing media. By contrast, the brush border lactate transport system does saturate in the presence of Na^+ but not in its absence.

The following observations could be taken as evidence for simple diffusion of L(+)lactate across the basolateral membrane: 1) no evidence for saturation of L(+)lactate, 2) analogues of L(+)lactate such as D(−)lactate, 2-thiolactate or 3-phenyllactate did not inhibit L(+)lactate uptake, 3) other analogues (3-thiolactate, 2-hydroxy-butyrate) had moderate inhibitory effects which could be due to electrical effects of those anions or the transmembrane electrical potential. However, the observed coupling of tracer L(+)lactate fluxes and the specific effect of phloretin on the uptake of L(+)lactate and not on that of D(−)lactate point towards a mechanism different from simple diffusion for the transport of L(+)lactate in basolateral membrane vesicles.

Driving Forces. No evidence was obtained for direct coupling of the L-lactate fluxes across the basolateral plasma membrane with those of Na^+. Both the unidirectional influx of ^{14}C-L(+)lactate and the net L(+)lactate uptake into basolateral vesicles equilibrated with NaCl or choline chloride were independent of the cation present. These observations indicate the absence of a direct Na^+ dependency of either the permeability of or any transport system for L(+)lactate present in the basolateral membrane vesicles.

However, the flux of lactate was accelerated by the dissipation of a sodium gradient (Fig. 1). In addition our observations with gramicidin and valinomycin (Fig. 1) indicate that increase in inside positivity (or decreases in inside negativity) promote the entry of L(+)lactate into the basolateral vesicles. Thus, if dissipation of a NaCl gradient results in a larger inside positive electrical potential (or a lesser inside negative potential) than dissipation of a KCl gradient, the L(+)lactate uptake could be accelerated by a Na^+ gradient. This would require that the Na^+ conductance exceeds the K^+ conductance of the membrane, opposite to the characteristics of the basolateral membrane in situ. This altered conductance could be an artefact due to the isolation procedure: the basolateral membrane preparation is known to include varying proportions of open flat membranes and vesicles of inside out and right side out orientation which may have altered ionic permeability [8]. Also, in the basolateral membrane vesicles uptake of lactate never exceeded the final equilibrium uptake values. This could be due to the absence of an adequate driving force capable of inducing concentrative lactate uptake (such as a sufficient electrical potential or an adequate chemical gradient of a counterfluxing anion) or to very rapid dissipation of these gradients due to a large passive permeability of the vesicles membrane. Our observations in vesicles equilibrated with Na^+ or choline chloride and those with valinomycin and gramicidin suggest however that the lactate uptake in basolateral membrane vesicles is not Na^+ dependent and thus that NaCl gradients could influence lactate uptake through electrical coupling of the lactate fluxes to electrical diffusion potential generated upon dissipation of the NaCl gradient. Alternatively if a Na-H exchange system were to occur in the basolateral membranes, dissipation of a NaCl gradient by promoting H^+ extrusion from the vesicles, could generate pH gradients which could induce non-ionic diffusion of lactic acid into the vesicles. However there is as yet no evidence for the existence of a Na-H exchange system in the basolateral membrane.

Thus, the major identified driving force for the transfer of L-lactate across the basolateral membrane in this study was the electrochemical potential gradient for L-lactate. However, the possibility of counter-flux of L(+)lactate with other anions or the influence of pH gradients have not been eliminated. This is again in contrast with the system in the luminal membranes where the electrochemical potential gradient for sodium was shown to be the major driving force for concentrative lactate transport.

Relationship to Lactate Handling by the Kidney and Intestine. Taken together, the characteristics of the brush border lactate sodium cotransport system, described before [1] and the characteristics of the L-lactate transport system present in basolateral plasma membrane, here described, the following model of lactate handling in renal cells can be suggested. Reabsorption of lactate from the tubular lumen across the brush border membrane can occur against an electrochemical potential gradient [5], the driving force resulting from the coupling with the dissipative flux of sodium across that membrane. Thus, the reabsorptive flux of lactate across the luminal membrane is largely independent of the cellular

lactate concentration and of the metabolic utilization of lactate [2].

By contrast, the direction of the net lactate flux across the peritubular membrane will depend on the direction of the existing electrochemical potential gradient for lactate across the basal-lateral membrane. The electrical potential across the basolateral membranes always favors lactate efflux from the cell. The lactate concentration gradient across this membrane will depend on the relative rates of lactate reabsorption through the luminal cotransport system and of intracellular lactate utilization (in addition to the blood lactate level). Either net transcellular flux of the lactate reabsorbed which did no enter metabolism could occur or, additional facilitated uptake of lactate into the cells from the peritubular spaces could prevail when the rate of lactate metabolism is relatively high [3]. In the latter case, the cystolic lactate level should be maintained at a sufficiently low level with respect to that in the peritubular fluid ($\simeq 1/15$), so as to determine a net electrochemical potential gradient for lactate directed towards the inside of the cell. This could occur when the rate of lactate utilization exceeds that of its reabsorption [3]. It remains to be see if factors other than the electrochemical potential gradient for lactate, such as anion exchanges [6] or H^+ lactate symport [10] could influence the lactate fluxes across the peritubular membrane of the cells. Thus, the proximal tubular cells could either use more lactate than that filtered, or conserve whatever lactate is reabsorbed and is not metabolized [2]. A similar system has been recently described in small intestinal cells [11]. Both in the proximal tubule and the small intestine, luminal transport of lactate is achieved through Na-coupled cotransport systems while transport at the basal-lateral membranes occurs through facilitated diffusion systems similar to that existing in red cells [4].

However, the proximal tubular lactate transport systems were not identical to those in intestine. While the renal cotransport of lactate with Na^+ at the brush border membrane was electrogenic [1] that in the intestinal microvilli was not [11]. Also, the inhibitor specificity of the lactate transport system in basolateral membranes of intestinal cells [11] was not the same as that in the BLV of renal proximal cells, here described. Isolation and characterization of the transport systems would be required to understand the molecular basis for these differences.

We wish to acknowledge the encouragement and support of Prof. Dr. K. J. Ullrich.

References

1. Barac-Nieto M, Kinne R, Murer H (1980) Lactate sodium cotransport in rat renal brush border membranes. Am J Physiol 239: F496–506
2. Brand PH, Cohen JJ, Bignall MC (1974) Independence of lactate oxidation from net Na^+ reabsorption in the dog kidney in vivo. Am J Physiol 227:1255–1262
3. Cohen JJ, Merkens LS, Peterson OW (1980) Relation of Na^+ reabsorption to utilization of O_2 and lactate in the perfused rat kidney. Am J Physiol 238: F-415–427
4. Deuticke B, Rickert F, Beyer E (1978) Stereoselective SH-dependent transport of lactate in mammalian erythrocytes. Biochem Biophys Acta 507:137–155
5. Hoehmann B, Frohnert PP, Kinne R, Baumann K (1977) Proximal tubular lactate transport in rat kidney. Pflügers Arch 370:156–171
6. Kastendieck E, Moll W (1977) The placental transfer of lactate and bicarbonate in the guinea-pig. Pflügers Arch 370:156–171
7. Keljo DJ, Kleinzeller A, Murer H, Kinne R (1978) Is hexokinase present in the basal lateral membranes of rat kidney proximal tubular epithelial cells? Biochem Biophys Acta 508:500–512
8. Kinne R, Murer H, Kinne-Saffran E, Thees M, Sachs G (1975) Sugar transport by renal plasma membrane vesicles. Characterization of the systems in the brush border microvilli and basal lateral plasma membranes. J Memb Biol 21:375–395
9. Lowry OH, Rosenbrough NJ, Farr AL, Randall J (1951) Protein measurements with the Folin phenol reagent. J Biol Chem 193:265–275
10. Spencer TL, Lehniger AL (1976) L-lactate transport in Ehrlich ascites tumor cells. Biochem J 154:405–415
11. Storelli C, Corcelli A, Cassano G, Hildmann B, Murer H, Lippe L (1980) Polar distribution of sodium-dependent and sodium independent transport systems for L-lactate in the plasma membrane of rat enterocytes. Pflügers Arch 388:11–16

Received May 21 / Accepted November 25, 1981