Renal transport of monocarboxylic acids

Heterogeneity of lactate-transport systems along the proximal tubule

Karl Evald JØRGENSEN and M. Iqbal SHEIKH*

Institute of Medical Biochemistry, University of Aarhus, DK-8000 Aarhus C, Denmark

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The characteristics of D- and L-lactate transport in luminal-membrane vesicles derived from whole cortex, from the pars convoluta and from the pars recta of rabbit kidney proximal tubule were studied. It was found that uptake of both isomers in vesicles from whole cortex occurred by means of dual electrogenic transport systems, namely a low-affinity system and a high-affinity system. Uptake of both isomers in vesicles from the pars recta was strictly Na^+ -dependent and is mediated via a single high-affinity common transport system. Vesicles from the pars convoluta contained a cation-dependent but Na^+ -unspecific low-affinity common transport system for these compounds. The physiological importance of this system is briefly discussed.

Lactate transport has been studied in tissues such as erythrocytes (Aubert & Motais, 1975; Deuticke, 1977, 1980, 1982; Deuticke *et al.*, 1978), liver cells (Schwab *et al.*, 1979), Ehrlich ascites cells (Spencer & Lehninger, 1976), small intestine (Hildmann *et al.*, 1980; Storelli *et al.*, 1980) and kidney tissue, where it has been shown to be confined exclusively to the proximal tubule (Dies *et al.*, 1969; Höhmann *et al.*, 1974). The mechanism of lactate re-absorption across the luminal membrane of the proximal tubule has been examined by using vesicles prepared from whole cortical tissue (i.e. containing vesicles from both the pars convoluta and the pars recta of the proximal tubule) of rat (Barác-Nieto *et al.*, 1980), rabbit (Nord *et al.*, 1983) and horse kidney (Mengual & Sudaka, 1983; Mengual *et al.*, 1983). The results of these studies indicated the presence of an Na^+ -dependent electrogenic transport process for L- and D-lactate in luminal-membrane vesicles. Furthermore, it was reported that L-lactate transport in these vesicle preparations occurred by means of a single strictly Na^+ -specific system.

More recently, Turner & Moran (1982) and Sheikh and co-workers have studied the characteristics of D-glucose (Kragh-Hansen *et al.*, 1984), L- and D-serine (Kragh-Hansen & Sheikh, 1984), L-phenylalanine (Kragh-Hansen *et al.*, 1984) and L-proline, hydroxy-L-proline and 5-oxoproline (Røigaard-Petersen & Sheikh, 1984) transport by rabbit luminal-membrane vesicles isolated from two

anatomically different regions of the proximal tubule, namely the pars convoluta and the pars recta. Interestingly, the results of all these studies revealed the existence of more than one transport system for the re-absorption of these compounds in the kidney tubule.

In the present investigation we examined the tubular localization of the Na^+ -dependent L- and D-lactate-transport system along the rabbit kidney proximal tubule by the use of luminal-membrane vesicles derived from the pars convoluta and the pars recta, using the method recently described from this laboratory (Kragh-Hansen *et al.*, 1984). By contrast with the previous observations, we now present clear evidence for the existence of dual electrogenic transport systems for lactate, which can be characterized in terms of affinity, specificity and cation-dependence, and are localized in luminal-membrane vesicles from two different regions of the proximal tubule.

Experimental

Preparation of membrane vesicles

Luminal-membrane vesicles were prepared from whole cortex, from the pars convoluta and from the pars recta of the proximal tubule of rabbit kidney by the method already described (Kragh-Hansen *et al.*, 1984). The purity of membrane preparations from the pars convoluta and the pars recta was examined by electron microscopy and by measuring specific activities of various markers as previously described (Sheikh *et al.*, 1982). The

* To whom correspondence should be addressed.

amount of protein was determined by the method of Lowry *et al.* (1951) as modified by Petersen (1977), with human serum albumin as standard.

Uptake experiments

Uptake of L- and D-lactate by various vesicle preparations was examined by a spectrophotometric method with potential-sensitive carbocyanine dye as previously described (Kragh-Hansen *et al.*, 1982a,b).

Results

Uptake of lactate by vesicles from whole renal cortex

In preliminary experiments (results not shown) we found that addition of L- and D-lactate to luminal-membrane vesicles/dye suspension in the presence of an Na^+ gradient (extravesicular > intravesicular) resulted in absorbance changes (ΔA) indicative of depolarizing event(s) (for details see Kragh-Hansen *et al.*, 1982a,b) strongly suggesting the existence of electrogenic transport system(s) for these compounds. Figs. 1(a) and 1(b) show the absorbance changes induced by addition of increasing concentration of L-lactate and D-lactate respectively to luminal-membrane vesicles from whole cortex, in the presence of an NaCl gradient. The insets in the Figures show Eadie-Hofstee analysis of the experimental data. By contrast with previous observations (Barnieto *et al.*, 1980; Nord *et al.*, 1983), curvilinear plots were obtained with both L-lactate and D-lactate, which indicates the presence of multiple transport systems in vesicles from whole cortex for the uptake of these compounds. The spectrophotometric data were analysed as already described (Kragh-Hansen & Sheikh, 1984). The following K_A values were obtained: for L-lactate, $K_A^1 = 0.25 \pm 0.02 \text{ mM}$ and $K_A^2 = 42.4 \pm 4.5 \text{ mM}$; for D-lactate, $K_A^1 = 0.34 \pm 0.02 \text{ mM}$ and $K_A^2 = 41.7 \pm 4.2 \text{ mM}$. We have also determined the K_A values for these compounds by using initial rates of uptake (4 s uptake value) instead of measuring the peak value of the 'overshoot'. This resulted in more-scattered data but gave very similar K_A values. Furthermore, we have previously tested the validity of the kinetic parameters obtained by spectrophotometric measurements by using the Millipore filtration technique and found that the two different methods are equally good for the determination of the kinetic parameters (Jrgensen *et al.*, 1983).

Uptake of lactate by vesicles from the pars recta

Figs. 2(a) and 2(b) show the absorbance changes induced by addition of L-lactate and D-lactate

Fig. 1. Kinetics of L-lactate and D-lactate uptake by luminal-membrane vesicles from whole renal cortex. The uptake of increasing concentrations of L-lactate (a) and D-lactate (b) by luminal-membrane vesicles prepared from whole cortex, as registered by the potential-sensitive dye 3,3'-diethyloxadicarbocyanine iodide, is shown. Common experimental conditions: protein concentration 0.25 mg/ml, pH 7.5, temperature 20°C, dye concentration 15 μM . The intravesicular medium was 310 mM-mannitol/15 mM-Hepes/Tris buffer [Hepes is 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid]. The results shown in the Figure are the absorbance changes obtained with an external medium of 155 mM-NaCl/15 mM-Hepes/Tris buffer minus the absorbance changes induced by adding a small volume of 15 mM-Hepes/Tris buffer alone (the medium of the solute's stock solutions). The spectrophotometer was operated in the dual-wavelength mode with 580 nm and 610 nm (reference wavelength). In the insets the results are shown in Eadie-Hofstee plots. v represents the absorbance change at substrate concentration $[S]$. Results are given as means $\pm$ S.D. for four experiments.

respectively to luminal-membrane vesicles from the pars recta in the presence of an NaCl gradient (curves 1) or a KCl gradient (curves 2). It is seen that both compounds depolarize the membrane vesicles to various extents in the presence of an

Fig. 2. Uptake of L-lactate and D-lactate by outer-medullary luminal-membrane vesicles

The uptake of 5 mM-L-lactate (a) and 5 mM-D-lactate (b) by luminal-membrane vesicles prepared from the pars recta of the proximal tubules is shown. The intravesicular medium was 310 mM-mannitol, whereas the external medium was 155 mM-NaCl (curves 1) or 155 mM-KCl (curves 2). In both intravesicular and extravesicular media 15 mM-Hepes/Tris was used as buffer system. First, membrane vesicles, dye and salt were mixed. Afterwards, L-lactate or D-lactate, at 0 min, was added, as indicated by the break in the curves. All the spectral curves were corrected for the effect of adding a small volume of 15 mM-Hepes/Tris buffer alone. (c) and (d) describe the uptake of increasing concentrations of L-lactate and D-lactate respectively in the presence of a 155 mM-NaCl gradient. In the insets the results are shown in Eadie-Hofstee plots. For further experimental details see Fig. 1 legend. Results are given as means $\pm$ S.D. for four experiments.

Na⁺ gradient. By contrast, no significant absorbance change was detected when NaCl was replaced by KCl, indicating that the uptake of lactate by these vesicle preparations is strictly Na⁺-dependent.

Figs. 2(c) and 2(d) show the optical response produced by various concentrations of L-lactate and D-lactate respectively in the presence of an Na⁺ gradient. The Na⁺-dependent uptake of these compounds shows a rapid increase at low concentrations in the medium (0–2 mM), whereas above 2 mM the increase in ΔA is less pronounced and approximately proportional to the increase in lactate concentration. The insets in Figs. 2(c) and 2(d) show Eadie-Hofstee analysis of the same data. A straight-line relationship is obtained for both L-lactate and D-lactate, suggesting that the Na⁺-dependent uptake of L- and D-lactate is mediated by means of a single transport system, with the following apparent K_A values (i.e.

substrate concentration that gives half-maximal uptake): for L-lactate, 0.26 ± 0.03 mM; for D-lactate, 0.38 ± 0.01 mM.

The question whether L-lactate and D-lactate are transported by a common transport system was studied in the following manner. L-Lactate and D-lactate were added in saturating concentrations either separately or jointly to vesicle/dye suspension, and the magnitudes of the dye response were compared. If L-lactate and D-lactate are transported by the same system the magnitude of ΔA (i.e. the maximal absorbance change observed by simultaneous addition of saturating concentrations of L- and D-lactate) should be approximately the same as ΔA_1 or ΔA_2 obtained when L- and D-lactate are added alone in saturating concentrations (for further discussion see Røigaard-Petersen & Sheikh, 1984). It was found that the magnitude of the maximal optical response induced by the simultaneous addition of L- and D-lactate did not

Fig. 3. Kinetics of L-lactate and D-lactate uptake by outer-cortical luminal-membrane vesicles

The uptake of increasing concentrations of L-lactate (a) and D-lactate (b) in the presence of a 155 mM-NaCl gradient is shown. (c) and panel (d) describe the uptake of L-lactate and D-lactate respectively in the presence of a 155 mM-KCl gradient instead of the NaCl gradient. In the insets the results are shown in Eadie-Hofstee plots. For further experimental details see Fig. 1 legend. Results are given as means $\pm$ S.D. for four experiments.

significantly differ from the maximal optical response induced by the addition of L- and D-lactate separately (results not shown).

Uptake of lactate by vesicles from the pars convoluta

We also studied the absorbance changes caused by the addition of L-lactate and D-lactate to vesicles derived from the pars convoluta in the presence of an NaCl or a KCl gradient. In contrast with the results obtained with vesicles from the pars recta, it was found that L- and D-lactate depolarize the luminal-membrane vesicles from the pars convoluta nearly to the same extent in the presence of an NaCl gradient and of a KCl gradient (results not shown).

Figs. 3(a), 3(b), 3(c) and 3(d) show ΔA induced by increasing concentrations of L-lactate and D-lactate under an NaCl gradient or a KCl gradient. It appears from the Figures that the cation-dependent uptake of lactate increases with increasing concentrations in the medium. Eadie-Hofstee analysis of the data (insets) indicates that L- and D-lactate transport in vesicles from the pars convoluta occurred via a single low-affinity cation-unspecific transport system, with following apparent K_A values: in the presence of an NaCl

gradient, for L-lactate, $K_A = 38.1 \pm 3.2$ mM, and for D-lactate, $K_A = 37.6 \pm 3.5$ mM; in the presence of a KCl gradient, for L-lactate, $K_A = 38.1 \pm 3.3$ mM, and for D-lactate, $K_A = 37.3 \pm 3.8$ mM.

The question whether L- and D-lactate are also transported by the same system in vesicles from the pars convoluta was examined in a similar way to that described for vesicles from the pars recta. The results of these experiments showed that cation-unspecific uptake of L- and D-lactate is mediated via a single common low-affinity transport system by vesicles prepared from the pars convoluta (results not shown).

Discussion

The results presented in this paper confirm and extend previous observations that L- and D-lactate are taken up by luminal-membrane vesicles by an electrogenic transport system. However, in contrast with the previous findings, the experiments now reported clearly demonstrate that the electrogenic uptake of L- and D-lactate is mediated via dual transport systems in membrane vesicles from whole renal cortex, namely a low-affinity and a high-affinity transport system. The tubular local-

ization of the transport systems was studied by the use of vesicles derived from the pars recta and the pars convoluta of the proximal tubule. In the pars recta transport of L- and D-lactate was strictly dependent on Na⁺ and occurred by means of a single high-affinity system. Cation-dependent but Na⁺-unspecific transport of low affinity for L- and D-lactate was exclusively localized to vesicles from the pars convoluta. In this connection it may be noted that Barác-Nieto *et al.* (1980) were unable to demonstrate any stimulation of L-lactate transport by luminal-membrane vesicles from rat kidney in the presence of artificially imposed pH gradients (extravesicular > intravesicular). Thus it seems improbable that the transient uptake of lactate can be energized by imposition of pH gradients.

Competition experiments revealed that transport systems for L- and D-lactate are common both in the pars convoluta and in the pars recta. The existence of a high-affinity transport system for these two isomers in luminal-membrane vesicles isolated from the pars recta observed in this study is in full agreement with expectation, since the luminal concentration of lactate, like that of other metabolites in the late proximal tubule, probably is low and therefore requires a high-affinity system for effective re-absorption of this compound (for further discussion see Røigaard-Petersen & Sheikh, 1984).

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