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Transport of pyruvate by luminal membrane vesicles from pars convoluta and pars recta of rabbit proximal tubule

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The characteristics of renal transport of pyruvate by luminal membrane vesicles from pars convoluta and pars recta of rabbit proximal tubule were studied. It was found that the uptake of pyruvate in these vesicle preparations occurred by means of multiple transport systems. An electrogenic and Na^+-requiring system confined to pars convoluta, exists for transport of pyruvate with an intermediate affinity, $K_A = 0.71 \pm 0.08$ mM. In vesicles from pars recta, the uptake of pyruvate was mediated by a dual transport system with a high ($K_{A1} = 0.30 \pm 0.05$ mM) and low affinity ($K_{A2} = 5.75 \pm 0.82$ mM). The relation of these three pyruvate transport systems to the transport of other monocarboxylates and dicarboxylates was determined by examination of the inhibitory effect of L-lactate, β-hydroxybutyrate and L-malate on the renal uptake of pyruvate. It was found that L-malate efficiently reduced the uptake of pyruvate by vesicles from pars convoluta, while addition of monocarboxylates (L-lactate, β-hydroxybutyrate) has no effect on the transport of pyruvate in this region of proximal tubule. Furthermore, it was observed that Na^+-dependent uptake of L-malate was specifically inhibited to about the same extent by 1 mM pyruvate. These findings strongly suggest that pyruvate and L-malate are taken up by the same transport system in vesicles from pars convoluta, which is different from that of L-lactate and β-hydroxybutyrate. Experiments designed to investigate the substrate specificity for the high and low affinity system for pyruvate in pars recta, revealed that monocarboxylates (L-lactate, β-hydroxybutyrate) substantially inhibit pyruvate transport at the low, but not the high substrate concentration, indicating that all monocarboxylates compete for the same high-affinity transport system. By contrast, L-malate at low concentrations preferentially inhibited the low-affinity system for pyruvate. This observation led us to suggest that L-malate and pyruvate share a common transport system in pars recta with low affinity for pyruvate and high affinity for L-malate.

Introduction

Tubular transport of organic acids against a concentration gradient across the luminal membranes of the renal proximal tubule generally occurs by Na^+-requiring and carrier-mediated systems [1–6]. As in the case of amino acid reabsorption these systems exhibit definite patterns of substrate specificity and differs in the various regions of the proximal tubule: In pars recta a common system with electrogenic and Na^+-requiring characteristics exist for transport of various monocarboxylates. This system has high affinity (K_m approx. 0.02 mM) for short chain fatty acids [1], and lower affinities (approx. 1 mM) for D- and L-lactate [2] and ketone bodies [3]. In pars convoluta the situation is more complex, since short

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chain fatty acids, D- and L-lactate, and ketone bodies appear to be transported by different transport systems. These generally are characterized by low substrate affinity and, although being Na^+ dependent, are electrically silent in the case of short chain fatty acids suggesting a 1 : 1 cotransport stoichiometry for Na^+ and these substrates [1–3].

Less information is available on the transport specificity of pyruvate and dicarboxylates. In studies on luminal membranes prepared from whole kidney cortex it has been found that dicarboxylates are transported by an electrogenic and Na^+-requiring system, distinct from that of monocarboxylates [7,8]. Furthermore, Nord et al. [9] reported that although pyruvate inhibits the transport of other monocarboxylates, it may also interact with a component of the dicarboxylic acid transporter.

As part of our ongoing studies on carboxylate reabsorption we here present data on the transport of pyruvate by luminal membranes from pars convoluta and pars recta of the proximal tubule of the rabbit kidney. This was done in order to resolve complexities, resulting from the presence of different transport systems in regionally specialized parts of the nephron. This approach resulted in a clearer picture of the relation of pyruvate transport to that of other monocarboxylates and to dicarboxylate transport.

Materials and Methods

Materials

Pyruvic acid, L-lactic acid, DL-β-hydroxyburyric acid, L-malic acid, Hepes, Trizma base and Trizma hydrochloride were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Radioactive [1-^{14}C]pyruvic acid (spec. act. 29 mCi/mmol) was obtained from Amersham International plc, Buckinghamshire, U.K., 3,3′-Diethyloxadicarbocyanide iodide was supplied by Eastman Kodac Co, Rochester, NY, U.S.A. These and all other reagents were of A.R. grade. All solutions were sterilized before use.

Preparation of luminal-membrane vesicles

Luminal-membrane vesicles were prepared from the pars convoluta ('outer cortex') and from the pars recta ('outer medulla') of the proximal tubules of rabbit kidney by the method previously described from this laboratory. The purity of membrane vesicle preparation was examined by electron microscopy and by measuring specific activities of various enzyme markers as previously described [10–12]. The protein concentration in the membrane fractions was determined by the method of Lowry et al. [13], as modified by Peterson [14], with human serum albumin (Sigma Chemical Co.) as standard.

Uptake experiments

The uptake of pyruvate was studied by Millipore filtration [15] and spectrophotometry [16]. The uptake of radioactive pyruvate was investigated by using a relatively simple, automated apparatus, originally described by Kessler et al. [17], which may be obtained from Innovac Labor, Adliswill, Switzerland. The apparatus consists of an electronic control unit, a vibrator for starting the reaction, and a stop solution injector. The individual measurements with the automated apparatus were performed as follows. A drop of 20 μl membrane vesicle suspensions and a drop of 20 μl incubation medium were placed close to but separate from each other at the bottom of a polystyrene test tube. The test tube was placed in a sled connected to the vibrator and the desired reaction time was set on the electronic control unit. The drops were mixed by switching the vibrator to the 'on' position and at the set time the reaction was stopped automatically by the addition of stop solution from the injector. After the adding of the stop solution the content of the test tube was rapidly filtered through a Sartorius membrane filter (0.45 μm, type SM 11306, Göttingen, F.R.G.) which was washed twice with 2.5 ml ice-cold stop buffer. The filter was dried overnight and the radioactivity was counted in a liquid scintillation counter (LKB-Wallac 1218 RackBeta) in Filter Count™ (Packard Instrument International S.A., Zürich, Switzerland). Correction for nonspecific binding to the filter and membrane vesicles was made by subtracting from all data the value of a blank obtained by filtering denatured membranes (boiled for 2 min) added to an incubation tube containing radioactive pyruvate.

The spectrophotometric measurements were

carried out as previously described from this laboratory [16], and details of the individual experiments are given in the legends to the Figures.

Calculations

The results of the saturation experiments were analyzed by using Michaelis-Menten kinetics. When data of the filtration experiments indicated uptake by more than one transport system the results were analyzed according to the following equation

$$\text{Uptake} = \frac{V_{max1}[S]}{K_{m1}+[S]} + \frac{V_{max2}[S]}{K_{m2}+[S]}$$

where K_m represents the substrate concentration that gives half-maximal uptake. V_{max} denotes maximal uptake and $[S]$ indicates initial concentration of substrate. Index 1 and 2 refer to the first and the second transport system, respectively. In case of transport via a single pathway the same equation without the second fraction was used. The spectrophotometric data were analyzed using an analogue relationship.

$$\Delta A = \frac{\Delta A_{max1}[S]}{K_{A1}+[S]} + \frac{\Delta A_{max2}[S]}{K_{A2}+[S]}$$

where ΔA is the absorbance change measured by addition of solute at concentration [S], ΔA_{max} is the maximal absorbance change and K_A is the monocarboxylic acid concentration producing a half-maximal absorbance. The various kinetic parameters for both types of uptake studies were calculated using iteration computer programs [18].

Results

Uptake of pyruvate by vesicles from whole cortex

In preliminary experiments (results not shown) we found that addition of pyruvate to luminal-membrane vesicle/dye suspension in the presence

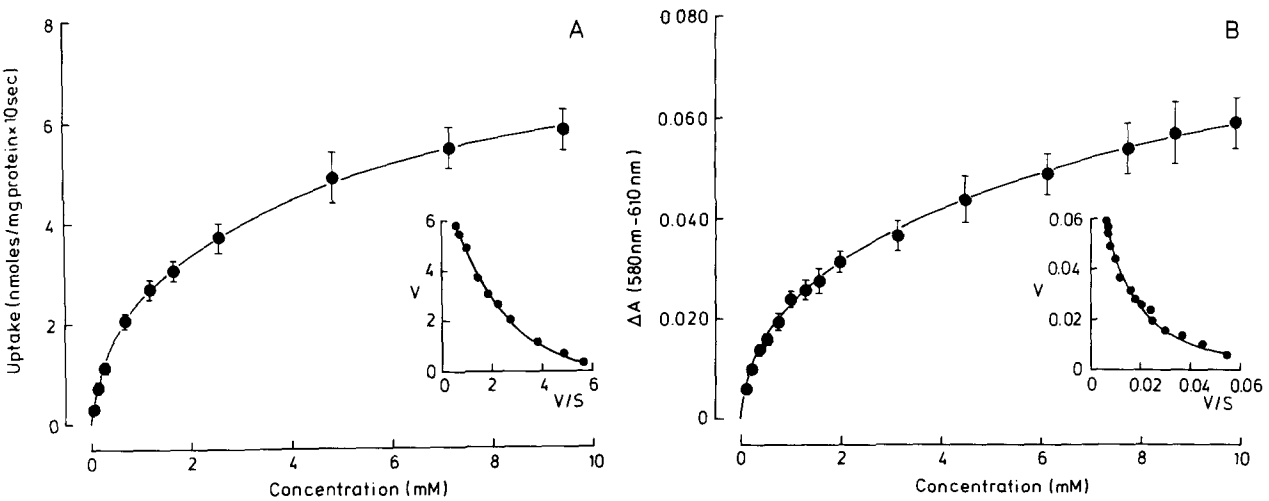

Fig. 1. Kinetics of pyruvate uptake by luminal-membrane vesicles from whole renal cortex. (A) Millipore filtration experiments. At time zero 20 μl of a concentrated membrane vesicle preparation (14–20 mg protein/ml) was added to 20 μl of uptake buffer containing [^{14}C]pyruvate, unlabelled pyruvate, 155 mM NaCl and 15 mM Hepes-Tris (pH 7.5). The intravesicular medium was 310 mM mannitol and 15 mM Hepes-Tris (pH 7.5). Temperature 20 °C. After 10 s the uptake was stopped by adding 1 ml of ice-cold stop buffer consisting of 155 mM NaCl in 15 mM Hepes-Tris (pH 7.5). In the inset the results are shown in an Eadie-Hofstee plot. V represents the rate of transport at substrate concentration S. (B) Spectrophotometric measurements. Common experimental conditions: protein concentration 0.3 mg/ml; pH 7.5; temperature 20 °C; dye concentration 15 μM. The intravesicular medium was 310 mM mannitol and 15 mM Hepes-Tris. The results shown in the figure are the absorbance changes obtained with an external medium of 155 mM NaCl in 15 mM Hepes-Tris. In the inset the results are shown in an Eadie-Hofstee plot. V represents the absorbance change at substrate concentration S. The spectrophotometer was operated in the dual wavelength mode with 580 nm and 610 nm (reference wavelength). All results are corrected for the Na^+-independent uptake of pyruvate, determined in parallel experiments in which Na^+ was replaced with choline. Results are given as mean values ± S.D. of three experiments.

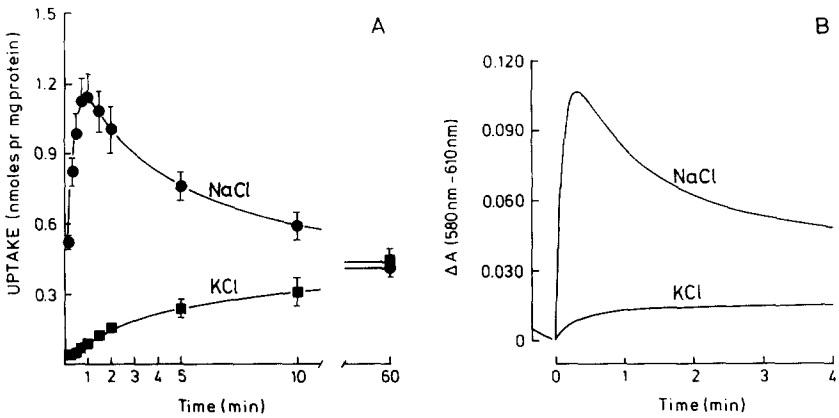

Fig. 2. Time course of pyruvate uptake by luminal-membrane vesicles from pars convoluta. (A) Millipore filtration experiments. 20 μl of the vesicle suspension was incubate at different time intervals in 20 μl incubation mixture consisting of 0.4 mM [^{14}C]pyruvate in 155 mM NaCl (●) or 155 mM KCl (■). In both intravesicular and extravesicular media 15 mM Hepes-Tris (pH 7.5) was used as buffer system. Results are given as mean values ± S.D. of three experiments. (B) Uptake of 3 mM pyruvate as studied by spectrophotometry. The intravesicular medium was 310 mM mannitol whereas the external medium was 155 mM NaCl or 155 mM KCl. In both intravesicular and extravesicular media 15 mM Hepes-Tris was used as buffer system. For further experimental details see Fig. 1 legend. Results are given as mean values ± S.D. of three experiments.

of Na^+ gradient (extravesicular > intravesicular) resulted in absorbance changes (ΔA) indicative of depolarizing event(s) (for details, see Ref. 16) strongly suggesting the existence of electrogenic transport system(s) for this compound. No depolarization was observed when NaCl was replaced by KCl or other cations plus chloride in the incubation medium indicating that the renal uptake of pyruvate is strictly Na^+ dependent. Fig. 1A describes the Na^+-dependent uptake of pyruvate at various medium concentrations as measured by Millipore filtration. The uptake of monocarboxylic acid shows a rapid increase at low medium concentrations (< 2 mM). At higher medium concentrations of pyruvate the increase in the uptake of this compound was less pronounced and approaching saturation at 10 mM. Inset shows Eadie-Hofstee analysis of the experimental data. A curvilinear plot was obtained, which suggests the presence of multiple transport systems in luminal-membrane vesicles from whole renal cortex for the transport of pyruvate. Fig. 1B shows the absorbance changes (ΔA) induced by addition of increasing concentraitons of pyruvate to suspensions of membrane vesicles and dye. The results obtained at increasing medium concentrations of pyruvate follow a curve of the same form as that shown in Fig. 1A. The spectrophotometric results have also been subjected to Eadie-Hofstee analysis (Fig. 1B inset). The spectrophotometric data give principally the same information as the filtration experiments, namely that pyruvate is taken up by more than one transport system.

Uptake of pyruvate by vesicles from pars convoluta

The time course for the uptake of [^{14}C]pyruvate by luminal-membrane vesicles from pars convoluta suspended in NaCl and KCl gradient is described in Fig. 2A. The initial pyruvate uptake was very rapid and linear by the first 10 s of incubation in the presence of an extravesicular > intravesicular gradient of 155 mM NaCl and reached a maximal value of 1.14 nmol/mg protein after approx. 1 min of incubation. This was followed by a slow but steady decrease in pyruvate uptake, reaching a steady-state level after 60 min of incubation. By contrast, no transient accumulation of pyruvate was observed when NaCl was replaced by equimolar concentration of KCl in the incubation medium. Upper curve and lower curve in Fig. 2B show the absorbance changes caused by the addition of pyruvate in the presence of NaCl and KCl gradient, respectively. It appears from the figure that the uptake of pyruvate is electrogenic and Na^+-dependent. Fig. 3 depicts ΔA values induced by various concentrations of pyruvate

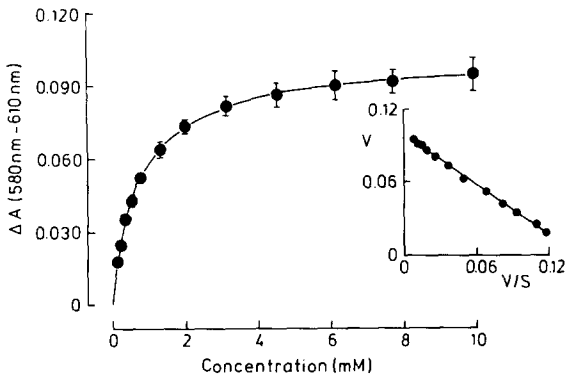

Fig. 3. Absorbance changes (ΔA) induced by increasing concentrations of pyruvate by luminal-membrane vesicles from pars convoluta as studied by spectrophotometry. The intravesicular medium was 310 mM mannitol and 15 mM Hepes-Tris (pH 7.5). The results shown in the figure are the absorbance changes obtained with an external medium of 155 mM NaCl/15 mM Hepes-Tris buffer. In the inset the results are shown in an Eadie-Hofstee plot. V represents the absorbance change at substrate concentration S. For further experimental details see Fig. 1 legend. Results are given as mean values ± S.D. of four experiments.

to the incubation system. The inset in Fig. 3 shows Eadie-Hofstee analysis of the experimental data, which indicates that Na^+-dependent uptake of this compound occurred via a single transport system with half-saturation value $K_A = 0.71 \pm 0.08$ mM.

Uptake of pyruvate by vesicles from pars recta

The mechanism of uptake of pyruvate in vesicles from pars recta was studied both by Millipore filtration and spectrophotometric method. Fig. 4A illustrates the uptake of radioactive pyruvate by these membrane vesicles during incubation for different lengths of time in the presence of NaCl or KCl gradient. The transient accumulation of pyruvate is only seen in the presence of Na^+ in the medium. Furthermore, the maximal uptake value (approx. 0.56 nmol/mg protein) of pyruvate observed at 1 min incubation period is nearly half of that obtained in vesicles from pars convoluta under same experimental conditions (compare upper curve of Fig. 2A with upper curve of Fig. 4A). Fig. 4B record the absorbance changes produced by pyruvate in the presence of NaCl or KCl gradient. According to the expectation, it is seen that depolarization of membrane vesicles only occurred in the presence of Na^+ gradient. The absorbance changes (ΔA) induced by various medium concentrations of pyruvate are shown in Fig. 5. Inset in the figure illustrates the Eadie-Hofstee analysis of the experimental data which suggest the existence of multiple transport systems in these vesicles for pyruvate. Computerized calculations gave the following half-saturation and ΔA_{max} values: $K_{A1} = 0.30 \pm 0.05$ mM, $K_{A2} = 5.75 \pm 0.82$ mM, $\Delta A_{max1} = 0.087 \pm 0.011$ absorbance unit/15 s per mg protein, and $\Delta A_{max2} = 0.122 \pm 0.014$ absorbance units/15 s per mg protein.

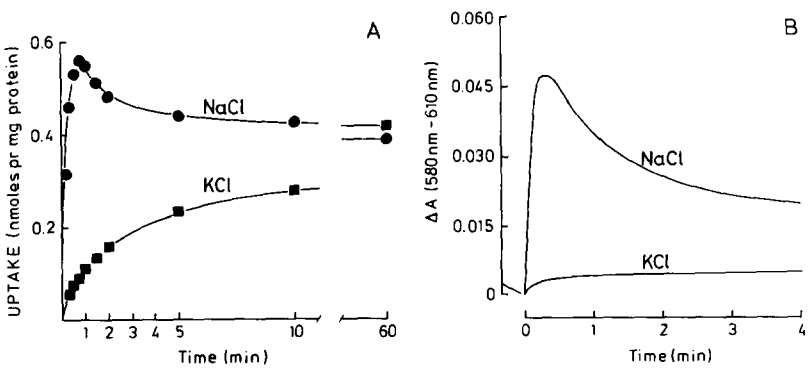

Fig. 4. Time course of pyruvate uptake by luminal-membrane vesicles from pars recta. (A) Millipore filtration experiments. The intravesicular medium was 310 mM mannitol whereas the incubation media were 0.4 mM [^{14}C]pyruvate in 155 mM NaCl (●) or 155 mM KCl (■). (B) Uptake of 3 mM pyruvate as registered by the spectrophotometric method. The intravesicular medium was 310 mM mannitol whereas the extravesicular medium was 155 mM NaCl or 155 mM KCl. For further experimental details see Fig. 2 legend. Results are given as mean values ± S.D. of three experiments.

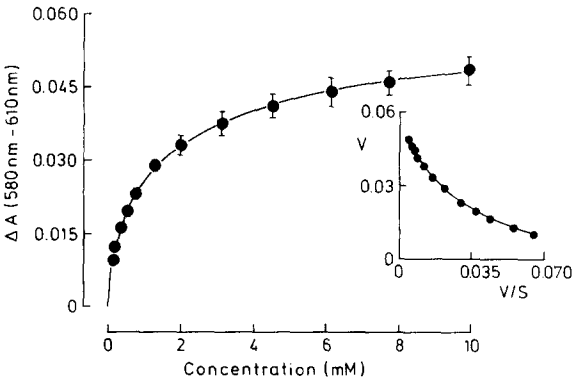

Fig. 5. Absorbance changes (ΔA) induced by increasing concentrations of pyruvate by luminal-membrane vesicles from pars recta as studied by spectrophotometry. Intra- and extravesicular media as described in Fig. 3. Results are given as mean values ± S.D. of four experiments.

Relation to transport of other monocarboxylates and L-*malate*

The results presented in the preceding sections reveal the existence of at least three different Na^+-dependent systems for the renal uptake of pyruvate in luminal-membrane vesicles derived from pars convoluta and pars recta of the rabbit kidney proximal tubule. The relation of these to the transport of other monocarboxylates and dicarboxylates was determined by examination of the inhibitory effect of two different monocarboxylates (L-lactate and β-hydroxybutyrate) and one dicarboxylate (L-malate) on pyruvate transport (upper half of Table I). These data show that L-malate preferentially inhibits Na^+-dependent transport in pars convoluta. The lower half of Table I shows that Na^+-dependent uptake of L-malate is inhibited to about the same extent by 1 mM pyruvate, while addition of L-lactate or β-hydroxybutyrate has no effect on the transport of dicarboxylate. These data lead to the conclusion that pyruvate and L-malate are taken up by the same transport system in vesicles from pars convoluta, which is different from that of L-lactate and β-hydroxybutyrate.

To examine the characteristics of L-malate uptake we follow dye potential changes, induced by addition of L-malate to vesicle suspensions. I both pars convoluta and pars recta absorbance change were observed that were indicative of depolarization event(s) in the presence of a Na^+ gradient (not shown). These findings are in accordance with earlier reports which showed that the uptake of dicarboxylates in luminal-membrane vesicles from whole renal cortex is Na^+ dependent and electrogenic [7,19–21]. Figs. 6A and 6B record the Na^+-dependent dye absorbance changes induced by different concentrations of L-malate in the presence of vesicles from pars convoluta and pars recta, respectively. Insets show Eadie-Hofstee analysis of the data (pars convoluta, $K_{A1} = 0.25 \pm 0.03$ mM; pars recta $K_{A2} = 0.28 \pm 0.02$ mM). This is consistent with L-malate being reabsorbed by means of a single transport system along the proximal tubule of the rabbit kidney.

To examine the substrate specificity for the high- and low-affinity system for pyruvate in pars recta, potential inhibitors were examined both at a low (0.3 mm) and a high (5 mM) concentration of pyruvate, while the concentrations of other carboxylates, tested as inhibitors (L-lactate, β-hydroxybutyrate, and L-malate) were chosen to correspond to the halfsaturation constant for their

TABLE I

EFFECT OF VARIOUS CARBOXYLIC ACIDS ON RATE OF Na^+-DEPENDENT UPTAKE OF PYRUVATE AND L-MALATE BY LUMINAL-MEMBRANE VESICLES FROM PARS CONVOLUTA

Results are given as mean values ± S.D. of at least three experiments. Final concentration of substrate in the incubation medium was 0.2 mM and of inhibitors 1.0 mM. For further experimental details see Methods.

Addition	[^{14}C]Pyruvate uptake (nmol/mg protein per 10 s)	Percent of control	P
None	0.282 ± 0.034	100	–
L-Lactate	0.268 ± 0.026	95	$0.6 < P < 0.7$
β-Hydroxybutyrate	0.285 ± 0.018	101	$0.9 < P$
L-Malate	0.164 ± 0.022	58	$P < 0.01$
Addition	**L-[^{14}C]Malate uptake (nmol/mg protein per 10 s)**	**Percent of control**	**P**
None	0.710 ± 0.076	100	–
L-Lactate	0.644 ± 0.082	91	$0.3 < P < 0.4$
β-Hydroxybutyrate	0.690 ± 0.064	97	$0.7 < P < 0.8$
Pyruvate	0.432 ± 0.058	61	$P < 0.01$

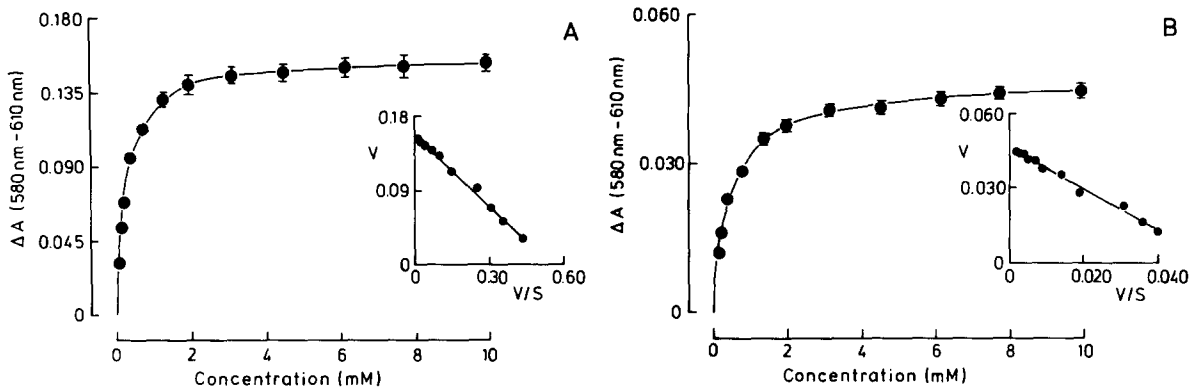

Fig. 6. Kinetics of L-malate uptake by luminal-membrane vesicles from pars convoluta and pars recta. Uptake of increasing concentrations of L-malate in the presence of a 155 mM NaCl gradient by luminal-membrane vesicles from pars convoluta (A) and from pars recta (B). In the inset the results are shown in an Eadie-Hofstee plot. For further experimental details see Fig. 1 legend. Results are given as mean values ± S.D. of three experiments.

TABLE II

MONO- AND DICARBOXYLATE INHIBITION OF Na^+-DEPENDENT UPTAKE OF PYRUVATE BY LUMINAL-MEMBRANE VESICLES FROM PARS RECTA

Results are given as mean values ± S.D. of at least three experiments. Final concentration of substrate in the incubation medium was either 0.3 mM or 5.0 mM and of inhibitors: L-lactate 0.3 mM, β-hydroxybutyrate 1.0 mM, and L-malate 0.2 mM. For further experimental details see Methods.

Inhibitor	[^{14}C]Pyruvate uptake, 0.3 mM			[^{14}C]Pyruvate putake, 5.0 mM		
	nmol/mg protein per 10 s	percent of control	P	nmol/mg protein per 10 s	percent of control	P
None	0.238 + 0.024	100	–	1.69 + 0.21	100	–
L-Lactate	0.123 + 0.026	52	$P < 0.01$	1.50 + 0.18	89	$0.2 < P < 0.3$
β-Hydroxybutyrate	0.111 + 0.019	47	$P < 0.01$	1.46 + 0.12	86	$0.1 < P < 0.2$
L-Malate	0.209 + 0.028	87	$0.1 < P < 0.2$	1,10 + 0.09	65	$0.01 < P < 0.02$

renal transport. It appears from Table II that L-lactate and β-hydroxybutyrate substantially inhibit pyruvate transport at the low, but not the high substrate concentration. This is consistent with the idea that all monocarboxylates compete for the same high affinity transport system (see Introduction). By contrast an inhibitory effect of 0.2 mM L-malate is observed at 5 mM pyruvate, suggesting that L-malate and pyruvate share a common transport system in pars recta with high affinity for L-malate and low affinity for pyruvate.

Discussion

The methods used to isolate highly purified luminal-membrane vesicles from pars convoluta and pars recta of rabbit kidney cortex (for a review, see Ref. 22) provided us with an opportunity to investigate the mechanism of reabsorption of various monocarboxylic acids [1–3] along the proximal tubule. In contrast to the previous findings [8,9] the results presented here demonstrate the presence of at least three Na^+-dependent transport systems for pyruvate. In vesicles from pars recta the uptake of pyruvate was mediated by a dual system, with K_A values of approx. 0.3 mM and 5 mM. The experiments designed to explore the effect of mono- and dicarboxylates on these two pyruvate transport systems showed that monocarboxylates namely L-lactate and β-hydroxybutyrate at their half-saturation concentrations (see Fig. 2c of Ref. 2; and Table II of Ref. 3)

efficiently inhibited the high-affinity transport of pyruvate, whereas L-malate at low concentrations was found to be a potent inhibitor of the low-affinity system that exists for the uptake of pyruvate in vesicles from pars recta. These results thus indicate that pyruvate is probably reabsorbed both by mono- and dicarboxylic acid transport systems in this region of rabbit proximal tubule. On the other hand the Na^+-dependent pyruvate transport system located in vesicles from pars convoluta seems to be the same as that responsible for the reabsorption of dicarboxylates, since L-malate effectively inhibited the uptake of pyruvate and vice versa. While neither L-lactate for β-hydroxybutyrate significantly inhibit the uptake of pyruvate nor the transport of L-malate by luminal-membrane vesicles prepared from pars convoluta (see Table I).

In conclusion, the results reported in this communication showed that pyruvate in contrast to other monocarboxylates is taken up by vesicles from two different segments of the nephron, namely pars convoluta and pars recta via three Na^+-dependent electrogenic transport processes. Two transport systems located in pars recta showed both features of mono- and dicarboxylate transport, and a single common transport system for pyruvate and L-malate exists in vesicles from pars convoluta.

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