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Characteristics of uptake of short chain fatty acids by luminal membrane vesicles from rabbit kidney

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The mechanisms of renal transport of short chain fatty acids by luminal membrane vesicles prepared from pars convoluta or pars recta of rabbit proximal tubule were studied by a Millipore filtration technique and by a spectrophotometric method using a potential-sensitive carbocyanine dye. Both luminal membrane vesicle preparations take up propionate and butyrate by strictly Na^+-dependent transport systems, although with different characteristics. The uptake of short chain fatty acids by membrane vesicles from the pars convoluta was insensitive to changes in membrane potential, which is indicative of electroneutral transport of these compounds. Furthermore, kinetic studies showed that the Na^+-dependent, but electrically silent transport of propionate is saturable ($K_m = 10.9 \pm 1.1$ mM and $V_{max} = 3.6 \pm 0.2$ nmol/mg protein per 20 s) and is unaffected by the presence of L- and D-lactate, indicating that these monocarboxylic acids did not share the same common transport system. In the luminal membrane vesicles from the pars recta, the uptake of propionate and butyrate was mediated by an Na^+-dependent electrogenic transport process, since addition of the organic compounds to these vesicle/dye suspensions depolarized the membrane vesicles and the renal uptake of propionate and butyrate was enhanced by K^+ diffusion potential induced by valinomycin. Competition experiments revealed that in contrast to the transport of propionate by vesicles from the pars convoluta, the Na^+-dependent electrogenic transport of short chain fatty acids in vesicles from the pars recta occurred via the same transport system that is responsible for the reabsorption of L- and D-lactate in this region of rabbit kidney proximal tubule.

Introduction

It is known that fatty acids are taken up by the mammalian kidney in vivo [1,2], by renal cortical slices in vitro [3] and by the perfused kidney [4]. However, the mechanism of transport of these monocarboxylic acids across the membranes of renal cells is not clear at present. Evidence of transport of fatty acids across the luminal membrane was obtained in microperfusion experiments by Ullrich et al. [5], who demonstrated that short chain fatty acids and their analogues, when added to perfusate at a concentration of 10 mM, efficiently inhibited the reabsorption of D-lactate by the proximal tubule of rat kidney. By contrast, Barac-Nieto et al. [6] were unable to demonstrate any inhibitory effect of propionate on the Na^+-dependent uptake of L-lactate by luminal membrane vesicles isolated from whole cortex of rat kidney.

In the present communication we have undertaken to investigate the existence of transport

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Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic aicd; Mes, 4-morpholineethanesulphonic acid.

systems for short chain fatty acids across luminal membrane vesicles, derived from the pars convoluta and pars recta of the proximal tubules. In both vesicle preparations we found that propionate and butyrate were transported by Na^+-dependent processes. However, there were distinct differences between transport systems of pars recta and pars convoluta with respect to the electrogenicity and inhibition by L- and D-lactate. These differences may in part account for the previously reported conflicting data on inhibition of L- and D-lactate reabsorption.

Materials and Methods

Materials

Valinomycin, Trizma base, Trizma hydrochloride, Hepes, Mes, D-glucose, propionic acid, and *n*-butyric acid were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Radioactive propionate (spec. act. 54 mCi/mmol) and butyrate (spec. act. 56 mCi/mmol) were purchased from Amersham, Buckinghamshire, U.K. 3,3′-diethyloxadicarbocyanine iodine 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 [7,8]. The purity of membrane vesicle preparations was investigated by electron microscopy and by measuring specific activities of various enzyme markers as previously described [1,9]. The protein concentration in the membrane fractions was determined by the method of Lowry et al. [10], as modified by Peterson [11], with human serum albumin as standard.

Uptake experiments

The uptake of propionate and butyrate was studied by Millipore filtration [12] and spectrophotometry [13]. The uptake of radioactive propionate and butyrate was investigated by using a relatively simple, automated apparatus, originally described by Kessler et al. [14], 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 suspension 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 addition of the stop solution the content of the test tube was rapidly filtered through a Sartorius membrane filter 0.45 μm, type SM 11106, 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 (Wallac LKB 1210 Ultrabeta) in Lumagel (Lumac, The Netherlands). Correction for non-specific 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 propionate or butyrate.

The spectrophotometric measurements were carried out as previously described [13], and details of the individual experiments are given in the legends to the figures.

Results

Uptake of short chain fatty acids by luminal membrane vesicles from proximal convoluted tubules

The time course of uptake of radioactive propionate and butyrate by luminal membrane vesicles isolated from pars convoluta was studied by Millipore filtration [12]. It appears from Fig. 1A and B that the presence of an inwardly directed Na^+ gradient markedly stimulated the accumulation of the organic acids in the vesicles, and the maximal concentration was reached at about 90–120 s of incubation. Thereafter propionate and butyrate concentrations decreased, indicating net efflux. After 60 min the level of the

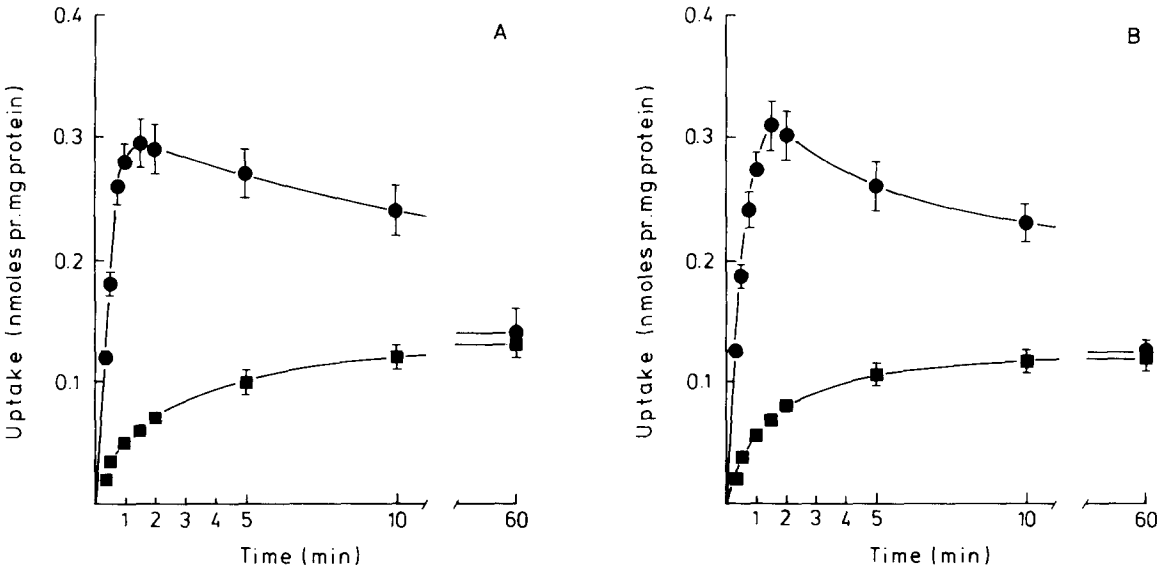

Fig. 1. Uptake of [^{14}C]propionate (panel A) and [^{14}C]butyrate (panel B) by luminal membrane vesicles prepared from pars convoluta of the proximal tubules as studied by Millipore filtration. Common experimental conditions: protein concentration 5.0 mg/ml; pH 7.5; temperature 20°C. The intravesicular medium was 310 mM mannitol, whereas the incubation media were 0.2 mM radioactive fatty acids, 155 mM NaCl (●) or 155 mM KCl (■). In both intravesicular and extravesicular media 15 mM Hepes-Tris was used as the buffer system. Results are given as mean values ± S.D. of three experiments.

organic acid uptake in the presence of Na^+ had fallen to the level attained in the absence of Na^+, suggesting that equilibrium was being approached between intra- and extravesicular concentrations of substrates. No transient uptake ('overshoot') of propionate and butyrate was observed in the presence of KCl gradient.

Fig. 2, curves 1, 2 and 3, illustrate the absorbance changes caused by the addition of 10 mM propionate, 10 mM butyrate and 10 mM D-glucose to the vesicle/dye suspension, respectively. No significant depolarization could be detected in the presence of short chain fatty acids under these conditions (see Fig. 2, curves 1 and 2). For the sake of comparison, the absorbance changes associated with addition of the same concentration of D-glucose (Fig. 2, curve 3) are shown. According to expectations, addition of D-glucose to dye/vesicle preparation resulted in a very pronounced optical change, which is indicative of electrogenic transport of this compound [13]. The results described in Figs. 1 and 2 strongly suggest that the uptake of short chain fatty acids in luminal membrane vesicles from pars convoluta is mediated by a strictly Na^+-dependent, but electroneutral transport process.

Table I shows the effect of K^+- and H^+-gradient on the Na^+-dependent uptake of radioactive propionate by the luminal membrane vesicles from

TABLE I

EFFECT OF K^+- AND H^+-GRADIENT ON RATE OF Na^+-DEPENDENT UPTAKE OF PROPIONATE BY LUMINAL MEMBRNAE VESICLES FROM PARS CONVOLUTA

Results are given as mean values ± S.D. of at least three experiments. For further experimental details see Results section.

	[^{14}C]Propionate uptake (nmol/mg protein per 20 s)	Percent of control	P
Control	0.122 ± 0.005	100	
K^+-gradient ($K^+_{in} > K^+_{out}$)	0.118 ± 0.008	97	$0.5 < P > 0.6$
pH-gradient ($pH_{in} > pH_{out}$)	0.124 ± 0.010	102	$0.7 < P < 0.8$

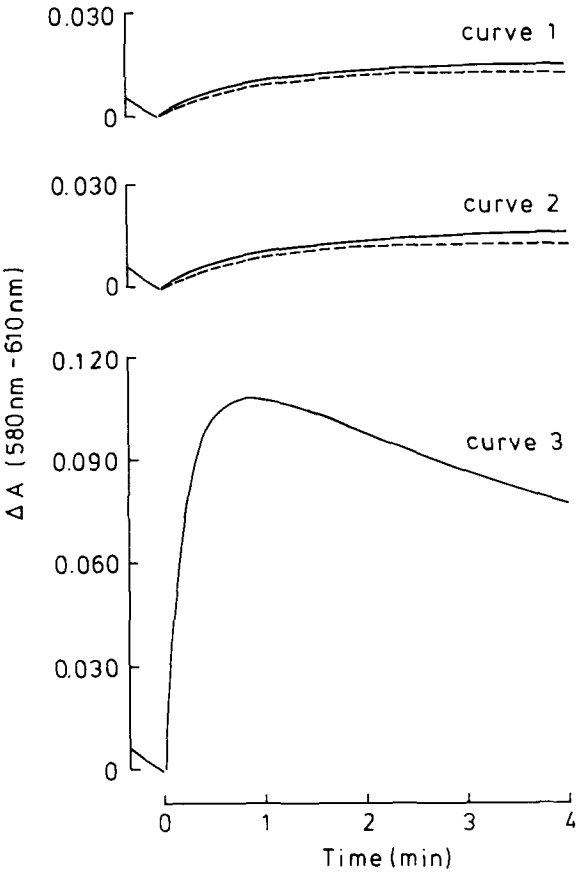

Fig. 2. Uptake of 10 mM propionate (curve 1), 10 mM butyrate (curve 2) and 10 mM D-glucose (curve 3) by luminal membrane vesicles prepared from pars convoluta of the proximal tubules as studied by spectrophotometry. Common experimental conditions: protein concentration 0.25 mg/ml; pH 7.5; temperature 20°C; dye concentration 15 μM. The intravesicular medium was 310 mM mannitol, whereas the external medium was 155 mM NaCl. In both intravesicular and extravesicular media 15 mM Hepes-Tris was used as the buffer system. The break in the curves, at 0 min, indicates addition of solute. Broken lines illustrate the time course without the addition of short chain fatty acids. The spectrophotometer was operated in the dual wavelength mode with 580 nm and 610 nm (reference wavelength).

the pars convoluta as measured by Millipore filtration technique. In a series of experiments the luminal membrane vesicles were preloaded with medium containing 155 mM KCl, 15 mM Hepes-Tris buffer (pH 7.5) and incubated in medium containing 200 μM radioactive propionate, 155 mM NaCl, 15 mM Hepes-tris buffer (pH 7.5). No stimulatory effect of a K^+ gradient on the Na^+-dependent uptake of the short chain fatty acids was observed. We have also studied the effect of inwardly directed hydrogen ion gradient on the Na^+-dependent uptake of radioactive propionate in these vesicle preparations. In these experiments the intravesicular medium was 310 mM mannitol, 15 mM Hepes-Tris buffer (pH 7.5), whereas the incubation medium consisted of 200 μM radioactive propionate, 155 mM NaCl, 15 mM Mes-Tris buffer (pH 5.5). The results of these experiments showed that the uptake of the fatty acid was unaltered in the presence of pH gradient (pH_{out} = 5.5 and pH_{in} = 7.5).

Fig. 3 depicts the uptake of radioactive propionate in the presence of a NaCl gradient at increasing concentrations of the organic acid in vesicles from pars convoluta. The Na^+-dependent uptake of fatty acid shows an increase at low medium concentrations. The renal accumulation of this organic compound increases slowly but

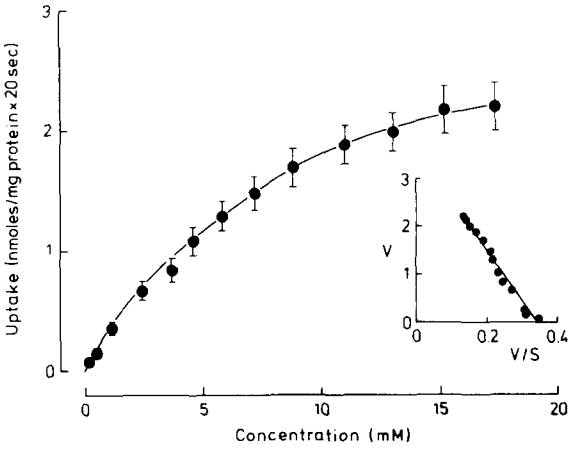

Fig. 3. Uptake of increasing concentrations of propionate by luminal membrane vesicles prepared from pars convoluta of the proximal tubules. At time zero 20 μl of a concentrated membrane vesicle suspension (14–20 mg protein/ml) was added to 20 μl of uptake buffer containing unlabelled propionate, [^{14}C]propionate, 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). After 20 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*. Results are given as mean values ± S.D. of four experiments.

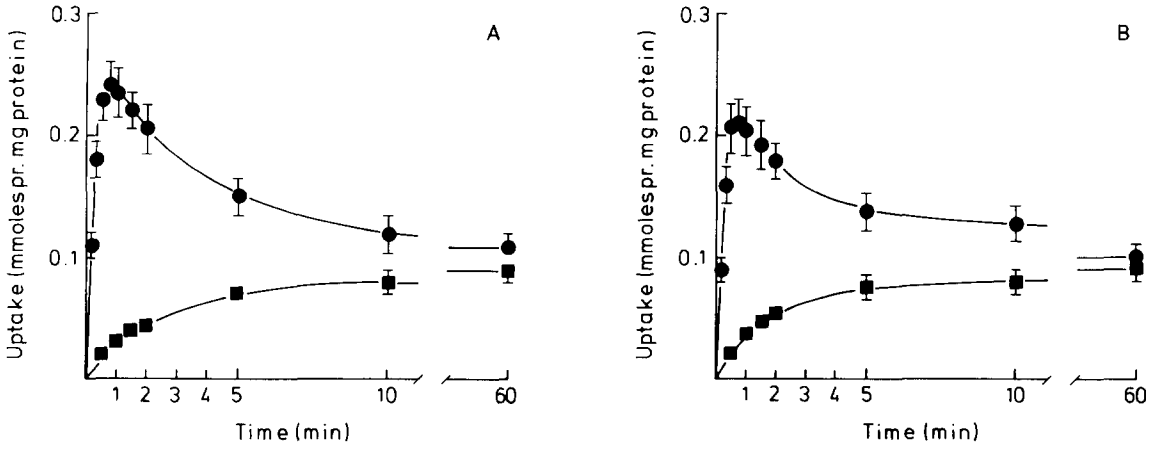

Fig. 4. Uptake of [^{14}C]propionate (panel A) and [^{14}C]butyrate (panel B) by luminal membrane vesicles prepared from pars recta of the proximal tubules as studied by Millipore filtration. The intravesicular medium was 310 mM mannitol, whereas the incubation media were 0.2 mM radioactive fatty acids, 155 mM NaCl (●) or 155 mM KCl (■). For further experimental details see Fig. 1 legend. Results are given as mean values ± S.D. of three experiments.

steadily at higher medium concentrations and approaches saturation at a fatty acid concentration of 20 mM. The inset in Fig. 3 illustrates an Eadie-Hofstee plot of the same experimental data. It is apparent from this plot that the renal uptake of propionate in the vesicles occurred via a single

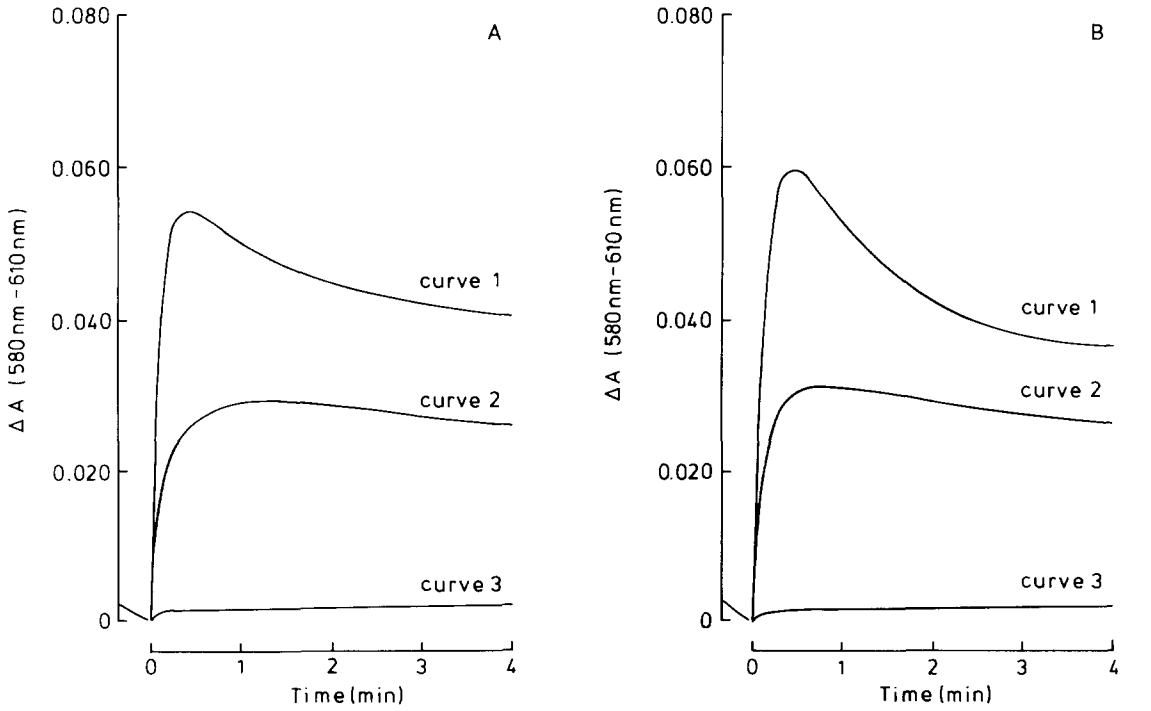

Fig. 5. Uptake of 0.37 mM propionate (panel A) and butyrate (panel B) by luminal membrane vesicles prepared from pars recta of the proximal tubules as studied by spectrophotometry. The intravesicular medium was 310 mM mannitol, whereas the external medium was 155 mM NaCl (curve 1), 103 mM Na_2SO_4 (curve 2) or 155 mM KCl (curve 3). In both intravesicular and extravesicular media 15 mM Hepes-Tris was used as the buffer system. For further experimental details see Fig. 2 legend.

transport system. The K_m and V_{max} values calculated from these experiments were as follows: propionate, $K_m = 10.9 \pm 1.1$ mM and $V_{max} = 3.6 \pm 0.2$ nmol/mg protein per 20 s.

The question as to whether the fatty acids are taken up by the same system which transports L- and D-lactate in luminal membrane vesicles from the pars convoluta was also examined. For this purpose radioactive propionate was chosen and the effect of either L- or D-lactate or butyrate (10 mM) on the initial uptake of this compound was determined. It is seen from Table II that the initial rate of propionate transport was uninfluenced by the addition of L- and D-lactate, indicating that these organic acids did not share the same transport system in vesicles from the pars convoluta. On the other hand, the values given in the table show that the presence of butyrate inhibits the uptake of propionate in these vesicles.

Uptake of short chain fatty acids by luminal membrane vesicles from proximal straight tubules

Fig. 4A and B shows the uptake pattern of radioactive propionate and butyrate, respectively, by luminal membrane vesicles from the pars recta. It appears from the figure that in the presence of an extravesicular > intravesicular Na^+ gradient, the initial rate of fatty acid uptake is much greater than that obtained in the presence of a K^+ gradient. Furthermore, transient overshoots are ob-

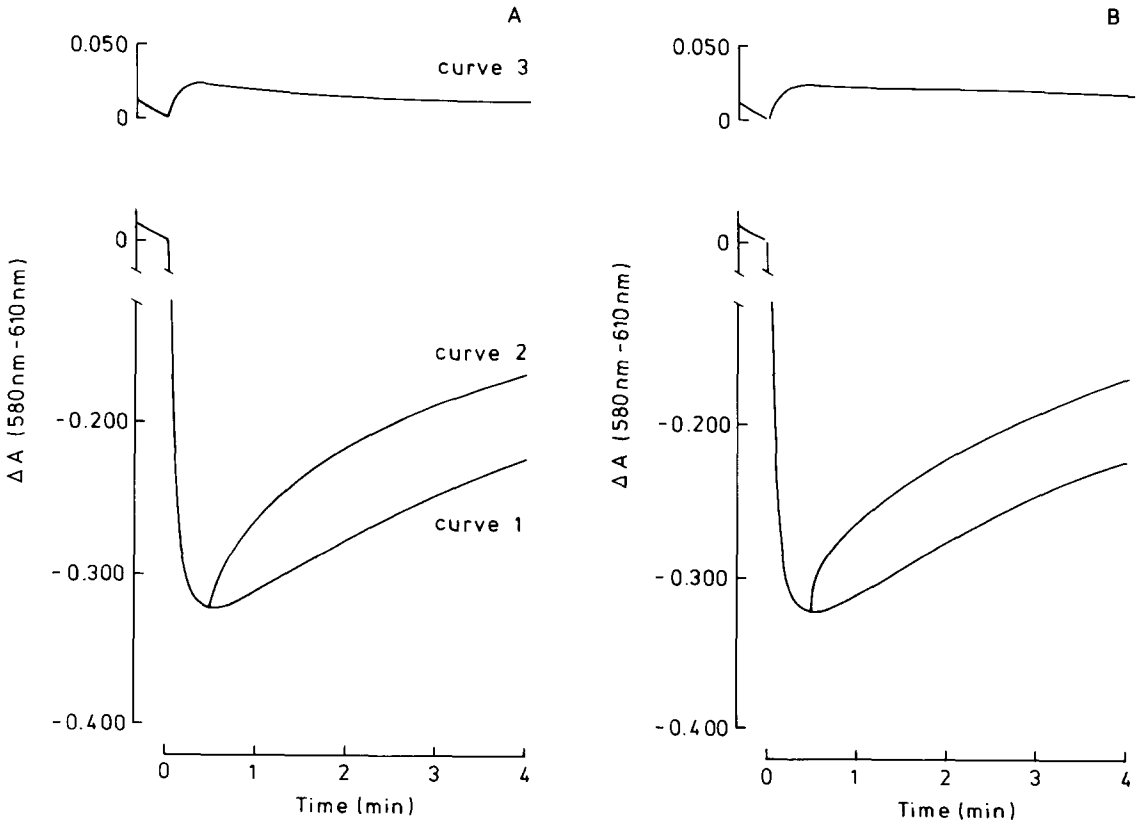

Fig. 6. Effect of valinomycin on the absorbance changes induced by addition of propionate (panel A) and butyrate (panel B) to luminal membrane vesicles prepared from pars recta of the proximal tubules. The membrane vesicles were preloaded for 1 h in 103 mM K_2SO_4, and the spectrophotometric experiments were carried out with an external medium of 103 mM Na_2SO_4. Curve 1 in each panel, addition of 5 μg valinomycin per mg protein; curve 2 in each panel, addition of valinomycin and, about 30 s later, 0.76 mM propionate or butyrate; curve 3 in each panel, addition of solute in the absence of valinomycin. For further experimental details see Fig. 2 legend.

TABLE II

EFFECT OF VARIOUS COMPOUNDS ON RATE OF Na^+-DEPENDENT UPTAKE OF PROPIONATE BY LUMINAL MEMBRANE VESICLES FROM PARS CONVOLUTA

Results are given as mean values ± S.D. of at least three experiments. Concentration of propionate in the incubation medium was 0.2 mM and of inhibitors 10 mM. For further experimental details see Fig. 3 legend.

Addition	[^{14}C]Propionate uptake (nmol/mg protein per 20 s)	Percent of control	P
None	0.120 ± 0.005	100	-
L-Lactate	0.115 ± 0.010	96	$0.4 < P < 0.5$
D-Lactate	0.122 ± 0.008	102	$0.7 < P < 0.8$
Butyrate	0.081 ± 0.010	68	$P < 0.01$

served only in the presence of Na^+. Fig. 5A and B records the absorbance changes induced by addition of propionate and butyrate, respectively, to vesicle/dye suspension in the presence of a NaCl gradient (curve 1), a Na_2SO_4 gradient (curve 2) or a KCl gradient (curve 3). It is seen that addition of fatty acids depolarized the membrane vesicles only in the presence of Na^+.

The effect of a K^+ diffusion potential generated by valinomycin on the Na^+-dependent uptake of propionate and butyrate in vesicles from the pars recta is described in Fig. 6A, B, respectively. In these experiments the vesicles were preloaded with K_2SO_4 and suspended in Na_2SO_4. This resulted in an outwardly directed K^+ gradient and inwardly directed Na^+ gradient. Addition of valinomycin under these conditions resulted in an approx. 2-fold increase in dye response caused by fatty acids (compare the difference between curves 1 and 2 with curve 3 of Fig. 6A and B). The results presented in Figs. 5 and 6 clearly demonstrated that the uptake of short chain fatty acids in vesicles from the pars recta has occurred by an Na^+-dependent and electrogenic process.

Fig. 7A and B shows the optical changes produced by various concentrations of propionate and butyrate, respectively, in the presence of an Na^+ gradient by luminal membrane vesicles from the pars recta. The Na^+-dependent uptake of fatty acids shows a rapid increase at low medium concentrations (less than 0.02 mM) and approaches saturation at a concentration of 1 mM. The insets show the Eadie-Hofstee analysis of the same experimental data. A straight line relationship is obtained in both cases, suggesting that the Na^+-

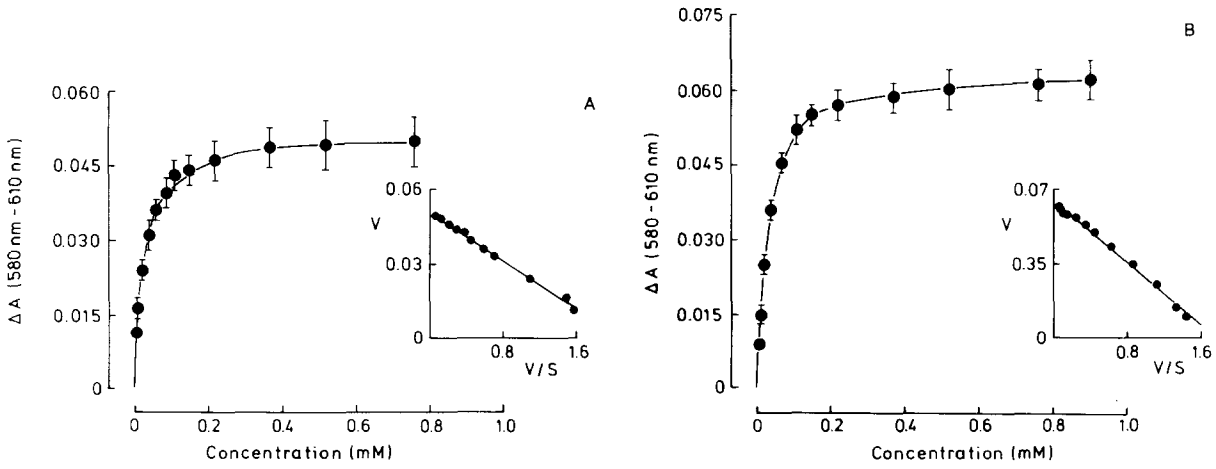

Fig. 7. Uptake of increasing concentrations of propionate (panel A) and butyrate (panel B) by luminal membrane vesicles prepared from pars recta of the proximal tubules as studied by spectrophotometry. The intravesicular medium was 310 mM mannitol and 15 mM Hepes-Tris (pH 7.5). The results shown in the 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 concentrations S. Further experimental details see Fig. 2 legend. Results are given as mean values ± S.D. of four experiments.

dependent uptake of fatty acids is mediated by means of a single transport system with the following K_a values: propionate, 0.020 ± 0.002 mM, butyrate, 0.025 ± 0.002 mM.

To test further the question as to whether L-lactate and short chain fatty acids share the same transport system in luminal membrane vesicles from the pars recta, spectrophotometric experiments were performed as described in previous papers [15–17]. The results of these experiments are plotted in Fig. 8A, B, C, D, E, F and G. It can be seen that the magnitude of the maximal optical response induced either by simultaneous addition of propionate and butyrate (Fig. 8E) or propionate and L-lactate (Fig. 8F) is approximately the same as that caused by the addition of propionate alone (Fig. 8A), strongly suggesting that all these monocarboxylic acids share the same transport system in luminal membrane vesicles isolated from pars recta of rabbit proximal tubule. Fig. 8G shows the absorbance changes produced by simultaneous addition of propionate and L-malate. It is seen that both substances when added alone induced maximal absorbance changes by approx. 0.055 (propionate, Fig. 8A) and approx. 0.027 (L-malate, Fig. 8D), but when applied together (Fig. 8G) the maximal absorbance change observed is approx. 0.075, indicating that mono- and dicarboxylic acids are transported by separate transport systems in luminal membrane vesicles from the pars recta.

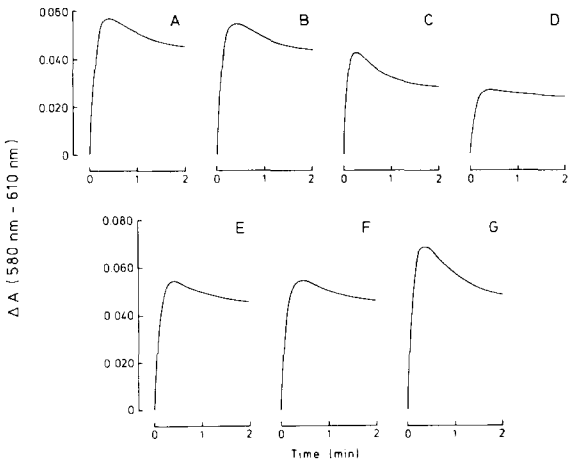

Fig. 8. Absorbance changes caused by addition of 1 mM propionate (A), 1 mM butyrate (B), 1 mM L-lactate (C), 1 mM L-malate (D), 1 mM propionate plus 1 mM butyrate (E), 1 mM propionate plus 1 mM L-lactate (F) or 1 mM propionate plus 1 mM L-malate (G) to luminal membrane vesicles prepared from pars recta of the proximal tubules. For further experimental details see Fig. 2 legend.

Discussion

The understanding of segment-specific kidney functions has been greatly advanced by the recent developments of methods for the isolation of highly purified luminal membrane vesicles from the pars convoluta and the pars recta of rabbit kidney proximal tubule (for a review see Ref. 18). Studies in our laboratory on the mechanism of renal transport of a wide variety of physiologically important compounds, including sugars [10,19], amino acids [7,8,15], monocarboxylic acids [16,17], and Na^+-H^+ exchange [20] in vesicles from these two distinct regions of proximal tubule clearly showed the existence of multiple transport systems for the reabsorption of these compounds that can be characterized in terms of affinity, specificity, cation dependence and the coupling ratio of different Na^+ solutes.

The experiments reported here demonstrate that both these luminal membrane vesicle preparations take up propionate and butyrate against a concentration gradient, although with different characteristics. Observations that the uptake of short chain fatty acids both in vesicles from the pars convoluta and from the pars recta is governed by an Na^+ gradient in the absence of other sources of energy established the existence of Na^+-fatty acid cotransport systems located along the proximal tubule. However, in vesicles from the pars convoluta the Na^+-dependent uptake of propionate and butyrate was insensitive to changes in membrane potential, which is indicative of electroneutral transfer of short chain fatty acids. These results further suggest that the coupling ratio of the Na^+-fatty acid cotransport in pars convoluta of rabbit proximal tubule is probably 1 : 1.

The characteristics of this Na^+-dependent, but electrically silent, transport system were studied by Millipore filtration technique. The observations that the initial rate of propionate transport was uninfluenced by the presence of L- and D-lactate

in the incubation medium strongly suggest the existence of a specific transport system for the reabsorption of short chain fatty acids in pars convoluta of rabbit proximal tubule.

On the other hand, our experiments showed that the luminal membrane vesicles from the pars recta possess an Na^+-dependent electrogenic transport system for short chain fatty acids. This conclusion is based on the following observations: (1) addition of propionate and butyrate to dye/vesicle suspensions in the presence of an Na^+ gradient caused transient depolarization of the membrane vesicles; (2) the rate of uptake of propionate and butyrate was enhanced by addition of valinomycin to K_2SO_4-loaded vesicles, which is indicative of electrogenic transport of these organic acids.

Another interesting feature of the present study is the observation that the uptake of propionate in vesicles from the pars recta is mediated by the same system which is responsible for the transport of lactate. On the basis of the above-mentioned findings, we emphasize the need in future studies to seriously consider that the pars recta and pars convoluta are to be considered as two structural entities with quite different functional properties. These will not be revealed in preparations derived from whole renal cortex, but will require the isolation of luminal membranes from discrete regions of the kidney.

Acknowledgements

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