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Characteristics of D-alanine transport by luminal membrane vesicles from pars convoluta and pars recta of rabbit proximal tubule

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Uptake of D-alanine against a concentration gradient has been shown to occur with isolated luminal-membrane vesicles from pars convoluta or pars recta of rabbit proximal tubule. Renal D-alanine transport systems, displaying the following characteristics, were shown: (1) In vesicles from pars convoluta, the uptake of D-alanine was mediated by both Na^+-dependent and Na^+-independent transport processes. It was found that an inwardly directed H^+-gradient could drive the transport of D-alanine into the vesicles both in the presence and absence of Na^+. Thus, in addition to Na^+, the transport of D-alanine is influenced by the H^+-gradient. (2) In vesicles from pars recta, the transient accumulation of D-alanine was strictly dependent on Na^+, since no 'overshoot' was ever observed in the absence of Na^+. Although the Na^+-dependent uptake of D-alanine was stimulated at acid pH, H^+ did not substitute for Na^+, as it apparently does in pars convoluta, but instead potentiated the Na^+ effect. (3) Addition of L-alanine to vesicle preparations, both from pars convoluta and from pars recta, specifically inhibited renal uptake of D-alanine. A comparison between the transport characteristics of D- and L-alanine indicated that these two isomers of alanine probably share common transport systems located along the proximal tubule of rabbit kidney.

Introduction

Earlier clearance studies have indicated that the renal amino acid transport systems prefer the naturally occurring L-isomers to the D-form [1]. It has been shown that the uptake of D-amino acids by rat and human kidney cortical slices [2] and by isolated perfused rabbit kidney tubules [3] can be inhibited by the L-isomers and vice versa, indicating that both types of stereo-isomers are transported by carrier-mediated processes. However, the uptake of D-alanine and of D-phenylalanine into isolated brush-border membrane vesicles apparently does not exhibit an 'overshoot' [4,5], nor do renal cortical slices accumulate D-lysine or D-valine to a higher concentration than present in the bath solution [2]. These observations have led Silbernagl [6] to suggest that the renal reabsorption of D-amino acids generally occurs by a carrier-mediated, but non-active transport.

Recently, we have been engaged in studying in detail the mechanism of L-alanine transport systems in luminal-membrane vesicles derived from pars convoluta and pars recta of rabbit proximal tubule [7,8]. We found that the uptake of L-alanine is mediated both by Na^+ and H^+ dependent electrogenic systems. In the present communication, we describe in similar experiments the characteristics of D-alanine transport by these vesicle preparations. The purpose of this study is (1) to examine whether this D-analogue of alanine can be taken up by membrane vesicles against a con-

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centration gradient, (2) the role of Na^+ and H^+ ion gradient on the uptake of D-alanine, (3) the tubular localization of D-alanine transport and finally, (4), to investigate whether D- and L-isomers of alanine share common transport system(s).

Materials and Methods

Materials

D-Alanine, L-alanine, and D-glucose, Trizma base, Trizma hydrochloride, Hepes, and Mes were purchased from Sigma Chemical Co., St. Louis, MO., U.S.A. Radioactive D-[^{14}C]alanine (specific activity 40 mCi/mmol) was obtained from Amersham International plc, Buckinghamshire, U.K. 3,3′-Diethyloxadicarbocyanine iodide was supplied by Eastman Kodac Co., Rochester, NY, U.S.A. These and all other reagents were of A.R. grade.

Preparation of luminal-membrane vesicles

Luminal-membrane vesicles were isolated from pars convoluta ('outer cortex') and from pars recta ('outer medulla') of the proximal tubule of rabbit kidney according to the method already described [9–11] and only briefly described here. The renal tissue was homogenized and luminal-membrane vesicles were prepared by differential centrifugation and Ca^{2+}-precipitation. Usually the vesicles were suspended in a solution containing 310 mM mannitol and 15 mM Hepes-Tris buffer (pH 7.5), but in a series of experiments luminal-membrane vesicles were prepared and suspended in a solution containing 310 mM mannitol and 15 mM Mes-Tris buffer (pH 5.5). The purity of the membrane vesicle preparation regarding the content of luminal vesicles was examined by electron microscopy [12] and by measuring specific activities of various enzyme markers as previously described [13]. Average enrichment in specific activity (final pellet/homogenate) of the luminal membrane marker leucine aminopeptidase was 17-fold (range 13–29), while that of basolateral marker, (Na^+/K^+)-stimulated ATPase, and that of the mitochondrial marker, succinate dehydrogenase, were both < 0.5. The amount of protein was determined by the method of Lowry et al. [14] as modified by Peterson [15] with serum albumin (Sigma Chemical Co., St. Louis, MO, U.S.A.) as a standard. All solutions used in this study were sterilized before use. The possible bacterial contamination of membrane vesicle preparations was examined by incubating the samples on blood-agar plates and by electron microscopy. No bacteria were found in these preparations.

Uptake of D-alanine by membrane vesicles

The uptake of D-alanine by luminal-membrane vesicles was studied by Millipore filtration [16] and by a spectrophotometric method with potential-sensitive carbocyanine dye as previously described [17]. The details of the individual experiments are given in the legends to the figures. Briefly, the uptake of radioactive D-alanine under various experimental conditions was studied as follows: 20 μl of luminal-membrane vesicle suspension were added at time zero to 100 μl of incubation medium. Transport of D-alanine in vesicles was stopped by addition of 1 ml ice-cold stop buffer. The resulting suspension 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 of 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 uptake data the value of a blank obtained by filtering denatured membranes (boiled for 2 min) added to an incubation tube containing radioactive D-alanine.

Calculations

The Michaelis-Menten kinetics of the uptake of various concentrations of L-alanine were analysed. Theoretical saturation curves were fitted to the experimental data by using a computer-analysed statistical iteration procedure [18].

Results

Uptake of D-alanine by luminal-membrane vesicles from pars convoluta

The characteristics of D-alanine transport in membrane vesicles from pars convoluta were studied by a spectrophotometric method and by a

rapid filtration technique. Fig. 1A and 1B show absorbance changes (ΔA) induced by the addition of D-alanine to vesicle-dye suspension in the presence of various Na^+- and K^+-salt gradients, respectively. It is seen that introduction of D-alanine to these vesicle preparations depolarizes the membrane potential to various extent both with Na^+- and K^+-salt gradients, in a manner which may be assumed to reflect the various permeabilities of the anions [17]. Fig. 1C records ΔA produced by D-alanine in the absence of Na^+ and K^+. In these experiments the luminal-membrane vesicles were prepared and equilibrated in a medium containing 310 mM mannitol dissolved in 15 mM Hepes-Tris buffer (pH 7.5). The potential-sensitive carbocyanine dye [17] was also dissolved in the same above-mentioned solution. 60 μl of the vesicle suspension (protein concentration 15 mg/ml) was added to 2.4 ml of the dye solution in the cuvette. Immediately after 80 μl of either stock solution of D-alanine (curve 1) or D-glucose (curve 2) was added, in order to obtain a final concentration in the cuvette of 4.5 mM of these organic solutes. It is apparent from the figure that addition of D-alanine, but not D-glucose, resulted in transient depolarization of luminal-membrane vesicles indicative of the Na^+- and K^+-independent electrogenic transport process for D-alanine uptake. Curve 3 shows the effect of FCCP on the rate of uptake of D-alanine in these vesicles. It is seen that ad-

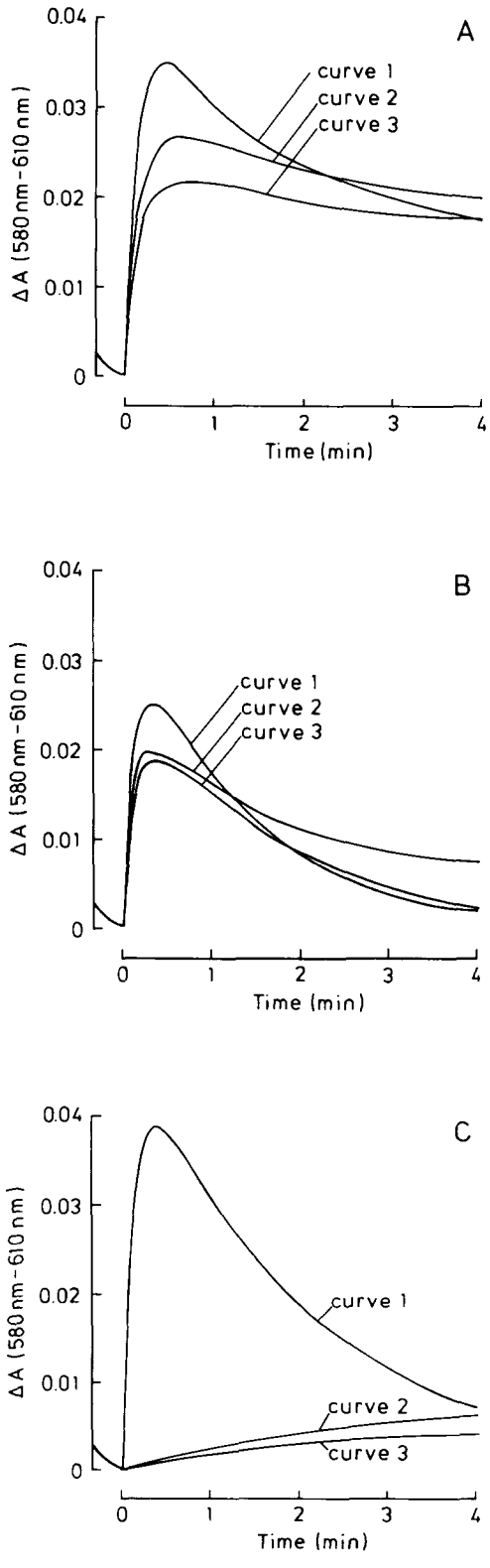

Fig. 1. Time course of D-alanine uptake by luminal-membrane vesicles from pars convoluta as registered by the potential-sensitive dye 3,3′-diethyloxadicarbocyanine iodide. Common experimental conditions were as follows: protein concentration 0.4 mg/ml, pH 7.5, temperature 20 °C and dye concentration 15 μM. The intravesicular medium was 310 mM mannitol and 15 mM Hepes-Tris. Panel A: Uptake of D-alanine at 4.5 mM in the presence of 155 mM NaCl (Curve 1), 103 mM Na_2SO_4 (Curve 2) or 155 mM sodium D-gluconate (Curve 3). Panel B: Uptake of D-alanine at 4.5 mM in the presence of 155 mM KCl (Curve 1), 103 mM K_2SO_4 (curve 2) or 155 mM potassium D-gluconate (Curve 3). Panel C: Uptake of D-alanine (Curve 1) and D-glucose (Curve 2) at concentrations of 4.5 mM in the presence of 310 mM mannitol. Curve 3 shows the effect of addition of 10 μM FCCP in the vesicle dye suspension before addition of D-alanine. In all extravesicular media 15 mM Hepes-Tris was used as buffer system. The spectrophotometer was operated in the dual wavelength mode with 580 nm and 610 nm (reference wavelength). The brake in the curves, at 0 min, indicates addition of solute.

dition of this ionophore to the dye-vesicle suspension abolished the transient depolarization of the membrane vesicles, suggesting that the Na^+-independent electrogenic uptake of D-alanine may be driven by H^+/D-alanine cotransport.

The pattern of uptake of radioactive D-alanine by luminal-membrane vesicles from pars convoluta is shown in Fig. 2A. The initial uptake of D-alanine shows a rapid increase in the presence of Na^+ gradient (curve 1). The uptake reaches a maximal value of 0.133 nmol/mg protein after approximately 1 min of incubation. Then follows a slow, but steady decrease in D-alanine uptake, which has reached an equilibrium state after 120 min. By contrast, no transient accumulation of D-alanine is observed when NaCl is replaced by an equimolar concentration of KCl in the incubation medium (Curve 2).

Fig. 2B shows the effect accomplished by H^+ and Na^+ gradient on the uptake of radioactive D-alanine by these membrane vesicle preparations. It appears that superposition of an H^+ gradient (extravesicular > intravesicular) resulted in an increase in the Na^+ dependent transient accumulation of D-alanine (compare curve 1 with curve 2). Curve 3 shows the Na^+-gradient-dependent up-

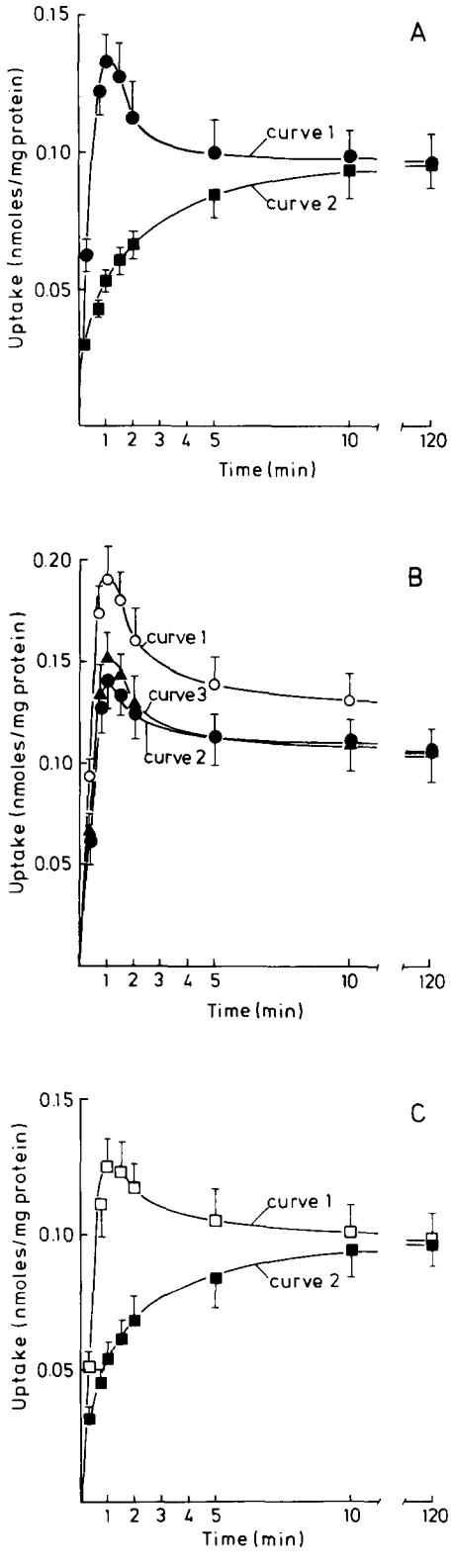

Fig. 2. Cation-dependent uptake of D-alanine by luminal-membrane vesicles from pars convoluta as studied by Millipore filtration. Panel A: a 20 μl portion of the vesicle suspension was incubated at different time intervals in 100 μl of incubation mixture consisting of 100 μM D-[^{14}C]alanine in 155 mM NaCl (Curve 1 (●)) or 155 mM KCl (Curve 2 (■)). In both intravesicular and extravesicular media 15 mM Hepes-Tris (pH 7.5) was used as buffer system. Panel B: Uptake of D-[^{14}C]alanine at 100 μM in the presence of 155 mM NaCl in 15 mM Mes-Tris buffer (pH 5.5) (Curve 1 (○)) or in 15 mM Hepes-Tris buffer (pH 7.5) (Curve 2 (●)). Curve 3 (▲) shows the effect of Na^+-gradient-dependent uptake of D-alanine at a low pH but in the absence of an H^+ gradient ($pH_{in} = pH_{out} = 5.5$). In these experiments the vesicles were preincubated with 310 mM mannitol and 10 μM FCCP in 15 mM Mes-Tris buffer (pH 5.5) for 30 min and then incubated in 155 mM NaCl, 100 μM D-[^{14}C]alanine, 10 μM FCCP in 15 mM Mes-Tris buffer (pH 5.5) at different time intervals. Panel C: The experimental conditions were essentially the same as in Panel B except that NaCl is replaced by KCl at pH 5.5 (Curve 1 (□)) and 7.5 (Curve 2 (■)). The composition of stop-buffers as well as washing solutions was the same as the various incubation media but without D-alanine used in different groups of experiments. Results are given as mean values ± S.D. of three experiments.

take of D-alanine at lower pH, but in the absence of H^+ gradient (i.e., $pH_{in} = pH_{out} = 5.5$) by luminal membrane vesicles. In these experiments the vesicles were preincubated for 30 min in a medium containing 310 mM mannitol, 15 mM Mes-Tris buffer (pH 5.5). The vesicle preparation was then centrifuged and resuspended in an appropriate amount of the above-mentioned medium in order to achieve a protein concentration of 15 mg/ml. 20 µl of these vesicle preparations were incubated in 100 µl of incubation medium containing radioactive D-alanine and 155 mM NaCl dissolved in 15 mM Mes-Tris buffer (pH 5.5). This procedure was performed to ensure that the uptake of D-alanine is now measured in the presence of an Na^+ gradient (extravesicular > intravesicular). It is seen that the H^+-gradient-dependent uptake of D-alanine is abolished under these experimental conditions.

The effect of H^+ and K^+ gradient on the renal uptake of radioactive D-alanine is shown in Fig. 2C. The purpose of performing these experiments was to examine whether an H^+ gradient in the absence of Na^+ can drive uphill transport of D-alanine in membrane vesicles from this segment of proximal tubule. It is interesting to note that inwardly directed H^+ gradient stimulated the uptake of D-alanine, resulting in an 'overshoot' (curve 1). By contrast, no transient accumulation of D-alanine was observed in the presence of K^+ gradient (curve 2), when the extravesicular pH was equal to intravesicular pH (i.e. pH 7.5).

Fig. 3A shows the uptake of radioactive D-alanine at increasing medium concentrations of the amino acid in the presence of an Na^+ gradient alone (curve 2) and in the presence of Na^+ gradient and H^+ gradient (curve 1). It is seen that superposition of an H^+ gradient in addition to an

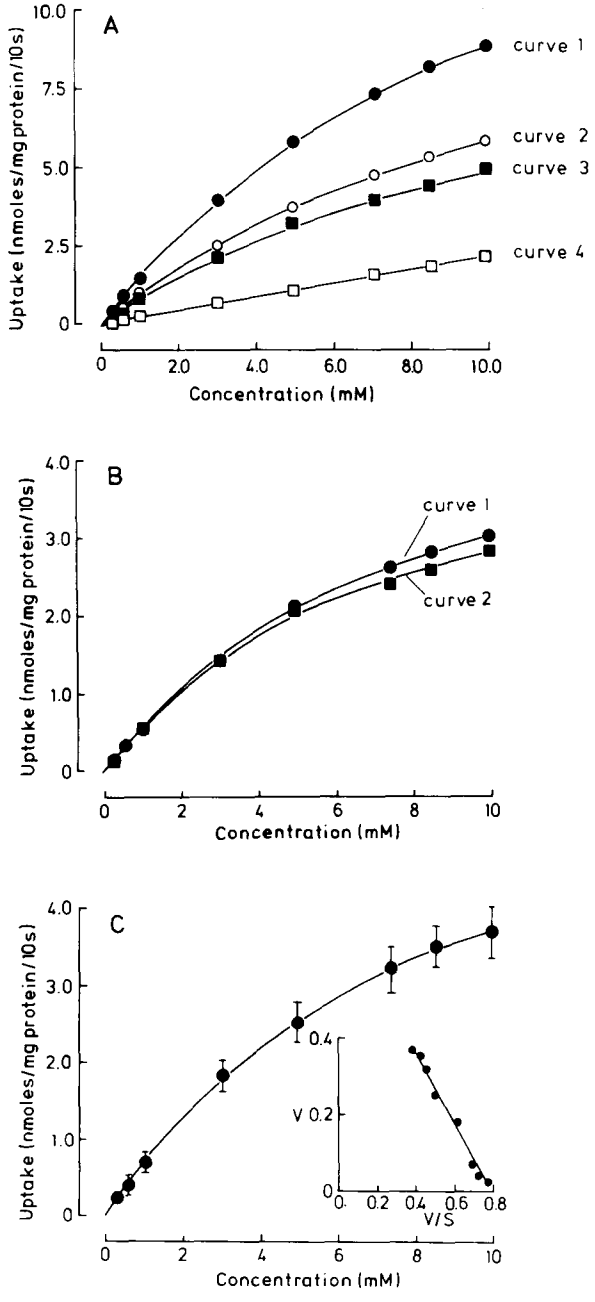

Fig. 3. Cation-dependent uptake of D-alanine at increasing concentrations by luminal-membrane vesicles from pars convoluta. Panel A: a 20 µl portion of the vesicle suspension prepared in 310 mM mannitol, 15 mM Hepes-Tris (pH 7.5) was added to 100 µl of incubation medium containing 155 mM NaCl in 15 mM Mes-Tris buffer (pH 5.5) (Curve 1 (●)), 155 mM NaCl in 15 mM Hepes-Tris buffer (pH 7.5) (Curve 2 (○)), 155 mM KCl in 15 mM Mes-Tris buffer (pH 5.5) (Curve 3 (■)), or 155 mM KCl in 15 mM Hepes-Tris buffer (pH 7.5) (Curve 4 (□)). The media contained 100 µM D-[^{14}C]alanine and various concentrations of unlabelled D-alanine ranging from 0.2 to 10 mM (final concentration). Panel B: Effect of H^+-gradient in the presence of Na^+ (Curve 1 (●)) and in the absence of Na^+ (Curve 2 (■)). Panel C: Uptake of increasing concentrations of D-alanine in the presence of an NaCl gradient alone. In the inset the results are shown in an Eadie-Hofstee plot.

TABLE I

EFFECT OF L-ALANINE AND D-GLUCOSE ON Na^+-DEPENDENT UPTAKE OF D-ALANINE BY LUMINAL-MEMBRANE VESICLES FROM PARS CONVOLUTA

Results are given as mean values ± S.D. of at least three experiments. Final concentrations of substrate in the incubation medium was 0.3 mM and of inhibitors 5.0 mM. For further experimental details see Methods.

Addition	Extravesicular pH 7.5			Extravesicular pH 5.5		
	D-[^{14}C]alanine uptake (nmol/mg protein per 30 s)	% of control	P	D-[^{14}C]alanine uptake (nmol/mg protein per 30 s)	% of control	P
None	0.926 ± 0.065	100	–	1.688 ± 0.128	100	–
L-Alanine	0.811 ± 0.078	88	$0.1 < P < 0.2$	0.791 ± 0.053	47	$P < 0.01$
D-Glucose	0.912 ± 0.092	98	$0.8 < P < 0.9$	1.637 ± 0.104	97	$0.6 < P < 0.7$

Na^+ gradient resulted in an increased uptake of D-alanine by these vesicles. Curve 4 illustrates the uptake of D-alanine in the presence of KCl gradient, but in the absence of Na^+ and H^+ gradients. The uptake of D-alanine exhibited simple diffusion properties, being proportional to medium concentration of amino acid under these conditions. Curve 3 depicts the uptake of D-alanine in the presence of an H^+ gradient alone. It is apparent from the curve that in the absence of Na^+, the H^+ gradient markedly enhanced the uptake of D-alanine in vesicles from pars convoluta. The effect of the H^+ gradient as such is the same as the difference between the uptake in an Na^+ plus H^+ gradient and Na^+ gradient alone as shown in Fig. 3B with $K_m = 7.86 \pm 0.38$ mM and $V_{max} = 5.25 \pm 0.21$ nmol/mg protein per 10 s. In the presence of an Na^+ gradient alone (i.e. curve 2 minus curve 4) the K_m was 6.77 ± 0.52 mM and $V_{max} = 6.19 \pm 0.16$ nmol/mg protein per 10 s (Fig. 3C).

Table I describes the effect of L-alanine and D-glucose on the Na^+-dependent uptake of radioactive D-alanine at extravesicular pH 7.5 (left column) and pH 5.5 (right column). The intravesicular pH was 7.5 in these experiments. Addition of L-alanine (5 mM) to the incubation medium resulted in moderate inhibition at pH 7.5, while the inhibition of D-alanine uptake was more pronounced at medium pH 5.5. D-Glucose did not inhibit the influx of D-alanine at either of these medium pH values.

Uptake of D-alanine by luminal membrane vesicles from pars recta

The patterns of uptake of radioactive D-alanine by luminal-membrane vesicles from pars recta of

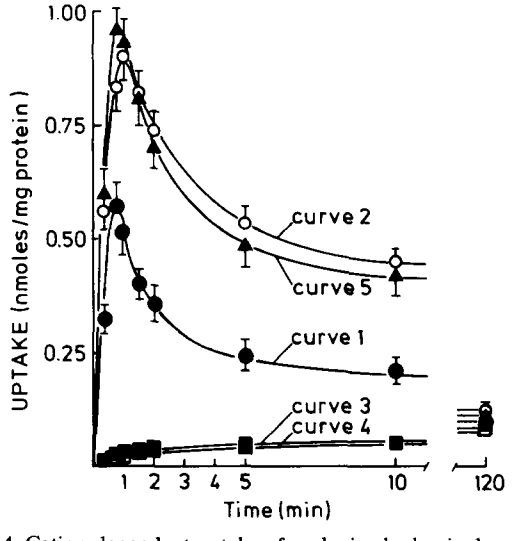

Fig. 4. Cation-dependent uptake of D-alanine by luminal-membrane vesicles from pars recta as studied by Millipore filtration. 20 µl of vesicle suspension were incubated at different time intervals in 100 µl of incubation mixture consisting of 155 mM NaCl, 100 µM D-[^{14}C]alanine in 15 mM Hepes-Tris buffer (pH 7.5) (Curve 1 (●)), or in 15 mM Mes-Tris buffer (pH 5.5) (Curve 2 (○)). Curves 3 and 4 show the effect of substituting NaCl by KCl in 15 mM Hepes-Tris buffer (pH 7.5) (Curve 3 (■)) or in 15 mM Mes-Tris buffer (pH 5.5) (Curve 4 (□)), respectively. Curve 5 (▲) shows the effect of Na^+-gradient-dependent uptake of D-alanine at a low pH but in the absence of an H^+ gradient ($pH_{in} = pH_{out} = 5.5$). Results are given as mean values ± S.D. of four experiments.

rabbit proximal tubule suspended in various incubation media are shown in Fig. 4. The presence of Na^+ gradient (curve 1) stimulated uptake of D-alanine in the membrane vesicles. The transient renal accumulation of D-alanine was maximal at approx. 1 min of incubation, and reached a value of approx. 0.6 nmol/mg protein which is approx. 4-fold of that obtained in vesicles from pars convoluta under the same experimental conditions (compare Curve 1 of Fig. 2 with Curve 1 of Fig. 4). Curve 2 shows the effect accomplished by H^+ plus Na^+ gradient on the uptake of D-alanine by these vesicle preparations. It is apparent from the experimental data that the superposition of H^+ gradient resulted in an approx. 2-fold increase in the Na^+-dependent transient accumulation of D-alanine. Curves 3 and 4 show the uptake of D-alanine in the presence of K^+ and H^+ plus K^+ gradients, respectively. No 'overshoot' was observed in the presence of K^+ or H^+ plus K^+ gradient. The results of these experiments clearly demonstrate that the transient uptake of D-alanine in membrane vesicles from pars recta is strictly dependent on the Na^+ gradient. To examine whether the Na^+-dependent transient accumulation of D-alanine in vesicles from pars recta can be obtained by a lower pH without pH gradient (i.e. $pH_{in} = pH_{out} = 5.5$), another series of experiments was performed. In these experiments luminal membrane vesicles from pars recta were prepared in medium containing 310 mM mannitol in 15 mM Mes-Tris buffer (pH 5.5). These vesicles were then incubated in a medium containing radioactive D-alanine and 155 mM NaCl dissolved in 15 mM Mes-Tris buffer (pH 5.5) (Curve 5 in Fig. 4). This procedure was performed to ensure that the uptake of D-alanine is measured only in the presence of an Na^+ gradient (extravesicular > intravesicular). The comparison between the results given in Curve 5 (lower pH without pH gradient) and Curve 2 (pH gradient) revealed that the magnitude of transient accumulation of D-alanine is not significantly different under these experimental conditions. These results thus strongly suggest that the lower pH but not the pH gradient itself is responsible for the stimulation of Na^+-gradient-dependent uptake of D-alanine in membrane vesicles from pars recta.

Fig. 5A records the absorbance changes (ΔA) induced by the addition of D-alanine to these

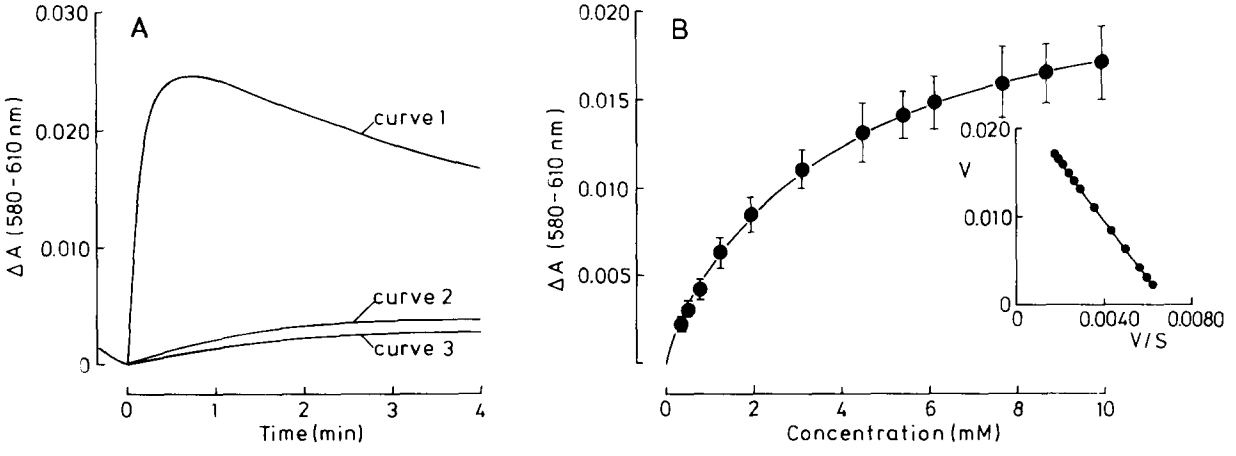

Fig. 5. Panel A: Time course of D-alanine uptake by luminal-membrane vesicles from pars recta as registered by the spectrophotometric method. The intravesicular medium was 310 mM mannitol whereas the external medium was 10 mM D-alanine, and 155 mM NaCl (Curve 1), or 155 mM KCl (Curve 2) or 310 mM Mannitol (Curve 3). In both intravesicular and extravesicular media 15 mM Hepes-Tris was used as buffer system. For further experimental details see Fig. 1 legend. Panel B: Absorbance changes (ΔA) induced by increasing concentrations of D-alanine by luminal-membrane vesicles from pars recta. The intravesicular medium was 310 mM mannitol in 15 mM Hepes-Tris buffer (pH 7.5). The results shown are the absorbance changes obtained with an external medium of 155 mM NaCl in 15 mM Hepes-Tris buffer (pH 7.5), corrected for absorbance changes obtained in the presence of an equimolar concentration gradient of KCl. 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.

vesicle-dye suspensions in the presence of NaCl gradient (Curve 1), KCl gradient (Curve 2) and mannitol (Curve 3). It is seen that introduction of D-alanine to the suspension depolarizes vesicle membrane only in the presence of Na^+ gradient. These results thus establish that the transport of D-alanine in vesicles from pars recta is a strictly Na^+-dependent electrogenic process, since no 'overshoot' was ever observed under KCl gradient or in mannitol. Fig. 5B shows the corresponding ΔA produced by addition of different concentrations of D-alanine to membrane vesicles and potential sensitive dye. Insert shows the Eadie-Hofstee analysis of the data. A straight line relationship is obtained suggesting that D-alanine transport in these luminal-membrane vesicles is mediated via a single Na^+-dependent electrogenic system, with $K_m = 3.2 \pm 0.28$ mM.

The effect of low concentrations of L-alanine (0.35 mM) and D-glucose (0.35 mM) on the Na^+-dependent uptake of radioactive D-alanine by these vesicle preparations was also examined. The results of these experiments showed that L-alanine reduced the uptake of D-alanine to $31 \pm 4\%$ of control value, whereas the uptake of D-alanine was unaffected by D-glucose (not shown).

Discussion

The data presented showed that D-alanine is taken up against a concentration gradient by isolated luminal-membrane vesicles from pars convoluta as well as pars recta, although with different transport characteristics. The uptake of D-alanine in vesicles from pars convoluta occurred by both Na^+-dependent and Na^+-independent transport processes. The results plotted in Fig. 2B and Fig. 2C provide direct evidence for coupling between D-alanine and H^+ fluxes both by the Na^+-dependent and Na^+-independent D-alanine transporters of membrane vesicles from pars convoluta. Thus, it was found that superposition of H^+ gradient (extravesicular > intravesicular) enhanced the renal uptake of D-alanine both in the presence and absence of Na^+. The unequivocal evidence supporting the existence of H^+/D-alanine cotransport in these vesicle preparations is the ability of an H^+ gradient to drive net D-alanine accumulation in the absence of other energy source (Curve 1 in Fig. 2C). These transport characteristics of D-alanine are similar to those of L-alanine uptake properties recently found in vesicles from pars convoluta of rabbit proximal tubule (for details see Ref. 7). Competition experiments showed that addition of L-alanine, but not D-glucose to the incubation medium inhibited the uptake of D-alanine, suggesting that these two isomers of alanine probably share a common transport system in pars convoluta.

In contrast to the above-mentioned findings the transient H^+-stimulated uptake of D-alanine in luminal-membrane vesicles obtained from pars recta is strictly dependent on Na^+ since no 'overshoot' was ever observed in the absence of Na^+. Although the Na^+-dependent uptake of D-alanine was stimulated at acid pH, H^+ did not substitute for Na^+, as it apparently does in pars convoluta, but instead potentiated the Na^+ effect (Curves 2 and 5 in Fig. 4). Similar results were obtained in the case of L-alanine uptake by luminal membrane vesicles from pars recta [8].

However, saturation kinetic experiments showed that in contrast to the uptake of L-alanine, the transport of D-alanine is mediated by a single system (inset in Fig. 5B), in vesicles from pars recta. To examine whether the uptake of D-alanine occurred via a high affinity L-alanine transport system, previously shown to exist in this region of proximal tubule (for details see Refs. 7 and 8), a series of experiments were performed. It is found that addition of L-alanine at a low concentration (0.35 mM) to the incubation medium effectively inhibited the uptake of D-alanine to these vesicle preparations. These results suggest that D-alanine is probably reabsorbed by L-alanine transport system (high affinity) in pars recta of rabbit proximal tubule. However, further both in vivo and in vitro kinetic studies are needed to confirm and establish this proposal.

Acknowledgements

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