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Renal transport of neutral amino acids

Demonstration of Na^+-independent and Na^+-dependent electrogenic uptake of L-proline, hydroxy-L-proline and 5-oxo-L-proline by luminal-membrane vesicles

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Uptake of L-proline, hydroxy-L-proline and 5-oxo-L-proline by luminal-membrane vesicles isolated either from whole cortex or from pars convoluta or pars recta of proximal tubules was studied by a spectrophotometric method. Uptake of L-proline and hydroxy-L-proline by vesicles from whole cortex was mediated by both Na^+-dependent and Na^+-independent, but electrogenic, processes, whereas transport of 5-oxo-L-proline in these vesicles was strictly Na^+-dependent. Eadie–Hofstee analysis of saturation-kinetic data suggested the presence of multiple transport systems in luminal-membrane vesicles from whole renal cortex for the uptake of all these amino acids. Tubular localization of the transport systems was studied by the use of vesicles derived from pars convoluta and from pars recta. In pars recta transport of all three amino acids was strictly dependent on Na^+ and occurred via a high-affinity system (half-saturation: 0.1–0.3 mM). Cation-dependent but Na^+-unspecific transport of low affinity for L-proline and hydroxy-L-proline was exclusively localized to the pars convoluta, which also contained a Na^+-preferring system of intermediate affinity (half-saturation: L-proline, 0.75 mM; hydroxy-L-proline, 1.3 mM). 5-Oxo-L-proline was transported by low-affinity and Na^+-dependent systems in both pars convoluta and pars recta. Competition experiments revealed that transport systems for L-proline and hydroxy-L-proline are common, but indicated separate high-affinity transport systems for 5-oxo-L-proline and L-proline in luminal-membrane vesicles from pars recta. The physiological importance of the presence of various neutral amino acid-transport systems in different segments of the proximal tubule is discussed.

Renal amino acid transport has been studied on isolated membrane fragments, in particular by the use of luminal-membrane vesicle preparations derived from whole kidney cortex (Sacktor, 1977, 1978). From these studies it was proposed that the reabsorption of neutral α-amino acids may be attributed to transport by one common system, except for glycine and L-proline, for which some evidence was obtained for additional separate transport systems (McNamara *et al.*, 1976). This proposal contrasts with the variety of separate amino acid-transport systems described in other cell systems (Christensen, 1975). These systems, designated L, A and ASC, are distinguished by their dependence on Na^+ and by the nature of the amino acids that they transport (Christensen, 1969, 1975). However, unambiguous discrimination between various transport systems can be difficult to obtain, and in the past renal investigators have relied heavily on competition studies for this purpose, i.e. specificity is determined on the basis of the inhibitory effect of different amino acids towards a single test amino acid (Fass *et al.*, 1977; Hammerman & Sacktor, 1977*a*,*b*). Competition as a single criterion is not well suited to reveal, for example, two separate transport systems for the same amino acids, and in some instances may even be an apparent phenomenon, since certain solutes may inhibit transport activity without themselves being transported by the system (Boezi & DeMoss, 1961; Hagihara *et al.*, 1963; Sheikh, 1976).

The primary purpose of this series of studies is to investigate systematically the mechanisms of transport of various neutral L- and D-amino acids

in highly purified luminal- and basolateral-membrane vesicles derived from rabbit kidney cortex (Sheikh *et al.*, 1982). We have developed methods especially for delineation and characterization of separate transport systems. The strategy is based on the use of rapid screening procedures to measure electrogenic transport of solutes by the use of a potential-sensitive dye, in conjunction with conventional transport studies by the Millipore-filtration technique (Kragh-Hansen *et al.*, 1982*a*,*b*; Jørgensen *et al.*, 1983). With this methodology a very differentiated pattern of amino acid transport systems is readily shown to exist, which can be characterized in terms of differences in affinity, specificity and cation-dependence. In the present paper we report clear evidence for the existence of two different Na^+-dependent systems and a unique Na^+-independent, but electrogenic, transport system for L-proline, hydroxy-L-proline and 5-oxo-L-proline, which are localized in luminal-membrane vesicles from two different regions of kidney cortex, namely pars recta and pars convoluta of the proximal tubule. The physiological importance of the arrangement of low- and high-affinity systems for amino acid reabsorption along the renal proximal tubule is discussed.

Experimental

Isolation of luminal-membrane vesicles

Luminal-membrane vesicles were isolated either from whole renal cortex or from pars convoluta or pars recta of proximal tubules of rabbit kidney as reported from this laboratory (Kragh-Hansen *et al.*, 1982*b*, 1984). Vesicles were suspended in solution containing 310mM-mannitol and 15mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid]/Tris buffer (pH7.5), and used within 6h after the preparation. The purity of membrane preparations from outer cortical tissue and outer medullary tissue was examined by electron microscopy as described in the preceding paper (Kragh-Hansen *et al.*, 1984) and by measuring specific activities of various enzyme markers as previously described (Sheikh *et al.*, 1982). The amount of protein in different fractions was determined by the method of Lowry *et al.* (1951) as modified by Peterson (1977).

Transport studies

The characteristics of uptake of L-proline, hydroxy-L-proline and 5-oxo-L-proline by luminal-membrane vesicles obtained either from renal cortex or from pars recta or pars convoluta of proximal tubules were studied by the spectrophotometric method previously described from this laboratory (Kragh-Hansen *et al.*, 1982*a*).

Results

Characteristics of uptake of amino acids by vesicles from whole renal cortex

Figs. 1(*a*), 1(*b*) and 1(*c*) respectively depict the pattern of uptake of L-proline, hydroxy-L-proline and 5-oxo-L-proline by luminal-membrane vesicles from whole renal cortex. Curves 1, 2 and 3 in Fig. 1 describe the absorbance changes (ΔA, i.e. $A_{580}-A_{610}$) caused by the addition of amino acids in the presence of NaCl, Na_2SO_4 and sodium gluconate respectively. Fig. 1 shows that addition of amino acids to the membrane–dye suspension in the presence of the various sodium salt gradients depolarizes the membrane vesicles. The magnitude of the spectral change ('overshoot') varies with the type of sodium salt anion and increases in the following order: gluconate$^-$ < SO_4^{2-} < Cl^-. Figs. 2(*a*), 2(*b*) and 2(*c*) record the spectral changes caused by the addition of L-proline, hydroxy-L-proline and 5-oxo-L-proline respectively to membrane–dye suspension in the presence of various potassium salt gradients. It is noteworthy that introduction of L-proline and hydroxy-L-proline to the membrane–dye suspension under various potassium salt gradients also depolarizes the membrane vesicles, although in the presence of KCl the depolarization response is less than in NaCl. By contrast, no depolarization is discernible when 5-oxo-L-proline is added to the membrane–dye suspension in the presence of a KCl gradient. Thus electrogenic transport of L-proline and hydroxy-L-proline is not exclusively dependent on co-transport with Na^+. This situation is different from that encountered with many other transported solutes, which require Na^+ specifically for demonstration of electrogenic transport. This is illustrated by curves 4 and 5 in Fig. 2(*a*), which, in accordance with previous findings (Kragh-Hansen *et al.*, 1982*a*), show that addition of D-glucose or L-phenylalanine to the vesicle–dye system did not depolarize the membrane vesicles in the absence of a NaCl gradient (presence of a KCl gradient). We also studied the rate of uptake of these compounds by vesicles in the presence of various tetraethylammonium salts or a choline chloride gradient and obtained results similar to those obtained in the presence of the potassium salts (not shown).

Figs. 3(*a*), 3(*b*) and 3(*c*) show the absorbance changes (ΔA) measured at the peak of the dye response 'overshoot' (approx. 15s) induced by addition of increasing concentrations of L-proline, hydroxy-L-proline and 5-oxo-L-proline respectively to membrane vesicles from whole renal cortex, in the presence of a NaCl gradient. The Na^+-dependent uptake of these amino acids shows a rapid increase at low concentrations in the medium (0.1–1mM), whereas above this value the increase in ΔA

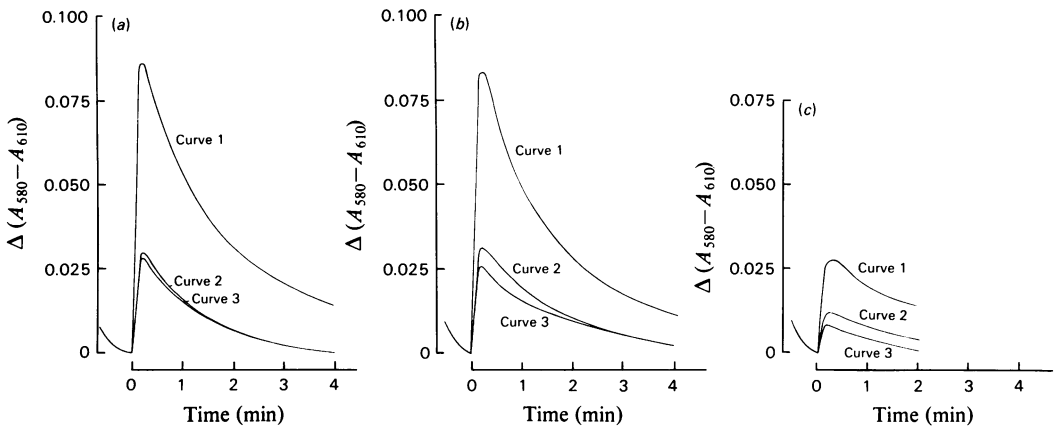

Fig. 1. *Uptake of* L-*proline, hydroxy-*L-*proline and 5-oxo-*L-*proline by renal luminal-membrane vesicles from whole cortex in the presence of a* Na^+ *gradient*

Uptake of L-proline (*a*), hydroxyproline (*b*) and 5-oxo-L-proline (*c*) by luminal-membrane vesicles prepared from whole cortex was studied by spectrophotometry. Common experimental conditions: the protein concentration was 0.2mg/ml, the pH7.5 and the temperature 20°C. The intravesicular medium was 310mM-mannitol, whereas the external media were 155mM-NaCl (curves 1), 103mM-Na_2SO_4 (curves 2) or 155mM-sodium D-gluconate (curves 3). In both intravesicular and extravesicular media 15mM-Hepes/Tris was used as buffer system. The dye concentration was 15μM. The break in the curves, at 0min, indicates the addition of solute. The final concentration of the solute was in all cases 5mM. All the spectral curves were corrected for the effect of adding a small volume of 15mM-Hepes/Tris buffer alone (the medium of the solute's stock solutions). The spectrophotometer was operated in the dual-wavelength mode of 580nm and 610nm (reference wavelength). The results are from representative experiments.

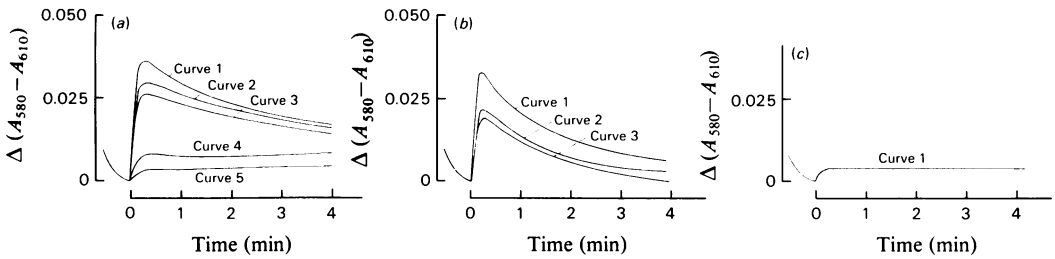

Fig. 2. *Uptake of* L-*proline, hydroxy-*L-*proline and 5-oxo-*L-*proline by renal luminal-membrane vesicles from whole cortex in the presence of a* K^+ *gradient*

Uptake of L-proline (*a*), hydroxy-L-proline (*b*) and 5-oxo-L-proline (*c*) by luminal-membrane vesicles prepared from whole cortex was studied by spectrophotometry. Experimental conditions were the same as given in Fig. 1 legend, except that the external media were KCl (curves 1), K_2SO_4 (curves 2) and potassium D-gluconate (curves 3). Curves 4 and 5 in panel (*a*) depict the uptake of L-phenylalanine and D-glucose, respectively, in the presence of 155mM-KCl in Hepes/Tris buffer.

is less pronounced and is approximately proportional to the increase in amino acid concentration. Insets in Fig. 3 show Eadie–Hofstee analysis of the experimental data. Curvilinear plots were obtained in all cases, which suggests the presence of multiple transport systems in luminal-membrane vesicles from whole renal cortex for the uptake of these amino acids. In the presence of a KCl gradient only low-affinity uptake of L-proline (Fig. 4*a*) and hydroxy-L-proline (Fig. 4*b*) is demonstrated, but no discernible dye response is detected for 5-oxo-L-proline (results not shown). The insets in Fig. 4 show Eadie–Hofstee analysis of the data. A straight-line relationship is obtained for both L-proline and hydroxy-L-proline, suggesting that the Na^+-independent uptake of these amino acids occurs via a single transport system. These results were analysed by a computer statistical procedure

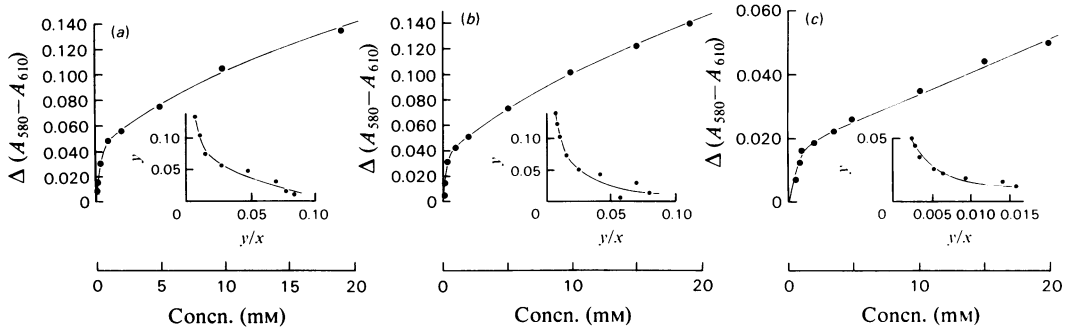

Fig. 3. *Kinetics of* L-*proline, hydroxy-*L-*proline and 5-oxo-*L-*proline uptake by luminal-membrane vesicles from whole cortex in the presence of a NaCl gradient*

Uptake of increasing concentrations of L-proline (*a*), hydroxy-L-proline (*b*) and 5-oxo-L-proline (*c*) by luminal-membrane vesicles prepared from whole cortex was measured. Insets show the same data in an Eadie–Hofstee-type plot. In the inset *y* represents $(A_{580}-A_{610})$, and *x* the concentration (mM) of the amino acids. The intravesicular medium was 310mM-mannitol/15mM-Hepes/Tris buffer. The results shown in the Figure are the absorbance changes obtained with an external medium of 155mM-NaCl/15mM-Hepes/Tris buffer, pH 7.5. The data are from a representative experiment.

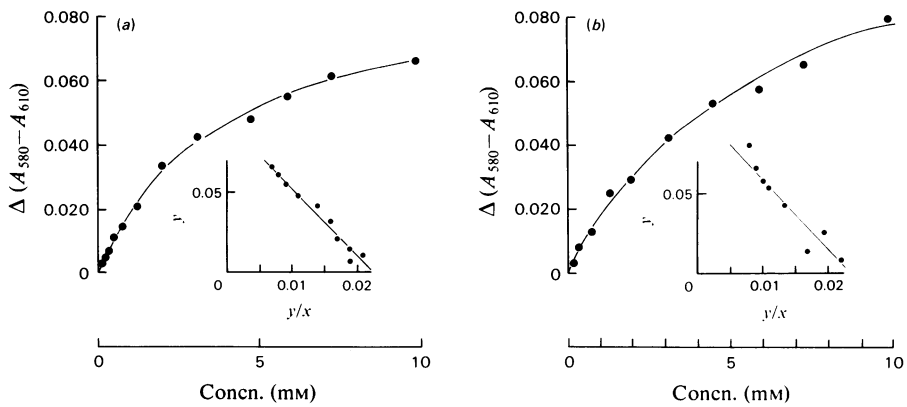

Fig. 4. *Kinetics of uptake of* L-*proline and hydroxy-*L-*proline by luminal-membrane vesicles from whole cortex in the presence of a KCl gradient*

Uptake of L-proline (*a*) and hydroxy-L-proline (*b*) under the same experimental conditions as for the results shown in Fig. 3, except for the external medium being 155mM-KCl/15mM-Hepes/Tris buffer, pH 7.5.

(Jacobsen *et al.*, 1982), in which an iteration program based on a least-squares procedure minimizes the square root of the sum of all square deviations in the ordinate direction. The apparent K_A values (i.e. substrate concentration that gives half-maximal uptake) for Na^+-independent uptake of amino acids were as follows: L-proline, 4.5mM; hydroxy-L-proline, 7mM.

Characteristics of uptake of amino acids by vesicles from pars recta

Figs. 5(*a*), 5(*b*) and 5(*c*) respectively show the absorbance changes (ΔA) produced by addition of L-proline, hydroxy-L-proline and 5-oxo-L-proline to membrane vesicles from pars recta in the presence of various sodium salts or a KCl gradient. Fig. 5 shows that all three compounds depolarize the membrane vesicles to various extents in the presence of different sodium salts. By contrast, no depolarization was observed when sodium salt was replaced by KCl, indicating that the uptake of amino acids by these vesicle preparations is exclusively Na^+-dependent.

Figs. 6(*a*), 6(*b*) and 6(*c*) record the optical response (ΔA) induced by increasing concentrations of L-proline, hydroxy-L-proline and 5-oxo-L-proline respectively in the presence of a Na^+ gradient. The rate of uptake of all three compounds

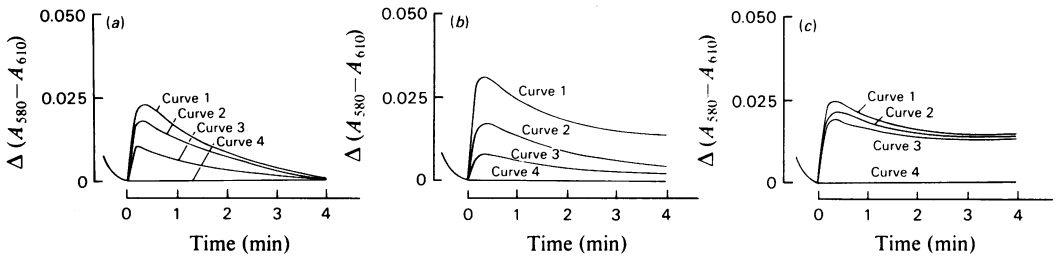

Fig. 5. *Uptake of* L-*proline, hydroxy*-L-*proline and 5-oxo*-L-*proline by renal luminal-membrane vesicles from pars recta*
Uptake of L-proline (*a*), hydroxy-L-proline (*b*) and 5-oxo-L-proline (*c*) by luminal-membrane vesicles prepared from pars recta of the renal proximal tubule was measured. Curves 1, 2, 3 and 4 show uptake with NaCl, Na_2SO_4, sodium D-gluconate and KCl as external medium, respectively. The final concentration of the amino acids was 3 mM. For other details see legend to Fig. 1.

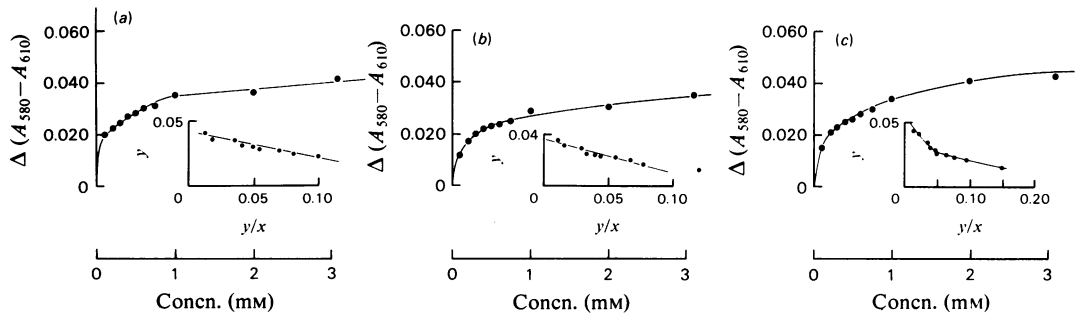

Fig. 6. *Kinetics of uptake of* L-*proline, hydroxy*-L-*proline and 5-oxo*-L-*proline by luminal-membrane vesicles from pars recta of the renal proximal tubule*
For details see legend to Fig. 3.

is very rapid at low concentrations and approaches saturation at higher concentrations of amino acids in the medium. The insets in Fig. 6 depict Eadie–Hofstee analysis of the experimental data, showing that the uptake of L-proline and hydroxy-L-proline occurs via a single individual transport system. The K_A values were calculated to be as follows: L-proline, 0.162 mM, hydroxy-L-proline, 0.26 mM. By contrast, the uptake of 5-oxo-L-proline seems to be mediated by two Na^+-specific transport systems, with K_A^1 and K_A^2 values of 0.1 mM and 3 mM respectively.

The question of whether these compounds are transported by a common system or by separate systems was investigated as follows. Two substrates, 1 and 2, were added in saturating concentrations either separately or jointly to vesicle–dye suspension and the magnitude of the dye response (ΔA) was compared. Ideally, if the transport of substrates 1 and 2 is completely independent of each other, the optical response (ΔA) should be the sum of their individual responses according to the following equation:

$$\sum \Delta A = \Delta A_1 + \Delta A_2$$

where $\sum \Delta A$ is the maximal absorbance change observed by simultaneous addition of saturating concentrations of two substrates, and ΔA_1 and ΔA_2 are the maximal absorbance changes induced by individual application of these compounds. However, in reality $\sum \Delta A$ will be less than the sum of ΔA_1 and ΔA_2, since both compounds are driven by the same electrochemical Na^+ gradient and each substrate decreases this gradient, resulting in decreased membrane potential across the luminal-membrane vesicles. To test this concept, a series of competition experiments was carried out with L-proline and D-glucose, which are shown to be transported by different carrier systems (Hammerman & Sacktor, 1977*b*). The results of these experiments revealed that L-proline (10 mM) and D-glucose (10 mM) when added alone induced maximal absorbance changes of 0.045 and 0.028 respectively, but when they were applied together the maximal absorbance change observed was 0.062 instead of 0.073 (result not shown). Similar experiments were performed with L-proline and 5-oxo-L-proline instead of D-glucose. The maximal absorbance changes (ΔA) produced by separate addition of L-proline (10 mM) and 5-oxo-L-proline (10 mM)

were 0.045 and 0.044 respectively. Simultaneous addition of these two compounds to membrane–dye suspension resulted in maximal absorbance change of 0.070 (result not shown). These results suggest that 5-oxo-L-proline, like D-glucose, is taken up by luminal-membrane vesicles from pars recta by a transport system different from that for L-proline transport. On the contrary, simultaneous addition of L-proline (10mM) and hydroxy-L-proline (10mM) at substrate concentrations approaching saturation did not significantly change the maximal absorbance values from those obtained by separate application of these compounds (results not shown), indicating that uptake of L-proline and hydroxy-L-proline in vesicles from pars recta occurs via a single common transport system.

Characteristics of uptake of amino acids by vesicles from pars convoluta

Figs. 7 and 8 show absorbance changes (ΔA) caused by the addition of amino acids by luminal-membrane vesicles from pars convoluta in the presence of various sodium salts and potassium salt gradients respectively. Application of L-proline and hydroxy-L-proline to these vesicles depolarizes the membrane potential to various extents both with sodium salt gradients and with different potassium salt gradients. By contrast, only a small degree of depolarization was observed when 5-oxo-L-proline was added to membrane–dye suspension in the presence of the KCl gradient (results not shown).

Fig. 9 shows absorbance changes (ΔA) caused by the addition of amino acids at increasing concentrations in the medium in the presence of a NaCl gradient. Insets in Fig. 9 depict Eadie–Hofstee analysis of the data. Curvilinear plots were obtained for L-proline and hydroxy-L-proline, suggesting the presence of multiple transport systems for the uptake of these compounds in membrane vesicles from pars convoluta. The results were analysed with the help of a computer program (Jacobsen *et al.*, 1982) according to the following equation:

$$\Delta A = \frac{\Delta A^1_{\text{max.}}[S]}{K^1_A + [S]} + \frac{\Delta A^2_{\text{max.}}[S]}{K^2_A + [S]}$$

where ΔA is the absorbance change measured by addition of amino acid at concentration [S], $\Delta A_{\text{max.}}$ is the maximal absorbance change, and K_A is the concentration of amino acid that produces a half-maximal absorbance change. Superscripts 1 and 2 refer to the first and the second transport systems respectively. The K_A values calculated are shown in Table 1, together with those obtained for pars recta. The affinity for Na^+-dependent transport of all three amino acids is highest in pars recta, whereas pars convoluta contains two transport systems, with an intermediate and a low affinity for proline and hydroxy-L-proline. For 5-oxo-L-proline, transport systems with high and intermediate affinity are found in pars recta, whereas pars convoluta only contains one, low-affinity, transport system.

Figs. 10(*a*) and 10(*b*) respectively record the absorbance changes (ΔA) caused by the addition of

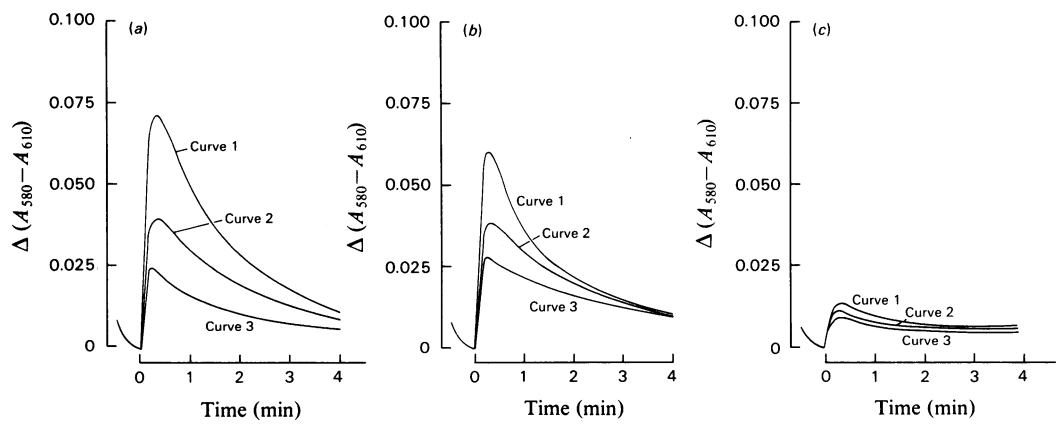

Fig. 7. *Uptake of L-proline, hydroxy-L-proline and 5-oxo-L-proline by renal luminal-membrane vesicles from pars convoluta in the presence of a Na^+ gradient*

Uptake of L-proline (*a*), hydroxy-L-proline (*b*) and 5-oxo-L-proline (*c*) by luminal-membrane vesicles prepared from pars convoluta of the renal proximal tubule was measured. Curves 1, 2 and 3 show uptake with NaCl, Na_2SO_4 or sodium D-gluconate as extravesicular medium, respectively. The final concentration of the amino acids was 5mM. For details see legend to Fig. 1.

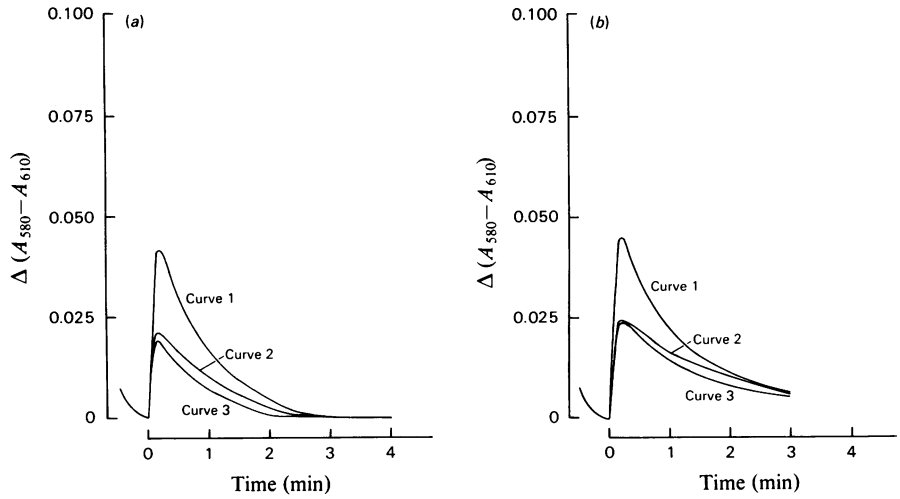

Fig. 8. *Uptake of* L-*proline and hydroxy-*L-*proline by renal luminal-membrane vesicles from pars convoluta in the presence of a* K^+ *gradient*

Uptake of L-proline (*a*) and hydroxy-L-proline (*b*) by luminal-membrane vesicles prepared from pars convoluta was studied by spectrophotometry. Experimental conditions were the same as given in Fig. 1 legend, except that the external media were KCl (curves 1), K_2SO_4 (curves 2) and potassium D-gluconate (curves 3).

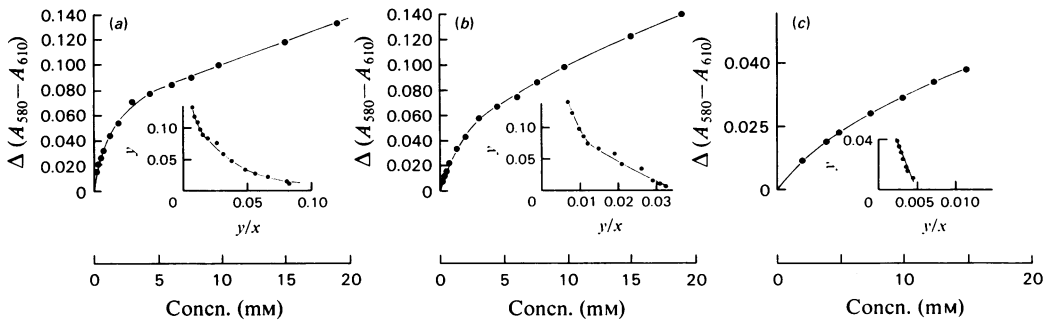

Fig. 9. *Kinetics of uptake of* L-*proline, hydroxy-*L-*proline and 5-oxo-*L-*proline by luminal-membrane vesicles from pars convoluta in the presence of a NaCl gradient*

Uptake of increasing concentrations of L-proline (*a*), hydroxy-L-proline (*b*) and 5-oxo-L-proline (*c*) by luminal-membrane vesicles prepared from pars convoluta of the renal proximal tubule was measured. For further details see legend to Fig. 3.

Table 1. *Affinities of various transport systems of pars convoluta and pars recta for* L-*proline, hydroxy-*L-*proline and 5-oxo-*L-*proline*

The values given in the Table are the half-saturation (K_A^1 and K_A^2) values (in mM) for all three amino acids.

		Pars convoluta		Pars recta	
Amino acid	Class	NaCl	KCl	NaCl	KCl
L-Proline	K_A^1	0.75	–	0.16	–
	K_A^2	9	4.5	–	–
Hydroxy-L-proline	K_A^1	1.3	–	0.26	–
	K_A^2	9	7	–	–
5-Oxo-L-proline	K_A^1	18	–	0.1	–
	K_A^2	–	–	3	–

L-proline and hydroxy-L-proline at increasing concentrations in the medium, but in the presence of a KCl gradient instead of a NaCl gradient as shown above. The insets in Fig. 10 show Eadie–Hofstee analysis of the experimental data, which indicates that Na^+-independent uptake of these compounds occurred via a single, low-affinity transport system (see Table 1).

Competition experiments were also performed by using luminal-membrane vesicles from pars convoluta in a similar way as with vesicles from pars recta. It was observed that the maximal absorbance changes produced by addition of saturating concentrations of L-proline plus hydroxy-

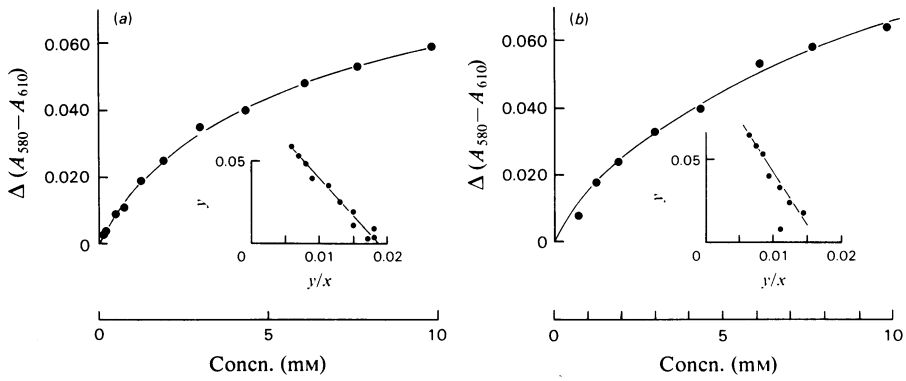

Fig. 10. *Kinetics of uptake of* L-*proline and hydroxy-*L-*proline by luminal-membrane vesicles from pars convoluta in the presence of KCl gradient*

Uptake of increasing concentrations of L-proline (*a*) and hydroxy-L-proline (*b*) by luminal-membrane vesicles prepared from pars convoluta of the renal proximal tubule was measured. The same experimental conditions were used as in the legend to Fig. 3, except for the extravesicular medium being 155mM-KCl/15mM-Hepes/Tris buffer, pH7.5.

L-proline and L-proline plus 5-oxo-L-proline are not significantly different from those caused by the addition of L-proline alone (results not shown), strongly suggesting the uptake of all three amino acids by vesicles from pars convoluta mediated by a common transport system.

Discussion

The results obtained in the present study concerning transport of L-proline, hydroxy-L-proline and 5-oxo-L-proline across the luminal-membrane vesicles of proximal tubule may be summarized as follows.

(1) The amino acids are transported in pars recta by Na^+-specific and high-affinity transport systems. L-Proline and hydroxy-L-proline apparently are taken up by a system different from that for 5-oxo-L-proline. In addition, 5-oxo-L-proline is transported in pars recta by a Na^+-dependent system of low affinity.

(2) Two electrogenic systems for transport of L-proline and hydroxy-L-proline exist in pars convoluta, with low and intermediate affinity. The transport system with intermediate affinity prefers Na^+, whereas the other system operates in the presence of a variety of cations and is not Na^+-specific.

(3) 5-Oxo-L-proline is transported in pars convoluta by a Na^+-specific, electrogenic and low-affinity system.

Previously, evidence for differentiated transport systems for L-proline in the proximal tubule was obtained by experiments *in vitro* on isolated renal tubules (Hillman *et al.*, 1968; Hillman & Rosenberg, 1969). Furthermore, studies on the tubular localization of reabsorption suggested involvement of the distal portion of the proximal tubule (perhaps referable to pars recta) in this process (Weise *et al.*, 1972). However, Hammerman & Sacktor (1977*b*) reported that L-proline transport in luminal-membrane vesicles from whole rabbit kidney cortex occurred by means of a single Na^+-specific system (K_m 0.22mM). On the other hand, McNamara *et al.* (1976) reported that Na^+-dependent uptake of L-proline by luminal vesicles isolated from whole rat kidney cortex was mediated by a dual system, with K_m values of 0.067mM and 5.26mM. The reason for this different result is not clear, but the possibility of a species or methodological difference is not excluded (e.g. if vesicles have been prepared from various depths of cortex). It is clear from our data that the two Na^+-dependent systems in pars recta and pars convoluta are different, but in both cases have a relatively high affinity for L-proline (< 1mM; see Table 1).

Ganapathy *et al.* (1982, 1983) studied the characteristics of uptake of 5-oxo-L-proline by luminal-membrane vesicles from whole rabbit renal cortex. They found that 5-oxo-L-proline was transported in these vesicles by a single Na^+-dependent electrogenic system, with an apparent K_m of 20.4mM (Ganapathy *et al.*, 1983). In accordance with their results, we found that electrogenic uptake of 5-oxo-L-proline in vesicles from whole cortex as well as from pars convoluta and pars recta was strictly Na^+-dependent. However, our experiments clearly showed that renal uptake of 5-oxo-L-proline occurred by multiple transport systems. Na^+-dependent uptake of 5-oxo-L-proline by vesicles from pars convoluta was

mediated by means of a single low-affinity system with K_A 18mM, which is in extremely good agreement with the K_m value reported by Ganapathy *et al.* (1983). Moreover, in contrast with the results of Ganapathy *et al.* (1983), we found that membrane vesicles from pars recta possessed two distinct Na^+-dependent and electrogenic transport systems for 5-oxo-L-proline, i.e. a very-high-affinity system with K_A^1 approx. 0.1mM and a relatively low-affinity system with K_A^2 approx. 3mM. It is noteworthy that at physiological pH 5-oxo-L-proline bears one negative charge, and therefore co-transport with more than one Na^+ ion is suggested by the present results. In contrast with pars recta, transport of 5-oxo-L-proline in pars convoluta appeared to occur in competition with L-proline and hydroxy-L-proline. Since electrogenic transport of 5-oxo-L-proline also was Na^+-specific in this segment, it is conceivable that the low-affinity transport occurs by the same system which exhibits intermediate affinity for L-proline and hydroxy-L-proline, but definitive data on this issue are lacking.

In the present paper electrogenic transport was identified by a positive dye response after addition of amino acid and by dependence on membrane potential (negative inside, created by addition of three anions of different epithelial permeabilities, i.e. $Cl^- > SO_4^{2-} >$ gluconate$^-$). On the basis of these criteria, L-proline and hydroxy-L-proline are transported by an electrogenic process in the presence of a variety of other cations than Na^+. This is different from the behaviour of most other neutral amino acids (Evers *et al.*, 1976; Hammerman & Sacktor, 1977*a*, 1978, 1982), and is also demonstrated by our previous findings on phenylalanine. However, we have made similar observations of Na^+-independent electrogenic transport of glycine (H. Røigaard-Petersen & M. I. Sheikh, unpublished work). The nature of these processes remains to be clarified, but the prevalence of genetic defects in the renal reabsorption of L-proline and glycine also suggests that particular features exist in the transport of these amino acids.

In conclusion, the results presented in this paper establish that L-proline, hydroxy-L-proline and 5-oxo-L-proline are reabsorbed by the proximal tubule of rabbit kidney via multiple transport systems. Owing to the inaccessibility of pars recta of proximal tubule to micro-puncture operations, little is known about the role of this nephron segment in amino acid reabsorption. Our studies on the mechanism of transport of L-phenylalanine and D-glucose (Kragh-Hansen *et al.*, 1984), as well as the results shown in the present paper on the uptake of L-proline, hydroxy-L-proline and 5-oxo-L-proline, strongly suggest that the high-affinity transport systems for neutral α-amino acids and glucose in this segment of nephron are mainly responsible for efficient reabsorption of very low luminal concentrations of these important metabolites that exist in this region of proximal tubule (for further discussion, see Kragh-Hansen *et al.*, 1984).

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