L-Tryptophan uptake by segment-specific membrane vesicles from the proximal tubule of rabbit kidney

Henrik JESSEN* and M. Iqbal SHEIKH

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

1. The mechanism of the renal transport of L-tryptophan by basolateral and luminal membrane vesicles prepared from either the pars convoluta or the pars recta of the rabbit proximal tubule was studied. 2. The uptake of L-tryptophan by basolateral membrane vesicles from the pars convoluta was found to be an Na⁺-dependent transport event. The Na⁺-conditional influx of the amino acid was stimulated in the presence of an inwardly directed H⁺ gradient. Lowering the pH without an H⁺ gradient had no effect, indicating that L-tryptophan is co-transported with H⁺. 3. On the other hand, no transient accumulation of L-tryptophan was observed in the presence or absence of Na⁺ in basolateral membrane vesicles from the pars recta. 4. In luminal membrane vesicles from the pars recta, the transient Na⁺-dependent accumulation of L-tryptophan occurred via a dual transport system. In addition, an inwardly directed H⁺ gradient could drive the uphill transport of L-tryptophan into these vesicles in both the presence and the absence of an Na⁺ gradient. 5. By contrast, the uptake of L-tryptophan by luminal membrane vesicles from the pars convoluta was a strictly Na⁺-dependent and electrogenic transport process, mediated by a single transport component. 6. Investigation of the coupling ratio in luminal membrane vesicles suggested that 1 Na⁺:1 L-tryptophan are co-transported in the pars convoluta. In the pars recta, examination of the stoichiometry indicated that approx. 1 H⁺ and 2 Na⁺ (high affinity) or 1 Na⁺ (low affinity) are involved in the uptake of L-tryptophan.

INTRODUCTION

Previous studies *in vitro* utilizing isolated renal tubules and cell preparations, and *in vivo* clearance and stop-flow experiments in dogs [1] and microperfusion in rats [2], have indicated that 85–90 % of the filtered L-tryptophan is actively re-absorbed by a single transport component in the renal tubules. Furthermore, the stop-flow measurements demonstrated that the transport of L-tryptophan is a bidirectional event, even though the re-absorption process occurs predominantly in the proximal tubule. Investigation of the structural specificity of the L-tryptophan transport mechanism by microperfusion showed that D-tryptophan, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid (5-HIAA) underwent only slight re-absorption from the proximal tubular lumen [3]. Moreover, lowering the pH resulted in an increase in the uptake of 5-HIAA. This effect was not observed with D-tryptophan or 5-hydroxytryptamine. Thus our knowledge of the exact nature of the renal handling of L-tryptophan, one of the amino acids essential for protein synthesis, is rather limited. Is the luminal transport of L-tryptophan in the pars convoluta different from that in the pars recta? If so, what is the energetic cost of re-absorbing L-tryptophan in the convoluted part compared with the straight part of the proximal tubule? Is the uptake of L-tryptophan a strictly Na⁺-dependent event, or can other cations drive the uphill transport of the amino acid? These questions need to be answered before we can understand the mechanism of re-absorption of L-tryptophan by the renal proximal tubule.

In previous reports, we have shown that purified renal membrane vesicles are very suitable for transport studies. We have provided evidence for the existence of multiple transport systems for the uptake of various neutral amino acids in luminal membrane vesicles from the pars convoluta and the pars recta of the proximal tubule from rabbit kidney. In addition to the Na⁺-dependent transport component(s), we have described an H⁺-

gradient-dependent co-transport system for L-proline, L- and D-alanine, glycine, taurine and β -alanine [4–11] which is exclusively confined to the pars convoluta. By contrast, the Na⁺-dependent uptake of L- and D-alanine and L-proline in luminal membrane vesicles from the pars recta is stimulated at lower pH, since H⁺ potentiates the Na⁺ effect without being co-transported [7,12,13]. In the present investigation, we report an H⁺-dependent co-transport system for L-tryptophan in luminal membrane vesicles from the straight part of rabbit proximal tubule. We demonstrate the existence of multiple Na⁺-dependent transport systems, and the kinetics, electrogenicity, pH effect, and stoichiometry of these systems are examined in the two different regions of the nephron. Finally, the secretory component of L-tryptophan movement in segment-specific basolateral membrane vesicles from the proximal tubule is studied.

EXPERIMENTAL

Materials

L-Tryptophan, Trizma base, Hepes and Mes were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Radioactive L-[5-³H]tryptophan (sp. radioactivity 29.5 mCi/mmol) was purchased from Amersham International, Amersham, Bucks., U.K. 3,3'-Diethyloxodicyanide iodide was supplied by Eastman Kodak Co., Rochester, NY, U.S.A. All reagents used in this study were of A.R. grade.

Preparation of luminal and basolateral membrane vesicles

Luminal and basolateral membrane vesicles were prepared from the pars convoluta (outer cortex) and from the pars recta (outer medulla) of the rabbit kidney proximal tubule by the method previously described [14]. The basolateral membrane fractions were separated from the luminal membrane vesicles by Ca²⁺ precipitation and differential centrifugation followed by a self-orienting Percoll (modified colloidal silica) gradient. The

Abbreviation used: 5-HIAA, 5-hydroxyindoleacetic acid.

* To whom correspondence should be addressed.

purity of membrane vesicle preparations was investigated by electron microscopy [15] and by measuring the specific activities of various enzyme markers, as previously described [15,16]. The protein concentration in the membrane fractions was determined by the method of Lowry *et al.* [17], as modified by Peterson [18], with BSA (Sigma) as a standard.

Uptake experiments

The uptake of radioactive L-tryptophan by various vesicle preparations was studied by Millipore filtration [19]: 20 μ l of luminal membrane vesicle suspension was added at time zero to 100 μ l of incubation medium containing radioactively labelled ligand and other constituents as required. Transport of L-tryptophan into vesicles was stopped by the addition of 1 ml of ice-cold stop solution consisting of either 155 mM-NaCl or 155 mM-KCl dissolved in 15 mM-Hepes/Tris buffer (pH 7.5), or in 15 mM-Mes/Tris buffer (pH 5.5) for the low-pH experiments. The resulting suspension was rapidly filtered through a prewetted Millipore filter (HAWP 0.45 μ m) which was washed twice with 2.5 ml of ice-cold buffer. The filter was dried and radioactivity was counted in a liquid scintillation counter (LKB-Wallac 1218 RackBeta) after addition of Filter Count (Packard Instrument International SA, Zürich, Switzerland). All uptake measurements were performed at room temperature (22 ± 2 °C). Correction for non-specific binding to the filter and membrane vesicles was made by subtracting from all uptake data the value of a blank obtained by the addition of ice-cold stop solution to incubation medium and vesicles before they were mixed and filtered through the filter. All experiments were carried out in triplicate, and each point in an experiment was performed in duplicate or triplicate. In the different series of experiments, the results of the individual experiments were very much alike. Therefore we have chosen to illustrate results of representative experiments, unless otherwise stated (S.D. of three experiments was approx. 10–15 %).

In a series of experiments in which the Na⁺ coupling ratio with L-tryptophan was examined, precautions were taken to ensure that any effects owing to variations in transmembrane electrical potential were minimized by short-circuiting the membrane.

Uptake of L-tryptophan by vesicle preparations was also examined by a spectrophotometric method with a potential-sensitive carbocyanine dye as previously described [20]. The principle of the spectrophotometric method is as follows. A 1.2 ml portion of a buffered aqueous solution of the potential-sensitive dye 3,3'-diethyloxodicarbocyanine iodide, 1.2 ml of a buffered salt solution and 60 μ l of membrane vesicle suspension were mixed in a 1 cm-path-length cuvette. The cuvette was placed in an Aminco DW-2a u.v.-visible spectrophotometer with a constant temperature in the sample compartment of 20 °C. The salt anions permeate into the vesicles faster than the salt cations, resulting in a slight reversible hyperpolarization of the membranes. The hyperpolarization was recorded on the spectrophotometer, and at its maximum a small volume of a stock solution of L-tryptophan or buffer was added, under magnetic stirring, through a small opening in the top of the sample compartment.

The details of the individual experiments are given in the legends to the Figures.

Calculations

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

$$\text{Uptake} = \frac{V_{\max,1}[S]}{K_{m1} + [S]} + \frac{V_{\max,2}[S]}{K_{m2} + [S]}$$

where K_m represents the substrate concentration that gives half-maximal uptake, $V_{\max}$ denotes maximal uptake and [S] indicates initial concentration of substrate. Subscripts 1 and 2 refer to the first and second transport system respectively. In the case of transport via a single pathway, the same equation without the second fraction was used. Theoretical saturation curves were fitted to the experimental data by using a computer-analysed statistical iteration procedure [21].

To determine the cation/L-tryptophan coupling ratio, we used the 'activation method' [22]. Here, one measures the stimulation of substrate (L-tryptophan) flux by increasing concentrations of activator (Na⁺ or H⁺). The data were analysed by the equation [23]:

$$\text{Flux} = V_{\max}[A]^n/(K_{0.5}^n + [A]^n)$$

The equation assumes the existence of n essential co-operative site(s) for the activator A per L-tryptophan site. According to this equation, a plot of flux/[A] ^{n} against flux for the correct value of n will yield a straight line with slope $1/K_{0.5}^n$.

RESULTS

Uptake of L-tryptophan by basolateral membrane vesicles from the pars convoluta

The time course of uptake of radioactive L-tryptophan by basolateral membrane vesicles from the convoluted part of the rabbit proximal tubule is illustrated in Fig. 1(a). The presence of an external Na⁺ gradient stimulated the transport of L-tryptophan into the vesicles (curve 2). A pH gradient (pH_{in} = 7.5, pH_{out} = 5.5) increased the Na⁺-gradient-dependent uptake of the amino acid (curve 1). This effect was not observed when the Na⁺-dependent influx was measured at lower pH, but in the absence of an H⁺ gradient (pH_{in} = pH_{out} = 5.5) (results not shown). When the extravesicular Na⁺ gradient was replaced by a K⁺ gradient, no 'overshoot' was found in the presence (curve 3) or the absence (curve 4) of an H⁺ gradient. Moreover, the above-mentioned H⁺-gradient-dependent extra uptake of L-tryptophan was not observed in the absence of Na⁺ (compare curves 3 and 4 in Fig. 1a). It has previously been described that significant binding of L-tryptophan to human placental brush-border membrane vesicles was found with longer incubations [24]. Preliminary results showed that a certain amount of the amino acid is also bound to renal membrane vesicles. Since the purpose of the present study is to characterize the transport mechanism for L-tryptophan, we have not investigated the binding of L-tryptophan in further detail. Nonetheless, the binding component comprises a major part of the total uptake of L-tryptophan in basolateral membrane vesicles compared with luminal membrane vesicles. Therefore, in order to ensure that a Na⁺ gradient was capable of producing an overshoot, we subtracted the uptakes in KCl, pH 7.5 from the uptakes in NaCl, pH 7.5. A similar approach was followed at pH 5.5, even though we did not find any significant pH-dependence of L-tryptophan binding to the different membrane vesicles in the pH range 5.5–7.5 (e.g. compare curves 3 and 4 of Fig. 1a). The data are given in Fig. 1(b). The presence of an inwardly directed sodium gradient resulted in a typical overshoot phenomenon (Fig. 1b, curve 2), where the uptake values were increased after imposition of an external pH gradient (Fig. 1b, curve 1).

Uptake of L-tryptophan by basolateral membrane vesicles from the pars recta

The uptake of radioactive L-tryptophan by renal basolateral membrane vesicles from the straight part of the proximal tubule as a function of time is given in Fig. 2. It is seen that, in the

Fig. 1. Time course of radioactive L-tryptophan uptake by basolateral membrane vesicles from the pars convoluta

(a) A 20 μ l portion of the vesicle suspension prepared in 310 mM-mannitol/15 mM-Hepes/Tris, pH 7.5, was incubated for different time intervals in 100 μ l of incubation mixture, consisting of 155 mM-NaCl in 15 mM-Hepes/Tris buffer, pH 7.5 (curve 2), of 155 mM-NaCl in 15 mM-Mes/Tris buffer, pH 5.5 (curve 1), of 155 mM-KCl in 15 mM-Hepes/Tris, pH 7.5 (curve 4), or of 155 mM-KCl in 15 mM-Mes/Tris, pH 5.5 (curve 3). Media contained 0.45 μ M-L-[5- 3 H]tryptophan and unlabelled L-tryptophan to give 25 μ M (final concentrations). (b) Calculated effect of an Na^+ gradient in the presence of an H^+ gradient [curve 1 minus curve 3 from (a); curve 1] and in the absence of an H^+ gradient [curve 2 minus curve 4 from (a); curve 2]. The data are from a representative experiment ($n = 3$).

presence of an Na^+ gradient (curve 2), an Na^+ plus H^+ gradient (curve 1) or a K^+ gradient (curve 3), the uptake of L-tryptophan did not exhibit a transient overshoot.

Uptake of L-tryptophan by luminal membrane vesicles from the pars convoluta

The time course of L-tryptophan uptake by luminal membrane vesicles is given in Fig. 3. The initial uptake of L-tryptophan was rapid in the presence of an inwardly directed Na^+ gradient and reached a peak value of 1 nmol/mg of protein within 1 min (curve 2). The intravesicular concentration of amino acid subsequently decreased, and after 120 min an equilibrium state was obtained. The maximal uptake of L-tryptophan was about 11 times that at 120 min. No concentrative uptake (overshoot) of

Fig. 2. Time course of radioactive L-tryptophan uptake by basolateral membrane vesicles from the pars recta

A 20 μ l portion of the vesicle suspension prepared in 310 mM-mannitol/15 mM-Hepes/Tris, pH 7.5, was incubated for different time intervals in 100 μ l of incubation mixture consisting of 155 mM-NaCl in 15 mM-Hepes/Tris buffer, pH 7.5 (curve 3, $\square$), of 155 mM-NaCl in 15 mM-Mes/Tris buffer, pH 5.5 (curve 1, $\bullet$), or of 155 mM-KCl in 15 mM-Hepes/Tris, pH 7.5 (curve 2, $\square$). Media contained 0.45 μ M-L-[5- 3 H]tryptophan and unlabelled L-tryptophan to give 25 μ M (final concentrations). The data are from a representative experiment ($n = 3$).

L-tryptophan was found in the presence of a K^+ gradient (curve 4). It has been reported that a number of amino acids [4–11] can be co-transported by a pH gradient (extravesicular > intravesicular) in brush-border membrane vesicles from the pars convoluta. However, we found that imposition of an inwardly directed H^+ gradient in the presence (curve 1) or absence (curve 3) of an Na^+ gradient had no significant effect on the influx of L-tryptophan in these vesicle preparations. The inset in Fig. 3 illustrates that the transport of 25 μ M-L-tryptophan was a linear function of time from 0 to 15 s, indicating that 15 s uptake values represent estimates of the initial rate of uptake (similar results were obtained for 5 mM-L-tryptophan; not shown).

The uptake of L-tryptophan was also studied by spectrophotometry using the potential-sensitive dye 3,3'-diethyloxadecarbocyanine iodide. Fig. 4 illustrates that only an inwardly directed NaCl gradient (curve 1) resulted in a depolarization of the membrane vesicles. No absorbance changes were recorded in the presence of a KCl (curve 2) gradient or mannitol (curve 3). This additional evidence demonstrates that the transport of L-tryptophan in vesicles from the pars convoluta is an electrogenic event, strictly dependent on Na^+ .

The kinetic parameters of the Na^+ -dependent L-tryptophan uptake were determined by measuring the initial uptake (15 s values) of radioactive L-tryptophan as a function of amino acid concentration. The values given in Fig. 5 have been corrected for non-saturable simple diffusion and binding by subtracting the uptakes measured in KCl from the uptakes measured in NaCl. The carrier-mediated uptake exhibited saturable behaviour, obeying Michaelis-Menten kinetics. The same data are analysed in the inset of Fig. 5 as an Eadie-Hofstee plot. The linearity of this plot indicates that a single carrier is involved in the Na^+ -dependent influx of L-tryptophan in luminal membrane vesicles from the pars convoluta. Computerized calculations gave the following kinetic parameters: K_m , 1.93 ± 0.31 mM; V_{max} , 19.72 ± 1.77 nmol/15 s per mg of protein.

Fig. 3. Time course of radioactive L-tryptophan uptake by luminal membrane vesicles from the pars convoluta

A 20 μ l portion of the vesicle suspension prepared in 310 mM-mannitol/15 mM-Hepes/Tris, pH 7.5, was incubated for different time intervals in 100 μ l of incubation medium containing 155 mM-NaCl in 15 mM-Hepes/Tris buffer, pH 7.5 (curve 2) or in 15 mM-Mes/Tris buffer, pH 5.5 (curve 1). Media contained 0.10 μ M-L-[5- 3 H]tryptophan and unlabelled L-tryptophan to give 25 μ M (final concentrations). Curves 4 and 3 show the effect of substituting NaCl by KCl in 15 mM-Hepes/Tris buffer, pH 7.5 (curve 4), or in 15 mM-Mes/Tris buffer, pH 5.5 (curve 3), respectively. The inset shows results for 15 s. The data are from a representative experiment ($n = 3$).

Fig. 4. Time course of absorbance changes caused by addition of L-tryptophan to luminal membrane vesicles from the pars convoluta

The potential-sensitive dye 3,3'-diethyloxadecarbocyanine iodide was used to measure absorbance changes. General experimental conditions were as follows: protein concentration, 0.4 mg/ml; pH 7.5; temperature, 20 $^{\circ}$ C; dye concentration, 15 μ M. The intravesicular medium was 310 mM-mannitol and 15 mM-Hepes/Tris. In all extravesicular media, 15 mM-Hepes/Tris was used as buffer system. The absorbance changes were caused by L-tryptophan at 1.8 mM in the presence of 155 mM-NaCl (curve 1), 155 mM-KCl (curve 2) or 310 mM-mannitol (curve 3). The start of the curves, at 0 min, indicates addition of solute. All the spectral curves were corrected for the effect of adding a small volume of 15 mM-Hepes/Tris buffer alone (the medium of solute stock solutions). The spectrophotometer was operated in the dual-wavelength mode at 580 nm and 610 nm (reference wavelength). The results shown are from a representative experiment ($n = 3$).

Fig. 5. Kinetics of L-tryptophan uptake by luminal membrane vesicles from the pars convoluta

Na^+ -dependent uptake of L-tryptophan is shown at increasing concentrations of the amino acid. The results are given as the uptakes measured in 155 mM-NaCl/15 mM-Hepes/Tris, pH 7.5, minus the uptakes in 155 mM-KCl/15 mM-Hepes/Tris, pH 7.5. The media contained 0.20 μ M-L-[5- 3 H]tryptophan and various concentrations of unlabelled L-tryptophan ranging from 0.01 to 10.0 mM (final concentration). In the inset the results are shown in an Eadie-Hofstee plot, where v represents the rate of transport at substrate concentration $[S]$. Results are given as mean values $\pm$ S.D. of three experiments.

The results presented above indicate that the presence of a Na^+ gradient is required for driving the electrogenic uphill transport of L-tryptophan in luminal membrane vesicles from the convoluted part of the proximal tubule. Therefore, in order to obtain further insight into the transport mechanism of L-tryptophan, we examined the Na^+ /L-tryptophan coupling ratio by measuring the initial uptake of L-tryptophan as a function of Na^+ concentration. In these experiments, the membrane vesicles were voltage-clamped and the incubation period was 15 s. The uptake values given in Fig. 6(a) have been corrected for the uptake obtained in the absence of a sodium gradient. The plot of L-tryptophan flux versus Na^+ concentration (0–200 mM) gave a simple hyperbolic curve, suggesting that one Na^+ ion participates in the transport of one molecule of L-tryptophan in luminal membrane vesicles from the pars convoluta. This is confirmed in Fig. 6(b), where we have plotted flux/ $[\text{Na}^+]^n$ against flux. On assuming that $n = 1$ a straight line is obtained, indicating a 1:1 stoichiometry. Accordingly, an n value of 2 yielded an exponential curve.

Uptake of L-tryptophan by luminal membrane vesicles from the pars recta

Fig. 7 shows the time course of radioactive L-tryptophan uptake by luminal membrane vesicles isolated from the pars recta of the rabbit proximal tubule. The influx of L-tryptophan exhibited a typical overshoot phenomenon in the presence of an inwardly directed NaCl gradient (curve 2). The peak value was reached at 3–5 min, and was about three times the 120 min value. Curve 1 illustrates the combined effect of Na^+ and H^+ gradients (extravesicular > intravesicular) on the accumulation of L-tryptophan by these vesicle preparations. The imposition of the H^+ gradient enhanced the Na^+ -dependent uptake of the amino acid (compare curves 1 and 2 in Fig. 7). We examined whether this pH effect on the Na^+ -dependent transport process for L-tryptophan in vesicles from the pars recta could be achieved by a lower pH without a pH gradient. At pH 5.5 no additional influx of L-tryptophan was observed under these experimental

Fig. 6. Na^+ -dependent L-tryptophan flux as a function of Na^+ concentration in luminal membrane vesicles from the pars convoluta

Vesicles were preincubated in 15 mM-Hepes/Tris buffer, pH 7.5, containing 100 mM-KSCN, 700 mM-mannitol and valinomycin at a concentration of 12.5 $\mu\text{g}/\text{mg}$ of protein. The incubation medium contained 15 mM-Hepes/Tris, pH 7.5, 0.77 μM -L-[5- ^3H]tryptophan, 100 mM-KSCN, 300 mM-mannitol and various concentrations of NaCl ranging from 0 to 200 mM (final concentration). Choline chloride was used to replace NaCl iso-osmotically to obtain the various Na^+ concentrations studied. The Na^+ -gradient-driven uptake values plotted have been corrected for the uptake of L-tryptophan in the absence of an Na^+ gradient. (a) Plot of flux versus Na^+ concentration. The results shown are the mean values $\pm$ S.D. of three experiments. (b) Plots of $\text{flux}/[\text{Na}^+]^n$ versus flux for $n = 1$ (●) and $n = 2$ (○). The units of $[\text{Na}^+]$ are M.

conditions compared with the Na^+ -dependent uptake measured at pH 7.5 (results not shown). No overshoot was found in the presence of a KCl gradient (Fig. 7, curve 4) when the extravesicular pH was equal to the intravesicular pH (i.e. pH = 7.5). Curve 3 of Fig. 7 illustrates the renal uptake of radioactive L-tryptophan in the presence of H^+ and K^+ gradients (extravesicular > intravesicular). It should be noted that imposition of an H^+ gradient (extravesicular > intravesicular) resulted in an increase in the accumulation rate of L-tryptophan under these experimental conditions (compare curves 3 and 4 in Fig. 7). As also demonstrated in the pars convoluta, the inset of Fig. 7 shows that the influx of L-tryptophan versus time gave a straight line relationship for the first 15 s of incubation. Therefore we have used 15 s uptake values as estimates of the initial rate of transport of the amino acid [similar results were obtained for 5 mM-L-tryptophan (not shown)].

Fig. 8 shows the absorbance changes caused by the addition of L-tryptophan to luminal membrane vesicles from the pars recta

Fig. 7. Time course of radioactive L-tryptophan uptake by luminal membrane vesicles from the pars recta

A 20 μl portion of the vesicle suspension prepared in 310 mM-mannitol/15 mM-Hepes/Tris (pH 7.5) was incubated at different time intervals in 100 μl of incubation mixture consisting of 155 mM-NaCl in 15 mM-Hepes/Tris buffer, pH 7.5 (curve 2), of 155 mM-NaCl in 15 mM-Mes/Tris buffer, pH 5.5 (curve 1), of 155 mM-KCl in 15 mM-Hepes/Tris, pH 7.5 (curve 4) or of 155 mM-KCl in 15 mM-Mes/Tris, pH 5.5 (curve 3). Media contained 0.10 μM -L-[5- ^3H]tryptophan and unlabelled L-tryptophan to give 25 μM (final concentrations). Inset, results are shown for 0–15 s. The data are from a representative experiment ($n = 3$).

Fig. 8. Time course of absorbance changes caused by addition of L-tryptophan to luminal membrane vesicles from the pars recta

L-Tryptophan absorbance changes as registered by the spectrophotometric method. The intravesicular medium was 310 mM-mannitol, whereas the external medium was 1.8 mM-L-tryptophan, plus 155 mM-NaCl (curve 1), 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. The results shown are from a representative experiment ($n = 3$). For further experimental details, see the legend to Fig. 2.

in the presence of an NaCl gradient (curve 1), KCl gradient (curve 2) or mannitol (curve 3). It is seen from Fig. 8 that L-tryptophan depolarized the membrane potential in the presence of an Na^+ gradient, indicating a net transfer of positive charge. By contrast, only a small degree of depolarization was observed when L-tryptophan was added to membrane dye suspension in the presence of the KCl gradient or mannitol.

Table 1. Michaelis–Menten constants for the uptake of L-tryptophan in luminal membrane vesicles from the pars recta

The Michaelis–Menten constants were determined by the computer-analysed statistical iteration procedure. The analysed Na^+ -dependent uptake values were the uptakes measured in 155 mM-NaCl/15 mM-Hepes/Tris (pH 7.5) minus the uptakes in 155 mM-KCl/15 mM-Hepes/Tris (pH 7.5). The media contained $0.20 \mu\text{M}$ -L-[5- ^3H]tryptophan and various concentrations of unlabelled tryptophan ranging from 0.01 to 10.0 mM (final concentration). The analysed H^+ -dependent uptake values were the uptakes measured in 155 mM-KCl/15 mM-Mes/Tris (pH 5.5) minus the uptakes in 155 mM-KCl/15 mM-Hepes/Tris (pH 7.5). The experimental conditions were essentially as mentioned above, except that the concentration of L-tryptophan ranged from 0.01 to 5.0 mM (final concentration). Results are given as mean values of three experiments, with s.d. values less than 12%.

Cation gradient	K_m (mM)	$V_{\max}$ (nmol/15 s per mg of protein)
Na^+	0.49	1.80
	9.18	12.88
H^+	0.81	0.82

Fig. 9. Na^+ -dependent L-tryptophan flux as a function of Na^+ concentration in luminal membrane vesicles from the pars recta

The preparation of vesicles and the composition of the incubation media were the same as described in the legends to Fig. 6. All uptake values were corrected for the uptake in the absence of an Na^+ gradient. (a) Plot of flux versus Na^+ concentration for $0.77 \mu\text{M}$ -L-[5- ^3H]tryptophan. The results shown are the mean values $\pm$ s.d. of three experiments. (b) Plots of flux/ $[\text{Na}^+]^n$ versus flux for $n = 1$ (●), $n = 2$ (○) and $n = 1.8$ (□) for the high-affinity system ($0.77 \mu\text{M}$ -tryptophan). The units of $[\text{Na}^+]$ are M.

Fig. 10. Effect of inwardly directed H^+ gradients on the uptake of L-tryptophan by luminal membrane vesicles from the pars recta

The vesicles were preincubated with 155 mM-KCl in 15 mM-Hepes/Tris (pH 7.5). The incubation media contained $0.75 \mu\text{M}$ -L-[5- ^3H]tryptophan and 155 mM-KCl in various Hepes/Tris or Mes/Tris buffer solutions, with pH ranging from 7.5 to 5.0. (a) Flux versus extravesicular pH (pH_0). The results given are the mean values of three experiments, with s.d. less than 15%. (b) Plots of flux/ $[\text{H}^+]$ versus flux for $n = 1$ (■) and $n = 0.8$ (□) are shown. All uptake values were corrected for the uptake in the absence of an H^+ gradient ($\text{pH}_{\text{in}} = \text{pH}_{\text{out}} = 7.5$).

We also examined the initial uptake of radioactive L-tryptophan at increasing medium concentrations of the amino acid in the presence of an Na^+ gradient alone and in the presence of an H^+ gradient alone (results not shown). The uptake values were corrected for passive diffusion and binding by subtracting the uptake values obtained in the presence of a KCl gradient (i.e. $\text{pH}_{\text{in}} = \text{pH}_{\text{out}} = 7.5$). We found that the Na^+ -gradient-dependent uptake of L-tryptophan occurred via a dual transport system, whereas the H^+ -gradient-dependent influx was mediated by a single transport component. The calculated K_m and $V_{\max}$ values for L-tryptophan transport in vesicles from the pars recta are given in Table 1.

To determine the Na^+/L -tryptophan stoichiometry in luminal membrane vesicles from the pars recta, the initial flux of $0.77 \mu\text{M}$ - and 5 mM -L-tryptophan was measured as a function of Na^+ concentration (0–200 mM). The experimental conditions were exactly the same as previously described for the pars convoluta. Fig. 9(a) shows that at $0.77 \mu\text{M}$ -L-tryptophan the uptake values exhibited a marked sigmoidal dependence on Na^+ concentration, suggesting that the Na^+/L -tryptophan stoichiometry is greater than one. Analysis of the data (Fig. 9b) illustrates that an n value of 1.8 yielded a straight line relationship, indicating that approximately 2 Na^+ are interacting with the carrier system per one molecule of L-tryptophan. In order to examine the stoichiometric properties of the Na^+ -dependent low-affinity transporter, we subtracted the activity of the high-affinity component

(Fig. 9a) from the total Na^+ -dependent uptake values obtained at 5 mM-L-tryptophan. The hyperbolic shape of the curve suggested a Michaelis-Menten-type dependence (results not shown). A further analysis of the corrected flux values demonstrated that an n value of 1 gave the best fit to these data, indicating a coupling stoichiometry of 1:1 (results not shown).

Finally, we investigated the coupling ratio of the H^+/L -tryptophan co-transport component in vesicles from the pars recta. The vesicles were preloaded with KCl, pH 7.5, and incubated in KCl at different pH values (5.0–7.5). The uptake of L-tryptophan increased as the magnitude of the H^+ gradient increased (extravesicular > intravesicular) (Fig. 10a). An analysis of the experimental data is performed in Fig. 10(b), where all uptake values were corrected for the uptake obtained in the absence of an H^+ gradient. The plot suggests that approx. one (0.8) H^+ ion participates in the transport of one molecule of L-tryptophan in luminal membrane vesicles from the pars recta.

DISCUSSION

Our present studies demonstrate that both Na^+ -dependent and Na^+ -independent systems mediate L-tryptophan transport in the proximal tubule of the rabbit kidney. In luminal membrane vesicles from the pars convoluta, transport of L-tryptophan was an electrogenic process, strictly dependent on Na^+ . Analysis of the saturation kinetic data showed that the influx of the amino acid occurred via a single transport component (half-saturation 1.93 mM), which corresponds very well with observations from microperfusion studies in the rat (half-saturation 4.1 mM [2]). Similar results have been obtained for L-phenylalanine [25], L- and D-serine [26], L-leucine, L-isoleucine and L-valine [27] in luminal membrane vesicles from the pars convoluta. However, re-absorption of some neutral amino acids can be driven both by an Na^+ gradient and an H^+ gradient, and can take place via one or more transport systems. This seems to be the case for L-proline [5], L- and D-alanine [6,7], glycine [8], β -alanine [10,11] and taurine [9]. Imposition of a pH gradient ($\text{pH}_{\text{in}} = 7.5$, $\text{pH}_{\text{out}} = 5.5$) did not significantly influence the uptake of L-tryptophan in this section of the proximal tubule. In luminal membrane vesicles from the pars recta, however, an H^+ gradient (extravesicular > intravesicular) was able to accelerate the influx of L-tryptophan in both the presence and the absence of an Na^+ gradient. Our results strongly indicate that this acceleration must be a result of H^+/L -tryptophan co-transport, since the presence of an inwardly directed H^+ gradient can drive net L-tryptophan accumulation in the absence of other energy sources (curve 3 of Fig. 7). This is supported by the fact that the H^+ -gradient-dependent extra uptake of L-tryptophan in addition to the Na^+ -gradient-dependent influx of amino acid could not be obtained at lower pH without an H^+ gradient (i.e. $\text{pH}_{\text{in}} = \text{pH}_{\text{out}} = 5.5$). Thus our data clearly suggest the existence of an H^+ -gradient-dependent transport system in luminal membrane vesicles from the pars recta (half-saturation 0.81 mM). We have also provided evidence for the existence of an electrogenic Na^+ -dependent dual transport system ($K_{m1} = 0.49$ mM; $K_{m2} = 9.18$ mM) in these vesicle preparations. It is interesting to note that the Na^+ conditional influx is mediated by high-capacity transport components compared with the H^+ -dependent transport system (see Table 1).

In order to estimate the energetic cost of re-absorbing L-tryptophan across the brush-border membrane, we used the 'activation method'. In the pars convoluta we found a Na^+/L -tryptophan stoichiometry of 1:1, whereas the data from pars recta revealed a coupling ratio of approx. 1 H^+ :1 L-tryptophan, 2 Na^+ :1 L-tryptophan (high affinity), and 1 Na^+ :1 L-tryptophan (low affinity). The activation method does not distinguish be-

tween catalytic and energetic couplings [23]. Nevertheless, we have shown that Na^+ -dependent uptake of L-tryptophan (zwitterion at pH 7.5) in both segments of the proximal tubule is an electrogenic process. Moreover, the H^+ -dependent influx of L-tryptophan could only be observed in the presence of an inwardly directed pH gradient as previously discussed. Thus it is most likely that these cations are co-transported with L-tryptophan along the proximal tubule of the rabbit kidney.

Finally, since the transport of L-tryptophan has been reported to be a bidirectional event, we examined the uptake of L-tryptophan by basolateral membrane vesicles from rabbit proximal tubule. In the pars convoluta the uptake of the amino acid was found to be an Na^+ -dependent transport process. Moreover, the Na^+ conditional transport of L-tryptophan was stimulated in the presence of an inwardly directed H^+ gradient, whereas lowering the pH without an H^+ gradient had no effect. Thus our results suggest that the H^+ -gradient-dependent uptake is not due to protonation of some specific membrane groups induced by the pH change, but rather represents an H^+/L -tryptophan co-transport event. By contrast, no active transport in the presence or absence of Na^+ was observed in basolateral membrane vesicles from the pars recta, indicating that the secretory component of L-tryptophan is exclusively confined to the convoluted part of the proximal tubule.

This study was supported in part by the Danish Medical Research Council, Aarhus University Research Foundation, Fonden til Lægevidenskabens Fremme, NOVO's Fond, Carlsbergfondet, and the Danish Biotechnology Programme.

REFERENCES

- Williams, W. M. & Huang, K. C. (1970) *Am. J. Physiol.* **219**, 1468–1474
- Chan, Y.-L. & Huang, K. C. (1971) *Am. J. Physiol.* **221**, 575–579
- Chan, Y.-L. & Huang, K. C. (1973) *Am. J. Physiol.* **224**, 140–143
- Røigaard-Petersen, H. & Sheikh, M. I. (1984) *Biochem. J.* **220**, 25–33
- Røigaard-Petersen, H., Jacobsen, C. & Sheikh, M. I. (1987) *Am. J. Physiol.* **253**, F15–F20
- Jørgensen, K. E. & Sheikh, M. I. (1987) *Biochem. J.* **248**, 533–538
- Jessen, H., Vorum, H., Jørgensen, K. E. & Sheikh, M. I. (1988) *Biochim. Biophys. Acta* **942**, 262–270
- Røigaard-Petersen, H., Jessen, H., Møllerup, S., Jørgensen, K. E., Jacobsen, C. & Sheikh, M. I. (1990) *Am. J. Physiol.* **258**, F388–F396
- Jessen, H. & Sheikh, M. I. (1991) *Biochim. Biophys. Acta* **1064**, 189–198
- Jessen, H., Jørgensen, K. E., Røigaard-Petersen, H. & Sheikh, M. I. (1989) *J. Physiol. (London)* **411**, 517–528
- Jessen, H. & Sheikh, M. I. (1991) *Biochem. J.* **277**, 891–894
- Vorum, H., Jessen, H., Jørgensen, K. E. & Sheikh, M. I. (1988) *FEBS Lett.* **227**, 35–38
- Røigaard-Petersen, H., Jacobsen, C. & Sheikh, M. I. (1988) *Am. J. Physiol.* **254**, F628–F633
- Sheikh, M. I. & Møller, J. V. (1987) in *Biochemical Toxicology: A Practical Approach* (Snell, K. & Mullock, B., eds.), pp. 153–182, IRL Press, Oxford
- Kragh-Hansen, U., Røigaard-Petersen, H. & Sheikh, M. I. (1985) *Am. J. Physiol.* **249**, F704–F712
- Sheikh, M. I., Kragh-Hansen, U., Jørgensen, K. E. & Røigaard-Petersen, H. (1982) *Biochem. J.* **208**, 377–382
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Peterson, G. L. (1977) *Anal. Biochem.* **83**, 346–356
- Hopfer, U., Nelson, K., Perrotto, J. & Isselbacher, K. J. (1973) *J. Biol. Chem.* **248**, 25–32
- Kragh-Hansen, U., Jørgensen, K. E. & Sheikh, M. I. (1982) *Biochem. J.* **208**, 359–368
- Jacobsen, C., Frich, J. R. & Steensgaard, J. (1982) *J. Immunol. Methods* **50**, 77–88
- Turner, R. J. & Moran, A. (1982) *J. Membr. Biol.* **67**, 73–80
- Turner, R. J. & Moran, A. (1982) *J. Membr. Biol.* **70**, 37–45

24. Ganapathy, M. E., Leibach, F. H., Mahesh, V. B., Howard, J. C., Devoe, L. D. & Ganapathy, V. (1986) *Biochem. J.* **238**, 201–208
25. Kragh-Hansen, U., Røigaard-Petersen, H., Jacobsen, C. & Sheikh, M. I. (1984) *Biochem. J.* **222**, 15–24
26. Kragh-Hansen, U. & Sheikh, M. I. (1984) *J. Physiol. (London)* **354**, 55–67
27. Jørgensen, K. E., Kragh-Hansen, U. & Sheikh, M. I. (1990) *J. Physiol. (London)* **422**, 41–54

Received 7 October 1991/10 February 1992; accepted 27 February 1992