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Renal transport of taurine in luminal membrane vesicles from rabbit proximal tubule

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The uptake of taurine by luminal membrane vesicles from pars convoluta and pars recta of rabbit proximal tubule was examined. In pars convoluta, the transport of taurine was characterized by two Na^+-dependent ($K_{m1} = 0.086$ mM, $K_{m2} = 5.41$ mM) systems, and one Na^+-independent ($K_m = 2.87$ mM) system, which in the presence of an inwardly directed H^+-gradient was able to drive the transport of taurine into these vesicles. By contrast, in luminal membrane vesicles from pars recta, the transport of taurine occurred via a dual transport system ($K_{m1} = 0.012$ mM, $K_{m2} = 5.62$ mM), which was strictly dependent on Na^+. At acidic pH with or without a H^+-gradient, the Na^+-dependent flux of taurine was drastically reduced. In both kind of vesicles, competition experiments only showed inhibition of the Na^+-dependent high-affinity taurine transporter in the presence of β-alanine, whereas there was no significant inhibition with α-amino acids, indicating a β-amino acid specific transport system. Addition of β-alanine, L-alanine, L-proline and glycine, but not L-serine reduced the H^+-dependent uptake of taurine to approx. 50%. Moreover, only the Na^+-dependent high-affinity transport systems in both segments specifically required Cl^-. Investigation of the stoichiometry indicated 1.8 Na^+ : 1 Cl^- : 1 taurine (high affinity), 1 Na^+: 1 taurine (low affinity) and 1 H^+: 1 taurine in pars convoluta. In pars recta, the data showed 1.8 Na^+ : 1 Cl^- : 1 taurine (high affinity) and 1 Na^+ : 1 taurine (low affinity).

Introduction

Taurine is the major free intracellular amino acid present in most mammalian tissue [1]. Substantial evidence now indicates that taurine plays an important functional role in retinal, in cardiac and skeletal muscles as well as serving tasks in endocrine function and in neurotransmission [1–3]. Renal responses to changes in dietary taurine intake suggest that this amino acid is a conserved metabolite and that the kidney plays a considerable role in whole body taurine homeostasis [4–6]. Studies on the mechanism of renal tubular transport of taurine showed that almost 90% of the filtered load is reabsorbed in the proximal tubule [7], by Na^+-dependent high-affinity transport in the brush-border membrane [8–10]. However, Na^+-dependent uptake of taurine by vesicles from pars convoluta or whole cortex also specifically requires Cl^- [11–13] which has been suggested to stimulate taurine flux primarily by facilitating the formation of the translocated solute carrier complex whereas the uphill transport of taurine is driven mainly by the Na^+-gradient, and is sensitive to the membrane potential owing to a negatively charged empty carrier [14]. However, in contrast to previous reports we observed in preliminary experiments that the uptake of taurine in vesicles from pars convoluta of rabbit proximal tubule was mediated by both a high- and low-affinity Na^+-dependent and an intermediate affinity Na^+-independent, but electrogenic transport systems. Concentrated uptake by the Na^+-independent system was found to be dependent on a H^+-gradient, which operates equally well both in the presence and absence of Na^+. In pars recta, the preliminary experiments indicated the existence of more than one Na^+-dependent transport component. Thus, taurine transport has not been studied in sufficient detail, also with regard to regional differences in the proximal tubule, cation and anion dependence, and stoichiometry, to permit conclusions at the present stage. The present investigation attempts to fill in this gap by providing

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transport data from supposedly transport homogeneous preparations of the kidney.

Materials and Methods

Materials

Taurine, β-alanine, L-alanine, L-proline, L-serine and glycine, Trisma base, Hepes, and Mes were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Radioactive [1,2-^{3}H]taurine (specific activity 1.30 TBq/mmol) was obtained from Amersham International plc. (Buckinghamshire, U.K.). These and all other reagents were of A.R. grade.

Preparation and enzymic characterization of renal luminal membrane vesicles

Luminal membrane vesicles were isolated from pars convoluta (cortex corticis) and from pars recta (outer stripe of outer medulla) of the proximal tubule of rabbit kidney according to the method already described [15]. Briefly, outer cortical tissue was obtained by cutting slices < 0.4 mm thick from the surface of the kidney. Strips of outer medulla approx. 1 mm thick (representing predominantly pars recta) were dissected from outer stripe of outer medulla. We always prepared luminal membrane vesicles from outer cortical and outer medullary tissue from the same kidneys and the two preparations were performed in parallel by use of the Ca^{2+}-precipitation procedure previously described by Sheikh and Møller [15]. 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 designed to test the effect of low pH 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 was examined by electron microscopy [16] and by measurement of specific activities of various enzyme markers as previously described [17]. Average enrichment in specific activity (final pellet/homogenate) of leucine aminopeptidase (a brush-border membrane marker) was 13-fold, while that of Na^+-, K^+-ATPase (a basolateral marker) and succinate dehydrogenase (a mitochondrial marker) both were < 0.5. The amount of protein was determined by the method of Lowry et al. [18] as modified by Peterson [19] 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. No bacterial contamination of membrane vesicle preparations was found after incubating the samples on blood-agar plates and by electron microscopy.

Uptake experiments

The uptake of taurine by various vesicle preparations was studied by Millipore filtration [20]. The details of the individual experiments are given in the legends to the figures. Briefly, the uptake of radioactive taurine was studied as follows: 20 μl of luminal membrane vesicle suspension were added at time zero to 100 μl of incubation medium containing radioactively labelled ligand and other constituents as required. Transport of taurine into vesicles was stopped by addition of 1 ml 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), in the low pH experiments. The resulting suspension was rapidly filtered through a Millipore filter (HAWP 0.45 μm) which was washed twice with 2.5 ml of ice-cold buffer. The filter was dried overnight and radioactivity was counted in a liquid scintillation counter (LKB-Wallac 1218 RackBeta) after addition of Filter Count™ (Packard Instrument International SA. Zürich, Switzerland). 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 filtering denatured membranes (boiled for 2 min) added to an incubation tube containing radioactive taurine.

In a series of experiments in which Na^+ coupling ratio with taurine was examined, precautions were taken to ensure that any effects due to variations in transmembrane electrical potential were minimized by short-circuiting the membrane. In these experiments 100 mM KSCN was present in both intravesicular and extravesicular media. Besides, valinomycin at a concentration of 12.5 mg/g protein was added as a stock solution of 25 g/l in ethanol. As shown by Turner and Moran [21], the presence of 100 mM KSCN equilibrium together with this concentration of valinomycin is sufficient to short-circuit transmembrane electrical potential differences.

In another series of experiments examining the stoichiometry of the Na^+/taurine cotransport mechanism for chloride, vesicles were preincubated with valinomycin (12.5 mg/g protein) and loaded with K-gluconate in order to clamp membrane potentials at potassium diffusion potential.

Calculations

The Michaelis-Menten kinetics of the uptake of various concentrations of taurine were analysed. Theoretical saturation curves were fitted to the experimental data using a computer-analysed statistical iteration procedure [22].

In order to determine the ion : taurine stoichiometry we used the 'activation method' [23]. Here, one measures the stimulation of substrate (taurine) flux by increasing concentrations of activator (Na^+, H^+ or Cl^-). The data were analyzed by the equation [24]:

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

The equation assumes the existence of n essential cooperative site(s) for the activator A per taurine site. According to this equation a plot of flux/$[A]^n$ against flux for the correction of n will yield a straight line with slope $1/K_{0.5}^n$.

Results

Cation dependence of taurine uptake by luminal membrane vesicles from pars convoluta

By the use of the spectrophotometric method [25] and with the experimental protocol recently described (for details see Fig. 1 of Ref. 26), we found that the rate of uptake of taurine in vesicles from pars convoluta was

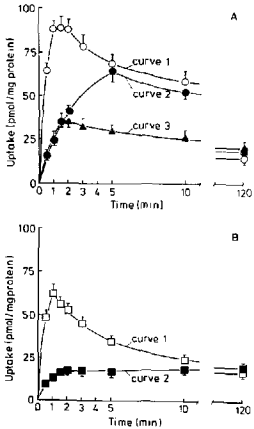

Fig. 1. Cation-dependent uptake of taurine by luminal membrane vesicles from pars convoluta as studied by Millipore filtration. (Panel A) 20 µl of vesicle suspension prepared in 310 mM mannitol, 15 mM Hepes-Tris (pH 7.5), were incubated at different time intervals in 100 µl of incubation medium containing 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 taurine at a low pH, but in the absence of a H^+ gradient ($pH_{in} = pH_{out} = 5.5$). In these experiments the vesicles were prepared in 310 mM mannitol, 15 mM Mes-Tris buffer (pH 5.5), and incubated in 155 mM NaCl in 15 mM Mes-Tris buffer (pH 5.5) at different time intervals. Media contained 0.2 µM [1,2-^{3}H]taurine and unlabelled taurine reaching 12 µM (final concentration). (Panel B) The experimental conditions were essentially the same as in Panel A except that NaCl is replaced by KCl at pH 5.5 (curve 1(□)) and 7.5 (curve 2 (■)) and all the vesicles were prepared in 310 mM mannitol, 15 mM Hepes-Tris (pH 7.5). Results are given as mean values ± S.D. of five experiments. In those cases where vertical bars are absent, standard deviations are smaller than the graphical representation of the mean.

electrogenic and mediated by both Na^+-dependent and Na^+-independent systems (not shown).

Figs. 1A and 1B summarize the results of a series of experiments in which the effect of various cations on the uptake of radioactive taurine was investigated. Fig. 1A describes the uptake of taurine by membrane vesicles in the presence of a Na^+-gradient (curve 2) and a Na^+ plus H^+ gradient (curve 1). Na^+-dependent uptake of taurine reaches an equilibrium value after 120 min. A maximal accumulation over the equilibrium value was observed after 5 min of incubation in the presence of a Na^+-gradient. Imposition of a H^+-gradient (extravesicular > intravesicular) in addition to Na^+ results in an increased transient uptake of taurine (compare curve 1 with curve 2) and maximal accumulation is observed already after 1 min of incubation. Curve 3 shows the Na^+-gradient-dependent uptake of taurine at lower pH, but in the absence of H^+-gradient (i.e. $pH_i = pH_0 = 5.5$) by luminal membrane vesicles. It is seen that the H^+-gradient dependent extra uptake of taurine is abolished under these experimental conditions.

The effect of H^+ and K^+ gradients on the renal uptake of radioactive taurine is shown in Fig. 1B. The purpose of performing these experiments was to examine whether a H^+ gradient in the absence of Na^+ can drive uphill transport of taurine in membrane vesicles from this segment of proximal tubule. It is interesting to note that inwardly directed H^+-gradient stimulated the uptake of taurine (curve 1) and maximal accumulation of amino acid is observed already after 1 min of incubation. By contrast, no transient accumulation of taurine was observed in the presence of a K^+-gradient (curve 2), when the extravesicular pH was equal to intravesicular pH (i.e. pH = 7.5).

In preliminary experiments we observed that the Na^+-dependent uptake of taurine at increasing medium concentrations of the amino acid (0.010–10 mM) by vesicles from pars convoluta occurred via a dual transport system. By contrast, the uptake of taurine in the presence of a KCl gradient ($pH_{out} = pH_{in} \simeq 7.5$) exhibited simple diffusion properties that were proportional to medium concentration of taurine under these conditions (not shown). We calculated the uptake of taurine in the presence of a Na^+-gradient alone, but for the sake of clear visualization the uptake values obtained at low medium taurine concentration (0.01–0.20 mM) are plotted in Fig. 2A, and a relatively high medium concentration (0.2–10.0 mM) is given in Fig. 2B. The following kinetic parameters were obtained from these data: $K_{m1} = 0.086 \pm 0.009$ mM, $K_{m2} = 5.41 \pm 0.48$ mM, and $V_{max1} = 0.12 \pm 0.03$ and $V_{max2} = 5.14 \pm 0.44$ nmol/mg protein per 20 s.

Fig. 2C depicts the uptake of taurine at increasing medium concentrations ranging from 0.010 to 10 mM in the presence of a H^+-gradient alone. It is apparent from the curve that in the absence of Na^+, the H^+ gradient

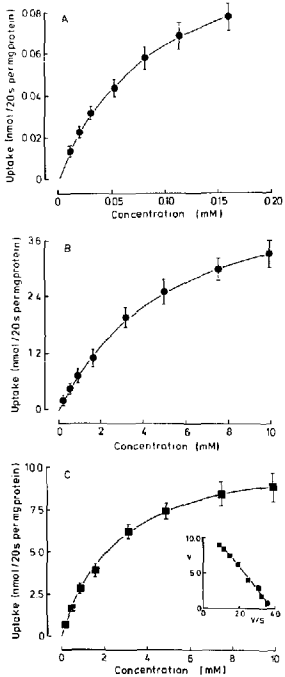

Fig. 2. Cation-dependent uptake of taurine at increasing concentrations by luminal membrane vesicles from pars convoluta. (Panel A) Uptake of increasing concentrations of taurine in the presence of a Na^+-gradient alone. The results are given as the uptakes measured in 155 mM NaCl, 15 mM Hepes-Tris (pH 7.5) minus the uptakes in 155 mM KCl, 15 mM Hepes-Tris (pH 7.5). The media contained 1 μM [1,2-^{3}H]taurine and various concentrations of unlabelled taurine ranging from 0.01 to 0.20 mM (final concentration). (Panel B) The experimental conditions were essentially the same as in Panel A except that the media contained 1 μM [1,2-^{3}H]taurine and various concentrations of unlabelled taurine ranging from 0.20 to 10.0 mM (final concentration). (Panel C) Uptake of increasing concentrations of taurine (final concentration 0.01–10.0 mM) in the presence of a H^+-gradient alone. The results are given as 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). In the inset the results are shown in an Eadie-Hofstee plot. V represents the rate of transport at substrate concentration S. Results are given as mean values ± S.D. of five experiments. In those cases where vertical bars are absent, standard deviations are smaller than the graphical representation of the mean.

markedly enhanced the uptake of taurine in vesicles from pars convoluta. The kinetic parameters calculated from these data were as follows: $K_m = 2.87 \pm 0.24$ mM and $V_{max} = 11.54 \pm 1.20$ nmol/mg protein per 20 s. Inset in the figure shows the Eadie-Hofstee analysis of the experimental data and a straight-line relationship is obtained for taurine transport, suggesting that the H^+-gradient dependent uptake of the amino acid occurs via a single transport system.

Effect of different neutral amino acids on the uptake of taurine by luminal membrane vesicles from pars convoluta

The uptake of radioactive taurine (1 μM) in the presence of a 129 mM NaCl gradient ($pH_{in} = pH_{out} = 7.5$) and unlabelled neutral amino acids was measured (not shown). The concentration of amino acids tested as inhibitor was chosen to correspond to the half-saturation constant for their renal transport [26,27–30]. It was found that only addition of β-alanine (2.7 mM) reduced the uptake of taurine to 24.2 ± 7.9% of the control value (20 s uptake value). L-Alanine (2.1 mM), L-proline (0.57 mM), glycine (6.7 mM), and L-serine (3.7 mM) did not significantly inhibit the influx of taurine (uptake 93.1 ± 8.2%, 95.1 ± 10.8%, 100.2 ± 10.0%, and 86.8 ± 10.1%, respectively). Furthermore, we investigated the effect of above-mentioned amino acids on the H^+-gradient dependent transport of taurine (not shown). Here, NaCl was replaced by KCl ($pH_{out} = 5.5$, $pH_{in} = 7.5$). The presence of β-alanine (3.9 mM), L-alanine (4.4 mM), L-proline (0.54 mM), or glycine (3.9 mM) decreased the uptake of taurine to 43.8 ± 10.2%, 44.8 ± 9.4%, 55.4 ± 9.5%, 58.0 ± 9.9%, respectively. On the other hand, L-serine (3.7 mM) did not change the uptake (i.e. 96.9 ± 6.9%). In both kinds of experiments, the gradient driven uptake values given have been corrected for a passive diffusion component. Results are given as mean values ± S.D. of five experiments.

Pattern of taurine uptake by luminal membrane vesicles from pars recta

By the use of the spectrophotometric method [25] we found that the rate of uptake of taurine in vesicles from pars recta was a strictly Na^+-dependent and electrogenic process (not shown).

Fig. 3 describes the uptake of radioactive taurine by luminal membrane vesicles from pars recta after incubation for different lengths of time in the presence of various cation gradients. The transient accumulation of taurine is only seen in the presence of Na^+-gradient alone (curve 1). Incubation at lower pH without H^+-gradient (curve 3) abolished the Na^+-dependent 'overshoot' of taurine in vesicles from pars recta. We have also studied the effect of combined gradient of Na^+ and H^+ ($pH_{out} = 5.5$, $pH_{in} = 7.5$) on the uptake of taurine in vesicles from pars recta. The results of these experiments showed that application of H^+-gradient

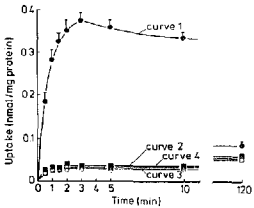

Fig. 3. Cation-dependent uptake of taurine by luminal membrane vesicles from pars recta as studied by Millipore filtration. 20 μl of vesicle suspension prepared in 310 mM mannitol, 15 mM Hepes-Tris (pH 7.5), were incubated at different time intervals in 100 μl of incubation mixture consisting of 155 mM NaCl in 15 mM Hepes-Tris buffer (pH 7.5) (Curve 1 (●)), of 155 mM KCl in 15 mM Hepes-Tris buffer (pH 7.5) (Curve 2 (■)), or of 155 mM KCl in 15 mM Mes-Tris (pH 5.5) (Curve 4 (□)). Curve 3 (○) shows the effect of Na^+-gradient dependent uptake of taurine at a low pH but in the absence of a H^+ gradient ($pH_{in} = pH_{out} = 5.5$). In these experiments the vesicles were prepared in 310 mM mannitol, 15 mM Mes-Tris buffer (pH 5.5), and incubated in 155 mM NaCl in 15 mM Mes-Tris (pH 5.5). Media contained 0.2 μM [1.2-^{3}H]taurine and unlabelled taurine reaching 75 μM (final concentrations). Results are given as mean values ± S.D. of five experiments. In those cases where vertical bars are absent, standard deviations are smaller than the graphical representation of the mean.

drastically reduced the renal accumulation of taurine (not shown).

In a series of experiments we found that the Na^+-dependent uptake of radioactive taurine at increasing medium concentrations of the amino acid (0.01–10 mM) by vesicles from pars recta was characterized by two transport systems (not shown). The uptake of taurine in the presence of a KCl gradient ($pH_{in} = pH_{out} = 7.5$) showed simple diffusion properties. The Na^+-gradient driven uptake values of taurine, corrected for the passiv diffusion component, resulted in the following kinetic parameters: $K_{m1} = 0.012 \pm 0.005$ mM, $K_{m2} = 5.62 \pm 0.55$ mM, and $V_{max1} = 0.28 \pm 0.03$ and $V_{max2} = 4.15 \pm 0.38$ nmol/mg protein per 20 s.

Effect of different neutral amino acids on the uptake of taurine in vesicles from pars recta

The effect of various neutral amino acids on the Na^+-dependent uptake of taurine in luminal membrane vesicles from pars recta was studied (not shown). The experimental conditions were the same as in pars convoluta. Only β-alanine (0.16 mM) reduced the uptake of taurine compared to the uptake in the absence of test compound (25.0 ± 10.5%). No effect was observed with L-alanine (0.24 mM), L-proline (0.16 mM), glycine (0.34 mM) or L-serine (0.37 mM) (i.e. uptake 90.1 ± 11.2%, 89.5 ± 9.9%, 94.2 ± 11.7%, and 99.7 ± 7.2%, respectively.

Anion dependence of taurine uptake by luminal membrane vesicles from pars convoluta

The use of different anions results in a variation of the membrane potential. Uptake in the presence of the relatively impermeable anion, gluconate, would be expected to be markedly reduced as compared to the uptake in the presence of a Cl^--gradient since the membrane potential is less negative and the transport of taurine is an electrogenic process. Using NO_3^-, which is a more permeable anion, one would expect an increased uptake of taurine because intravesicularly it is more negatively charged. This is also the case if one examines the Na^+-dependent low-affinity and the H^+-dependent transport systems which confirm the electrogenicity of these components (Table I). However, the uptake of taurine at 1 μM is reduced again, indicating the presence of a high-affinity taurine transport system dependent on both Na^+ and Cl^- ions. The same system was found to be stimulated by potassium diffusion potential caused by the presence of valinomycin (not shown).

Stoichiometry of Na^+, H^+ and Cl^- dependent taurine transport in vesicles from pars convoluta

Fig. 4A shows the results of a representative experiment where the initial flux of 1 μM taurine was measured as a function of Na^+ concentration over the range 0 to 200 mM. The sigmoidal shape of the plot of taurine uptake vs. sodium concentration suggests that more than one Na^+ is associated with the transport process. Fig. 4B shows two plots for the data of Fig. 4A assuming n values of 1 and 2 (see calculations). The inset in Fig. 4B shows a series of plots with values of n ranging from 1.5 to 1.9. A value of n of 1.8 provided the best fit

TABLE I

Effect of anions on the uptake of taurine in luminal membrane vesicles from pars convoluta

Vesicles were prepared in a solution containing 310 mM mannitol and 15 mM Hepes-Tris (pH 7.5). Measuring the Na^+-dependent uptake of taurine, the incubation media contained 15 mM Hepes-Tris (pH 7.5), 155 mM of one of the anions listed below, as Na^+ salt, 1 μM [1.2-^{3}H]taurine and unlabelled taurine reaching the final concentration as shown in the table. Measuring the H^+-dependent uptake of taurine, the experimental conditions were essentially the same as mentioned above except that Na^+ is replaced by K^+ and the incubation media contained 15 mM Mes-Tris (pH 5.5). Solute uptakes at 20 s were normalized to uptake observed in the presence of NaCl or KCl, respectively. Results are given as mean values ± S.D. of five experiments.

Anion	Solute uptake relative to that in Cl^- medium		
	Na^+-dependent uptake		H^+-dependent uptake
	1 μM taurine	3 mM taurine	1 μM taurine
Cl^-	1.00	1.00	1.00
NO_3^-	0.69 ± 0.08	1.26 ± 0.11	1.44 ± 0.13
Gluconate	0.28 ± 0.05	0.65 ± 0.06	0.13 ± 0.04

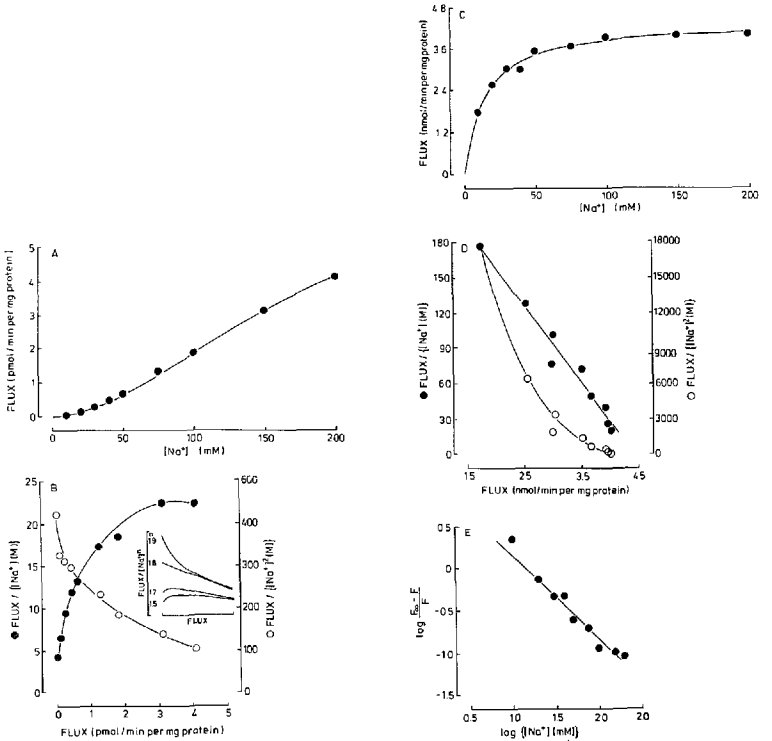

Fig. 4. Effect of Na^+ concentration on the uptake of taurine by luminal membrane vesicles from pars convoluta. Vesicles were preincubated in 15 mM Hepes-Tris buffer (pH 7.5), containing 100 mM KSCN, 700 mM mannitol and valinomycin at a concentration of 12.5 mg/g protein. The incubation media contained 15 mM Hepes-Tris, 100 mM KSCN, 300 mM mannitol and various concentrations of NaCl ranging from 0 to 200 mM (final concentration). Choline replaced sodium isosmotically to obtain the various sodium concentrations studied. The composition of the stop buffer was 15 mM Hepes-Tris (pH 7.5), 400 mM mannitol and 300 mM NaCl. The uptake was studied at two different concentrations of taurine. All uptake values were corrected for uptake in the absence of a Na^+ gradient. (Panel A) Plot of flux versus Na^+ concentration for 1 μM [1,2-^{3}H]taurine. (Panel B) Plots of flux/$[Na^+]^n$ versus flux for $n = 1$ (●) and $n = 2$ (○) for the high-affinity system (1 μM). The inset shows a series of plots of flux/$[Na^+]^n$ against flux for different values of n ranging from 1.5 to 1.9. The units of $[Na^+]$ are M. (Panel C) Plot of flux versus Na^+ concentration for 1 μM [1,2-^{3}H]taurine and unlabelled taurine reaching a final concentration at 3 mM. The curve shows the difference between flux at 3 mM and 1 μM. (Panel D) Plots of flux/$[Na^+]^n$ versus flux for $n = 1$ (●) and $n = 2$ (○) for the low-affinity system (3 mM minus 1 μM). (Panel E) Double-logarithmic (Hill-type) plot. F is the uptake at a given Na^+ concentration and F_∞ is the maximal uptake at the saturating Na^+ concentration (the intercept of the regression line with the abscissa in Panel D). The results shown are from a representative experiment ($n = 4$).

to these data, suggesting that approx. two Na^+ ions are involved in the translocation of taurine in vesicles from pars convoluta.

In order to study the coupling ratio of Na^+ to both the high-affinity and low-affinity transporters we measured the initial flux of 1 μM and 3 mM taurine. It

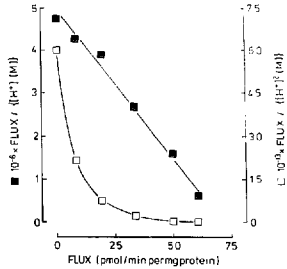

Fig. 5. Effect of H^+ gradient (extravesicular > intravesicular) on the uptake of taurine by luminal membrane vesicles from pars convoluta. The vesicles were preincubated with 155 mM KCl in 15 mM Hepes-Tris (pH 7.5). The incubation media contained 0.6 μM [1,2-^{3}H]taurine and 155 mM KCl in various Hepes-Tris or Mes-Tris buffer solutions with pH ranging from 7.5 to 5.0. The quantities plotted in the figure are the H^+ gradient dependent component of flux, i.e. all uptake values were corrected for the uptake in the absence of a H^+ gradient ($pH_{out} = pH_{in} = 7.5$). Plots of flux/$[H^+]$ vs. flux for $n = 1$ (■), $n = 2$ (□) are shown. The results shown are from a representative experiment ($n = 4$).

appears from the Figure that the apparent K_m for the overall transport of taurine with respect to Na^+ depends on the taurine concentration. The initial flux measurements of radioactive taurine carried out at 1 μM concentration in the medium represent the transport of this amino acid mediated predominantly by a high-affinity system, whereas the influx values obtained at 3 mM taurine concentration may represent both the high-affinity and low-affinity transport of this compound. An attempt is made to assess the low affinity component of taurine by subtracting the activity of the high-affinity component from the total Na^+-dependent transport activity at different concentrations of Na^+ (Fig. 4C). The corrected flux values show a Michaelis-Menten type dependence on Na^+ concentration. Fig. 4D indicates that an n value of 1 provides the best fit to these data. The experimental data are reanalyzed in Fig. 4E on a log-log (Hill-type) plot. The numerical value of the slope of the least-squares fit to these points which gives the Na^+/taurine stoichiometry is 0.98 ± 0.06, again indicating a 1 : 1 interaction.

The stoichiometry of the H^+/taurine cotransport system was also investigated (Fig. 5). The initial flux of taurine was measured in the presence of different mag-

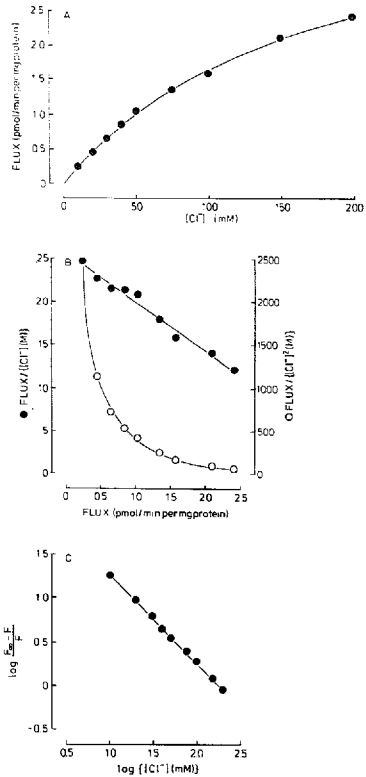

Fig. 6. Effect of Cl^- concentration on the uptake of taurine by luminal membrane vesicles from pars convoluta. Vesicles were preincubated in 20 mM Hepes-Tris buffer (pH 7.5), containing 180 mM K-gluconate, 50 mM Na-gluconate and valinomycin at a concentration of 12.5 mg/g protein. The incubation media contained 50 mM Na-gluconate, 1 μM [1,2-^{3}H]taurine and various concentrations of *N*-metyl-D-glucamine chloride ranging from 0 to 200 mM (final concentration). Sulphate replaced chloride isosmotically to obtain the various chloride concentrations studied. Uptake measured in the absence of chloride was subtracted from all points. The composition of the stop buffer was 20 mM Hepes-Tris (pH 7.5), 200 mM mannitol and 300 mM NaCl. (Panel A) Plot of flux versus Cl^- concentration. (Panel B) Plots of flux/$[Cl^-]^n$ versus flux for $n = 1$ (●) and $n = 2$ (○). The units of $[Cl^-]$ are M. (Panel C) Double-logarithmic (Hill-type) plot. F is the uptake at a given chloride concentration and F_∞ is the maximal uptake at a saturating chloride concentration (the intercept of the regression line with the abscissa in Panel B). The results shown are from a representative experiment ($n = 4$).

nitudes of the H^+-gradient (extravesicular > intravesicular). It is seen that assuming $n = 1$ a straight line relationship between flux/$[H^+]^n$ against flux is obtained, suggesting that one H^+ ion may be involved in the translocation of taurine in these vesicle preparations.

The stoichiometry of the Na^+/taurine cotransport mechanism for chloride was determined (Fig. 6). The Cl^--dependent taurine transport shows a Michaelis-Menten type dependence, indicating a 1 Cl^- : 1 taurine stoichiometry (see Figs. 6A and 6B). Fig. 6C shows a log-log (Hill-type) plot. The numerical value of the slope of the least squares fit to these points which gives the Cl^-/taurine stoichiometry is 0.99 ± 0.01, again indicating a 1 : 1 interaction.

Anion dependence of taurine uptake by luminal membrane vesicles from pars recta

Table II shows the effect of various sodium salts on the uptake of taurine at a concentration of 1 μM and 1 mM. The results show, as also seen in pars convoluta, the presence of a high-affinity taurine transport system dependent on both Na^+ and Cl^- (and stimulated by potassium diffusion potential caused by addition of valinomycin, not shown), whereas the low-affinity Na^+-dependent taurine uptake is not affected by Cl^-.

Stoichiometry of Na^+ and Cl^--dependent taurine transport in vesicles from pars recta

Initial flux rates of 1 μM and 1 mM taurine were measured as a function of sodium concentration. Furthermore, the stoichiometry of the Na^+/taurine cotransport mechanism for chloride was investigated. Similar types of curves were obtained with the pars recta as those with the pars convoluta presented in Figs. 4 and 6, indicating the following coupling ratio: 1.8 Na^+ : 1 Cl^- : 1 taurine (high affinity) and 1 Na^+ : 1 taurine (low affinity) (not shown).

TABLE II

Effect of anions on the sodium-gradient dependent uptake of taurine in luminal membrane vesicles from pars recta

Vesicles were prepared in a solution containing 310 mM mannitol and 15 mM Hepes-Tris (pH 7.5). The incubation media contained 15 mM Hepes-Tris (pH 7.5), 155 mM of one of the anions listed below, as Na^+ salt, 1 μM [1,2-^{3}H]taurine and unlabelled taurine reaching the final concentration as shown in the table. Solute uptakes at 20 s were normalized to uptake observed in the presence of NaCl. Results are given as mean values ± S.D. of five experiments.

Anion	Solute uptake relative to that in Cl^- medium	
	1 μM taurine	1 mM taurine
Cl^-	1.00	1.00
NO_3^-	0.48 ± 0.09	1.36 ± 0.17
Gluconate	0.10 ± 0.04	0.52 ± 0.08

Discussion

In this communication, we demonstrate the presence of multiple transport systems for the uptake of taurine in highly purified membrane vesicles from pars convoluta and pars recta. In contrast to previous reports [5,8–13], the uptake of taurine in vesicles from pars convoluta was found to be mediated by two Na^+-dependent and one Na^+-independent, but electrogenic processes. The Na^+-dependent transport of taurine occurred via a dual transport system, namely a high-affinity (half-saturation 0.086 mM) and a low-affinity system (half-saturation 5.41 mM). The K_m value for the high-affinity system found in our vesicle preparations is in good agreement with the K_m values (0.023–0.042 mM) found in rat renal cortex brush-border membrane vesicles by various investigators [5,10,12,13]. However, it should be noted that these measurements were performed on vesicles prepared from whole cortex. Furthermore, we have demonstrated that an inwardly directed H^+-gradient enhance both the Na^+-dependent and Na^+-independent components of taurine transport. The Na^+-dependent initial and maximal uptake was increased in the presence of a H^+-gradient. In the absence of Na^+, H^+-gradient was able to drive uphill transport in membrane vesicles from pars convoluta (curve 1 of Fig. 1B). These findings strongly indicate the presence of a novel H^+-dependent transport system. Evidence for a similar proton gradient-dependent transport component has recently been reported for the uptake of L-proline [28], L- and D-alanine [26,31], β-alanine [29] and glycine [27] in luminal membrane vesicles from pars convoluta of rabbit proximal tubule.

In contrast to the above-mentioned findings, the uptake of taurine in vesicles from pars recta was strictly dependent on Na^+ and mediated by a high-affinity (half-saturation 0.012 mM) and a low-affinity (half-saturation 5.62 mM) transport system. Therefore, it is interesting to note that the substrate specificity at low concentrations of taurine is different from that of pars convoluta. Since the plasma concentration of taurine is normally 50 μM, it is reasonable to assume that the filtered taurine is predominantly reabsorbed by the high-affinity transport system in both segments of the proximal tubule. Lowering pH without a H^+-gradient completely abolished the Na^+-dependent transient accumulation of taurine and superposition of a H^+-gradient (extravesicular > intravesicular) drastically reduced the uptake of taurine in vesicles from pars recta. This phenomenon has also been reported for β-alanine [29] from this laboratory, and we have proposed that the reduction of the transport activity at pH 5.5 is based on changes in the protonation state of some critical amino acid residues involved in the translocation of β-amino acids. Incidentally, these results exclude any major contamination of pars recta with membrane fragments from

pars convoluta. Also, these findings emphasize the importance of the use of highly purified luminal membrane vesicles from the two anatomically distinct segments of proximal tubule in studying the mechanism of various solute transports along the nephron.

In order to characterize further the taurine transport systems, we studied the effect of different amino acids on the uptake of taurine in vesicles from pars convoluta and pars recta of rabbit proximal tubule. In pars convoluta, competition experiments showed only inhibition of the uptake of taurine by the Na^+-gradient dependent high-affinity transport system in the presence of β-alanine, whereas there was no appreciable inhibition with α-amino-acids (L-alanine, L-proline, glycine and L-serine). These observations suggest, as previously demonstrated *in vivo* and *in vitro* studies [5,8–13,32–34], the existence of a Na^+-dependent β-amino-acid-specific transport system. By contrast, we found that the H^+-gradient dependent uptake of taurine was reduced to approx. 50% in the presence of β-alanine, L-alanine, L-proline and glycine. There was no effect of L-serine. Accordingly, we have previously shown that the uptake of L-serine in vesicles from pars convoluta and pars recta was strictly Na^+-dependent [30]. In vesicles from pars recta, the uptake of taurine by the Na^+-dependent high-affinity transport system was drastically reduced by β-alanine, while addition of L-alanine, L-proline, glycine and L-serine had no significant effect. As in pars convoluta, the competition experiments indicate the existence of a Na^+-dependent β-amino-acid-specific transport system in vesicles from pars recta.

It has previously been reported that the Na^+-dependent uptake of β-amino acids by renal brush-border membrane vesicles from rat, rabbit and dog [11–14] specifically required Cl^-. Therefore, we have investigated the anion dependence of taurine uptake in both segments of the proximal tubule. We found that only the Na^+-dependent high-affinity transport system was stimulated by Cl^-, whereas there was no effect on the low-affinity transporter both in pars convoluta and pars recta. Nor was there any effect of Cl^- on the H^+-dependent transport of taurine in pars convoluta. Attempts to calculate the stoichiometry of the Na^+/Cl^-/taurine, Na^+/taurine and H^+/taurine transporters located in pars convoluta and pars recta by using the 'activation method' showed 1.8 Na^+ : 1 Cl^- : 1 taurine (Na^+-dependent, high affinity), 1 Na^+ : 1 taurine (Na^+-dependent, low affinity) and 1 H^+ : 1 taurine. The 2 Na^+ : 1 Cl^- : 1 taurine cotransport mechanism is in good agreement with earlier studies in renal brush-border membrane vesicles from rabbit [14] and dog [11] outer cortex or from whole cortex of rat [12,13]. It is known [23] that the 'activation method' does not distinguish whether the stimulation produced by these ions is a result of cotransport with taurine (energetic coupling) or interaction with the carrier in some other way which leads to an enhancement of the uptake of taurine (catalytic coupling). However, Wolff and Kinne [14] have demonstrated in rabbit renal outer cortical brush-border membrane vesicles that taurine translocation is energetically activated by Cl^-; i.e. that Cl^- is cotransported, even though a Cl^--gradient alone was unable to produce an overshoot. Furthermore, we found that both the Na^+-dependent and Na^+-independent transport of taurine in various preparations is an electrogenic process (taurine is predominantly a zwitterion at pH 7.5). Consequently, it is reasonable to conclude that one H^+ and approx. two Na^+, one Cl^- or one Na^+, respectively, are cotransported with taurine in the proximal tubule of rabbit kidney with transfer of a net positive charge.

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