Characterization of sodium-dependent and sodium-independent nucleoside transport systems in rabbit brush-border and basolateral plasma-membrane vesicles from the renal outer cortex

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The transport of uridine into rabbit renal outer-cortical brush-border and basolateral membrane vesicles was compared at 22 °C. Uridine was taken up into an osmotically active space in the absence of metabolism for both types of membrane vesicles. Uridine influx by brush-border membrane vesicles was stimulated by Na^+ , and in the presence of inwardly directed gradients of Na^+ a transient overshoot phenomenon was observed, indicating active transport. Kinetic analysis of the saturable Na^+ -dependent component of uridine flux indicated that it was consistent with Michaelis–Menten kinetics (K_m 12 ± 3 μM , $V_{\max}$ 3.9 ± 0.9 pmol/s per mg of protein). The sodium:uridine coupling stoichiometry was found to be consistent with 1:1 and involved the net transfer of positive charge. In contrast, uridine influx by basolateral membrane vesicles was not dependent on the cation present and was inhibited by nitrobenzylthioinosine (NBMPR). NBMPR-sensitive uridine transport was saturable (K_m 137 ± 20 μM , $V_{\max}$ 5.2 ± 0.6 pmol/s per mg of protein). Inhibition of uridine flux by NBMPR was associated with high-affinity binding of NBMPR to the basolateral membrane (K_d 0.74 ± 0.46 nM). Binding of NBMPR to these sites was competitively blocked by adenosine and uridine. These results indicate that uridine crosses the brush-border surface of rabbit proximal renal tubule cells by Na^+ -dependent pathways, but permeates the basolateral surface by NBMPR-sensitive facilitated-diffusion carriers.

INTRODUCTION

Transcellular transport in renal tubules involves the movement of solutes across two barriers: the brush-border (apical) and the basolateral (serosal) plasma membranes. For many solutes the transport properties of brush-border and basolateral membranes differ, resulting in vectorial transport of solutes, for example Na^+ -coupled systems for hexoses at the brush-border membrane surface and a facilitated-diffusion system at the basolateral membrane surface [1]. For nucleosides, relatively few studies have been performed on the transport characteristics of the renal proximal tubules. In 1982, measurements of the renal clearance of adenosine in humans and mice led to the hypothesis that an active transport system exists in the kidney with the function of re-absorbing the filtered adenosine, a nucleoside that has been postulated to play a role in the intrinsic control of glomerular filtration rate and renin release [2,3]. Subsequent studies with rat renal brush-border membrane vesicles demonstrated that the uptake of adenosine and a variety of purine and pyrimidine nucleosides did not proceed by classical facilitated diffusion, as seen in most cell types [4,5], but rather by Na^+ -coupled transport systems [6–8]. Moreover, recent studies have shown that in addition to Na^+ stimulating uridine transport, K^+ gradients (out > in) can also provide a driving force for the active transport of uridine in rat renal brush-border

membrane vesicles [8]. The K^+ -dependent uridine uptake was not due to substitution of K^+ for Na^+ in the Na^+ -dependent system, as stimulation rather than inhibition was observed when both Na^+ and K^+ were present extravesicularly. One possible explanation for these findings is that there are two separate carrier proteins, one driven by Na^+ and the other driven by K^+ . Interestingly, the stoichiometry for Na^+ :uridine is 1:1, whereas that for K^+ :uridine is 3:2 (C.-W. Lee, C. I. Cheeseman & S. M. Jarvis, unpublished work). It is not known if similar co-transport systems for nucleosides exist in the kidneys of other species. Moreover, no studies have been performed on uridine uptake by renal basolateral membrane vesicles.

In the present paper, we compare the influx of uridine by rabbit renal brush-border and basolateral membrane vesicles prepared from the proximal tubule. Our results demonstrate the presence of a Na^+ -coupled system for uridine uptake in brush-border membrane vesicles. In contrast, uridine uptake in basolateral membrane vesicles is not stimulated by Na^+ and can be inhibited by the specific facilitated-diffusion nucleoside transport inhibitor, nitrobenzylthioinosine (NBMPR). These results indicate that the nucleoside transport properties of the apical and basolateral membranes of rabbit renal proximal tubule cells are different. Preliminary reports of some of these results have been published [9,10].

Abbreviations used: NBMPR, nitrobenzylthioinosine; IC_{50} , concentration causing half-maximal inhibition.

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MATERIALS AND METHODS

Preparation of brush-border membrane vesicles

Male New Zealand White rabbits (2.0–2.5 kg) were killed by an intravenous injection of sodium pentobarbital (Nembutal; 50 mg/kg; Ceva, Watford, Herts., U.K.), and the kidneys were removed, decapsulated and placed on ice. The outer cortex was dissected, homogenized with a Polytron on setting 5 for 30 s repeated four times, and brush-border membrane vesicles were prepared by a Mg^{2+} precipitation technique [11]. The purity of the preparation was assessed by measuring the enrichment of the brush-border membrane enzyme, alkaline phosphatase, as compared with the homogenate [12]; the enrichment was 8–14-fold. The vesicles were generally resuspended in vesicle suspension medium, composed of 300 mM-mannitol and 5 mM-Tris/HCl, pH 7.4, and used within 24 h of preparation.

Preparation of basolateral membrane vesicles

Basolateral membrane vesicles were prepared as described previously [13], with the following modifications. Rabbit kidney cortical tissue was obtained as above and homogenized at a ratio of 50 ml of homogenizing medium (250 mM-sucrose/2 mM-Hepes/Tris, pH 7.4) per 5 g wet wt. of tissue with a glass-Teflon Potter homogenizer (30 strokes at 1500 rev./min). The homogenate was centrifuged at 1000 *g* for 10 min and the resultant supernatant was re-centrifuged at 22000 *g* for 20 min. The pellet was composed of three layers, and the light upper layer was carefully removed and mixed with Percoll and buffer to give a final concentration of 12 % Percoll (maximum 10 ml per 5 g wet wt. of tissue). A self-orientating Percoll gradient was established by centrifuging the Percoll solution in 15 ml tubes in a Beckman JA 20.1 rotor at 44000 *g* for 35 min. Two distinct bands of turbidity were observed: the upper band represented the basolateral membranes. The membrane bands were carefully removed from the Percoll gradient with a syringe and needle and diluted with 85 mM-sucrose/85 mM-KCl/25 mM-Hepes/Tris, pH 7.4, to a volume of 10 ml. The membrane suspension was centrifuged at 160000 *g* for 30 min to remove the Percoll, and the membrane pellet lying on top of the glassy Percoll pellet was resuspended in 100 mM-sucrose/100 mM-KCl/2 mM-Hepes/Tris, pH 7.4, and used on the same day of preparation. The purity of the basolateral membrane preparation was assessed by the activities of marker enzymes [12,14]. Na^+/K^+ -ATPase, a basolateral membrane enzyme, was enriched 16.5-fold over the homogenate. There was some contamination by alkaline phosphatase (brush-border enzyme), with an enrichment of 2.2-fold. These results are similar to those obtained previously [13], and that earlier study also indicated that nearly 90 % of the basolateral vesicles are in a right-side-out orientation. Interestingly, specific [3H]NBMPR-binding activity was also enriched 12.8-fold over the homogenate.

Uptake of uridine by membrane vesicles

The uptake of [3H]uridine (25 μ Ci/ml) at 22 °C was measured by an inhibitor-stop filtration technique as described previously [8]. Briefly, a 10 μ l portion of the vesicle preparation (100–200 μ g of protein) and 20 μ l of the incubation medium, containing [3H]uridine and appropriate salt concentrations (with choline chloride sub-

stituted to maintain osmolarity), were pipetted separately at the bottom of plastic test tubes. In inhibition studies, test compounds and [3H]uridine were added simultaneously, except for NBMPR, diprydamole and dilazep, which were preincubated with the vesicles for 20 min. Transport was initiated by vigorous mixing with a vortex mixer. Short incubation times were timed with a metronome. At specified times, 1.0 ml of ice-cold stop solution (100 mM-mannitol, 100 mM-NaCl, 5 mM-Tris/HCl, pH 7.4, and 1 mM-phlorhizin for brush-border membrane vesicles and 10 μ M-NBMPR for basolateral membranes) was added to terminate transport. The suspension was immediately filtered through a Gelman nitrocellulose filter (Gelman Sciences, Northampton, U.K., 0.45 μ m) under suction. The filter was washed with 5.0 ml of ice-cold stop solution, dissolved in 4.0 ml of Optiphase T (LKB Scintillation Products), and the radioactivity was determined in a Beckman LS 7800 liquid-scintillation counter with automatic quench correction and d.p.m. conversion. A blank value for transport was obtained by filtering the transport medium without the membrane vesicles. This value was subtracted from measurements of uridine associated with the membrane vesicles to determine uptake rates. The final compositions of the incubation media are given in the Figure legends.

Uridine metabolism

Membrane vesicles were incubated with 5 μ M-[3H]uridine at 22 °C, and the reaction was terminated as described above. The filter was immediately shaken for 30 min at 22 °C in 250 μ l of 2 M-NH₃ [15]. The NH₃ extract (30 μ l) was spotted on to either a cellulose-coated plate impregnated with a fluorescent indicator (Eastman, New York, NY, U.S.A.; 0.16 mm thick) or a silica-gel-coated plate containing a fluorescent indicator (Eastman; 0.1 mm thick). Each spot was overlaid with 5 μ l of a standard solution containing uridine, uracil, UMP, UDP and UTP. The solvent system was 0.55 M-LiCl in 0.2 % formic acid and butan-1-ol saturated with water for the cellulose and silica-gel plates respectively. After drying, the zones bearing the standards were localized under u.v. light (R_f values of 0.83, 0.67 and 0.94 for uridine, uracil and uridine nucleotides respectively for the solvent 0.55 M-LiCl in 0.2 % formic acid, and R_f values of 0.26, 0.42 and 0 for uridine, uracil and uridine nucleotides respectively for the solvent butan-1-ol saturated with water). The rest of the lane was equally divided into individual zones (1 cm). Each zone was cut from the plate and the strips were soaked in 1 ml of water to extract the radioactivity before addition of 8 ml of scintillation fluid.

[3H]NBMPR-binding assay

Equilibrium [3H]NBMPR-binding assays were initiated by adding 10 μ l samples of membrane vesicles (50–100 μ g of protein) to graded concentrations of [3H]NBMPR (0–10 nM) in a total volume of 1 ml at 22 °C in the absence and presence of 10 μ M-NBMPR. When inhibitors of binding activity were evaluated, the test compounds were added simultaneously with [3H]NBMPR. After 30 min incubation the samples were rapidly filtered through Whatman GF/B filters that had been pre-soaked for at least 1 h in 0.3 % (v/v) polyethylenimine and washed twice with 4 ml of ice-cold suspension medium. The filters were counted for 3H radioactivity in 3 ml of Optiphase RIA (LKB

Scintillation Products). Specific binding is defined as the difference between membrane content of [^3H]NBMPR in the presence and absence of $10\text{ }\mu\text{M}$ -NBMPR.

Data analysis

All transport and binding experiments were carried out in triplicate. The errors shown in the Tables and Figures are standard deviations unless noted otherwise. In least-squares fits to the data, points were weighted according to the inverse of their relative experimental errors.

Chemicals

[5,6- ^3H]Uridine (sp. radioactivity 40–50 Ci/mmol) and [^3H]NBMPR (sp. radioactivity 23 Ci/mmol) were purchased from New England Nuclear, Stevenage, Herts., U.K., and Moravsek Biochemicals, Brea, CA, U.S.A., respectively. NBMPR, valinomycin, furosemide, dipyrindamole, phlorrhizin and harmaline were obtained from Sigma Chemical Co. (Poole, Dorset, U.K.). Percoll was purchased from Pharmacia (Milton Keynes, U.K.). Dilazep was generously given by Hoffman-La Roche and Co., Basle, Switzerland. All reagents were of analytical grade.

RESULTS

Brush-border membrane vesicles

A previous study [8] had demonstrated that ice-cold stop solution containing 1 mM -phlorrhizin was necessary to prevent the rapid loss of radiolabelled uridine from rat renal brush-border membrane vesicles during filtration and washing. Preliminary studies with rabbit renal-cortical brush-border vesicles also established that this stop solution was effective at preventing the loss of radiolabel from the membrane vesicles. Thus the phlorrhizin-containing stop solution was employed in this study for use with the brush-border membrane vesicles.

Control experiments confirmed that no significant metabolism of uridine occurs within 30 min of incubating rabbit renal brush-border membrane vesicles with [^3H]uridine; $>90\%$ of the radioactivity co-chromatographed with uridine on the t.l.c. plate. Thus uridine metabolism does not complicate the present study of uridine transport by the brush-border membrane vesicles.

The time course of uridine uptake by rabbit renal brush-border membrane vesicles in the presence of various cations is shown in Fig. 1. Marked stimulation of uridine uptake was observed in the presence of an inwardly directed 100 mM - NaNO_3 electrochemical gradient with a transient overshoot of the intravesicular uridine concentration above its equilibrium value. Accumulation of uridine reached a maximum value at about 60 s and then declined slowly, indicating uridine efflux. In the absence of a Na^+ gradient, i.e. Na^+ at equilibrium across the vesicle, no overshoot of uridine was observed (see Fig. 1). This overshoot phenomenon indicates that rabbit renal brush-border membrane vesicles are capable of catalysing the concentrative uptake of uridine in the presence of an inwardly directed Na^+ electrochemical gradient, suggesting that uridine uptake is coupled to that of Na^+ . Na^+ -stimulated uptake of uridine was a linear function of time for the first 6 s of incubation. The uptake at 2–3 s was therefore taken to approximate the initial rate of uridine influx.

Fig. 1. Time course of uridine uptake by rabbit renal brush-border membrane vesicles

Vesicles were resuspended in media containing either 300 mM -mannitol and 5 mM -Tris/HCl (pH 7.4) or 150 mM - NaNO_3 and 5 mM -Tris/HCl (pH 7.4). Then $10\text{ }\mu\text{l}$ portions of vesicles were incubated with $20\text{ }\mu\text{l}$ of media containing (final concns.) $10\text{ }\mu\text{M}$ -[^3H]uridine, 5 mM -Tris/HCl (pH 7.4) and either 100 mM - NaNO_3 or 100 mM - KNO_3 . Uridine uptake was determined either in the presence of an inwardly directed gradient of Na^+ ($\bullet$) or K^+ ($\blacksquare$) or with Na^+ equilibrated across the vesicle ($\circ$).

Table 1. Effect of various univalent cations on uridine uptake by rabbit renal brush-border membrane vesicles

Samples ($10\text{ }\mu\text{l}$) of the vesicle suspension were incubated with $20\text{ }\mu\text{l}$ of medium containing 100 mM of the chloride salt listed in the table, 5 mM -Tris/HCl (pH 7.4) and $5\text{ }\mu\text{M}$ -[^3H]uridine (final concns.) The uptake of uridine was measured after 30 s of incubation. Values are the means $\pm$ s.d. of triplicate estimates: * these results are not significantly different from each other, as determined by Student's *t* test.

Cation	Uridine uptake (pmol/30 s per mg of protein)
Na^+	39.3 ± 11
K^+	$4.0 \pm 0.9^*$
Li^+	$3.3 \pm 0.3^*$
Cs^+	$2.8 \pm 0.1^*$
Choline $^+$	$3.0 \pm 0.2^*$

In contrast with Na^+ , inwardly directed gradients of KNO_3 , KCl and choline chloride failed to stimulate the uptake of uridine, and no overshoot was observed (Figs. 1 and 2; Table 1; results not shown). The lack of effect by KNO_3 contrasts with previous studies with rat renal

Fig. 2. Effect of various anions on Na^+ -dependent uridine uptake by rabbit renal brush-border membrane vesicles

Vesicles were incubated with $5 \mu\text{M}$ - $[\text{H}]$ uridine in the presence of inwardly directed gradients of 100 mM- NaNO_3 (●), 100 mM- NaCl (○), 50 mM- Na_2SO_4 (■) or 100 mM- KCl (□). Values are the means of triplicate estimates, expressed as a percentage of the 30 min uptake value for KCl (8.1 pmol/mg of protein). For clarity, the errors on the data points have been omitted, but were similar to those observed in Fig. 1.

brush-border membrane vesicles that demonstrated a significant increase in uridine influx in the presence of a 100 mM- KCl gradient (out > in) relative to choline chloride, and moreover a transient overshoot [8]. Other cations, e.g. Li^+ and Cs^+ , were also completely ineffective in stimulating uridine uptake, demonstrating the high Na^+ -specificity for uridine uptake by rabbit brush-border membrane vesicles (Table 1). In further experiments the Na^+ -dependent component of uridine transport was calculated as that in the presence of NaCl minus that in the presence of choline chloride. In other experiments in which the equilibrium value for uridine uptake was measured as a function of extravesicular osmolarity, it was shown that the uptake of uridine occurred into an osmotically reactive intravesicular space (results not shown).

The effect of membrane potential on uridine transport was examined in a number of different but complementary ways. Fig. 2 compares the effects of a 100 mM- NaNO_3 or 50 mM- Na_2SO_4 gradient relative to a 100 mM- NaCl gradient on the uptake of uridine. As the permeability of the membrane to anions increased ($\text{NO}_3^- > \text{Cl}^- > \text{SO}_4^{2-}$) [16], both the initial rate of uridine uptake and the magnitude of the transient overshoot increased. Similar experiments with NaSCN and NaI also demonstrated an increased initial rate of uridine uptake and an enhanced uridine overshoot compared with NaCl (results not shown). These results indicate that modification of the membrane potential with anions of differing permeability alters the rate of Na^+ -dependent

Fig. 3. Effect of valinomycin-induced K^+ -diffusion potentials on Na^+ -dependent uptake of uridine by rabbit renal brush-border membrane vesicles

Vesicles were prepared in 150 mM-mannitol, 50 mM- K_2SO_4 and 1 mM-Hepes/Tris, pH 7.4 (●, ▲), or 50 mM-mannitol, 50 mM- Na_2SO_4 , 50 mM- K_2SO_4 and 1 mM-Hepes/Tris, pH 7.4 (■), and preincubated with valinomycin at $12.5 \mu\text{g}/\text{mg}$ of protein for 1 h. The incubation media contained (final concns.) 150 mM-mannitol, 50 mM- Na_2SO_4 , 1 mM-Hepes/Tris, pH 7.4, and $7 \mu\text{M}$ - $[\text{H}]$ uridine (●, ■), or 50 mM-mannitol, 50 mM- Na_2SO_4 , 50 mM- K_2SO_4 , 1 mM-Hepes/Tris, pH 7.4, and $7 \mu\text{M}$ - $[\text{H}]$ uridine (▲).

uridine uptake, consistent with the idea that transport of uridine is an electrogenic process.

In the second series of experiments, the role of both the Na^+ concentration gradient and the membrane potential in Na^+ -dependent uridine transport was assessed by conducting experiments under various conditions in the presence of valinomycin (Fig. 3). Curve A illustrates the time course of uridine uptake driven both by a Na^+ concentration gradient ($[\text{Na}^+]_o > [\text{Na}^+]_i$) and a K^+ diffusion potential in the presence of valinomycin ($[\text{K}^+]_i > [\text{K}^+]_o$, inside negative). Relative to curve B (Na^+ chemical gradient, but in the absence of membrane potential) the initial rate of uridine uptake and the peak of the overshoot were significantly increased. The finding that the uptake values for uridine were similar at 30 min indicates that the enhancement in the uptake of uridine could not be attributed to changes in the intravesicular space. The results shown by curve B also demonstrate that a Na^+ chemical gradient independently can support the uphill transport of uridine. Similarly, in the presence of only a membrane potential but in the absence of a Na^+ chemical gradient ($[\text{Na}^+]_i = [\text{Na}^+]_o$, curve C), concen-

Fig. 4. Concentration-dependence of Na^+ -dependent uridine uptake by rabbit renal brush-border membrane vesicles

Vesicles were prepared in 300 mM-mannitol, 100 mM-KSCN, 10 mM-Tris/Hepes, pH 7.4, and $12.5 \mu\text{g}$ of valinomycin/mg of protein, so that the membrane potential across the vesicle membrane could be short-circuited. Initial $[^3\text{H}]$ uridine uptake rates (2 s) in the presence of 100 mM-KSCN and either 100 mM-NaCl (●) or 100 mM-choline chloride (○) were determined. The Na^+ -dependent flux was taken as the rate in the presence of NaCl minus the rate in presence of choline chloride for each concentration of uridine. The kinetic constants of saturable Na^+ -dependent uridine uptake were determined by non-linear least squares fit of the Michaelis-Menten equation by using the computer program Hypmic [19], and gave a K_m value of $11.6 \pm 4 \mu\text{M}$, with a $V_{\max}$ of 3.2 ± 0.4 pmol/s per mg of protein.

trative uptake of uridine was also observed, demonstrating that membrane potential independently can provide the energy to accumulate uridine against a concentration gradient provided that Na^+ is present. These experiments further support the view that Na^+ -dependent uridine transport is an electrogenic system, involving the net transfer of positive charge.

The concentration dependence of uridine uptake was studied by varying the extravesicular uridine concentration (0–50 μM) in the presence of 100 mM-NaCl or 100 mM-choline chloride and with the membrane potential difference across the vesicle membrane being short-circuited by using valinomycin and K^+ [17,18]. Fig. 4 shows that uptake of $[^3\text{H}]$ uridine in the presence of choline chloride was linear with increasing uridine concentration. In contrast, uridine transport in the presence of extravesicular NaCl could be resolved into two components: (i) a linear component, and (ii) a saturable component. The kinetic constants of Na^+ -dependent

Fig. 5. Na^+ -dependent uridine flux as a function of the Na^+ concentration

$[^3\text{H}]$ Uridine (5 μM) uptake was measured in the presence of various extravesicular concentrations of NaI (0–100 mM). Choline replaced Na^+ iso-osmotically to obtain the various Na^+ concentrations studied. Uptake was measured after 3 s incubation. Inset: plot of $[\text{Na}^+]/\text{flux}$ versus $[\text{Na}^+]$. A least-squares fit to this plot yields $K_{\text{Na}} = 15 \pm 3$ mM, with $r = 0.995$. Linearity of the plot is indicative of the involvement of one Na^+ ion per uridine molecule transported (also see the text).

Table 2. Effect of nucleosides, transport inhibitors and glucose on Na^+ -dependent uridine influx by rabbit renal brush-border membrane vesicles

The uptake of uridine (3 s) was initiated by addition of brush-border membrane vesicles to medium containing (final concns.) 10 μM - $[^3\text{H}]$ uridine, 100 mM-NaI or 100 mM-choline iodide and test compound. For NBMPR, dipyrindamole and dilazep, vesicles were preincubated with these compounds for 20 min before addition of $[^3\text{H}]$ uridine. Results are the means of three separate experiments and expressed as a percentage of the control flux (uptake in the presence of NaI minus that in the presence of choline iodide).

Inhibitor (μM)	Uridine influx (%)
Thymidine (50)	59 ± 6
Deoxyuridine (50)	22 ± 15
5-Iodo-2'-deoxyuridine (50)	37 ± 6
Deoxycytidine (50)	64 ± 19
Inosine (100)	61 ± 10
Deoxyinosine (100)	81 ± 2
Adenosine (100)	26 ± 7
Guanosine (100)	57 ± 10
Deoxyguanosine (100)	57 ± 14
NBMPR (3)	109 ± 8
Dipyridamole (3)	108 ± 9
Dilazep (3)	121 ± 5
Furosemide (100)	122 ± 19
Harmaline (100)	59 ± 13
Glucose (1 mM)	89 ± 8

Fig. 6. Time course of uridine uptake by rabbit renal basolateral membrane vesicles

Vesicles were incubated with $5 \mu\text{M}$ - ^3H uridine in the presence of inwardly directed gradients of 100 mM -NaCl (Δ), 100 mM -choline chloride ($\blacktriangle$) or 100 mM -choline chloride plus $10 \mu\text{M}$ -NBMPR ($\circ$).

transport were determined after subtraction of the linear component estimated in the presence of choline. The saturable component of Fig. 4 conformed to simple Michaelis-Menten kinetics, and the mean values for the kinetic constants from three separate experiments determined by least-squares analysis by using the computer program Hypmic [19] were $12.0 \pm 3.2 \mu\text{M}$ for the apparent K_m , with a $V_{\max}$ estimate of $3.9 \pm 0.9 \text{ pmol/s per mg of protein}$ (mean $\pm$ S.E.M.).

The Na^+ -dependency of uridine uptake was explored further by measuring the Na^+ activation curve at a fixed uridine concentration. Increasing the concentration of NaI in the extravesicular medium (iso-osmolarity maintained with choline iodide) produced a hyperbolic stimulation in the rate of uridine transport, consistent with a Na^+ :uridine stoichiometry of 1:1 (Fig. 5). The data were also fitted to the Hill equation [20]:

$$\text{Flux} = V_{\max} \cdot [\text{Na}]^h / (K_{\text{Na}}^h + [\text{Na}]^h)$$

where K_{Na} is the $[\text{Na}]$ giving $0.5 V_{\max}$, and h is the Hill coefficient. The Hill coefficient for three separate experiments was 0.98 ± 0.1 , with K_{Na} values of $10.1 \pm 4.7 \text{ mM}$. This result would also suggest one Na^+ -binding site on the carrier.

The effect of a variety of extravesicular compounds on Na^+ -dependent uridine uptake ($10 \mu\text{M}$) was examined (Table 2). A variety of purine and pyrimidine nucleosides at 50 – $100 \mu\text{M}$ inhibited the initial rate of Na^+ -dependent

Fig. 7. Concentration-dependence of ^3H NBMPR binding to rabbit renal basolateral membrane vesicles

Membrane vesicles were incubated with graded concentrations of ^3H NBMPR for 30 min at 22°C in the presence and absence of $10 \mu\text{M}$ -NBMPR, and the difference between membrane content of ^3H NBMPR in the presence and absence of NBMPR was plotted. Inset: Scatchard plot of the data. A least-squares fit to this plot gives equilibrium binding constants of $K_d = 1.4 \pm 0.2 \text{ nM}$ and $B_{\max} = 1.6 \pm 0.2 \text{ pmol/mg of protein}$, with $r = 0.976$.

uridine uptake. D-Glucose (1 mM), the facilitated-diffusion nucleoside transport inhibitors NBMPR, NBTGR, dilazep and dipyridamole (all at $3 \mu\text{M}$) and the diuretic drug furosemide were without effect. In contrast, harmaline at 0.1 mM , an inhibitor of Na^+ binding in some co-transporter systems [21,22], caused about 50% inhibition of Na^+ -dependent uridine uptake.

Basolateral membrane vesicles

The time course of uridine uptake ($5 \mu\text{M}$) by rabbit basolateral membrane vesicles in the presence of inwardly directed Na^+ and choline gradients is shown in Fig. 6. In contrast with brush-border membrane vesicles (Fig. 1), no significant difference in the time course of uptake was observed for the two cations. Moreover, the uptake of uridine was inhibited by $10 \mu\text{M}$ -NBMPR, decreasing the approximate initial rate of influx by over 90%. Control experiments established that, as with rabbit renal brush-border vesicles, > 90% of radioactivity associated with the basolateral vesicles after 5 min incubation co-chromatographed with uridine on t.l.c. plates. A further difference between the brush-border and basolateral membrane vesicles in their transport of uridine is the much lower rates of uptake observed with basolateral vesicles (compare Figs. 1 and 6), leading to a low signal-to-noise ratio and thus a limit on the precision of quantitative studies on the initial rates of nucleoside uptake by basolateral vesicles using the present methodology. Despite this problem, the kinetic constants of approximate initial rates of uridine uptake (5 s incubation) by basolateral membrane vesicles were deter-

Fig. 8. Effect of uridine on specific [^3H]NBMPR binding to rabbit renal basolateral membrane vesicles

The reciprocals of [^3H]NBMPR bound to specific sites in the presence of graded concentrations of uridine (mM: ■, 0; △, 1.0; □, 2.0; ▲, 4.0) at 22 °C are plotted against the respective reciprocals of the free equilibrium concentrations of [^3H]NBMPR. Inset shows a plot of the slope of the double-reciprocal plot versus uridine concentration (mM). Apparent inhibition constant was 0.69 mM.

mined. In the presence of 10 μM -NBMPR uridine uptake was linear with concentration, but in the absence of inhibitor both a saturable and a linear component were observed (results not shown). The NBMPR-sensitive saturable component was determined by the difference in uptake rates in the presence and absence of 10 μM -NBMPR, and least-squares fit of data from three separate experiments gave an apparent K_m of 137 ± 20 μM , with a $V_{\max}$ estimate of 5.2 ± 0.6 pmol/s per mg of protein. To investigate further the properties of the nucleoside transporter at the basolateral membrane surface, and having demonstrated that NBMPR can block uridine transport, [^3H]NBMPR binding to basolateral membrane vesicles was used as a ligand probe for the transporter.

Fig. 7 shows the concentration-dependence of specific [^3H]NBMPR binding to rabbit renal basolateral membrane vesicles, where membrane-associated binding is plotted against the equilibrium free concentration of the inhibitor. Specific [^3H]NBMPR binding was saturable, and a Scatchard plot of the binding data (inset to Fig. 7) revealed a linear relationship, indicating a single population of high-affinity NBMPR-binding sites with an apparent K_d of 1.4 ± 0.2 nM and a $B_{\max}$ (maximal binding) of 1.6 ± 0.2 pmol/mg of protein. Basolateral preparations from three separate rabbits gave a mean apparent K_d of 0.76 ± 0.46 nM, with a $B_{\max}$ of 1.4 ± 0.9 pmol/mg of protein.

A variety of nucleosides were tested for their ability to inhibit the specific binding of [^3H]NBMPR. Dose-response curves for inhibition of site-specific NBMPR binding (1.5 nM) revealed that adenosine was the most effective inhibitor of the nucleosides tested, with IC_{50} values of 0.3, 1.3, 0.9 and 1.0 mM for adenosine, inosine, uridine and thymidine respectively. This result is con-

sistent with the relative affinities of these nucleosides for NBMPR-sensitive transporters [4,5]. Addition of deoxycoformycin (10 μM), an inhibitor of adenosine deaminase [23], further potentiated the effectiveness of adenosine as an inhibitor of [^3H]NBMPR binding (IC_{50} 0.2 mM), suggesting metabolism of adenosine by the basolateral vesicles. In other experiments, the nature of the inhibition by adenosine plus deoxycoformycin and uridine was explored by using various concentrations of both nucleoside and [^3H]NBMPR (Fig. 8). In both cases the inhibition profiles were consistent with a competitive type of inhibition, with apparent K_i values of 690 and 140 μM for uridine and adenosine respectively (adenosine results not shown). Plots of apparent K_d values versus initial uridine concentrations were linear (inset to Fig. 8), providing further evidence of simple competitive inhibition of binding activity.

DISCUSSION

Previous studies have shown that inwardly directed gradients of Na^+ or K^+ can cause a transient accumulation of uridine in rat renal brush-border membrane vesicles, suggesting active transport by one of two different nucleoside transport systems, one requiring Na^+ [7,8] and the other K^+ as transport coupler [8]. The present results extend these observations for renal outer-cortical brush-border membrane vesicles prepared from rabbit. Moreover, the present data also demonstrate for the first time that uridine transport at the basolateral surface of rabbit kidney proximal cells occurs by a NBMPR-sensitive facilitated-diffusion system.

In our rabbit renal outer-cortical brush-border membrane vesicle preparation uridine is not metabolized and is taken up into an osmotically active space. Uridine uptake was markedly enhanced by Na^+ , but showed no stimulation with other univalent cations tested (Fig. 1 and Table 1). A transient overshoot phenomenon was observed in the presence of a Na^+ gradient (out > in), indicating active Na^+ -dependent transport of uridine. Elimination of the Na^+ gradient abolished the overshoot (Fig. 1). Similar results have been reported for rat renal brush-border membrane vesicles, with the major exception that K^+ gradients (out > in) could also drive the movement of uridine into the vesicle against its concentration gradient, leading to the suggestion of a K^+ -dependent uridine transporter distinct from the Na^+ -dependent system [8]. No evidence for a K^+ -dependent uridine carrier in rabbit renal brush-border membrane vesicles was obtained in the present study. Preliminary results have also indicated that bovine brush-border membrane vesicles lack a K^+ -dependent system [24]. It is possible that a K^+ -dependent uridine transporter is present in rabbit renal brush-border membrane vesicles, but that the activity is too low to be detected.

Kinetic analysis of the Na^+ -dependent component of uridine zero-trans influx indicated a saturable component obeying Michaelis-Menten kinetics, with K_m 12 ± 3 μM and $V_{\max}$ 3.9 ± 0.9 pmol/s per mg of protein as measured at 100 mM-NaCl, 22 °C and with the membrane potential difference across the vesicle membrane at 0. This K_m value is similar to that estimated for Na^+ -dependent uridine influx by rat renal and rabbit intestinal brush-border membrane vesicles [24,25]. However, the $V_{\max}$ value is nearly 20-fold less than that of rat renal brush-border membrane vesicles [24]. A variety of purine and

pyrimidine nucleosides (Table 2) were able to inhibit Na^+ -dependent uridine transport, suggesting that these nucleosides may be substrates for the carrier. However, it is noteworthy that some nucleosides were notably less potent inhibitors of uridine influx than were others. A recent report [26] has suggested that in mouse intestinal enterocytes two Na^+ -dependent systems with differing substrate specificities are responsible for the transport of uridine. Further studies are required to test this possibility with renal brush-border membrane vesicles. The facilitated-diffusion nucleoside transport inhibitors NBMPR, dilazep and dipyridamole had no effect on Na^+ -dependent uridine uptake by rabbit renal brush-border membrane vesicles.

If Na^+ -dependent uridine transport is electrogenic, there will be a net transfer of positive charge across the membrane, and thus a change in membrane potential should affect the transport process. The results of the experiments illustrated in Figs. 2 and 3 confirm this postulate. For example, an inside-negative membrane potential stimulated uridine influx even in the absence of a Na^+ gradient. Studies with the potential-sensitive fluorescent dye 3,3'-dipropylthiadicarbocyanine iodide also indicate that uridine transport by rat renal brush-border membrane vesicles is electrogenic (C.-W. Lee, C. I. Cheeseman & S. M. Jarvis, unpublished work). The Na^+ :uridine coupling stoichiometry, determined by the activation method, yielded a minimum stoichiometry of 1 Na^+ :1 uridine, similar to that proposed for rat renal and rabbit intestinal brush-border membrane vesicles [8,24,25].

The transport characteristics of uridine influx by rabbit renal basolateral membrane vesicles differed significantly from those described above for brush-border membrane vesicles. First, uridine transport is not stimulated by Na^+ (Fig. 6). Second, in contrast with brush-border membrane vesicles, uridine influx in basolateral membrane vesicles was inhibited by NBMPR. This inhibition by NBMPR was associated with high-affinity binding of [^3H]NBMPR to the membrane vesicles. The apparent dissociation constant for [^3H]NBMPR binding, $0.74 \pm 0.46 \text{ nM}$, is similar to values reported for other mammalian tissues [4,5]. Adenosine and uridine blocked [^3H]NBMPR binding in a competitive manner. Adenosine was a more potent inhibitor than uridine (apparent K_i values 140 and $690 \mu\text{M}$), consistent with the relative affinities of these two nucleosides for NBMPR-sensitive facilitated-diffusion transporters [4,5]. Third, estimates of the affinity of the NBMPR-sensitive component of uridine influx by basolateral membrane vesicles (apparent K_m $137 \pm 20 \mu\text{M}$) were 10-fold less than for the Na^+ -dependent system at the brush-border surface. The discrepancy between the apparent K_m value for uridine influx and the apparent K_i value for uridine inhibition of specific [^3H]NBMPR binding has been noted previously [4]. However, in human erythrocytes the apparent K_m value for uridine equilibrium exchange ($760 \mu\text{M}$) is higher than the apparent K_m for zero-trans influx ($170 \mu\text{M}$), but similar to the apparent K_i value for inhibition of NBMPR binding by uridine [27,28]. We have no information at present on the kinetic constants of uridine equilibrium exchange by renal basolateral membrane vesicles. Interestingly, the V_{max} for both systems was similar, although obviously the Na^+ -dependent system is saturated at much lower uridine concentrations. Taken together, these results strongly suggest that uridine crosses the basolateral

membrane surface of rabbit renal proximal cells by a NBMPR-sensitive facilitated-diffusion system, with properties similar to those seen in many other animal cells.

In conclusion, the present results have established the presence of high-affinity Na^+ -coupled uridine transport at the brush-border membrane surface of rabbit renal proximal-tubule cells. In contrast, uridine transport at the basolateral surface is accomplished by a NBMPR-sensitive facilitated-diffusion system. The presence of these two different transport systems on opposite sides of the renal tubule cell would be ideally suited to vectorial transport of uridine from the lumen of the kidney to the blood. Whether such vectorial transfer occurs *in vivo* is unknown, but may be unlikely, owing to the rapid metabolism of nucleosides by most mammalian cells.

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