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Nucleoside transport in brush border membrane vesicles from human kidney

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The goal of this study was to elucidate the mechanisms of nucleoside transport in the brush border membrane of the human kidney. [^{3}H]Uridine was transported into brush border membrane vesicles (BBMV) from human kidney via Na^+-independent and Na^+-dependent processes. The Na^+-dependent transport was saturable ($K_m = 4.76 \pm 0.39$ μM; $V_{max} = 6.42 \pm 0.17$ pmol/mg proteins per s) and was *trans*-stimulated by unlabeled uridine. Structural analogs of uridine (100 μM), 2'-deoxyuridine (2-dU) and dideoxyuridine (ddU), significantly inhibited Na^+-uridine uptake into BBMV. Previous studies have suggested that Na^+-nucleoside co-transport occurs via two major systems (Vijayalakshmi et al. (1988) J. Biol Chem. 263, 19419–19423). One system (cit) is generally pyrimidine-selective; thymidine serves as a model substrate. The other system (cif) is generally purine-selective; formycin B serves as a model substrate. Uridine and adenosine are substrates of both systems. Thymidine and cytidine (100 μM), but not formycin B (100 μM) inhibited Na^+-uridine uptake. In addition, [^{3}H]thymidine exhibited an Na^+-driven overshoot phenomenon whereas [^{3}H]formycin B did not. Na^+-thymidine uptake was inhibited by (100 μM) adenosine, uridine, guanosine, but not by formycin B and inosine. Further studies demonstrated that guanosine *trans*-stimulated thymidine uptake suggesting that guanosine and thymidine share a common transporter in the human renal BBMV. A different pattern was identified in BBMV from the rabbit kidney where both [^{3}H]thymidine and [^{3}H]formycin B as well as [^{3}H]uridine exhibited a transient Na^+-driven overshoot phenomenon. Collectively, these data sugest that in rabbit renal BBMV both cif and cit systems are present whereas in human renal BBMV, there appears to be a single concentrative Na^+-nucleoside cotransport system that interacts with uridine, cytidine, thymidine, adenosine and guanosine but not with formycin B and inosine. The system is similar to the previously described cit system except that guanosine is also a substrate.

Introduction

Nucleoside transport systems are present ubiquitously in mammalian plasma membranes, facilitating the transmembrane movement of nucleosides both into and out of cells. Because nucleosides are synthesized de novo in most animal cells, transport of nucleosides across the plasma membrane generally represents a salvage pathway. However, some cells require intracellular transport of nucleosides to support anabolic processes (e.g., intestinal mucosa, bone marrow, and some central nervous system cells). Conversely, in all cells nucleosides need to be released to prevent excess accumulation of nucleotide breakdown products. Thus, virtually all cells require transmembrane movement of nucleosides.

Nucleosides are transported via multiple mechanisms. Equilibrative (facilitated diffusion) and Na^+-dependent concentrative (energy-dependent) systems have been characterized in a number of cells and cell membranes including renal [1–8] and intestinal brush border membrane vesicles [9], intestinal epithelial cells [10–12], spleen cells [13,14], and leukemia cells [15–18]. Two facilitated diffusion transporters for nucleosides have been described. Both are characterized by broad substrate specificity, low affinity, and high capacity but are distinguished by their sensitivity to nitrobenzylmercaptopurineriboside (NBMPR). The NBMPR-insensitive equilibrative transporter (termed 'ei' [12]) is not inhibited by concentrations as high as 10 μM, whereas the NBMPR-sensitive system (termed 'es' [12]) is inhibited by concentrations as low as 0.1 nM. Concentrative

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systems are unaffected by high concentrations of NBMPR, but are dependent on extracellular Na^+.

Recently, in mouse intestinal epithelial cells, Vijayalakshmi and Belt described two Na^+-dependent transport systems characterized by different substrate specificities [12]. Formycin B, guanosine, and inosine were substrates for one system whereas thymidine and cytidine were substrates for the other transport system. Because of this substrate specificity, these investigators proposed a 'cif' (concentrative, insensitive to NBMPR, and utilizes formycin B as a substrate) and 'cit' (concentrative, insensitive to NBMPR, and utilizes thymidine as a substrate) nomenclature. More simply, one system is pyrimidine-selective and the other is purine-selective although certain nucleosides such as adenosine, 2′-deoxyadenosine and uridine are substrates for both systems.

There is considerable evidence that concentrative Na^+-dependent nucleoside transport systems are present in mammalian kidney. Both pyrimidine- and purine-selective Na^+-dependent nucleoside transport systems have been identified in brush border membrane vesicles (BBMV) prepared from the renal cortex of rat [1] and cow [3]. These nucleoside transport systems exhibit characteristics similar to the cit and cif systems proposed by Vijayalakshmi and Belt [12]. In the rabbit renal brush border membrane, Na^+-dependent uridine transport appears to have a lower capacity than that observed in the rat [7]. Uridine is transported in basolateral membrane vesicles from rabbit kidney via an NBMPR-sensitive facilitated-diffusion system [7]. The different characteristics of nucleoside transport in the two membranes is conducive to the transport of nucleosides in the reabsorptive direction from the tubule into the blood and suggests that the systems function in concert in nucleoside salvage for the body.

To date, little is known about nucleoside transport in the human kidney. Kuttesch and Nelson studied the renal clearance of 2′-deoxyadenosine and adenosine in a child with adenosine deaminase deficiency and in adults receiving deoxycoformycin (an adenosine deaminase inhibitor) [19]. The study demonstrated that adenosine is actively reabsorbed and suggested that the kidney may play a role in nucleoside salvage for the body.

The overall goal of this study was to determine if an Na^+-dependent nucleoside transport system is present in the human renal brush border membrane and to elucidate its characteristics. We also characterized the substrate specificities of the nucleoside transport system in the BBMV from rabbit kidney and compared these data with those obtained in human renal BBMV. Our results suggest that a single Na^+-dependent nucleoside transport system is present in the human renal brush border membrane. The data represent the first demonstration of an Na^+-nucleoside transport system in human epithelium. The substrate specificity of the system differs from previously described cif and cit systems.

Materials and Methods

Human kidney tissue. Human kidneys deemed unsuitable for transplant by the Organ/Tissue Transplant Services at the University of California, San Francisco were donated to our laboratory for research purposes (Table I). All kidneys had been perfused according to transplant protocol and were placed on ice during transport to our laboratory. The capsid and surrounding tissue were removed upon arrival. The cortex was isolated and weighed. Unless brush border membrane vesicles were prepared immediately, the cortex was divided into 15–30-g portions, frozen in liquid nitrogen, and stored at −70°C. Figure legends and Results describe if studies were conducted in fresh or frozen tissue.

Rabbit kidney tissue. New Zealand White male rabbits weighing 2–3 kg were given 40–50 mg/kg of ketamine subcutaneously. Approximately 15–20 min later, the animals were decapitated. After the kidneys were dissected, each was perfused with 20–30 ml of ice-cold buffer (pH 7.4) consisting of 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes), 150 mM KCl, and 5 mM ethyleneglycol bis-(β-aminoethyl ether)-N,N′-tetraacetic acid (EGTA) (HK-

TABLE I

Characteristics of kidney donors

Donor No.	Age (y)	Sex	Associated medical problems	Drugs received prior to procurement of kidney
1	44	M	hypertension	dyazide, enalapril, dopamine
2	49	F	hypertension, intracerebral bleed	clonidine, dyazide
3	9	F	hypertension, intracerebral bleed	phenobarbital. DDAVP, dopamine, ranitidine, cefotaxime diazepam, furosemide
4	46	M	cardiac arrest	dopamine
5	34	M	intracerebral bleed, IHSS	dopamine, gentamicin, kefzol
6	51	M	coronary artery disease, peripheral vascular disease, chronic obstructive lung disease	diltiazem, dipyridamole, isosorbide dinitrate pronestyl, pepcid
7	54	F	atherosclerosis, cardiac arrest,	dopamine, lidocaine, DDAVP, sodium bicarbonate
8	13	F	developmental delay, seizures	[a]

[a] Not available.

EGTA buffer). The cortex was dissected free from the medulla and stored in ice-cold HK-EGTA buffer. Subsequent steps for vesicle preparation were the same for human and rabbit kidneys.

Preparation of brush border membrane vesicles. BBMV were prepared by divalent (Mg^{2+}) cation precipitation and differential centrifugation [20], as modified in our laboratory [21–24]. For studies involving frozen human kidney, the required amount of tissue was thawed at room temperature.

Protein concentration was measured by the method of Bradford using the Bio-Rad protein assay kit; bovine serum albumin was the standard. The purity of the BBMV preparations was monitored by noting the enrichment of enzyme markers of specific cellular membranes. The activity of maltase and γ-glutamyltransferase (markers of the brush border membrane), acid phosphatase (marker of lysosomal membranes), glucose-6-phosphatase (marker of endoplasmic reticulum membranes) and the Na^+/K^+-ATPase (marker of basolateral membranes) was measured in the final pellet and compared to the activity determined in the initial homogenate. The activities of the enzymes associated with the BBMV (all determined in preparations from fresh kidneys) were enriched as compared to the activities in the corresponding homogenates [24].

Transport studies. The uptake of [^{3}H]uridine (27.1 Ci/mmol), [^{3}H]thymidine (2 Ci/mol), and [^{3}H]formycin B (7 Ci/mmol) at 22°C was measured by an inhibitor-stop filtration technique as described previously [2]. A 10 μl aliquot of BBMV suspension (10–20 mg/ml protein) was added to 40 μl of medium containing [^{3}H]uridine, [^{3}H]thymidine, or [^{3}H]formycin B in buffer (10 mM Hepes, 150 mM KCl or NaCl (pH 7.4) with KOH or NaOH) and incubated for various times as described in the figure legends. For Michaelis-Menten kinetic studies, uptake of [^{3}H]uridine was measured at 5 s. For studies on the inhibition of [^{3}H]uridine or [^{3}H]thymidine uptake, test compounds were added to the reaction mixture and uptake was measured at 5 s. For counterflux studies, Na^+ (150 mM) was equilibrated across the vesicular membrane and the vesicles were preincubated with unlabeled uridine (50 μM) for 2 h. The uptake of [^{3}H]uridine into the loaded BBMV was compared to the uptake into unloaded (control) BBVM at various times. For all studies, the uptake of the tritiated nucleoside was initiated using a vortex mixer with no substrate-vesicular contact prior to uptake measurement (zero-*trans* conditions). At the end of each incubation, the uptake was stopped by adding 3 ml of ice-cold stop solution (150 mM KCl or NaCl, 10 mM Hepes, and 1 mM phloridzin (pH 7.4) and filtering the suspension under vacuum through a membrane filter (0.3 μm, PH type, Millipore Corp). The filter was then washed three times with 3 ml of ice-cold stop solution, and placed into 5 ml of scintillant (Cytoscint-ES, ICN). Radioactivity associated with the filter was measured by liquid scintillation counting (LS 1801, Beckman Instruments). In each set of experiments, the radioactivity measured after filtering the transport medium without any membrane vesicles is subtracted from radioactivity associated with the uptake of [^{3}H]uridine into BBMV to obtain the reported uptake values.

Data analysis. Kinetic parameters for the transport of uridine were obtained by fitting data to Michaelis-Menten model, including a linear, nonsaturable transport component:

$$\text{rate} = [(V_{max} * C)/(K_m + C)] + (K_{ns} * C)$$

where V_{max} is the maximal rate of transport, K_m represents the concentration where rate of transport is half of V_{max}, K_{ns} is the coefficient for the linear, nonsaturable component, and C is the concentration of uridine in the extravesicular solution. The FIT FUNCTION on the PROPHET computer system was used for fitting the data (weighted). This program is an iterative nonlinear least-squares regression program that allows weighting of data based upon variance (Prophet Statistics, National Institute of Health). Data points were determined in triplicate. Unless otherwise specified, experiments were repeated using three different kidney preparations. Data are expressed as mean ± S.D. of a representative experiment or as the mean ± S.E. of three replicate experiments. Data were analyzed by analysis of variance and the Student-Newman-Keuls test.

Chemicals. [*methyl*-^{3}H]Thymidine (2 Ci/mmol) and [^{3}H]formycin B (7 Ci/mmol) were purchased from Moravek Biochemical, INC, Brea, CA, and [5-^{3}H] uridine (27.1 Ci/mmol) was purchased from NEN Research Products, Wilmington, DE. All other chemicals were purchased from Sigma, St. Louis, MO or Aldrich, Milwaukee, WI and were the highest grade available.

Results

Time course of uridine uptake

Uridine transiently accumulated in human renal BBMV against its concentration gradient in the presence of a 150 mM inwardly-directed Na^+ gradient (Fig. 1). An apparent maximum overshoot was obtained at 60 s and tapered to an equilibrium value. In the absence of a Na^+ gradient, uridine accumulated to a similar equilibrium value and no overshoot was observed (Fig. 1). There was no significant difference between the equilibrium values of uridine uptake in the presence and absence of Na^+ suggesting that there was little or no difference in the integrity and size of the vesicles under both conditions. NBMPR (10 μM) did not significantly affect the uptake of uridine in the

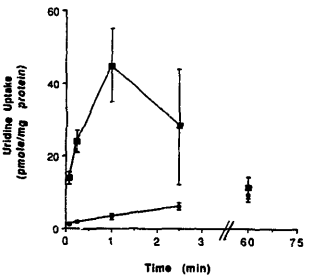


Fig. 1. [^{3}H]Uridine uptake into human renal BBMV as a function of time. The data represent the uptake of uridine in the presence (squares) or absence (circles) of an inwardly-directed Na^+ gradient. The total concentration of uridine was 5 μM. Data are from three experiments (mean ± S.E.) in fresh human kidney BBMV preparations.

absence of Na^+ (data not shown), suggesting that an NBMFR-sensitive (es) equilibrative transport system is not present in renal brush border membrane of the human.

Uridine uptake was measured at equilibrium in human BBMV in a media containing an impermeant, sucrose, at various concentrations and uptake was plotted as the reciprocal of the extravesicular osmolarity. This experiment suggests that uridine accumulated into an osmotically reactive intravesicular space with minimal binding (15%) to the vesicular membrane (results not shown).

Uridine counterflux studies

To determine whether the transport of uridine in human BBMV involves a carrier-mediated process, counterflux (*trans*-stimulation) experiments were performed [25]. In these studies, the Na^+ concentration was the same across the vesicular membrane (Fig. 2). The uptake of [^{3}H]uridine at each time point was significantly greater in the vesicles which had been pre-loaded with uridine when compared to the unloaded vesicles. The uptake of [^{3}H]uridine into the pre-loaded vesicles reached an apparent maximum at 5 min.

Kinetic studies

To determine the kinetics of Na^+-uridine transport in human renal BBMV it is important to measure uptake at a time when the initial rate of transport can be accurately assessed. Since the Na^+-dependent uptake of uridine was linear for the first 15 s (data not shown), 5 s was chosen to determine the initial rate of

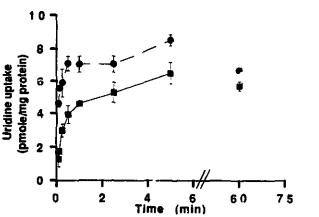


Fig. 2. The uptake of [^{3}H]uridine (5 μM) in human renal BBMV in which Na^+ had been equilibrated across the vesicular membrane ($[Na^+]_i = [Na^+]_o$). Squares represent data obtained in unloaded vesicles and circles represent data obtained in vesicles initially incubated with 50 μM of uridine for 2 h. Data are from one representative experiment (mean ± S.D.) obtained from frozen human kidneys.

transport. In the presence of Na^+, the transport process could be resolved into a linear and a saturable component (Fig. 3). The linear component was negligible and represented a small fraction of Na^+-uridine uptake (Table II). For three separate experiments, the K_m (mean ± S.E.) was 4.76 ± 0.39 μM and the V_{max} was 6.42 ± 0.17 pmol/mg protein per s (Table II). There was no statistically significant difference between the values obtained in freshly prepared renal BBMV and the values obtained in renal BBMV that had been frozen.

Inhibition studies

The effect of several structural analogs (Fig. 4) of uridine on the Na^+-dependent transport of uridine was assessed in BBMV from human kidney. The uptake of uridine after 5 s was determined in the presence and

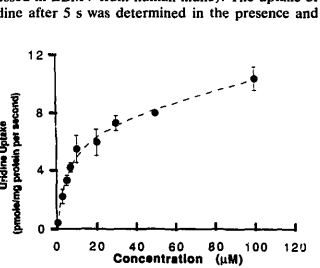
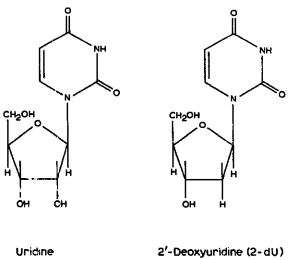


Fig. 3. Initial rate of Na^+-uridine transport in human renal BBMV (5 s) as a function of concentration. The curve represents the computer generated fit to the equation: rate = $[(V_{max} * C)/(K_m + C)] + (K_{ns} * C)$. Data are from one representative experiment (mean ± S.D.) of triplicate determinations from fresh human renal BBMV preparations (Experiment No. 2 from Table II).



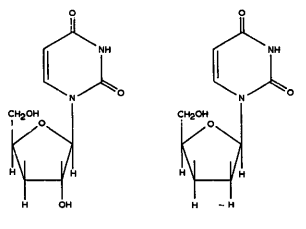


Fig. 4. Structures of compounds tested as inhibitors of Na^+-uridine uptake.

TABLE II

Computer generated parameters of uridine transport

Data are mean ± S.D. of computer fit as described in the text. Experiments 1 and 2 were done on fresh human renal BBMV preparations whereas Experiment 3 was performed on renal BBMV that had been frozen.

Experiment	K_m (μM)	V_{max} (pmol/mg protein per s)	K_{ns} (l/mg protein per s)
1	5.30 ± 0.92	6.48 ± 0.80	0.06 ± 0.01
	$P < 0.001$	$P < 0.0005$	$P < 0.003$
2	4.99 ± 0.84	6.68 ± 0.61	0.04 ± 0.01
	$P < 0.001$	$P < 0.0001$	$P < 0.005$
3	4.00 ± 0.21	6.10 ± 0.23	0.04 ± 0.01
	$P < 0.0001$	$P < 0.0001$	$P < 0.001$

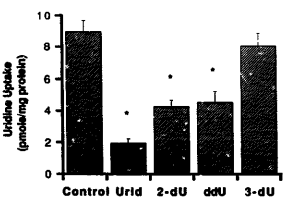


Fig. 5. Na^+-uridine uptake at 5 s in human renal BBMV in the absence (control) or presence of the structural analogs (100 μM) shown in Fig. 4. Data (mean ± S.E.) are from three separate experiments (human renal BBMV were prepared from frozen human kidneys). Uridine (Urid), 2'-deoxyuridine (2-dU) and dideoxyuridine (ddU) produced significant inhibition of uridine uptake in human renal BBMV versus control ($P < 0.0001$).

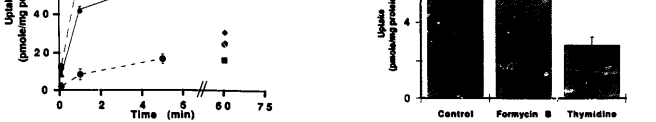


Fig. 6. (A) The time course of the uptakes of [^{3}H]thymidine (squares), [^{3}H]uridine (triangles) and [^{3}H]formycin B (circles) in human renal BBMV in the presence of an inwardly-directed 150 mM Na^+ gradient. Data are from one representative experiment (mean ± S.D.). (B) The uptake of uridine in human renal BBMV at 5 s in the presence of an inwardly-directed Na^+ gradient into human renal BBMV. Control bar repesents uptake of uridine in the absence of unlabeled nucleosides. Concentration of unlabeled nucleosides was 100 μM. Data represent mean ± S.E. from three separate experiments (from three different frozen human kidneys). Thymidine produced significant inhibition versus control ($P < 0.01$).

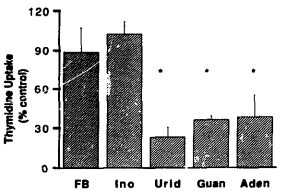


Fig. 7. The uptake of thymidine (with an Na^+ gradient) in human renal BBMV at 5 s in the presence of several nucleosides (100 μM). Data are from three separate experiments (from three different frozen human kidneys). Values are given in % with respect to the Na^+-dependent thymidine transport control. Urid (uridine), Guan (guanosine), and Aden (adenosine) produced significant inhibition ($P < 0.01$) whereas FB (formycin B) and Ino (inosine) did not.

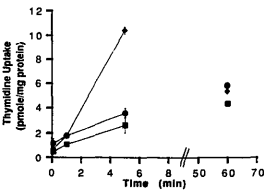


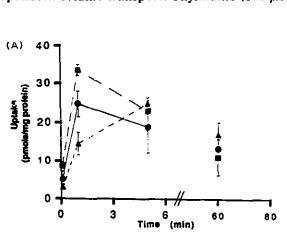
Fig. 8. The uptake of [^{3}H]thymidine (5 μM) in human renal BBMV in which Na^+ had been equilibrated across the vesicular membrane ($[Na^+]_i = [Na^+]_o$). Circles represent data obtained in unloaded vesicles; squares represent data obtained in vesicles initially incubated with 50 μM of inosine for 2 h; and diamonds represent data obtained in vesicles initially incubated with 50 μM of guanosine for 2 h. Data are from one representative experiment (mean ± S.D.) obtained from frozen human kidneys.

absence of the structural analogs (100 μM). Uridine, 2′-deoxyuridine (2-dU) as well as dideoxyuridine (ddU) were all potent inhibitors of Na^+-dependent uridine uptake whereas 3′-deoxyuridine (3-dU) had no inhibitory effect (Fig. 5).

Substrate specificity studies

To determine the type(s) of Na^+-dependent nucleoside transport systems present in the human renal brush border membrane, we compared the uptake of [^{3}H]uridine, [^{3}H]thymidine, and [^{3}H]formycin B in the presence of Na^+ (Fig. 6A). Both uridine and thymidine transiently accumulated in the vesicles against their concentration gradients in the presence of an initial inwardly-directed Na^+ gradient whereas no overshoot phenomenon was observed for formycin B.

The specificity of the transporter in the human brush border membrane was further investigated by studying the effects of thymidine and formycin B on Na^+-dependent uridine transport. Thymidine (100 μM) significantly inhibited uridine uptake to less than 50% of control whereas formycin B (100 μM) had no significant inhibitory effect (Fig. 6B). Cytidine and guanosine (100 μM), but not inosine, also significantly inhibited uridine uptake to approx. 50% of control (data not shown). Because no overshoot was observed during the uptake of formycin B and because formycin B did not inhibit the uptake of uridine, further experiments were conducted to define the substrate specificity of Na^+-dependent nucleoside transport system. The effect of formycin B, inosine, uridine, guanosine, and adenosine on [^{3}H]thymidine uptake (Fig. 7) were measured. Although guanosine significantly inhibited the uptake of [^{3}H]uridine and [^{3}H]thymidine, inosine had no effect. In addition, uridine and adenosine significantly decreased the uptake of [^{3}H]-thymidine, but formycin B had no effect. To determine whether guanosine and thymidine may share the same transporter a counter-flux experiment was performed in human renal BBMV



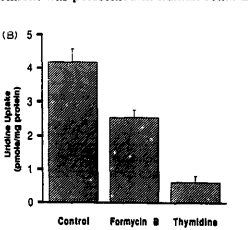


Fig. 9. (A) The time course of the uptakes of [^{3}H]thymidine (squares), [^{3}H]uridine (circles) and [^{3}H]formycin B (triangle) in rabbit renal BBMV in the presence of a 150 mM inwardly-directed Na^+ gradient. (B) The 5-s uptake of Na^+-uridine in the absence (control) and presence of thymidine (100 μM) or formycin B (100 μM). These experiments were performed only once (mean ± S.D.) on freshly prepared rabbit kidney BBMV.

either unloaded (control) or loaded with guanosine or inosine. Guanosine but not inosine was able to *trans*-stimulate the uptake of thymidine Fig. 8) which suggests that guanosine and thymidine but not inosine share the same transporter.

In contrast, in rabbit renal BBMV, uridine, thymidine and formycin B accumulated in the vesicles against a concentration gradient in the presence of an initial inwardly-directed Na^+ gradient (Fig. 9A). Both thymidine and uridine reached an apparent maximum overshoot at about 60 s whereas formycin B reached its apparent maximum overshoot at 5 min. In addition, both formycin B and thymidine inhibited the Na^+-dependent uptake of uridine into rabbit renal BBMV. Thymidine inhibited Na^+-uridine uptake to about 10% of control whereas formycin B inhibited Na^+-uridine uptake to about 60% of control (Fig. 9B).

Discussion

Although the liver appears to be primarily responsible for nucleoside homeostasis in the body [26–28], the recent identification of secondarily-active nucleoside transport systems in mammalian kidney [2,3,6,7] suggests that the kidney also plays a role in nucleoside homeostasis. Although de novo synthesis of pyrimidines seems to occur in almost all mammalian cells, purine biosynthetic pathways are absent in several cell types including leukocytes [29], erythrocytes [30,31], intestinal epithelial cells [32], and platelets [33]. The plasma is the major source of nucleosides for these cells which are dependent upon nucleoside salvage pathways [34,35]. Similarly, there is growing evidence that in cells in which pyrimidines can be synthesized via de novo pathways, salvage pathways are generally preferred [36].

Imbalances in nucleoside supply are often associated with serious consequences [37–39]. It is likely that the precise control of plasma and intracellular nucleoside levels requires both 'local' control mechanisms (e.g. in individual cells and tissues) as well as more 'central' control mechanisms (e.g., at sites of absorption and excretion) [40]. The overall goal of this study was to elucidate the mechanisms by which nucleosides are transported across the brush border membrane of the human kidney. A major finding of the study was that an Na^+-dependent nucleoside transport system is present in BBMV from human kidney, similar to previous findings in BBMV from the rabbit [7,9], rat [2,6] and bovine kidney [3]. Data demonstrating that unlabeled uridine can *trans*-stimulate [^{3}H]-uridine uptake (Fig. 2) suggest that Na^+-uridine transport in human renal BBMV occurs via a carrier-mediated process. This counterflux phenomenon has been demonstrated for uridine transport in BBMV from rat kidney [4], and in whole cells [41].

We directly examined the kinetics of the Na^+-dependent component of uridine zero-*trans* influx in human renal BBMV and found a saturable component which could be described by Michaelis-Menten kinetics (Fig. 3). The K_m (4.76 μM) is lower than the K_m obtained in BBMV from other mammalian species (rat = 9.7 μM [1], rabbit = 12.0 μM [7], cow = 11.8 μM [42]) suggesting that uridine has a somewhat higher affinity for the Na^+-driven nucleoside transport system in human BBMV. The K_m is consistent with uridine concentrations in human plasma (3.1 μM) [43] suggesting that the system would be efficient in the reabsorption of uridine. The V_{max} (6.42 pmol/mg protein per s) in human renal BBMV is comparable to that obtained in bovine BBMV [3,42], about twice that observed in BBMV from rabbit kidney [7,42], and approximately one-fourth that observed in BBMV from rat kidney [1].

Recently, two Na^+-dependent nucleoside transport mechanisms with differing substrate specificities have been described in mouse intestinal epithelial cells [12]. In general, the cif system is purine-selective and formycin B serves as the model substrate. The cit system is pyrimidine-selective and thymidine is the model substrate. However, the systems exhibit some substrate overlap. For example, uridine and adenosine are transported equally well by both systems. In our studies in human renal BBMV, the uptake of Na^+-uridine was inhibited by the pyrimidine nucleosides, cytidine and thymidine, two nucleosides that have been shown to interact selectively with the cit system [12]. Furthermore, the uptake of thymidine, but not formycin B, exhibited a transient overshoot in the presence of an inwardly-directed Na^+ gradient suggesting that a pyrimidine-selective, but not a purine-selective, system is present (Fig. 6A) in the human renal brush border membrane.

We directly examined the effects of inosine, formycin B and guanosine on the uptake of the thymidine. The data demonstrated that guanosine (100 μM) inhibits Na^+-thymidine uptake whereas inosine and formycin B do not (Fig. 7). The finding that inosine and formycin B do not inhibit either Na^+-thymidine or Na^+-uridine uptake suggests that uridine and thymidine share a common transport system that excludes formycin B and inosine. The system is similar to the cit system described previously [12], except that guanosine is also a substrate as demonstrated by its ability to both *cis* inhibit (Fig. 7) and *trans*-stimulate (Fig. 8) Na^+-thymidine transport. Studies in other tissues have suggested that guanosine and thymidine do not share a common Na^+-nucleoside transport system [12]. Teleologically, salvage of these nucleosides by the kidney seems appropriate since these compounds are the fundamental components of nucleic acids.

In contrast to our data in human renal BBMV, our data in rabbit renal BBMV suggest that both cit and cif

nucleoside transport systems are present. At concentrations of 100 μM, thymidine and formycin B would be expected to be potent inhibitors of the cit and cif systems, respectively. At this concentration, thymidine inhibited the uptake of uridine to about 10% of control whereas formycin B inhibited uridine uptake to 60% of control suggesting a preponderance of the cit in comparison to the cif system in the rabbit renal brush border membrane. These data are consistent with previous data in rat [1] and bovine BBMV which suggest that separate cit and cif nucleoside transport systems are present.

Many nucleoside analogs used clinically (e.g., dideoxyinosine (ddI) and dideoxycytidine (ddC)) as antiviral agents are modified at the ribose group. Similar to previous studies in BBMV of several species [1,7,42] and in murine splenocytes [13] we observed that 2-dU significantly inhibited Na^+-uridine uptake in human renal BBMV whereas 3-dU had no inhibitory effects. The finding that ddU significantly inhibited Na^+-uridine uptake was unexpected since previous studies have suggested that dideoxynucleosides do not interact with concentrative nucleoside transport systems. These data together with data demonstrating that guanosine inhibits both Na^+-uridine and Na^+-thymidine transport demonstrate important differences in nucleoside transport systems in tissues from other mammalian species.

Whether a system analogous to that identified in this study in the brush border membrane of the human kidney exists in other human epithelia is not known. In fact, evidence in the rabbit does not support the notion that all epithelia in a given species have a single type of concentrative nucleoside transporter. For example, data in this study suggest that both a Na^+-dependent purine-selective and a Na^+-dependent pyrimidine-selective system are present in renal epithelium of the rabbit. In contrast, we have recently demonstrated in choroid plexus epithelium of the rabbit that a unique Na^+-dependent nucleoside transport system which is neither purine- nor pyrimidine-selective is present (Wu, X., Hui, A.C. and Giacomini, K.M., unpublished results). Thus, in the same species, epithelia which are closely-related functionally and structurally have notably different concentrative nucleoside transport systems.

In conclusion, this is the first demonstration of Na^+-dependent nucleoside transport in human epithelium. There are important differences in the substrate specificity of nucleoside transport across the brush border membrane of human kidneys in comparison to nucleoside transport across the renal brush border membrane of other mammalian species. Specifically, there appears to be a single Na^+-nucleoside transport system which is similar to the previously described cit-system in terms of its substrate selectivity except that it also transports guanosine. Further studies are being conducted to ascertain the pathways of nucleoside transport across the basolateral membrane of the human kidney and to determine how they work in concert with the pathway identified in the brush border membrane.

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