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Characterization of sodium-dependent nucleoside transport in rabbit intestinal brush-border membrane vesicles

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The characteristics of uridine transport were studied in rabbit intestinal brush-border membrane vesicles. Uridine was taken up into an osmotically active space in the absence of metabolism and there was no binding of uridine to the membrane vesicles. Uridine uptake was markedly enhanced by sodium, but showed no significant stimulation by other monovalent cations tested. Kinetic analysis of the sodium-dependent component of uridine flux indicated a single system obeying Michaelis-Menten kinetics (K_m value of 6.4 ± 1.4 μM with a V_{max} of 9.1 ± 3.6 pmol/mg protein per s as measured under zero-trans conditions with a 100 mM NaCl gradient at 24°C). A variety of purine and pyrimidine nucleosides were able to inhibit sodium-dependent uridine transport, suggesting that these nucleosides are also permeants for the same system. Consistent with this suggestion was the finding that these nucleosides also stimulated uridine efflux from the brush-border membrane vesicles. The sodium:uridine coupling stoichiometry was found to be 1:1 as measured by the activation method. From these results it is concluded that a broad specificity sodium-dependent nucleoside transporter is present at the brush-border membrane surface of rabbit enterocytes.

Introduction

The transport of nucleosides across animal cell membranes is mediated by a number of different pathways. The most widely studied of these pathways is the non-concentrative facilitated diffusion transport system present in human erythrocytes (for a review see Refs. 1 and 2). The human erythrocyte nucleoside transporter accepts a wide variety of purine and pyrimidine nucleosides as permeants, is kinetically symmetrical in fresh cells, and is completely sensitive to inhibition by NBMPR and a number of other potent inhibitors, such as dilazep and dipyridamole [1–5]. This nucleoside transporter, designated as NBMPR-sensitive, is present in a wide variety of cells [1,2], and has been identified as a band 4.5 glycoprotein (apparent M_r 66 000–45 000) in mammalian erythrocytes [6–8]. In addition, non-concentrative nucleoside transporters that are insensitive to inhibition by concentrations of NBMPR as high as 1–10 μM also exist [9–14]. These NBMPR-insensitive facilitated diffusion nucleoside transporters also have a broad specificity and, in some cell types, the affinity of the carrier for certain nucleosides differs significantly from the NBMPR-sensitive system [11,13,14]. The proportion of NBMPR-sensitive and -insensitive transport in a particular cell type varies widely.

The third major class of nucleoside transporters are sodium-dependent systems recognized in renal BBMVs [15,16], murine splenocytes [17], freshly isolated guinea-pig enterocytes [18] and cultured rat intestinal epithelial cells [19]. A major difficulty of studying the transport functions of small intestinal epithelium using intact cells is that the transport properties of both the brush-border and the basolateral surface are determined simultaneously. Results obtained are, in consequence, difficult to interpret. The use of plasma-membrane vesicles prepared from each membrane surface has been extremely important in characterizing the basic mechanisms of epithelial solute transport and overcoming part of this difficulty [20]. In addition, membrane vesicles have the advantage that metabolism of the solute by cytoplasmic components is generally absent and the intravesicular and extravesicular fluid composition can be varied at will. In this report, the properties of uridine transport by rabbit intestinal BBMVs are described.

Abbreviations: NBMPR, 6-[4-nitrobenzyl)thio]-9-β-D-ribofuranosylpurine (nitrobenzylthioinosine); BBMV, brush-border membrane vesicle; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid.

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Materials and Methods

Isolation of BBMVs

Intestinal BBMVs were isolated from rabbit small intestine by the calcium precipitation method [21]. The final pellet was resuspended in 300 mM mannitol/10 mM Hepes/Tris (pH 7.5) and used on the same day of preparation for the transport assays. The membranes were, on average, enriched 8-fold in alkaline phosphatase (range 6–12-fold) and 9-fold in sucrase (range 7–13) compared to the initial homogenate.

Uridine transport

Uridine uptake at room temperature (24°C) was measured by a rapid quench, rapid filtration technique. 10 μl of the vesicle suspension (50–100 μg of protein) were rapidly mixed with 20 μl of a solution containing mannitol, buffer, [^{3}H]uridine (25 μCi/ml) and further additions as indicated in the legends. Short incubation times were timed using a metronome. The incubation was terminated by the addition of 1 ml of ice-cold stop solution containing 100 mM mannitol/200 mM NaCl/1 mM phloridizin/1 mM Hepes-Tris (pH 7.5). The diluted vesicles were quickly filtered through a pre-wetted nitrocellulose filter (pore size 0.45 μm). The filter was subsequently washed once with 5 ml of the stop solution and dissolved in 4 ml of Optiphase T scintillation fluid (LKB). The time required for filtration and washing was about 5 s. Blank values for uptake assays, due to trapping radioactivity on the nitrocellulose filter and binding to the vesicles, were determined using BBMVs simultaneously exposed to stop solution and [^{3}H]uridine at 4°C and were filtered immediately.

To test for the ability of compounds to accelerate the efflux of [^{3}H]uridine from BBMVs, BBMVs were preloaded with 5 μM [^{3}H]uridine in the presence of 100 mM NaSCN for 20 s in a total vol of 20 μl. Exchange was then initiated by the addition of 0.5 ml of test compound (10 μM–1 mM) in NaSCN buffer. Transport was terminated as described above after 5, 10 and 20 s. In control experiments, 0.5 ml of 100 mM NaSCN or 100 mM KSCN in 10 mM Hepes/Tris (pH 7.5) was added.

The intravesicular volume of the BBMVs was determined from the distribution ratio at equilibrium (30 min) of [^{3}H]glucose. The uptake of [^{3}H]glucose was determined as described above for [^{3}H]uridine.

All experiments were carried out in triplicate. The errors shown in the tables and figures are standard deviations. In least-squares fits to the data, points were weighted according to the inverse of their relative experimental errors.

Uridine metabolism

BBMVs were incubated for 10 s, 10 min and 20 min with 5 μM [5,6 – ^{3}H]uridine in the presence of a 100 mM NaCl gradient (out > in). The incubation was terminated as described above and the filter stirred for 30 min at room temperature with 250 μl of 2M NH_4OH to extract the radioactivity. The extract (50 μl) was cochromatographed with standards (uridine, uracil, UMP, UDP and UTP) on silica-gel-coated plates impregnated with fluorescent indicator (Whatman, 0.25 mm). The chromatogram was run with the solvent system *n*-butanol saturated with water (R_F values of 0.5, 0.4, 0.03, 0 and 0 for uracil, uridine, UMP, UDP and UTP, respectively). The zones containing the standards were localized under ultraviolet light and scraped into scintillation vials. The rest of the lane was equally divided into individual zones (1 cm). Radioactivity in the silica powder was extracted with 1 ml of water with shaking for 1 h before the addition of scintillation fluid.

Materials

[5,6 – ^{3}H]Uridine (47.1 Ci/mmol) and [^{3}H]glucose (40 Ci/mmol) were obtained from New England Nuclear Research Products. NBMPR and dilazep were generous gifts from Professor A.R.P. Paterson, Cancer Research Group, University of Alberta, Edmonton and Hoffmann La Roche (Vaudreuil, Canada), respectively. All other reagents were of analytical grade.

Results

The stop solution

Accurate determination of the rates of uridine uptake by use of the rapid-filtration technique depends critically on the ability of the stop solution to prevent both influx and efflux of radioactivity from the vesicles after its addition. Preliminary studies thus investigated the time-course of release of radioactivity from BBMVs into various ice-cold stop solutions (Table I). Brush-border vesicles were incubated for 10 s with 10 μM uridine in the presence of 100 mM NaCl gradient (out > in) and then diluted 33-fold into various ice-cold stop solutions that were filtered immediately or after varying periods of time. Stop solution A (containing 1 mM phloridizin) prevented the loss of intravesicular radioactivity, when the time between dilution and filtration was delayed. In contrast, ice-cold stop solutions B (containing 1 mM $HgCl_2$) and C (no addition) resulted in a steady loss of radioactivity from the vesicles. Thus, the phloridizin-containing stop solution was employed in this study.

Metabolism of uridine

Control experiments confirmed that more than 95% of the intravesicular radioactivity cochromatographed with uridine, demonstrating that [^{3}H]uridine was not metabolized by the BBMVs.

Cation dependence of uridine uptake

The time-course of uridine uptake (4.4 μM) by rabbit intestinal BBMVs is shown in Fig. 1, in the presence of

TABLE I

Effect of various stop solutions on uridine uptake

BBMVs were incubated with 10 μM [^{3}H]uridine in the presence of a 100 mM NaCl gradient (out > in) for 10 s at 24°C. The incubation was terminated by the addition of various ice-cold stop solutions: 100 mM mannitol/200 mM NaCl/1 mM Hepes-Tris (pH 7.5)/1 mM phloridizin (A), 100 mM mannitol/200 mM NaCl/1 mM Hepes-Tris (pH 7.5)/1 mM $HgCl_2$ (B) or 100 mM mannitol/200 mM NaCl/1 mM Hepes-Tris (pH 7.5) (C). Vesicles were diluted 33-fold into the stop solution and then filtered immediately (zero time) or left in the stop solution for varying times (15–60 s) and then filtered. Values are the means of triplicate estimates expressed as a percentage of the zero-time stop solution A (11.7 pmol/mg protein).

Stop solution	Uridine uptake (% of zero time)			
	15 s	30 s	45 s	60 s
A (+ 1 mM phloridizin)	95	107	99	130
B (+ 1 mM $HgCl_2$)	44	80	73	62
C (no addition)	86	80	72	71

either inwardly directed gradients of 100 mM NaCl or 100 mM choline chloride. Marked stimulation of uridine uptake was observed in the presence of the Na^+ electrochemical gradient, with a transient overshoot of the intravesicular uridine concentration above its equilibrium value. The uptake of uridine was maximum at between 10 and 20 s. When Na^+ was at equilibrium across the BBMV, no overshoot of uridine was observed (data not shown). This overshoot phenomenon indicates that the BBMV preparation is capable of catalyzing the concentrative uptake of uridine in the presence of an inwardly directed sodium chloride gradient, suggesting that uridine uptake is coupled to that of sodium. In the presence of an initial choline chloride gradient, [^{3}H]

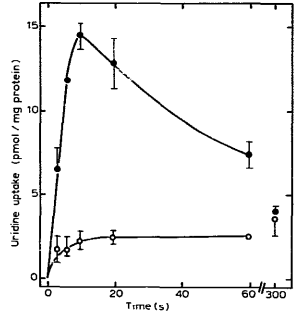

Fig. 1. Time-course of uridine uptake into rabbit intestinal BBMVs. Vesicles were incubated with 4.4 μM [^{3}H]uridine in the presence of inwardly directed gradients of 100 mM NaCl (●) or 100 mM choline chloride (○).

TABLE II

Effect of various monovalent cations on uridine uptake

10 μl of the vesicle suspension were incubated with 20 μl of incubation medium containing 100 mM of the chloride salt listed in the table, 10 mM Hepes/Tris (pH 7.5)/5 μM [^{3}H]uridine (final concentrations). The uptake of uridine was measured after 6 s of incubation. Values are means ± S.D. of triplicate estimates.

Cation	Uridine uptake (pmol/mg protein per s)
Na^+	1.04 ± 0.11
Li^+	0.16 ± 0.06
$Choline^+$	0.13 ± 0.08
Cs^+	0.09 ± 0.04
K^+	0.08 ± 0.05
none (buffered mannitol)	0.09 ± 0.09

uridine uptake increased over time and no overshoot was apparent. After 5 min, the intravesicular concentration of uridine had reached equilibrium with the extravesicular concentration (the intravesicular volume for the BBMV preparation used in Fig. 1 was 0.82 μl/mg protein, yielding an intravesicular uridine concentration, after 5 min incubation of 4.5 μM, identical to that of the extravesicular uridine concentration). Na^+-stimulated uptake of uridine was a linear function of time for the first 6 s of incubation. The uptake at 2 s was therefore taken to approximate the initial rate of uridine influx. Preliminary data [1] have also demonstrated that adenosine uptake by rabbit intestinal BBMVs is markedly stimulated by Na^+. However, in contrast to uridine, 5 μM [^{3}H]adenosine was metabolized by the BBMVs (85% of the radioactivity comigrating with inosine after 20 s uptake). Further studies were therefore performed with the non-metabolized nucleoside, uridine.

The specificity of extravesicular Na^+ in stimulating uridine uptake in BBMVs was studied by comparing the uptake of uridine in the presence of 100 mM gradients of chloride salts of various cations. Table II demonstrates that when a 100 mM Na^+ gradient was replaced by gradients of K^+, Cs^+ or choline, uridine uptake was reduced at least 10-fold with no significant difference between the various cations. A small enhancement in the rate of uridine flux was observed with a 100 mM Li^+ gradient, but this was not significant. These results demonstrate the high sodium specificity for uridine uptake by BBMVs and, in further experiments, the sodium-dependent component of uridine transport was calculated as that in the presence of NaCl minus that in the presence of choline chloride.

Effect of extravesicular osmolarity on uridine uptake by BBMVs

The effect of extravesicular osmolarity on the equilibrium value of uridine uptake by BBMVs (20 min) is

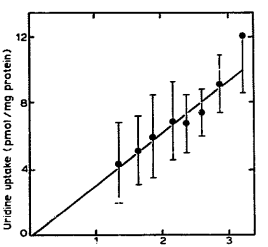

Fig. 2. Effect of extravesicular osmolarity on equilibrium uptake of uridine. Vesicles were prepared in 300 mM mannitol/10 mM Hepes/Tris (pH 7.5) and suspended in a buffer containing varying concentrations of cellobiose to vary the extravesicular osmolarity. Uptake of 5 μM [^{3}H]uridine at the extravesicular osmolarity was determined after incubation for 20 min and plotted as a function of the reciprocal of the extravesicular osmolarity. Data reported are the means of two separate experiments. The regression line was calculated by the least-squares method (regression coefficient 0.948).

shown in Fig. 2. The osmolarity of the extravesicular medium was varied by changing the concentration of cellobiose. The uptake of uridine was inversely proportional to the extravesicular osmolarity and the intercept on the vertical axis at infinite extravesicular osmolarity (zero intravesicular space) was not significantly different from zero. These results suggest that uridine associated with the BBMVs is due to transport of uridine across the membrane into an osmotically sensitive intravesicular space and, furthermore, there is no significant binding of uridine to the membrane. Previous experiments with other solutes, for example, glycine and glucose, have shown a similar behaviour [22,23].

Kinetics of uridine transport

The concentration dependence of sodium-dependent uridine influx is illustrated in Fig. 3. Uridine influx was saturable and the linearity of the s/v vs. s plot indicates that the data conform to simple Michaelis-Menten kinetics. Least-squares analysis of the s/v vs. s plot yielded an apparent K_m value of 4.3 ± 0.9 μM with an apparent V_{max} of 7.7 ± 0.5 pmol/mg protein per s. The mean values of the kinetic constants from three separate experiments were 6.4 ± 1.1 μM for the K_m with a V_{max} estimate of 9.1 ± 3.6 pmol/mg protein per s (mean $\pm$ S.E.). Uridine influx in the presence of choline chloride as a function of solute concentration was linear (data not shown), suggesting that uptake in the presence of choline represents simple diffusion.

The range in V_{max} values and uptake rates from one experiment to another was approx. 4-fold. Possible reasons for this variation are animal variation, differential membrane damage, such as proteolysis, and oxidation of the membrane proteins during BBMV preparation, possible dietary effects on the expression of the transport protein and heterogeneity of transport from the villus to the crypt of the rabbit small intestine. The digestive and absorptive function of the small intestine changes markedly along the villus crypt axis [24].

Inhibition of transport by nucleosides

The substrate specificity of uridine uptake by intestinal brush-border membranes was investigated by two different, but complementary, approaches. In the first study, the ability of nucleosides to inhibit uridine influx into BBMVs in the presence of 100 mM extravesicular NaCl or choline chloride was examined. Table III shows that both purine and pyrimidine nucleosides are effective inhibitors of sodium-dependent uridine influx. In contrast, glucose (1 mM) and the facilitated diffusion nucleoside transport inhibitors, NBMPR, dilazep and dipyridamole (all at 10 μM) failed to inhibit uridine influx in the presence of either NaCl or choline chloride. Similarly, the nucleoside competitors had no effect on uridine uptake in the presence of choline chloride (data not shown). In the second approach, membrane vesicles were preloaded with [^{3}H]uridine (5 μM) and then the efflux of [^{3}H]uridine into 100 mM NaSCN buffer alone (i.e., equal concentration of Na across the BBMV) or 100 mM NaSCN buffer containing nucleoside was examined. All of the nucleosides tested stimu-

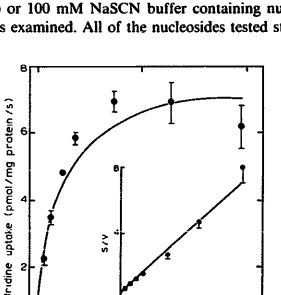

Fig. 3. Concentration dependence of sodium-dependent uridine influx by rabbit intestinal BBMVs. Membrane vesicles were incubated with [^{3}H]uridine (final concentrations 0–50 μM) in the presence of 100 mM NaCl or choline chloride. Initial rates were calculated from the uptake at 2 s. The Na^+-dependent flux was taken as the rate in the presence of NaCl minus that in the presence of choline chloride for each concentration of substrate. Inset: s/v vs. s plot of the data. A least-squares fit to this plot yields $K_m = 4.3 \pm 0.9$ μM (S.E.) with $V_{max} = 7.7 \pm 0.5$ pmol/mg protein per s, with $r = 0.993$. These kinetic parameters were used to draw the curve shown.

lated the rate of [^{3}H]uridine efflux, indicating carrier-mediated exchange of the nucleosides. For example, the percentage of [^{3}H]uridine retained in the BBMVs after 10 s efflux into 10 μM adenosine and thymidine was 51 and 73%, respectively, as compared to buffer alone. NBMPR (10 μM) had no effect on the rate of uridine efflux and when a Na^+ gradient was imposed by initiating efflux by adding 100 mM KSCN, the rate of efflux was stimulated 5-fold. These results demonstrate that uridine efflux is not mediated by the NBMPR-sensitive non-concentrative nucleoside carrier and is Na^+-dependent. Furthermore, the results also suggest that Na^+-dependent transport of the nucleosides tested is by a single common carrier, although the results do not exclude the possibility of other Na^+-dependent nucleoside pathways. Consistent with this suggestion was the finding that 1 mM glucose, which is transported by a Na^+-dependent system in rabbit intestinal BBMVs [25], failed to exchange with [^{3}H]uridine, demonstrating that the exchange observed was not a non-specific effect due to Na^+ cycling.

Effect of sodium on uridine transport

The sodium dependence of uridine uptake was explored further by measuring the initial flux of uridine (5 μM) as a function of the extracellular Na^+ concentration (0–100 mM). Fig. 4A demonstrates that there was a hyperbolic relationship between uridine flux and Na^+ concentration, suggesting a minimum sodium : uridine stoichiometry of 1 : 1. The data were also analyzed using the Hill equation [26].

$$\text{flux} = V_{max}[Na]^n/(K_{Na}^n + [Na]^n)$$

where K_{Na} is the [Na] giving 50% of V_{max} and n is the Hill coefficient. A plot of flux/$[Na]^n$ against flux for the correct value of n will yield a straight line. Fig. 5B shows the results of such a plot of the data in Fig. 5A assuming $n = 1$. A straight line was observed with $n = 1$ and $K_{Na} = 14 \pm 4$ mM; a result consistent with a single Na^+ binding site on the carrier.

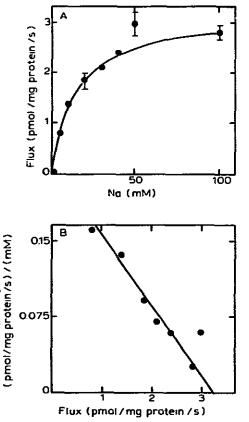

Fig. 4. Sodium-dependent uridine flux as a function of the sodium concentration. [^{3}H]Uridine (5 μM) uptake was measured in the presence of varying extravesicular concentrations of NaCl (0–100 mM). Choline replaced sodium isosmotically to obtain the various sodium concentrations studied. Uptake was measured after 2 s incubation. Panel A: plots of uridine uptake vs. sodium concentration. Panel B: plot of flux/$[Na]^n$ vs. flux for $n = 1$. Linearity of the plot is indicative of the involvement of one sodium ion per uridine molecule transported (also see text).

TABLE III

Effect of nucleosides, transport inhibitors and glucose on sodium-dependent uridine influx

The uptake of uridine at 22°C was initiated by addition of BBMVs to medium containing (final concentration) 5 μM [^{3}H]uridine/100 mM NaCl or choline chloride and test compound. For NBMPR, dilazep and dipyridamole, BBMVs were preincubated with these compounds for 5 min before addition of [^{3}H]uridine. Influx was terminated after 2 s and the Na^+-dependent component of uptake calculated as that in the presence of NaCl minus that in the presence of choline chloride. The test compounds had no effect on uridine influx in the presence of choline chloride (data not shown). Values are shown as a percentage of the control flux and are the mean values from at least three separate experiments. Control Na^+-dependent flux value 5.2±0.6 pmol/mg protein per s. n.d., not determined.

Inhibitor	% Uridine influx	
Final inhibitor concentration (μM)	10	50
Adenosine	47± 5	13± 1
2-Chloroadenosine	52± 9	33± 4
Inosine	48± 3	20± 1
Deoxyinosine	50± 4	23± 1
2-Deoxyuridine	72± 3	36± 2
5-Fluorouridine	69± 4	29± 3
Thymidine	73± 8	44±12
Cytidine	92± 6	70± 3
NBMPR	107±18	n.d.
Dilazep	109± 5	n.d.
Dipyridamole	100± 6	n.d.
Glucose (1 mM)	104± 5	

Discussion

Previous reports [18,19] have shown that guinea-pig enterocytes and cultured rat intestinal cells possess active transport systems for nucleosides. The present results extend these observations and further suggest that the sodium-dependent uridine transporter is located at the brush-border membrane surface of the rabbit intestine. This does not exclude the possibility of active

nucleoside transport systems at the basolateral membrane surface of the intestinal cell and to date there have been no direct studies on nucleoside transport at the basolateral side.

Measurements of nucleoside transport by intact cells is often complicated by rapid intracellular metabolism of the nucleoside. However, by using BBMVs, we were able to demonstrate no metabolic conversion of the [^{3}H]uridine. We also found that uridine is taken up into an osmotically active space, indicating that we are indeed measuring the transport of uridine and not its binding to the BBMV (Fig. 2). Uridine uptake was markedly enhanced by sodium (Fig. 1 and Table II) but showed little if any stimulation by the other monovalent cations tested in Table II. Similar results have recently been reported for Na^+-dependent uridine influx by outer cortical rabbit renal BBMVs [18]. In contrast, a K^+-dependent uridine transport system as well as a Na^+-dependent uridine carrier have been suggested to be present in rat renal BBMVs [27].

Kinetic analysis of the sodium-dependent component of uridine flux indicated a single transport system obeying Michaelis-Menten kinetics with K_m value of 6.4 ± 1.1 μM and V_{max} = 9.1 ± 3.6 pmol/mg protein per s as measured under zero-trans conditions at 100 mM NaCl and 24°C (Fig. 3). This K_m is close to that estimated for Na^+-dependent uridine transport by renal BBMVs (Refs. 15, 16 and Lee et al., unpublished results), but is about 20-fold lower than that observed for the facilitated-diffusion nucleoside carriers [1,2]. A variety of purine and pyrimidine nucleosides (Table III) were able to inhibit Na^+-dependent uridine transport by rabbit intestine BBMVs, suggesting that these nucleosides are substrates of the same system. Inhibition by these nucleosides is unlikely to be due to secondary effects, i.e., transport by a different system that eliminates the electrochemical gradient thereby decreasing uridine influx, since D-glucose, which is known to be transported in a Na^+-dependent manner with a V_{max} approx. 16-fold greater than the Na^+-dependent uridine V_{max} [25], had no inhibitory effect on uridine influx. Moreover, the nucleosides that inhibited Na^+-dependent uridine influx also stimulated uridine efflux demonstrating that these nucleosides are transported on a common system with a broad specificity. These findings contrast with those conducted on the properties of Na^+-dependent uridine uptake by rat and rabbit renal cortical BBMVs and murine splenocytes (Refs. 17, 28 and Lee et al., unpublished results). In rat and rabbit renal cortical BBMVs, Na^+-dependent uridine influx is inhibited by pyrimidine nucleosides, adenosine and adenosine analogues (Ref. 28 and Lee et al., unpublished results). Inosine was a poor inhibitor of uridine transport. However, Na^+-dependent uridine transport by murine splenocytes was inhibited by purine nucleosides, including inosine, but pyrimidine nucleosides had no effect [17]. Therefore, it seems likely that there exist a number of Na^+-dependent nucleoside transporters with different substrate specificities that may depend on both the species and the tissue. Alternatively, some of the differences reported in substrate specificity might reflect metabolism of the competitors, and further comparative studies will be required before coming to any firm conclusion on the number of different Na^+-dependent nucleoside transporters. One property that does appear to be common to all the Na^+-dependent nucleoside transporters studied to date is that they are resistant to inhibition by the facilitated-diffusion nucleoside transport inhibitors, NBMPR, dilazep and dipyridamole (see Table III) [15,19].

The sodium : uridine coupling stoichiometry was determined by the activation method and yielded a minimum stoichiometry of 1 Na^+ : 1 uridine, similar to that proposed for the uridine cotransporter in rabbit and rat renal cortical BBMVs [27,28]. Hill plots gave a slope of 1, indicating a single class of noninteracting Na^+ sites. A more direct method to measure the stoichiometry, such as the 'static head method', as introduced by Turner and Moran [29], was not possible due to the relatively low uridine transport rate and hence a poor signal-to-noise ratio when concentrations of both uridine and sodium were varied.

In conclusion, the present results have established the presence of a high-affinity, broad specificity sodium cotransporter system for nucleosides in rabbit intestinal BBMVs. Further studies are now required to characterize nucleoside and nucleobase transport across the intestinal basolateral membrane to further understand the mechanism involved in the transfer of nucleosides from the lumen of the gut to the blood.

Acknowledgements

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