Biochimica et Biophysica Acta, 942 (1988) 139–149
Elsevier

BBA 74030

Na^+- and K^+-dependent uridine transport in rat renal brush-border membrane vesicles

Chee Wee Lee [a], Chris I. Cheeseman [a] and Simon M. Jarvis [b]

[a] *Department of Physiology, University of Alberta, Edmonton (Canada) and* [b] *Biological Laboratory, University of Kent, Canterbury (U.K.)*

(Received 23 September 1987)
(Revised manuscript received 8 February 1988)

Key words: Uridine transport; Nucleoside transport; (Kidney brush-border vesicle)

The transport of uridine into rat renal brush-border membrane vesicles was investigated using an inhibitor-stop filtration method. Uridine was not metabolized under these conditions. The rapid efflux of intravesicular uridine was prevented by adding 1 mM phloridzin to the ice-cold stop solution. In the presence of inwardly directed gradients of either Na^+ or K^+, zero-trans uridine uptake exhibited a transient overshoot phenomenon indicating active transport. The overshoot was much more pronounced with Na^+ than K^+ and it was not observed when either Na^+ or K^+ was at equilibrium across the membrane. The K^+-induced overshoot was not due to the presence of a membrane potential alone, as an inwardly directed gradient of choline chloride failed to produce it. The amplitude of the overshoot was increased by raising either the Na^+ or K^+ concentration outside the membrane or by using more lipophilic anions (reactive order was $NO_3^- > SCN^- > Cl^- > SO_4^{2-}$). Zero-trans efflux studies showed that the uridine transport is bidirectional. Li^+ could substitute poorly for Na^+ but not at all for K^+. Stoichiometries of 1:1 and > 1:1 were observed for Na^+:uridine and K^+:uridine coupling, respectively. A preliminary analysis of the interactions between Na^+ and K^+ for uridine uptake showed complex interactions which can best be explained by the involvement of two different systems for nucleoside transport in the rat renal brush-border membrane, one requiring Na^+ and the other K^+ as transport coupler.

Introduction

A large number of nucleoside analogues have been synthesized and tested for antitumor, antiviral and antiparasitic activity [1–4]. A major obstacle to the clinical use of these substances is toxicity to the host tissues. The kidney is particularly sensitive to therapeutic treatment of inhibitors of adenosine deaminase which results in increased concentrations of adenosine and 2′-deoxyadenosine in serum [5,6]. Both nucleosides are potentially cytotoxic [7]. Nephrotoxicity has also been observed when the nucleoside analogues such as tubercidin and 2′-deoxycoformycin have been administered to experimental animals [5,8]. In addition, the physiological nucleoside, adenosine, may play a role in the intrinsic control of glomerular filtration rate and renin release [9,10]. The mechanism(s) by which the kidney handles high concentrations of nucleosides in the filtrate will therefore be an important factor in the therapeutic administration of nucleoside drugs.

Measurements of the renal clearance of adenosine in humans and mice led in 1982 to the

Correspondence: C.I. Cheeseman, Department of Physiology, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

hypothesis that an active transport system exists in the kidney with the function of reabsoring the filtered adenosine [11]. Subsequent studies with rat renal brush-border membrane vesicles demonstrated that adenosine uptake and the uptake of a variety of purine and pyrimidine nucleosides did not proceed by classical facilitated diffusion, as seen in most cell types [12–15], but rather by an active concentrative process which required the presence of an Na^+ gradient (out > in) as the driving force [16–18]. Such ion gradients are maintained in the living cell by Na^+/K^+-ATPase. However, Na^+ gradients cannot be maintained across isolated brush-border membrane vesicles because the Na^+/K^+-ATPase is present only in the basolateral membrane of the tubular cell, not in the brush-border membrane [19]. Therefore, the 'overshoot' uptake phenomenon observed in brush-border membrane vesicles can be abolished by dissipating the driving force. This stimulation of nucleoside uptake by an Na^+ gradient is not a consequence of the Na^+–H^+ exchange across the membrane, as an H^+ gradient (in > out or out > in) did not affect the uptake of adenosine in the presence of an Na^+ gradient. This suggests that Na^+ interacts directly with the nucleoside carrier, rather than indirectly through the formation of an H^+ gradient [16]. Since Na^+-nucleoside cotransport is accompanied by a net transport of positive charges from the medium into the vesicles, the nucleoside influx can be stimulated by an inside-negative membrane potential [17]. It is not known whether there is a single Na^+-dependent nucleoside carrier with a broad specificity as observed for the facilitated diffusion nucleoside transport systems [13–15,20] or whether there are a number of different active nucleoside carriers. In addition to the capacity for concentrative transport, the nucleoside carrier(s) in the renal brush-border membranes also has a 20–100-fold greater affinity for nucleosides [16,17] when compared to the nitrobenzylthioinosine-sensitive and -insensitive facilitated diffusion nucleoside transport systems [13–15,20].

Clearly the proximal tubule of the kidney is equipped with a system(s) for the reabsorption of nucleosides which differ from the facilitated diffusion nucleoside carrier of most other cell types. To obtain a more complete view on this system(s), transport characteristics such as membrane potential dependency, monovalent cation specificity and Na^+ : nucleoside stoichiometry and substrate structural specificity should be investigated. In this report are presented the results of a study on the anion and cation dependency of uridine transport by rat renal cortical brush-border membrane vesicles. In addition to an Na^+-dependent uridine transport system, the existance of a separate active transporter requiring K^+ as a transport coupler is also proposed.

Materials and Methods

Preparation of brush-border membrane vesicles

Rat renal cortical brush-border membrane vesicles were prepared by an $MgCl_2$-precipitation method [21]. The purity of the preparation was assessed by the specific activity of alkaline phosphatase [22] and ouabain-sensitive Na^+/K^+-ATPase [23] in the vesicles as compared to the initial homogenate. Alkaline phosphatase (a brush-border membrane marker) was enriched 10-fold, while ouabain-sensitive Na^+/K^+-ATPase (a basolateral membrane marker) was reduced by 10- to 12-fold. The purified vesicles were suspended in an ice-cold medium containing 300 mM mannitol and 5 mM Tris-HCl at pH 7.4. They were used on the same day of preparation. Protein was assayed according to Lowry et al. [24] with bovine serum albumin as a standard.

Uptake studies

The uptake of [^{3}H]uridine (50 μCi/ml) was measured using an inhibitor-stop filtration method. A 10 μl aliquot of brush-border membrane vesicle suspension (30–80 μg protein) and a 20 μl aliquot of incubation medium, containing [^{3}H]uridine and appropriate salt concentrations (with choline chloride substituted to maintain osmolarity) were placed separately on opposite sides of a polyethylene culture tube (16 × 100 mm) and preincubated at 22° C for 5 min. The uptake of [^{3}H]uridine was initiated by continuous mixing with a vortexer such that the brush-border membrane vesicles and the incubation medium came into contact. The detailed final compositions of the incubation media are given in the figure legends. At the initiation of the incubation, the vesicles did not

contain any substrate and hence the uptake was measured under 'zero-trans' conditions. All experiments were performed at room temperature (22–24° C). After an appropriate time interval, the uptake was terminated by addition of 1 ml of ice-cold stop solution (100 mM mannitol/100 mM NaCl/1 mM phloridzin/5 mM Tris-HCl (pH 7.4)). The suspension was immediately filtered through an MSI cellulose filter (pore size 0.45 μm, Fisher Scientific) under suction. The filter was subsequently washed once with 5 ml of ice-cold stop solution and dissolved in 4 ml of Scinti Verse Bio-HP (Fisher Scientific) after being dried. The radioactivity was determined using an LKB/Wallac 1217 liquid scintillation counter with automatic quench correction and disintegration per minute conversion. The entire stopping and washing process took less than 10 s and control experiments (see Results) established that no significant loss of [^{3}H]uridine from the vesicles occurs during this procedure. The radioactivity retained on the filter in the absence of membrane vesicles was used as the blank value for the uptake assays. These blanks were subtracted from measurements of uridine associated with the brush-border membrane vesicles to determine uridine uptake rates.

Efflux studies

Brush-border membrane vesicles were pre-equilibrated with incubation medium containing (final concentrations) 50 μM [^{3}H]uridine, 100 mM mannitol, 5 mM Tris-HCl (pH 7.4) and 100 mM of either $NaNO_3$, KNO_3 or choline chloride for 20 min at room temperature. The efflux was then initiated by mixing a 20 μl aliquot of the above vesicle suspension with 1 ml of incubation medium but lacking [^{3}H]uridine. This resulted in a 50-fold dilution of the vesicles and their preincubation medium to produce conditions approaching those required for 'zero-trans' efflux. After an appropriate time interval, the reaction was stopped and washed once with 5 ml of ice-cold stop solution. The radioactivity retained on the filter was determined as described in the preceding section. For measuring the total initial intravesicular [^{3}H]uridine concentration, the reaction was initiated by mixing the vesicle suspension with 1 ml of ice-cold stop solution and filtering immediately, other procedures remained unchanged.

The intravesicular volume was determined by measuring the intravesicular concentration of [^{3}H]glucose (100 μM) at equilibrium (90 min incubation). The mean value of the intravesicular volume obtained was 1.81 ± 0.10 μl/mg protein from 21 determinations.

Uridine metabolism

Brush-border membrane vesicles were incubated for 10 s, 1 and 30 min with 5 μM [^{3}H]uridine at 22° C in the presence of either an Na^+ or a K^+ gradient (out > in). The reaction was terminated as described above and the filter was stirred for 30 min at room temperature in 250 μl of 2 M NH_4OH. The NH_4OH extract (50 μl) was then chromatographed on silica-gel-coated plates impregnated with fluorescent indicator (Whatman K6F silica gel, 20 cm × 20 cm × 250 μm). The standards (uridine, uracil, UMP, UDP and UTP) were co-chromatographed with the samples. The solvent system used for the chromatographic analysis was *n*-butanol (saturated with water). After drying, the zones bearing the standards were localized under ultraviolet light (R_F values of 0.4, 0.5, 0.03, 0 and 0 for uridine, uracil, UMP, UDP and UTP, respectively). The rest of the lane was equally divided into individual zones (1 cm). Each zone was scraped from the plate into a scintillation vial. Radioactivity in the powder was extracted with 1 ml of water with shaking for at least 15 min before the addition of scintillation cocktail.

Chemicals

[5′-H^3]Uridine (25 Ci/mmol) and [^{3}H]glucose (40 Ci/mmol) were purchased from Moravek Biochemicals, Brea, CA, and Amersham, Oakville, Ontario, respectively. Phloridzin was obtained from Sigma Chemical Co. All reagents were of analytical grade.

Results

Stop solution

The rapid filtration technique used here to measure solute transport by renal brush-border membrane vesicles is valid only if all transport ceases after the addition of the stop solution and no loss of radioactivity from the vesicles occurs during filtration. Therefore, in preliminary experi-

ments, the effect of different stop solutions on the retention of [^{3}H]uridine by the rat renal brush-border membrane vesicles was tested. Renal brush-border membrane vesicles were incubated with [^{3}H]uridine in the presence of an Na^+-gradient (out > in) for 10 s then subsequently diluted 1 : 33 into various stop solutions (see Fig. 1). These diluted vesicles were then filtered immediately or after a 5 to 60 s delay. Fig. 1 shows that using an ice-cold stop-solution containing 1 mM phloridzin, a Na^+-dependent glucose transport inhibitor [25,26], total retention of radioactivity was observed for at least 1 min after addition of the phloridzin stop solution. In contrast, ice-cold stop solution containing 1 mM $HgCl_2$ or buffer alone resulted in rapid loss of radioactivity from the vesicles. The rate of uridine loss was further enhanced if stop solution at room temperature was used. Thus, the ice-cold stop solution containing 1 mM phloridzin was routinely used in the present study. These results, however, were not in agreement with those reported by Le Hir and Dubach [16–18] that ice-cold buffer solution alone was able to prevent the rapid efflux of nucleosides from rat renal brush-border membrane vesicles. The reason for these differences are unknown, but it is clear that a stop solution containing NaCl and phloridzin is necessary for uridine transport studies using our renal brush-border membrane vesicle preparation.

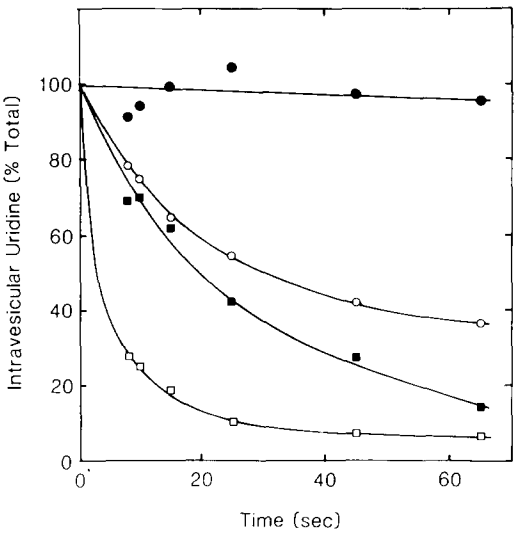

Fig. 1. Effect of various stop solutions on uridine uptake by rat renal brush-border membrane vesicles. 10 μl of the vesicle suspension were incubated with 20 μl of incubation medium containing (final concentrations) 5 μM [^{3}H]uridine, 5 mM Tris-HCl (pH 7.4) and 100 mM NaCl for 10 s at 22°C. Transport was stopped by adding 1 ml of stop solution (Tris-HCl buffer (pH 7.4)/100 mM mannitol/100 mM NaCl) at room temperature (□), ice-cold stop solution (■), ice-cold stop solution containing 1 mM $HgCl_2$ (○) or ice-cold stop solution containing 1 mM phloridzin (●). The suspension was poured onto the filter at different times after being stopped and subsequently washed once with 5 ml of respective solution. The time points indicated in the figure were corrected for the time taken (about 5 s) between stopping and the completion of the washing. The radioactivity retained by the vesicles at each time point is expressed as percentage of the radioactivity retained when the incubation medium was filtered immediately with ice-cold stop solution containing 1 mM phloridzin (90 pmol/mg protein).

Metabolism

Control experiments confirmed that no significant metabolism of uridine occurs within the first minute of incubating renal brush-border membrane vesicles with [^{3}H]uridine in the presence of either Na^+- or K^+-gradient (out > in) and only 2% of the intravesicular radioactivity was recovered in the uracil fraction after 30 min (data not shown). Therefore, uridine metabolism does not complicate the present study of uridine transport by rat renal brush-border membrane vesicles.

Time-course of uridine influx

The time-course of [^{3}H]uridine uptake by rat renal brush-border membrane vesicles is illustrated in Fig. 2. In the presence of an initial 100 mM NaCl gradient (out > in) a transient overshoot of the intravesicular uridine concentration above its equilibrium value was observed. The initial rate of uridine uptake (2 s) was about 300-times the initial rate in the absence of the gradient. Accumulation of uridine reached a maximum value at about 15 s and then decreased afterwards, indicating uridine efflux. In the absence of NaCl or in the presence of a 100 mM choline chloride gradient no transient overshoot was observed (Fig. 2). However, a 100 mM KCl gradient (out > in) produced a significant increase in uridine influx relative to choline chloride and, moreover, a transient overshoot was also observed (Fig. 2). The maximal accumulation of uridine with an initial 100 mM KCl gradient was 35 pmol/mg protein compared to 120 pmol/mg pro-

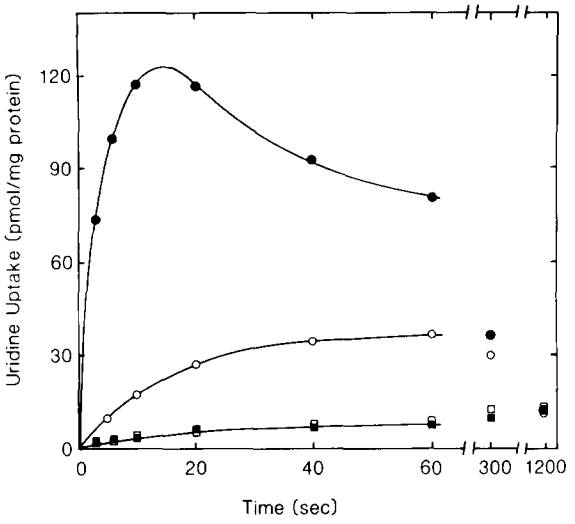

Fig. 2. Time-course of uridine uptake by rat renal brush-border membrane vesicles. 10 μl of the vesicle suspension were incubated with 20 μl of media containing (final concentrations) 5 μM [^{3}H]uridine, 5 mM Tris-HCl (pH 7.4) and either 100 mM NaCl (●), 100 mM KCl (○), 100 mM choline chloride (■), or 200 mM mannitol (□).

tein with an initial 100 mM NaCl gradient. 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 (data not shown). The superimposition of the choline chloride and mannitol curves (Fig. 2) rules out the possibility that the effect of a Cl^--generated membrane potential alone is an explanation for the overshoot seen in the presence of K^+. Thus, the above overshoot phenomena indicate that rat renal brush-border membrane vesicles are capable of catalyzing the concentrative uptake of uridine in the presence of inwardly directed transient electrochemical gradients formed by NaCl or KCl. Due to the absence of a stimulatory effect of choline chloride on uridine uptake and high solubility of this compound, choline chloride was used in this study to measure cation-gradient-independent uridine transport and to maintain isoosmolarity of the incubation medium.

Effect of anions

The effect of various anions on Na^+- and K^+-dependent uptake of [^{3}H]uridine is shown in Fig. 3. In the presence of an Na^+ gradient (out > in) (Fig. 3A), the anions increased the amplitude of the overshoot in the following order: $NO_3^- > SCN^- > Cl^- > SO_4^{2-}$. A similar order was also

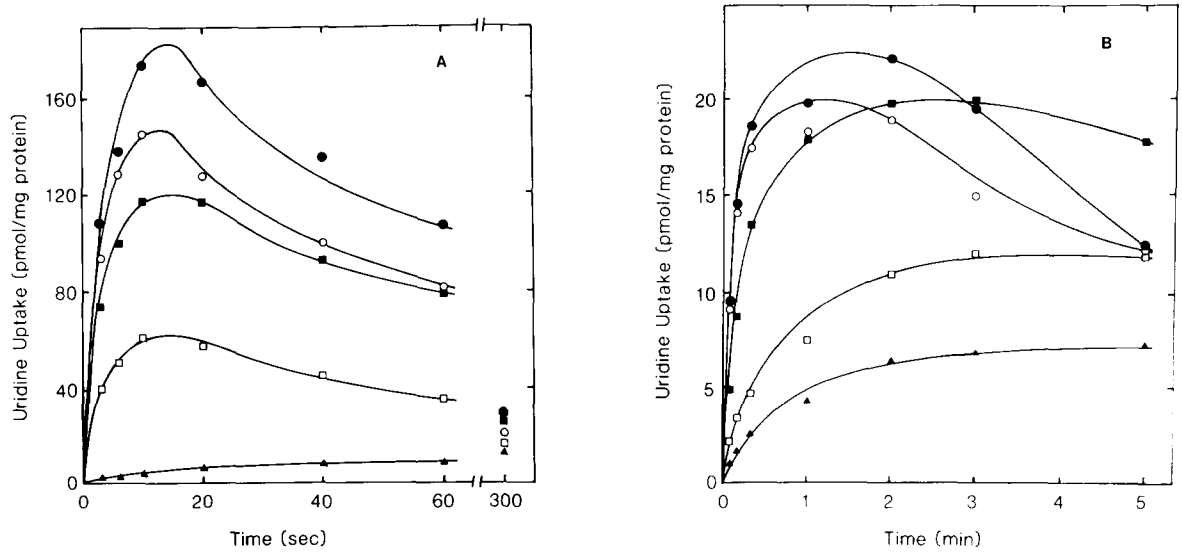

Fig. 3. Effect of anions on Na^+- (A) and K^+-dependent (B) uridine uptake by rat renal brush-border membrane vesicles. Experimental conditions were as described in the legend of Fig. 2. The gradients (out > in) were generated with either (final concentrations) 100 mM $NaNO_3$ or KNO_3 (●), 100 mM NaSCN or KSCN (○), 100 mM NaCl or KCl (■), 50 mM Na_2SO_4 or K_2SO_4 (□) or 100 mM choline chloride (▲).

found in the presence of a K^+ gradient (out > in) (Fig. 3B). This anion effect is similar to that which would be predicted from the order of diffusibility of these ions and the resultant magnitude of the membrane potential ($SCN^- > NO_3^- > Cl^- > SO_4^{2-}$) [27], with the exception that the amplitude of the overshoot was higher with NO_3^- than SCN^- in this system. Therefore in subsequent experiments, NO_3^- salts were used to generate the chemical gradient.

Effect of salt concentrations

Increasing the concentration of $NaNO_3$ or KNO_3 in the extravesicular medium (isoosmolarity maintained with choline chloride) resulted in the amplitude of the overshoot for uridine uptake being increased for both Na^+ (Fig. 4A) and K^+ (Fig. 4B). Moreover, the initial rate of uridine uptake was increased and the Na^+ and K^+ dependence of approximate initial rates of uridine uptake (2 s for Na^+ and 5 s for K^+) was explored further by measuring the Na^+ and K^+ activation curves at a fixed uridine concentration (Fig. 5). As the extracellular Na^+ concentration increased from 0 to 200 mM, there was a hyperbolic activation of uridine uptake that conformed to simple saturation kinetics (K_{Na} 18 ± 4 mM) consistent with a single Na^+-binding site on the uridine transporter. In contrast, the K^+ activation curve for uridine uptake was sigmoidal, suggesting a K^+ : uridine coupling stoichiometry of more than 1 : 1. An approximate estimate for the K_K was 280 ± 70 mM. Further studies are currently being performed to quantify the precise coupling ratio of these systems using voltage clamped vesicles.

Interactions between Na^+ and K^+

The above results show that both an Na^+ and a K^+ gradient (out > in) may elicit active transport of uridine into renal brush-border membrane vesicles. Thus, in further experiments the possible interactions between Na^+ and K^+ when these ions are both present in the incubation medium on the same or opposite sides of the membrane were investigated. Fig. 5 shows that the approximate initial rates of Na^+-dependent uridine uptake can be enhanced by the addition of a K^+ gradient (out > in). Greatest stimulation by K^+ was observed at extravesicular Na^+ concentrations ranging from 10 to 40 mM. The stimulation, however, was not profound at extravesicular Na^+ concentrations above 100 mM. The K_{Na} value in the

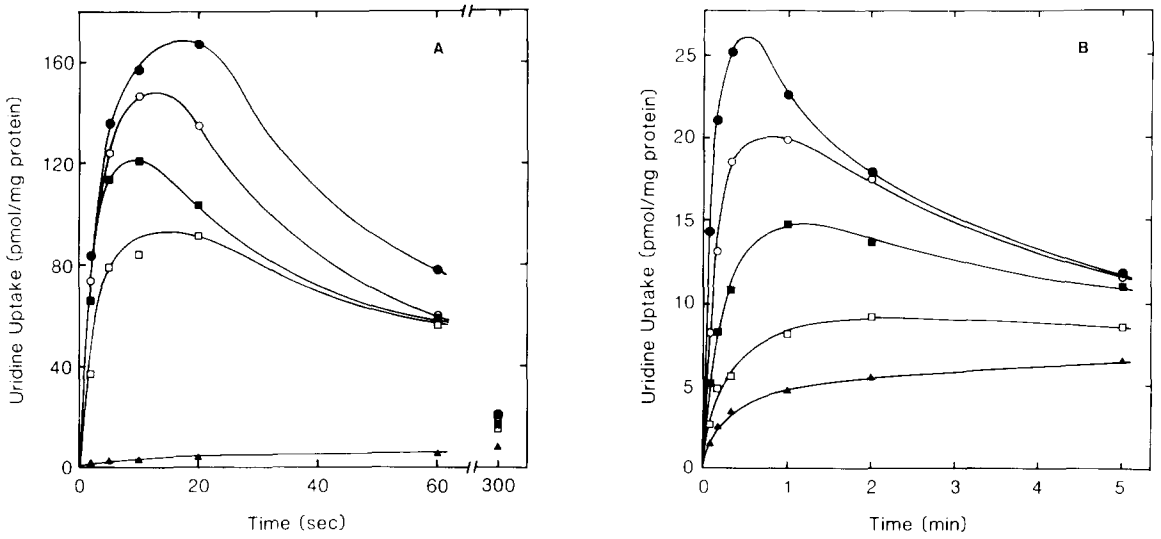

Fig. 4. Effect of external salt concentrations on Na^+- (A) and K^+-dependent (B) uridine uptake by rat renal brush-border membrane vesicles. 10 μl of the vesicle suspension were incubated with 20 μl of media containing (final concentrations) 5 μM [^{3}H]uridine, 5 mM Tris-HCl (pH 7.4) and 200 mM total salt concentrations. Variable external Na^+ and K^+ concentrations from 0 to 200 mM were obtained by substitution with choline chloride. Final external Na^+ or K^+ concentrations (mM) were 200 (●), 100 (○), 50 (■), 10 (□) and 0 (▲).

presence of a 100 mM K^+ gradient was decreased 3-fold compared to the absence of K^+ (6 ± 1 and 18 ± 4 mM, respectively). Likewise, increasing the extravesicular K^+ concentration from 100 to 200 mM did not further stimulate the initial rate of uridine uptake. Therefore, a Na^+ and K^+ concentration of 20 and 100 mM, respectively, were used in subsequent experiments.

The stimulatory effect of K^+ on the Na^+-dependent uridine uptake is further illustrated in Fig. 6 (Na^+: out > in; K^+: out > in; curve a). Both the initial rate and the amplitude of uptake were significantly higher than the uptake in the presence of Na^+ gradient alone (Na^+: out > in; curve b). This suggests that Na^+ and K^+ are not interacting at the same carrier site. However, when K^+ is equilibrated on both sides of the membrane (Na^+: out > in; K^+: out = in; curve c), uridine uptake is greatly reduced. The inhibitory effect

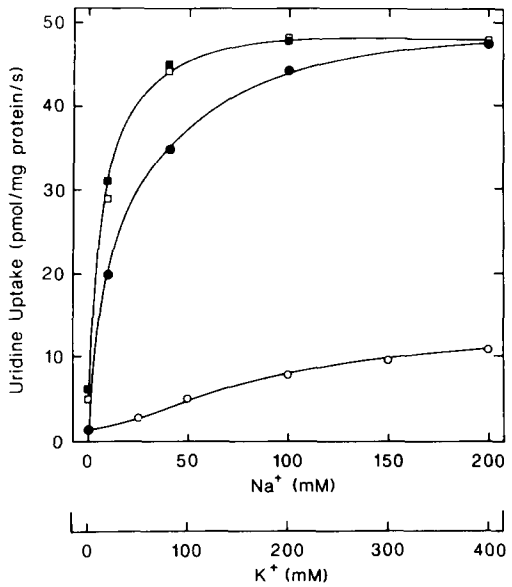

Fig. 5. Na^+ and K^+ activation of uridine uptake. 10 μl of the vesicle suspension were incubated with 20 μl of media containing either (final concentrations) 200 mM KNO_3 (■), 100 mM KNO_3 plus 100 mM choline chloride (□) or 200 mM choline chloride (●), 5 μM [^{3}H]uridine, 5 mM Tris-HCl buffer (pH 7.4) and variable Na^+ concentrations. Effect of external K^+ concentration alone (○) on uridine uptake was also determined. Variable external Na^+ and K^+ concentrations from 0 to 200 mM and to 400 mM, respectively, were obtained by substitution with choline chloride. Approximate initial rates of uridine uptake were estimated from 2 (●, □, ■) and 5 s (○) uptake intervals.

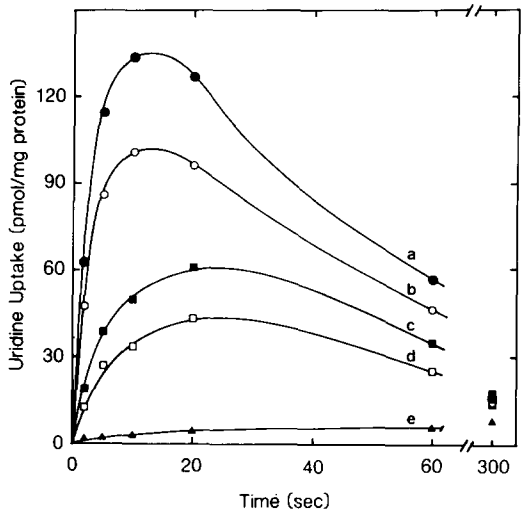

Fig. 6. Interactions between Na^+ and K^+ for uridine uptake by rate renal brush-border membrane vesicles. Vesicles were resuspended in media containing 300 mM mannitol, 5 mM Tris-HCl (pH 7.4), and 100 mM of either KNO_3 or choline chloride. 10 μl of the vesicles were incubated with 20 μl of media containing (final concentrations) 5 μM [^{3}H]uridine, 5 mM Tris-HCl (pH 7.4) and either 20 mM $NaNO_3$ plus 100 mM KNO_3 or 20 mM $NaNO_3$ plus 100 mM choline chloride or 120 mM choline chloride. Symbols have the following meanings: inward Na^+ and K^+ gradient (●, curve a); inward Na^+-gradient (○, curve b); inward Na^+ gradient with K^+ equilibrated (■, curve c); inward Na^+ gradient with outward K^+ gradient (□, curve d); choline chloride (▲, curve e).

was further increased when an outwardly directed K^+ gradient was present (Na^+: out > in; K^+: out < in; curve d).

Under Na^+-equilibrated conditions (Fig. 7), no net transport was observed in an outwardly directed K^+ gradient (Na^+: out = in; K^+: out < in). Also, an inwardly directed K^+ gradient with an outwardly directed Na^+ gradient (Na^+: out < in; K^+: out > in) was unable to increase uptake above the simple diffusion level (choline chloride: out = in). These two situations were therefore not plotted in Fig. 7. However, uridine uptake was elicited with either Na^+ or K^+ equilibrated on both sides of the membrane (Na^+: out = in; curve c, or K^+: out = in; curve d), and reached, the equilibrium concentration rapidly at 10 and 120 s for Na^+- and K^+-equilibrated membranes, respectively, with no overshoot observed (compared with over 5 min for simple diffusion). These results suggest that both Na^+ and K^+ can stimulate uridine transport

and the overshoot phenomenon is observed only if an inwardly directed gradient of either Na^+ or K^+ is imposed. No difference in uridine uptake was observed in vesicles equilibrated with only Na^+ (Na^+: out = in; curve c) and those equilibrated with both Na^+ and K^+ (Na^+: out = in; K^+: out = in, data not shown). As already described in Fig. 6, an overshoot induced by the presence of an inwardly directed Na^+ gradient can be greatly reduced when K^+ was present on the inside. It was also observed that when vesicles were equilibrated with Na^+ the amplitude of overshoot induced by K^+ gradient was reduced (Na^+: out = in; K^+: out > in; curve b; Fig. 7). These observations can best be explained by postulating the involvement of more than one transport system for uridine in renal brush-border membranes. The inhibition of K^+-dependent uridine influx by intravesicular Na^+ could be due to the rapid removal of intravesicular uridine via the Na^+-dependent transport system. Similarly, inhibition of Na^+-dependent uridine influx by intravesicular K^+ could be due to K^+-dependent efflux of intravesicular uridine.

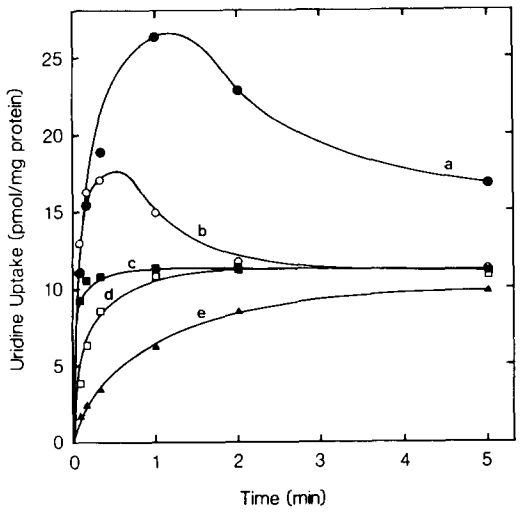

Fig. 7. Interactions between Na^+ and K^+ for uridine uptake by rat renal brush-border membrane vesicles. Vesicles were resuspended in media containing 300 mM mannitol, 5 mM Tris-HCl (pH 7.4), and either 20 mM $NaNO_3$ plus 100 mM KNO_3 or 20 mM $NaNO_3$ plus 100 mM choline chloride or 120 mM choline chloride. 10 μl of the vesicles were incubated with 20 μl of media containing (final concentrations) 5 μM [^{3}H]uridine, 5 mM Tris-HCl (pH 7.4) and either 20 mM $NaNO_3$ plus 100 mM KNO_3 or 20 mM $NaNO_3$ plus 100 mM choline chloride or 100 mM KNO_3 plus 20 mM choline chloride or 120 mM choline chloride. Eight combinations were generated and analysed for uridine uptake. Only the significant results have been plotted. The experiment was performed in the same day on the same preparation of vesicles. The symbols have the following meanings: inward K^+ gradient (●, curve a); inward K^+ gradient with Na^+ equilibrated (○, curve b); Na^+ equilibrated (■, curve c); K^+ equilibrated (□, curve d), choline chloride (▲, curve e).

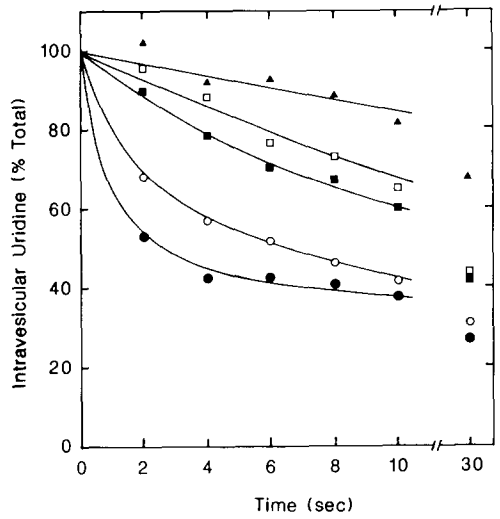

Fig. 8. Time-course of uridine efflux by rat renal brush-border membrane vesicles. Experimental conditions were as described in the text. Symbols have the following meanings: outward Na^+ gradient (●); Na^+ equilibrated (○); outward K^+ gradient (■); K^+ equilibrated (□); and choline chloride equilibrated (▲).

Time-course of uridine efflux

Efflux experiments were performed to provide further evidence that uridine transport is bidirectional and that it is the rapid removal of the intravesicular uridine by the presence of the intravesicular Na^+ or K^+ that caused the decreased in the magnitude of uridine overshoot observed in Figs. 6 and 7. A higher concentration of uridine (50 μM) was used in these studies to improve the signal-to-noise ratio. Fig. 8 shows that both Na^+ and K^+ (out = in) stimulated the efflux of intravesicular uridine, although Na^+ had a greater effect. When the cation gradient was oriented in to out, the rate of uridine efflux was further increased, although again Na^+ had a larger effect than K^+.

Effect of lithium and rubidium on uridine uptake

To investigate further the possible involvement of other monovalent cations in eliciting uridine transport, both $LiNO_3$ and $RbNO_3$ were used to generate an electrochemical gradient for uridine uptake. Fig. 9 shows that both Li^+ and Rb^+ were able to induce active transport of uridine, but were less potent than Na^+ and K^+ in producing an overshoot. It is possible that the active transport of uridine elicited by Li^+ and Rb^+ could be via the Na^+- and K^+-uridine cotransport systems, respectively. Lineweaver-Burks plot (Fig. 10) of Na^+-dependent uridine uptake rates versus sodium concentrations showed that Li^+-gradient (out > in) increased the slope of the regression line with no effect on the *y*-intercept, suggesting that Li^+ is acting as an apparent competitive inhibitor of Na^+-dependent uridine transport by competing with Na^+ on the same binding site. In contrast, Li^+ had little effect on the K^+-dependent system (data not shown). It is likely that the molecular size of Li^+ is too small to emulate K^+ in the K^+-dependent system; hence it was ineffective in competing with K^+ for the ligand-binding site. Similarly, Rb^+ only inhibited K^+-dependent uridine transport and not the Na^+-dependent system (data not shown).

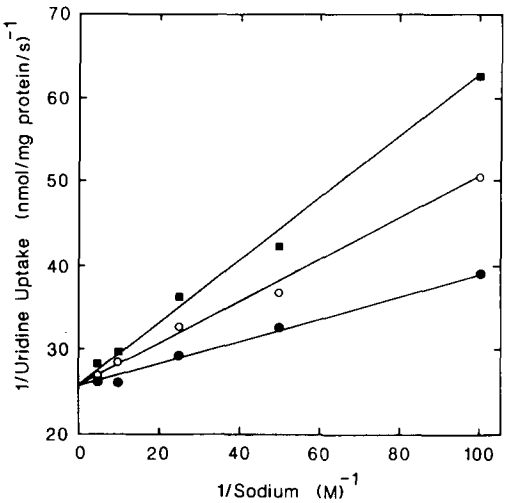

Fig. 10. Lineweaver-Burk plot of extravesicular Li^+ concentrations on Na^+-dependent uridine uptake by rat renal brush-border membrane vesicles. 10 μl of the vesicle suspension were incubated with 20 μl of media containing either (final concentrations) 200 mM $LiNO_3$ (■), 100 mM $LiNO_3$ plus 100 mM choline chloride (○) or 200 mM choline chloride (●), 5 μM [^{3}H]uridine, 5 mM Tris-HCl (pH 7.4) and 200 mM salt concentrations. Variable external Na^+ concentrations from 0 to 200 mM were obtained by substitution with choline chloride. Approximate initial rates of uridine uptake were taken at 2 s.

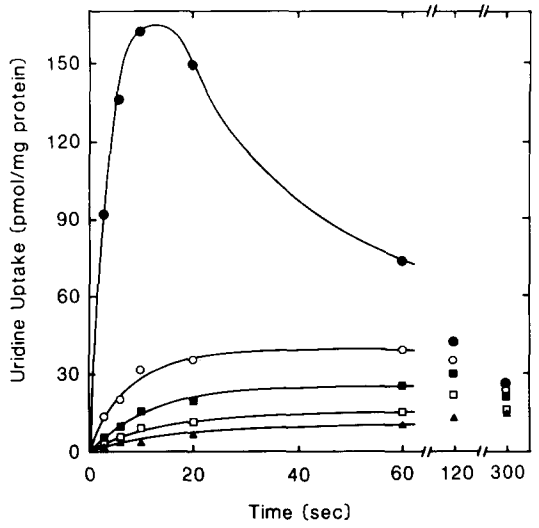

Fig. 9. Time-course of cation-dependent uridine transport by rat renal brush-border membrane vesicles. 10 μl of the vesicle suspension were incubated with 20 μl of media containing (final concentrations) 5 μM [^{3}H]uridine, 5 mM Tris-HCl (pH 7.4) and 100 mM of either $NaNO_3$ (●), KNO_3 (○), $LiNO_3$ (■), $RbNO_3$ (□), or choline chloride (▲).

Discussion

Intracellular metabolism of nucleosides often makes the characterization of the mechanisms of their transport across cell membranes difficult [13,14]. It has been demonstrated that adenosine is rapidly metabolized during uptake into renal brush-border membrane vesicles [16]. However, metabolism of uridine was not observed under the present experimental conditions. In this paper, we therefore did use uridine as the permeant to investigate nucleoside transport in rat renal brush-border membrane vesicles.

The present results demonstrate that in the presence of an Na^+ or K^+ gradient (out > in), uridine uptake showed a transient overshoot phenomenon, indicating active Na^+- and K^+-dependent transport of uridine (Fig. 2). Shunting of the driving force by equilibration of Na^+ and K^+ on

both sides of the membrane abolished the overshoot (Fig. 7). In addition, uridine was taken up into an osmotically active intravesicular space, efflux from which was blocked by phloridzin and by reducing the temperature. Therefore, an alternative explanation that the uridine overshoot phenomenon reflects cation dependent uridine binding to the vesicle membrane is not valid. Although evidence for an Na^+-dependent uptake of nucleosides by renal brush-border membrane vesicles [16–18], isolated intestinal epithelial cells [28], murine splenocytes [29] and cultured intestinal IEC-6 cells [30] has been documented, this is the first time to our knowledge that evidence for a K^+-dependent uptake of uridine has been presented. Our results also show that the K^+-dependent uptake of uridine cannot be explained due to the action of a membrane potential induced by Cl^-, as choline chloride failed to elicit increased uptake as compared to mannitol (Fig. 2). Thus, the above overshoot phenomena indicate that the rat renal brush-border membrane vesicle preparation is capable of catalyzing the concentrative uptake of uridine in the presence of inwardly directed transient electrochemical gradients formed by Na^+ and K^+.

One can predict that if Na^+- and K^+-uridine cotransport systems are electrogenic there will be a net transfer of positive charges across the membrane. Therefore any change in membrane potential will affect the transport process. The membrane potential dependency of uridine transport in Na^+- and K^+-gradient (out > in) conditions was analysed with different anions. Fig. 3A and 3B show the amplitude of the overshoot was increased by the use of more lipophilic anions. The reactive order of $NO_3^- > SCN^- > Cl^- > SO_4^{2-}$ observed is similar to that which would be predicted from the order of membrane diffusibility of these ions and the resultant reduction of the membrane potential [27] with the exception of the NO_3^-. These findings suggest that the transport of uridine in rat renal brush-border membrane vesicles is an electrogenic process.

The relationship between approximate initial rates of uridine uptake and extravesicular Na^+ or K^+ concentrations showed that there is a higher affinity for Na^+ than K^+ for uridine transport (K_{Na} 18 mM vs. K_K 280 mM, Fig. 5). It is possible, therefore, that either the ligand binding site on the uridine carrier has a different affinity for the two cations or, alternatively, there are two uridine transport systems, one requiring Na^+ and the other K^+. The latter suggestion is favoured by us and the evidence supporting the presence of two uridine transporters in rat renal brush-border membrane vesicles is discussed below.

Studies have shown that high external K^+ concentration can lead to an inhibition of Na^+-dependent transport processes [31–34]. The inhibitory effect of high external K^+ concentration appears to be due to the fact that K^+ is competing with Na^+ for the Na^+-dependent transport system. However, such inhibition was not observed in our system. Instead, when both Na^+ and K^+ were present outside the membrane, uridine uptake was stimulated. This finding rules out the possible competition between these ions for a common binding site on a single carrier. Also with vesicles adjusted to isoosmolarity with choline chloride, it appears that K^+ at the same concentration on both sides of the membrane could effectively reduce the Na^+-dependent accumulation of uridine. This could be due to the rapid removal of intravesicular uridine via the K^+-dependent system. Control experiments showed that in the presence of salt but absence of driving force (out = in), uridine equilibrated rapidly across the membrane (Fig. 7). This rapid equilibration of uridine across the membrane was further illustrated in efflux experiments (Fig. 8), and suggests that uridine transport is bidirectional and Na^+ and K^+ are mandatory for the transport process. Both Li^+ and Rb^+ were also able to elicit active transport of uridine (Fig. 9), and it would appear that Li^+ can substitute for Na^+ (Fig. 10) and Rb^+ for K^+ (data not shown).

Further evidence suggesting that these two uridine transport systems are separate entities comes from the studies on the activation of uridine transport by Na^+ and K^+. The curve for Na^+ activation of uridine transport was hyperbolic and conformed to simple saturation kinetics suggesting that the Na^+ : uridine coupling stoichiometry is 1 : 1 (Fig. 5). In contrast, the K^+ activation of uridine transport curve was sigmoidal, indicating that the K^+ : uridine coupling stoichiometry is likely to be greater than 1 : 1.

In conclusion, we have demonstrated that both

Na^+- and K^+-gradient (out > in) can provide the driving force for the active transport of uridine in rat renal brush-border membrane vesicles. The physiological significance of K^+-dependent transport of uridine into renal proximal tubule is not clear. However, it is possible that this system could be involved in an excretory process. Clearly, detailed kinetic analysis and the permeant specificity of these two active uridine transporters should be performed in the future to gain a more comprehensive view of active nucleoside transport in the kidney.

Acknowledgements

The authors thank Dr. D.D. Maenz for many helpful discussions. This work was supported by Alberta Heritage Foundation for Medical Research (AHFMR), Medical Research Council (Canada) and Medical Research Council (U.K.). C.W.L. is a research student of AHFMR.

References

1 Lynch, T.P., Jakobs, E.S., Paran, J.H. and Paterson, A.R.P. (1981) Cancer Res. 41, 3200–3204.
2 Smyth, J.F., Paine, R.M., Jackman, A.L., Harrap, K.R., Chassin, M.M., Adamson, R.H. and Johns, D.G. (1980) Cancer Chemother. Pharmacol. 5, 93–101.
3 Field, A.K., Davies, M.E., De Witt, C., Perry, H.C., Liou, R., Germershausen, J., Karkas, J.D., Ashton, W.T., Johnston, D.B.R. and Tolman, R.L. (1983) Proc. Natl. Acad. Sci. USA 80, 4139–4143.
4 El Kouni, M.H., Diop, D. and Sungman, C. (1983) Proc. Natl. Acad. Sci. USA 80, 6667–6670.
5 Grever, M.R., Siaw, M.F.E., Jacob, W.F., Neidhart, J.A., Miser, J.S., Coleman, M.S., Hutton, J.J. and Balcerzak, S.P. (1981) Blood 57, 406–417.
6 Mitchell, B.S., Koller, C.A. and Heyn, R. (1980) Blood 56, 556–559.
7 Fox, I.H. and Kelley, W.N. (1978) Annu. Rev. Biochem. 47, 655–686.
8 Bisel, H., Ansfield, F.J., Mason, J.H. and Wilson, W.L. (1970) Cancer Res. 30, 76–78.
9 Spielman, W.S. and Thompson, C.I. (1982) Am. J. Physiol. 242, F423–F435.
10 Spielman, W.S., Arend, L.J. and Forrest, J.N. (1987) in Topics and Perspective in Adenosine Research (Gerlach, E. and Becker, B., eds.), pp. 249–260, Springer-Verlag, Berlin.
11 Kutterch, J.F. and Nelson, J.A. (1982) Cancer Chemother. Pharmacol. 8, 221–229.
12 Berlin, R.D. and Oliver, J.M. (1975) Int. Rev. Cytol. 42, 287–336.
13 Paterson, A.R.P., Kolassa, N. and Cass, C.E. (1981) Pharmac. Ther. 12, 516–536.
14 Plagemann, P.G.W. and Wohlhueter, R.M. (1980) Curr. Top. Membr. Transp. 14, 225–330.
15 Young, J.D. and Jarvis, S.M. (1983) Biosci. Rep. 3, 309–322.
16 Le Hir, M. and Dubach, U.C. (1984) Pflueger Arch. 401, 58–63.
17 Le Hir, M. and Dubach, U.C. (1985) Pflueger Arch. 404, 238–243.
18 Le Hir, M. and Dubach, U.C. (1985) Eur. J. Clin. Invest. 15, 121–127.
19 Ernst, S.A. and Millis, J.W. (1980) J. Histochem. Cytochem. 28, 72–77.
20 Jarvis, S.M. (1987) in Topics and Perspective in Adenosine Research (Gerlach, E. and Becker, B., eds.), pp. 102–117, Springer-Verlag, Berlin.
21 Biber, J., Stieger, B., Haase, W. and Murer, H. (1981) Biochim. Biophys. Acta 647, 169–176.
22 Lansing, A.I., Belkhode, M.L., Lynch, W.E. and Lieberman, I. (1967) J. Biol. Chem. 242, 1772–1775.
23 Schwartz, A., Julius, J.C. and Harigaya, S. (1969) J. Pharmacol. Exp. Ther. 168, 31–41.
24 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
25 Aronson, P.S. (1978) J. Membr. Biol. 42, 81–98.
26 Toggenburger, G., Kessler, M., Rothstein, A., Semenza, G. and Tannenbaum, C. (1978) J. Membr. Biol. 40, 269–290.
27 Murer, H. and Hopfer, U. (1974) Proc. Natl. Acad. Sci. USA 71, 484–488.
28 Schwenk, M., Hegazy, E. and Lopez del Pino, V. (1984) Biochim. Biophys. Acta 805, 370–374.
29 Darnowski, J.W., Holdridge, C. and Handschumacher, R.E. (1987) Cancer Res. 47, 2614–2619.
30 Jakobs, E.S. and Paterson, A.R.P. (1986) Biochem. Biophys. Res. Commun. 140, 1028–1035.
31 Crane, R.K., Forstner, G. and Eichholz, A. (1965) Biochim. Biophys. Acta 109, 467–477.
32 Eddy, A.A., Mulcahy, M.F. and Thomson, P.J. (1967) Biochem. J. 103, 863–876.
33 Kipnis, D.M. and Parrish, J.E. (1965) Fed. Proc. 24, 1051–1059.
34 Nathans, D., Tapley, D.F. and Ross, J.E. (1960) Biochim. Biophys. Acta 41, 271–282.