Interaction of Nucleoside Analogues with the Sodium–Nucleoside Transport System in Brush Border Membrane Vesicles from Human Kidney

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Received January 16, 1992; accepted August 28, 1992

The therapeutic efficacy of nucleosides and nucleoside analogues as antitumor, antiviral, antiparasitic, and antiarrhythmic agents has been well documented. Pharmacokinetic studies suggest that many of these compounds are actively transported in the kidney. The goal of this study was to determine if therapeutically relevant nucleosides or analogues interact with the recently characterized Na^+-driven nucleoside transport system of the brush border membrane of the human kidney. Brush border membrane vesicles (BBMV) were prepared from human kidney by divalent cation precipitation and differential centrifugation. The initial Na^+-driven ^{3}H-uridine uptake into vesicles was determined by rapid filtration. The effect of several naturally occurring nucleosides (cytidine, thymidine, adenosine), a pyrimidine base (uracil), a nucleotide (UMP), and several synthetic nucleoside analogues [zidovudine (AZT), cytarabine (Ara-C), and dideoxycytidine (ddC)] on Na^+–uridine transport was determined. At a concentration of 100 μM the naturally occurring nucleosides, uracil, and UMP significantly inhibited Na^+–uridine transport, whereas the three synthetic nucleoside analogues did not. Adenosine competitively inhibited Na^+–uridine uptake with a K_i of 26.4 μM (determined by constructing a Dixon plot). These data suggest that naturally occurring nucleosides are substrates of the Na^+–nucleoside transport system in the renal brush border membrane, whereas synthetic nucleoside analogues with modifications on the ribose ring are not. The K_i of adenosine is higher than clinically observed concentrations and suggests that the system may play a physiologic role in the disposition of this nucleoside.

KEY WORDS: nucleoside; nucleoside transport; brush border membrane vesicles; sodium ion dependence; adenosine, (human kidney).

INTRODUCTION

The efficacy of nucleoside analogues and naturally occurring nucleosides as antitumor, antiviral, antiparasitic, and antiarrhythmic agents has been well documented. Because of their polar nature, nucleosides and many nucleoside analogues are transported across cellular membranes by specific transport systems. These transport systems mediate the initial absorption, the delivery toward a site of action, and the eventual excretion (in particular, via the kidney) of nucleosides and nucleoside analogues. Understanding the mechanisms which control transport across renal epithelia may allow manipulating the balance between reabsorption and excretion of these agents, which may decrease toxicity or facilitate nucleoside salvage.

Systems for nucleoside transport have been characterized in a variety of tissues and membranes and can be divided into two major categories: equilibrative (facilitated diffusion) and Na^+-dependent concentrative (energy dependent). Recently, Vijayalakshmi *et al.* (1) described two Na^+-dependent nucleoside transport systems in mouse intestinal epithelial cells. Formycin B, guanosine, and inosine were substrates of one system, whereas thymidine and cytidine were substrates of a separate transporter. Although the systems exhibit some overlapping specificities, in general, one system is pyrimidine selective and the other is purine selective. A similar pattern has been described in brush border membrane vesicles (BBMV)[3] from the rat and cow (2,3). Whether analogues of naturally occurring nucleosides follow this same pattern is not completely established.

Recent studies from this laboratory have demonstrated that there is a single Na^+-dependent system for nucleoside transport in the brush border membrane of the human kidney. Uridine, thymidine, adenosine, and guanosine, but not formycin B or inosine, interact with this system (4). The goal of this study was to determine if therapeutically relevant nucleosides or analogues interact with this recently characterized renal Na^+-dependent nucleoside transport system in the human. We studied the effect of several nucleosides and nucleoside analogues, a pyrimidine base, and a nucleotide on the Na^+-driven transport of ^{3}H-uridine in BBMV from the human kidney. In addition, we determined the K_i of adenosine in interacting with the Na^+–nucleoside transport system. We selected adenosine as a compound for further study because this nucleoside has become an important pharmacologic agent in the treatment of various cardiovascular disorders.

METHODS

When the Organ/Tissue Transplant Services at the University of California, San Francisco, obtained a kidney which was subsequently deemed unsuitable to transplant, the tissue was denoted for research studies. BBMV were prepared by divalent (Mg^{2+}) cation precipitation and differential centrifugation, modified in our laboratory (5–7). The final pellet of BBMV was resuspended in buffer (pH 7.4) consisting of 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and 150 mM KCl.

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 BBMV was monitored by noting the enrichment of enzymes known to be associated with this cellular membrane in comparison to enrichment of other enzymes known to be markers of other cellular membranes (8,9). The activities of markers of the brush border membrane, γ-glutamyl transferase and malt-

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[3] Abbreviations used: AZT, zidovudine; Ara-C, cytarabine; ddC, dideoxycytidine; UMP, uridine monophosphate; BBMV, brush border membrane vesicles.

0724-8741/93/0300-0423$07.00/0

ase, were enhanced approximately 7- and 10-fold, respectively (ratio of the activity in the final pellet to the activity in the initial homogenate). In contrast, the activity of Na^+–K^+ ATPase, a marker of the basolateral membrane, was not enhanced (11).

The uptake of ^{3}H-uridine (27 Ci/mmol) was measured at room temperature by an inhibitor-stop filtration technique as described previously (4,9). A 5-sec incubation period was selected since at this time Na^+-dependent uptake of uridine is in the linear range (4). A 10-μL aliquot of BBMV (10–20 mg/mL protein) was added to 40 μL of reaction medium containing ^{3}H-uridine (radiolabeled plus unlabeled uridine, 5 μ*M*) in NaCl (150 m*M*)–HEPES (10 m*M*) buffer, pH 7.4, in the presence or absence of one of the test compounds (100 μ*M*). After a 5-sec incubation, the uptake was stopped by adding 3 mL of ice-cold stop solution (150 m*M* NaCl, 10 m*M* HEPES, and 1 m*M* phloridzin, pH 7.4) and filtered under vacuum through a membrane filter (0.3 mm, 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 was measured by liquid scintillation counting (LS 1801, Beckman Instruments). For each set of experiments, an estimate of the radioactivity associated with the filter itself was made by conducting control experiments under identical conditions, but without the BBMV. This radioactivity was subtracted from the radioactivity associated with the filter plus BBMV to obtain the actual uptake into the BBMV. In general, the radioactivity associated with the filter accounted for less than 20% of the total uptake.

Data points were determined in triplicate. Experiments were repeated at least three times using vesicles prepared from different kidneys. Data are expressed as the mean ± SE. Data were analyzed by multiple regression analysis (10).

To determine the K_i of adenosine, the uptake of uridine at two concentrations (2.8 and 10.3 μ*M*) was measured in the absence and the presence of various concentrations of adenosine. By constructing a Dixon plot (12), the mechanism of interaction was noted and the K_i was obtained.

RESULTS AND DISCUSSION

The structural specificity of the nucleoside transporter was investigated by measuring the initial rate of the Na^+-driven uptake of ^{3}H-uridine in the absence and presence of a series of compounds (Fig. 1). Consistent with transport via a Na^+-driven system, uridine uptake at 5 sec was 4.75 ± 1.2 pmol/mg protein in the presence of Na^+ and 1.78 ± 0.09 pmol/mg protein in the absence of Na^+. Previously, we observed that unlabeled uridine (100 μ*M*) inhibited Na^+-dependent ^{3}H-uridine uptake to approximately 22% of control values at 5 sec (4). Although in several species separate purine- and pyrimidine-selective transport systems for nucleosides have been identified, recent data from this laboratory suggest that a single Na^+-driven nucleoside transport system exists in the human renal brush border membrane (4). With the exception that guanosine is also a substrate, the system appears similar to the previously described pyrimidine-selective transport system, which transports pyrimidine nucleosides as well as adenosine (1). Consistent with our

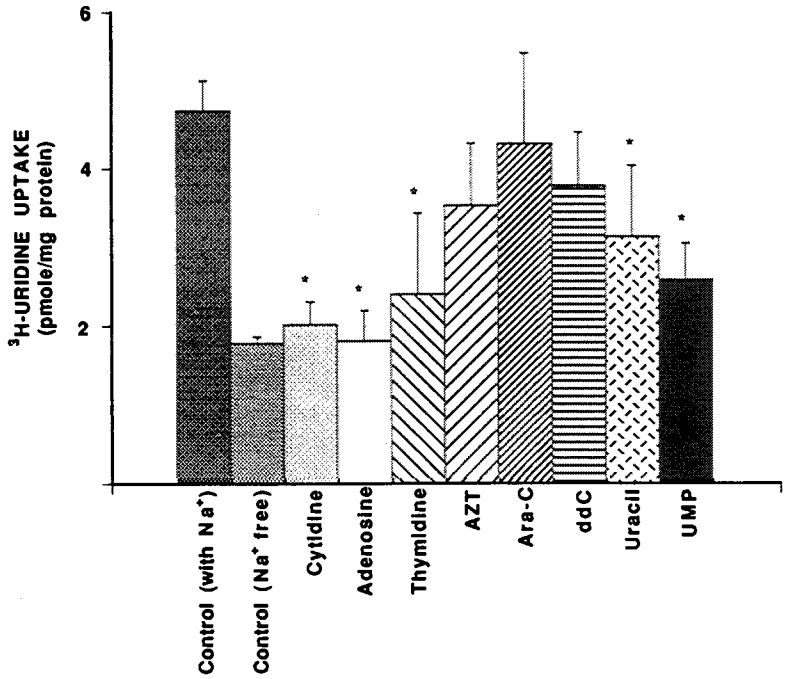
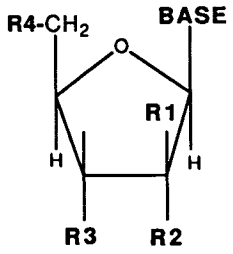


Fig. 1. Effect of nucleosides (adenosine, thymidine, cytidine) and nucleoside analogues (AZT, ddC, Ara-C), a pyrimidine base (uracil), and a nucleotide (UMP) on the Na^+-driven uptake of ^{3}H-uridine (5 μ*M*) at 5 sec in BBMV from human kidney. Data were obtained in the presence of a 100 μ*M* concentration of each compound. Controls were obtained in the presence and absence of Na^+. Each column represents the mean ± SE of data from three experiments. The asterisk indicates statistical significance ($P \leq 0.05$) from the control (with Na^+).

previous study in human renal BBMV (4), in this study we observed that, at a concentration of 100 μ*M*, the naturally occurring pyrimidine nucleosides (thymidine and cytidine), as well as the purine nucleoside (adenosine), significantly inhibited the Na^+-driven uptake of ^{3}H-uridine. The data suggest that both ribo (cytidine and adenosine)- and deoxyribo (thymidine)-nucleosides interact with the Na^+–nucleoside cotransporter in the brush border membrane of the human kidney. Ribo and deoxyribonucleosides have been shown previously to interact with renal Na^+–nucleoside cotransport systems (2,4,13).

Our data suggest that a ribose moiety is important, although not an absolute, requirement for nucleoside transport. That is, uracil (100 μ*M*), the nucleobase component of uridine, significantly inhibited the initial Na^+-driven uptake of ^{3}H-uridine, but to a lesser extent than did the naturally occurring nucleosides. Although a ribose (or deoxyribose) moiety is not an absolute requirement for transport, we observed that certain modifications on the ribose ring rendered compounds inactive in interacting with the Na^+-nucleoside cotransporter. At a concentration well above therapeutic concentrations, none of the synthetic nucleoside analogues [zidovudine (AZT), cytarabine (Ara-C), dideoxycytidine (ddC)] inhibited Na^+-driven uridine uptake. Each of these analogues has modifications on the 2′ or 3′ positions of the ribose ring (Fig. 2). In AZT, the azide group in place of a hydroxyl at the 3′ position seems to have a critical structural impact on the ability of this compound to interact with the human renal Na^+–nucleoside transporter. This finding is consistent with recent data obtained in bovine renal BBMV for AZT (3). Previous data from this laboratory demonstrating that 3′-deoxyuridine did not inhibit Na^+–uridine uptake into BBMV from human kidney (4) and data in rat macrophages for 2′,3′-dideoxyadenosine (14) also support the general conclusion that a hydroxyl group at the 3′ position is required. However, one notable exception to the requirement of a 3′-hydroxyl group is that in our previous study, 2′,3′-dideoxyuridine (100 μ*M*) inhibited Na^+–uridine uptake (4). Possibly, the dideoxy derivative of uridine is able to inhibit ^{3}H-uridine uptake, but the dideoxy derivative of other nucleosides cannot. The ability to interact with the Na^+–nucleoside cotransport system of the human renal brush border membrane also is impaired by adding a hydroxyl group at the 2′ position *trans* to the hydroxyl at the 3′ carbon (arabinose ring rather than ribose, Ara-C) or by removing a hydroxyl from both the 2′ and the 3′ positions of the ribose (ddC) (Fig. 1).



	R1	R2	R3	R4
AZT	H	H	N_3	OH
ddC	H	H	H	OH
Ara-C	OH	H	OH	OH
UMP	H	OH	OH	PO_4

Fig. 2. Structure of the nucleoside. The substitutions on the ribose ring for the nucleoside analogues and for UMP are noted.

The Na^+-driven nucleoside transport system in the brush border membrane of the human kidney functions in the active reabsorption of nucleosides. The finding that the synthetic nucleoside analogues Ara-C, ddC, and AZT did not interact with the system is consistent with clinical pharmacokinetic studies which suggest that these compounds are not actively reabsorbed (15,16).

Previously, adenosine was shown to be actively reabsorbed in humans who have adenosine deaminase deficiency and in humans in whom adenosine deaminase activity was pharmacologically suppressed with deoxyformycin (17). The data in the present study suggest that the mechanism by which adenosine may be actively reabsorbed in humans is via interaction with the Na^+-driven nucleoside transport system (Fig. 3). Importantly, adenosine competitively inhibited Na^+–uridine uptake with a K_i of 26.4 μ*M*. This value is considerably higher than the plasma concentrations measured in humans (0.2 μ*M*) (16), suggesting that at physiologic adenosine levels, the system for reabsorption across the renal brush border membrane would not be saturated and therefore would function as a highly efficient salvage pathway. Similarly, at these low plasma concentrations, adenosine would not inhibit the reabsorption of other nucleosides which share the same Na^+-driven transporter.

In summary, this study demonstrates that the naturally occurring nucleosides (cytidine, thymidine, and adenosine) as well as the nucleobase (uracil) and the nucleotide (UMP) inhibit Na^+-driven uridine transport in human renal BBMV. The data suggest that these compounds are substrates for the Na^+-driven nucleoside transport system. Synthetic nucleosides including AZT, ddC, and Ara-C do not interact with the system. The interaction of adenosine with the concen-

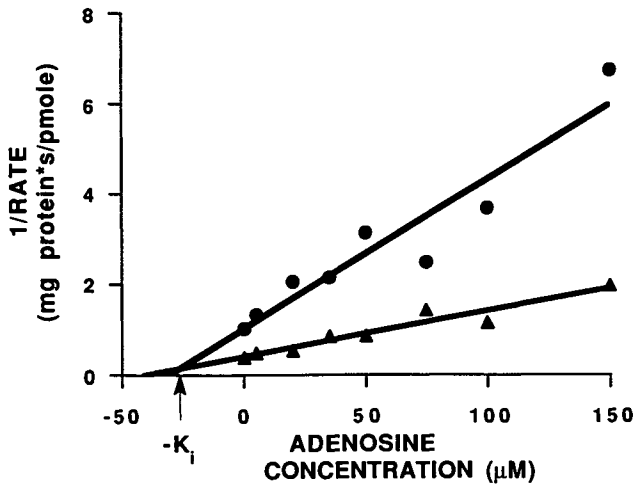


Fig. 3. Dixon plot: reciprocal of the rate of ^{3}H-uridine transport at 5 sec as a function of the concentration of adenosine. The concentration of uridine (labeled plus unlabeled) was 2.8 μ*M* (●) and 10.3 μ*M* (▲). Each data point represents the mean of triplicate determinations from a single experiment in BBMV from human kidney. The K_i determined from this experiment is 26.4 μ*M*.

trative, Na^+-driven nucleoside transport system may be responsible for the active reabsorption of adenosine in humans.

ACKNOWLEDGMENTS

We acknowledge David F. Teitel, M.D., for his valuable suggestions. We also acknowledge the Organ/Tissue Transplant Service at the University of California, San Francisco, for the generous donation of human kidneys.

This work was supported by grants from the National Institutes of Health (GM 36780 and GM 26691).

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