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Transport of the Antiviral Nucleoside Analogs 3′-Azido-3′-deoxythymidine and 2′,3′-Dideoxycytidine by a Recombinant Nucleoside Transporter (rCNT) Expressed in *Xenopus laevis* Oocytes

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SUMMARY

Expression screening in *Xenopus* oocytes has been used to isolate a cDNA from rat jejunal epithelium encoding an intestinal/kidney Na^+-dependent nucleoside transporter protein named rCNT1 [*J. Biol. Chem.* 269:17757–17760 (1994)]. rCNT1 is predicted to have 648 amino acid residues (relative molecular mass, 71,000) with 14 potential transmembrane domains and belongs to a new family of transporter proteins. Recombinant rCNT1 transports physiological pyrimidine nucleosides and adenosine. In the current investigation, functional expression in *Xenopus* oocytes was used to determine whether recombinant rCNT1 also transports antiviral pyrimidine nucleoside analogs. The recombinant protein mediated Na^+-dependent transport of both 3′-azido-3′-deoxythymidine (AZT) and 2′,3′-dideoxycytidine (ddC). Apparent K_m values of ′0.5 mM were obtained for both [^{3}H]AZT and [^{3}H]ddC influx compared with 37 μM for [^{3}H]uridine influx, with V_{max}/K_m ratios of 0.048, 0.039, and 0.57 for AZT, ddC, and uridine, respectively. Extracellular AZT and ddC stimulated rCNT1-mediated efflux of [^{3}H]uridine from preloaded oocytes. These experiments provide direct evidence for Na^+-dependent transport of AZT and ddC and suggest that members of the cNT family may be involved in the intestinal absorption and renal handling of pyrimidine nucleoside analogs used to treat acquired immune deficiency syndrome.

Mammalian cells transport physiological nucleosides and many nucleoside antimetabolites by both active and equilibrative NTs. Equilibrative NTs are widely distributed and can be divided into two classes (*es* and *ei*) on the basis of their sensitivity (*es*) or resistance (*ei*) to inhibition by nitrobenzylthioinosine (6-[(4-nitrobenzyl)thio]-9-β-D-ribofuranosylpurine (1). Active, Na^+-linked NTs were first detected in intestinal and renal epithelia but are also expressed in many different mammalian tissues and cell types, including choroid plexus, liver, lymphocytes, splenocytes, macrophages, and murine and human leukemic cells (1–12). Those so far characterized can be divided into three principal classes on the basis of their substrate specificity. NTs designated N1 or *cif* are generally purine specific, although they also transport uridine. NTs designated N2 or *cit* are generally pyrimidine specific, although they also transport adenosine. The third class of NTs, designated N3 or *cib*, have the ability to transport a wide range of both purine and pyrimidine nucleosides. A human kidney N2/*cit*-type process that transports pyrimidine nucleosides, adenosine, and guanosine has been given the designation N4 (13), although it otherwise exhibits the characteristics of an N2/*cit* process.

The pyrimidine nucleoside analog AZT (zidovudine) is used widely in the treatment of AIDS (14). The drug, administered orally, is absorbed efficiently from the gastrointestinal tract by unknown mechanism(s) (15–18) and is excreted in the urine either unchanged or as the glucuronide conjugate (19). On entry into cells, AZT is phosphorylated to AZTTP (20, 21) and incorporated into viral DNA by HIV reverse transcriptase (20, 22). Its unreactive 3′-azido group terminates DNA chain elongation by preventing formation of additional 5′-3′-phosphodiester linkages, thereby inhibiting viral DNA replication. AZT treatment delays onset of early symptoms of HIV infection and prolongs survival of HIV-infected patients (23–25).

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ABBREVIATIONS: AIDS, acquired immune deficiency syndrome; HIV, human immunodeficiency virus; NT, nucleoside transporter; AZT, 3′-azido-3′-deoxythymidine; ddC, 2′,3′-dideoxycytidine; ddI, 2′,3′-dideoxyinosine; AZTTP, AZT triphosphate; MBM, modified Barth's medium; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

Most studies of AZT membrane permeability have focused on nonepithelial cells. AZT is not transported by equilibrative NTs and has been reported to enter human erythrocytes, lymphocytes, macrophages, and bone marrow progenitor cells, mainly by passive diffusion across the lipid bilayer of the plasma membrane (26–28). The rate of entry of AZT into human erythrocytes is much less (<2%) than that of thymidine (26), and achievable intracellular concentrations of AZTTP in human immune effector cells *in vitro* are much less than the K_m of AZTTP for HIV reverse transcriptase (28). It is likely, therefore, that slow entry of AZT into HIV-infected cells reduces its therapeutic effectiveness.

Two other nucleoside analogs, ddC and ddI, also have application in the treatment of HIV infection (14). In contrast to AZT, ddC and ddI are low affinity equilibrative NT substrates (29–31), and some ddI transport may also occur via nucleobase carriers (31). However, both ddC and ddI are less hydrophobic than AZT and enter human erythrocytes, macrophages, and lymphocytes more slowly than AZT (26–31). Achievable intracellular concentrations of ddC triphosphate and ddI triphosphate in lymphocytes are correspondingly lower than those obtained for AZTTP (28). Therefore, a low rate of transmembrane passage compromises the therapeutic effectiveness of each of the currently available antiviral nucleoside analogs.

We used expression screening in *Xenopus* oocytes to isolate a cDNA from rat jejunal [1]epithelium encoding a pyrimidine-selective (N2/*cit*) Na^+-dependent NT called rCNT1[1] (32). The cDNA sequence of rCNT1 predicts a protein of 648 amino acids (relative molecular mass, 71,000) with 14 potential transmembrane domains. rCNT1, which is also expressed in kidney, is the first mammalian representative of a new transporter gene family that includes the NUPC proton/nucleoside symporter from *Escherichia coli* (33). rCNT1 and NUPC are structurally unrelated to a putative Na^+-linked NT (SNST1) with high homology to the rabbit intestinal Na^+-dependent glucose transporter SGLT1 (34). The conclusion that SNST1 is an NT protein was based on expression studies in *Xenopus* oocytes that demonstrated low levels of apparent N3/*cib* transport activity (34). Attempts to achieve higher levels of SNST1 expression in various heterologous expression systems have been disappointing, and the presence of the SNST1 protein in renal epithelia has not been demonstrated (35). Thus, the role of SNST1 in nucleoside physiology is uncertain. Recently, a cDNA encoding a second mammalian NT (SPNT) belonging to the rCNT1/NUPC family was isolated from rat liver (36) and, subsequently, from rat jejunum[2]. This protein (rCNT2 in our nomenclature) mediates purine-selective N1/*cif* transport activity in oocytes and is predicted to have 662 amino acid residues, 64% of which are identical to those of rCNT1.

In our original description of the rCNT1 cDNA (32), we described results of a limited series of functional expression experiments in *Xenopus* oocytes that demonstrated that recombinant rCNT1 also transports antiviral pyrimidine nucleoside analogs. The objective of the current study was to undertake a detailed analysis of this phenomenon. We used a variety of criteria, including cation dependence, saturability, *cis*-inhibition, and *trans*-acceleration, to confirm that rCNT1 mediates Na^+-dependent transport of both AZT and ddC and to define the kinetic characteristics of that transport. Our results suggest that transporter-mediated processes may be involved in the intestinal absorption and renal handling of pyrimidine nucleoside analogs used to treat AIDS.

[1] Previously designated as $cNT1_{rat}$.

Materials and Methods

***In vitro* transcription.** Plasmid pQQH1, containing a 2.4-kb insert encoding rCNT1 in the vector pGEM-3Z (Promega, Madison, WI), was isolated by expression selection from a rat jejunal cDNA library (32). Plasmid pAN1, containing a 2.6-kb insert encoding rCNT2, was obtained by reverse transcriptase-polymerase chain reaction amplification of rat jejunal total RNA using primers flanking the SPNT open reading frame (36) and ligation of the product into the vector pGEM-T (Promega).[2] pQQH1 and pAN1 were linearized with *Xba*1 and *Sal*1, respectively, and transcribed with T7 RNA polymerase in the presence of the m^7GpppG cap (Ambion, Austin, TX) using the MEGAscript (Ambion) transcription system.

Preparation of stage VI *Xenopus* oocytes. Healthy stage VI oocytes of *Xenopus laevis*, treated with collagenase to remove follicular layers (11), were maintained at 18° in MBM for 24 hr until injection with 10 nl of rCNT1 or rCNT2 RNA transcript (1 ng/nl) or 10 nl of water. Injected oocytes were incubated for 3 days at 18° with a daily change of MBM before the assay of transport activity. We established that 3 days gives maximum expression of rCNT1 transport activity. The presence of recombinant rCNT1 protein in 3-day postinjection oocyte membranes has been demonstrated by Western analysis with antipeptide cNT1-specific rabbit polyclonal antibodies.[3]

Transport assays. The uptake of AZT, ddC, and uridine by oocytes injected with rCNT1 RNA transcript, rCNT2 transcript, or water was traced with [*methyl*-^{3}H]AZT, [5,6-^{3}H]ddC, or [5,6-^{3}H]uridine (Moravek Biochemicals, Brea, CA) (20 μCi/ml), respectively. Assays were performed at 20° on groups of 10–12 oocytes in transport buffer (0.2 ml) containing either 100 mM NaCl or 100 mM choline chloride and 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM HEPES, pH 7.5 (32). At the end of the incubation, extracellular radioactivity was removed by six rapid washes in the appropriate ice-cold transport buffer. Control experiments, in which oocytes were mixed with [^{3}H]uridine in ice-cold transport buffer and immediately washed, demonstrated that the six-wash protocol removed all detectable extracellular radioactivity. Individual oocytes were dissolved in 0.5 ml of 5% (w/v) sodium dodecyl sulfate for quantification of oocyte-associated ^{3}H by liquid scintillation counting (LS 6000 IC; Beckman Instruments, Mississauga, Ontario, Canada). In competition experiments, nonradioactive nucleosides and nucleoside analogs (Sigma Chemical, St. Louis, MO) were added to oocytes at the same time as the ^{3}H permeate. In experiments to test the effect of probenecid on transport activity, oocytes were preincubated with the drug for 1 hr at 20° before the addition of ^{3}H permeate. Initial rates of uridine uptake were determined using a 1-min incubation period (32). Initial rates of AZT and ddC uptake were measured using a longer incubation period of 10 min (see Results).

For efflux studies, groups of 20 rCNT1 RNA transcript-injected oocytes were preloaded with 10 μM [^{3}H]uridine (20 μCi/ml) in 0.2 ml of NaCl transport buffer at 20° for 30 min, followed by six rapid ice-cold washes in NaCl transport buffer to remove extracellular [^{3}H]. One group of 20 oocytes was processed to determine the time 0 [^{3}H]uridine (typically, 50–60 pmol/oocyte) (32). The remaining groups of oocytes were each resuspended in 1 ml of NaCl transport buffer (with or without 1 mM or 5 mM nonradioactive nucleoside/nucleoside analog) at 20° to initiate efflux. At predetermined time

[2] S. Y. M. Yao, A. M. L. Ng, M. W. L. Ritzel, C. E. Cass, and J. D. Young. Transport of adenosine by recombinant N1/cif and NZ/cit sodium nucleoside cotransporters from rat jejunum expressed in *Xenopus* oocytes, submitted for publication.

[3] S. R. Hamilton and S. Y. M. Yao, unpublished observations.

intervals, duplicate 5-μl samples of transport buffer were added to 0.50-ml portions of 5% sodium dodecyl sulfate, and ^3H was counted as described above for uptake studies. It has been established that uridine is only slowly metabolized by oocytes (29). [^3H]Uridine composed the majority (77%) of intracellular ^3H in oocytes loaded with 10 μM extracellular [^3H]uridine for 30 min at 20° (time 0 in the efflux experiments), and of the remaining 23%, 22% was in UMP/UDP/UTP (and therefore unlikely to contribute to ^3H efflux) and only 1% was uracil.

Data analysis. Results for uptake experiments are mean ± standard error for 10–12 individual oocytes. Kinetic constants (apparent K_m and V_{max} values) for AZT and ddC influx were determined by nonlinear regression analysis (Enzfitter, Elsevier-Biosoft, Cambridge, UK). Data for efflux experiments are presented as plots of [^3H]uridine efflux (percent) versus time for groups of 20 oocytes. Each experiment was performed at least twice on different batches of oocytes.

Results

Inhibition of uridine influx by nucleoside analogs. It was reported that AZT and ddC at a concentration of 100 μM had no effect on Na^+-dependent uridine influx (5 μM) in brush border membrane vesicles from human kidney (13), whereas another study found that at the higher concentration of 1 mM, AZT significantly inhibited Na^+-dependent uridine influx (0.2 μM) in *Xenopus* oocytes injected with rabbit intestinal mRNA (37). In contrast, the same concentration of ddC had no effect on thymidine influx (50 μM) in oocytes injected with rabbit choroid plexus mRNA (system N3) (12). On the other hand, 1 mM ddC inhibited uridine influx (0.6 μM) mediated by the N3-type Na^+-dependent NT, SNST1 (34).

Table 1 (experiment 1) shows the effects of 1 mM and 5 mM AZT and ddC on the initial rate of uridine influx (10 μM, 1 min at 20°) in *Xenopus* oocytes injected with rCNT1 RNA transcript. Uridine uptake was reduced by 83% and 75% of control fluxes in the presence of 5 mM AZT and ddC, respectively, and by 55% and 52%, respectively, when the analog concentrations were reduced to 1 mM. Consistent with the lack of inhibition of rCNT1-mediated uridine and thymidine transport by inosine (32), 5 mM ddI inhibited cNT1-mediated uridine uptake by only 8%, and 1 mM ddI had no effect (Table 1, experiment 2).

Uptake of AZT and ddC. The finding that AZT and ddC inhibited rCNT1-mediated uridine influx (Table 1, experiment 1) did not in itself establish that these analogs are permeates of recombinant rCNT1. For example, although AZT is not itself a permeate of the human equilibrative NTs, it inhibited transport of 1 μM thymidine, with an IC_{50} of 1 mM (26). We therefore examined uptake of radioactive AZT and ddC uptake by RNA transcript-injected and water-injected oocytes (Fig. 1, A and B). Influx of both [^3H]AZT and [^3H]ddC (10 μM, 20°) into the RNA-injected oocytes was relatively rapid and Na^+ dependent, demonstrating substantial rCNT1-mediated transport of both analogs. After 10 min, uptake values for RNA-injected oocytes were 2.29 ± 0.13 and 0.87 ± 0.14 pmol/oocyte for AZT and ddC, respectively, compared with values for water-injected oocytes of 0.48 ± 0.03 and 0.010 ± 0.002 pmol/oocyte, respectively. Ratios for rCNT1-mediated uptake to basal uptake rates were therefore 3.8- and 66-fold for AZT and ddC, respectively. AZT had a 4.8-fold higher basal uptake rate compared with that of ddC, probably because of its greater lipophilicity (26). Uptake of AZT and ddC by RNA transcript-injected and water-injected oocytes was approximately linear during this time interval

TABLE 1

Effects of nucleoside analogs on rCNT1-mediated uridine influx

Oocytes injected with either 10 nl of rCNT1 RNA transcript (1 ng/nl) or 10 nl of water were incubated for 3 days at 18°c in MBM. Uridine influx (10 μM, 20°c, 1 min flux) was determined in NaCl transport buffer. Each value is the mean ± standard error of 10–12 oocytes. Experiments 1 and 2 were performed on different occasions using oocytes from two different frogs.

Inhibitor	Uridine uptake	
	pmol/oocyte · min⁻¹	%
Experiment 1		
Control	11.30 ± 0.68	100 ± 6.8
AZT (1 mM)	5.09 ± 0.68	45 ± 2.7
AZT (5 mM)	1.92 ± 0.18	17 ± 1.6
ddC (1 mM)	5.39 ± 0.27	48 ± 2.4
ddC (5 mM)	2.81 ± 0.08	25 ± 0.7
Experiment 2		
Control	5.80 ± 0.21	100 ± 3.6
ddI (1 mM)	5.65 ± 0.38	97 ± 6.6
ddI (5 mM)	5.33 ± 0.24	92 ± 4.1

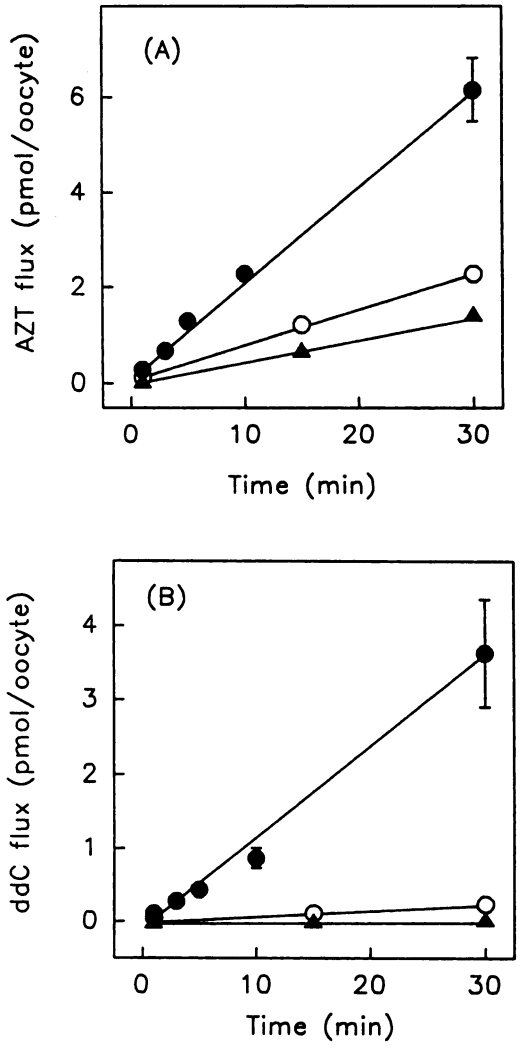

Fig. 1. Time course of AZT (A) and ddC uptake (B) by *Xenopus* oocytes. Oocytes injected with either 10 nl of water alone (▲) or 10 nl of water containing 10 ng of rCNT1 RNA transcript (●, ○) were incubated for 3 days at 18° in MBM. Fluxes (10 μM, 20°) were determined in transport buffer containing 100 mM NaCl (●, ▲) or 100 mM choline chloride (○). Each value is the mean ± standard error of 10–12 oocytes.

(10 min) and thus was used as the incubation period for subsequent initial rate measurements of AZT and ddC uptake.

In contrast to rCNT1, recombinant purine-selective rCNT2 did not transport AZT or ddC (10 μM, 20°) when expressed in oocytes under the same experimental conditions; in a representative experiment, influx of AZT was 0.37 ± 0.02 and 0.39 ± 0.02 pmol/oocyte/10 min in oocytes injected with rCNT2 RNA transcript or water, respectively, and 0.031 ± 0.003 and 0.026 ± 0.006 pmol/oocyte/10 min, respectively, for ddC.

Concentration dependence of AZT and ddC influx. Fig. 2, A and B, shows the concentration dependence of AZT and ddC influx in RNA transcript- and water-injected oocytes measured over the concentration range of 2.5 μM to 1 mM. rCNT1-mediated influx of AZT and ddC (uptake in RNA-injected oocytes minus uptake in water-injected oocytes) was saturable and conformed to simple Michaelis-Menten kinetics. Three independent experiments gave mean ± standard error apparent K_m values of 549 ± 98 μM and 503 ± 35 μM for AZT and ddC, respectively (Table 2). The corresponding V_{max} values were 26.4 ± 6.7 and 19.8 ± 4.6 pmol/oocyte/min, respectively. In contrast, influx of AZT and ddC in water-injected oocytes was approximately linear over the concentration range studied, indicating little or no endogenous mediated transport of AZT and ddC in oocytes. As was previously shown for uridine (32), influx of AZT and ddC into RNA-injected oocytes under Na^+-free conditions was (i) approximately linear with concentration and (ii) greater than AZT and ddC influx in water-injected oocytes. This difference represents uncoupled AZT and ddC transport (slippage) by the recombinant transporter.

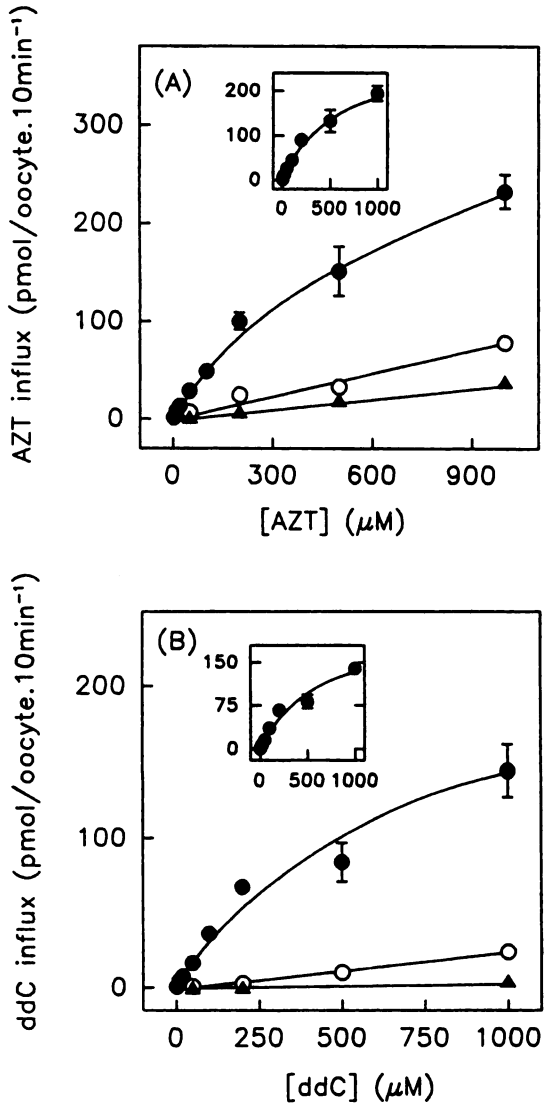

Fig. 2. Concentration dependence of rCNT1-mediated AZT (A) and ddC (B) influx. Oocytes injected with either 10 nl of water alone (▲) or 10 nl of water containing 10 ng of rCNT1 RNA transcript (●, ○) were incubated for 3 days at 18° in MBM. Influx of AZT and ddC (10 μM to 1 mM, 20°, 10 min flux) was determined in transport buffer containing 100 mM NaCl (●, ▲) or 100 mM choline chloride (○). Each value is the mean ± standard error of 10–12 oocytes. *Insets*, rCNT1-mediated uptake of AZT and ddC, respectively (influx in RNA transcript-injected oocytes in NaCl transport buffer minus influx in water-injected cells). Mean apparent K_m and V_{max} values for three independent experiments, determined by nonlinear regression analysis (Enzfitter) are presented in Table 2.

Inhibition experiments. rCNT1 is selective for physiological pyrimidine nucleosides and adenosine (29). Different nonradioactive nucleosides (1 mM) were tested for their abilities to inhibit rCNT1-mediated influx of [^{3}H]AZT and [^{3}H]ddC (10 μM, 10 min at 20°) by RNA-injected oocytes (Fig. 3, A and B). As shown in Fig. 3A, rCNT1-mediated influx of [^{3}H]AZT was strongly inhibited by thymidine (94% inhibition), cytidine (87%), uridine (89%), and adenosine (88%) but not by inosine or guanosine. A similar inhibition pattern was obtained for ddC influx (Fig. 3B).

Efflux experiments. The ability of extracellular nonradioactive AZT and ddC to cause *trans*-acceleration of rCNT1-mediated [^{3}H]uridine efflux was assessed (Fig. 4). Oocytes expressing the recombinant rCNT1 transporter were preloaded with 10 μM [^{3}H]uridine as described in Materials and Methods under conditions where the majority of the accumulated intracellular radioactivity remained as unmetabolized uridine. Release of this internalized [^{3}H]uridine from oocytes suspended in NaCl transport buffer was then measured under various conditions, producing the efflux time courses shown in Fig. 4. Efflux of intracellular [^{3}H]uridine against the Na^+ electrochemical gradient was slow (1% in 10 min). The addition of 1 mM uridine to the extracellular medium increased uridine efflux 14-fold, and the addition of 5 mM AZT and ddC stimulated efflux by 11- and 7-fold, respectively. Tested as a negative control, the purine nucleoside guanosine (1 mM) did not significantly stimulate uridine efflux (data not shown). That AZT and ddC caused *trans*-acceleration of rCNT1-mediated uridine efflux indicated that both antiviral nucleoside analogs function as rCNT1 permeates.

Effect of probenecid on recombinant rCNT1. Probenecid, an organic anion transport inhibitor that has been

TABLE 2

Kinetic parameters of rCNT1-mediated AZT, ddC, and uridine transport

Kinetic parameters for AZT and ddC influx (mean ± standard error) were calculated from three independent experiments using different preparations of oocytes. Apparent K_m and V_{max} values for rCNT1-mediated uridine transport were calculated from the data presented in Fig. 1a of Ref. 32.

Substrate	K_m	V_{max}	V_{max}/K_m
	μM	pmol/oocyte · min^{-1}	
AZT	549 ± 98	26.4 ± 6.6	0.048
ddC	503 ± 35	19.8 ± 4.6	0.039
Uridine	37 ± 7	20.8 ± 1.1	0.570

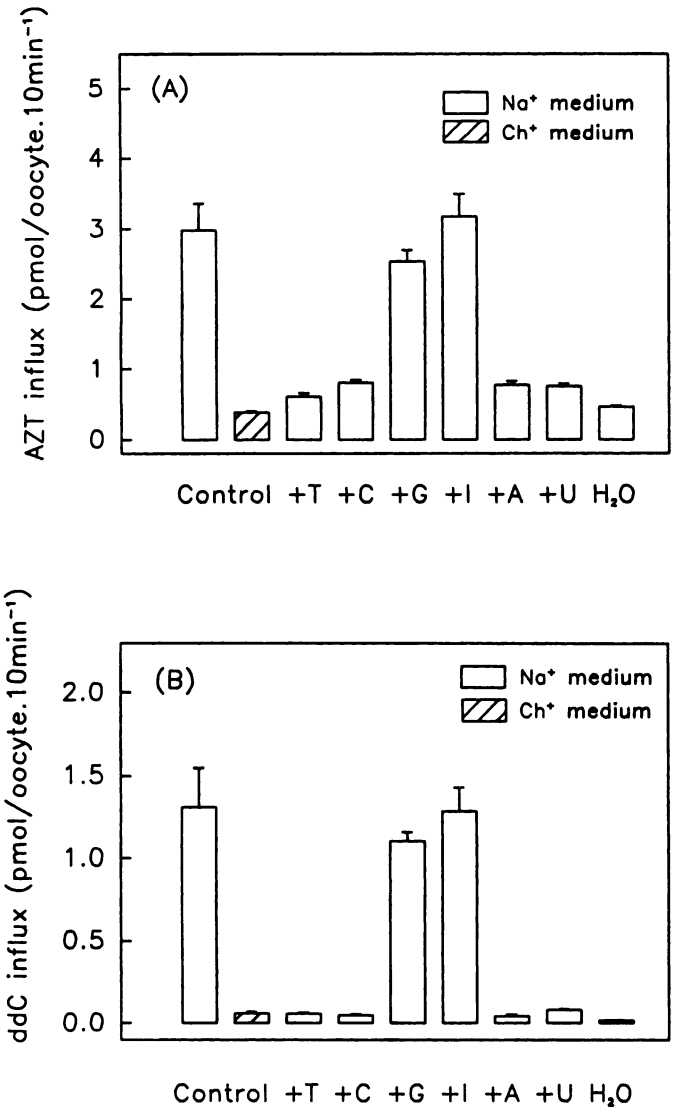

Fig. 3. Inhibition of rCNT1-mediated AZT (A) and ddC (B) influx by physiological nucleosides. AZT and ddC influx (10 μM, 10 min flux at 20°) was measured in rCNT1 RNA transcript-injected oocytes in the absence (control) or presence of 1 mM of nonradioactive nucleosides (*T*, thymidine; *C*, cytidine; *G*, guanosine; *I*, inosine; *A*, adenosine; *U*, uridine). Nonradioactive nucleosides were added to oocytes at the same time as [^{3}H]AZT or [^{3}H]ddC. H_2O, water-injected oocytes. Fluxes were not corrected for the contribution of endogenous transport activity. Each value is the mean ± standard error of 10–12 oocytes.

shown to modify AZT pharmacokinetics (38), was tested for possible interactions with rCNT1. Concentrations of probenecid in the range 10 μM of 1 mM had no significant effect on the initial rate of uridine influx (10 μM, 20°) (data not shown).

Discussion

A common structural feature of AZT, ddC, and ddI is the absence of the ribose 3′-hydroxyl group, which greatly reduces their ability to be transported by equilibrative NTs (39), and despite pharmacokinetic evidence to the contrary (38, 40), transmembrane movements of AZT in animal cells was thought to occur primarily by passive diffusion. Recombinant rCNT1 has high affinity for physiological pyrimidine nucleosides (32) and thus might be expected to transport AZT if the missing 3′-hydroxyl group is not required for permeant interaction with

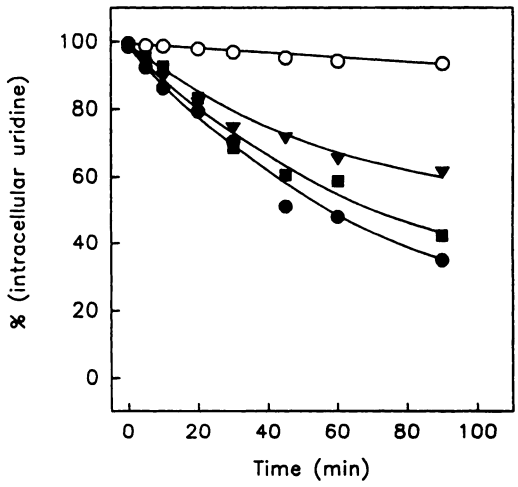

Fig. 4. *Trans*-stimulation of rCNT1-mediated uridine efflux by extracellular AZT, ddC, and uridine. Efflux of [^{3}H]uridine from CNT1 RNA transcript-injected oocytes preloaded with 10 μM [^{3}H]uridine for 30 min at 20° was measured at 20° on groups of 20 oocytes suspended in NaCl transport buffer alone (○) or in NaCl transport buffer containing 5 mM AZT (▼), 5 mM ddC (■), or 1 mM uridine (●).

the transporter. The results presented here unequivocally demonstrate that recombinant rCNT1 transports AZT.

AZT is absorbed efficiently by the gastrointestinal systems of humans and rats (15–18) and is administered orally. Plasma concentrations of AZT (therapeutic range, 6–10 μM) are determined primarily by intestinal absorption and not by drug elimination (17). The apparent affinity of recombinant rCNT1 for AZT (K_m = ~0.5 mM, Table 2) is within the range of anticipated luminal concentrations of AZT during oral administration in humans, suggesting a role for the human equivalent of this transporter in intestinal absorption of AZT. The presence of rCNT1 transcripts in kidney (32) suggests that the NT may also mediate renal AZT reabsorption. Probenecid, a drug that decreases renal, cerebrospinal fluid, and brain extracellular fluid clearance of AZT by mechanisms that may involve inhibition of organic anion secretion mechanisms (38), had no effect on rCNT1 transport activity.

Recombinant rCNT1 also transported ddC. The apparent K_m for ddC, which was similar to that for AZT (Table 2), was lower (~1/40) than the reported K_m of 23 mM for ddC transport by the human erythrocyte *es* NT (31). The V_{max} values for AZT and ddC influx were similar to that for uridine, the "preferred" substrate of the recombinant transporter. The V_{max}/K_m ratios for AZT, ddC, and uridine were 0.048, 0.039, and 0.57, respectively, indicating that rCNT1 is ~1–10th as "efficient" with respect to AZT and ddC transport as it is for uridine transport. As expected from previous studies of AZT permeation in blood cells (23–25), passive diffusion was greater for AZT than for ddC, although it accounted for only 20% of total AZT influx (10 μM) in RNA transcript-injected oocytes. The corresponding diffusional contribution to ddC uptake was 2%. Carrier-mediated transport was therefore the major route of uptake for both drugs. Consistent with the previously established preference for pyrimidine nucleosides (29), the purine nucleoside analog ddI did not inhibit rCNT1-mediated uridine transport (Table 1) and thus is unlikely to be a CNT1 permeate. Conversely, purine-selective rCNT2, which is expressed in both jejunum[2] and liver (36), did not transport either AZT or ddC.

Our results indicate that rCNT1 is more tolerant than equilibrative NTs of structural modifications at the 3′-hydroxyl group of the ribose ring because both AZT and ddC were accepted as permeates. rCNT1 transcripts have been found in rat intestine and kidney (32), and it is likely that the human homologs are involved in the intestinal absorption and renal handling of two of the principal drugs used in AIDS therapy in humans. The availability of a cDNA encoding an NT tolerant of the sugar structural modifications that confer antiviral activity (rCNT1) in combination with an efficient expression system (the *Xenopus* oocyte) provides a new tool for drug development. Our demonstration that recombinant rCNT1 exhibited *trans*-acceleration (Fig. 4) provides a rapid and powerful methodology to screen nucleoside analogs as CNT1 permeates without the need to undertake expensive and time-consuming preparation of radiolabeled compounds.

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