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Review

Functional and Molecular Characteristics of Na^+-dependent Nucleoside Transporters

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Nucleoside transporters play a critical role in the absorption, disposition, and targeting of therapeutically used nucleosides and nucleoside analogs. This review is focused on the Na^+-dependent, concentrative nucleoside transporters which are found in a variety of cells including renal, intestinal and hepatic epithelia. Five major Na^+-dependent nucleoside transporter subtypes have been characterized in isolated tissue preparations: N1 is purine selective; N2 is pyrimidine selective and N3–N5 exhibit variable selectivity for both purine and pyrimidine nucleosides. The recent cloning of N1 and N2 nucleoside transporters has provided the first information on the molecular function and structure of concentrative nucleoside transporters. In this manuscript we review the characteristics of the various subtypes of nucleoside transporters and the molecular structure, functional properties, and tissue distribution of the cloned Na^+-dependent nucleoside transporters. In addition, the interactions of nucleosides and nucleoside analogs with the cloned transporters in mammalian and amphibian expression systems are presented. Mammalian expression systems may be particularly useful during drug development in screening potential compounds for improved bioavailability and tissue specific targeting. Finally, we present our view of future areas of study in the field of nucleoside transporters.

KEY WORDS: nucleoside transporters; review; nucleoside analogs; expression systems; adenosine; intestinal absorption.

Nucleoside analogs (Table I), including zidovudine (AZT) and didanosine (ddI) (Fig. 1) are currently being used in the treatment of patients with Human Immunodeficiency Virus (HIV) (1). Other nucleoside analogs, such as cladribine (2CdA) and cytosine arabinoside (AraC), are important agents in cancer chemotherapy (2–4). The endogenous nucleoside, adenosine, has significant cardiac effects, and is used clinically in the treatment of cardiac arrhythmias (5–8). Because nucleosides and many nucleoside analogs are hydrophilic, specialized transporters are necessary for the movement of these compounds across cell membranes. Two major classes of plasma membrane nucleoside transporters in mammalian cells have been described in the literature: equilibrative (9–13) and concentrative (14–17).

This review focuses mainly on the concentrative, Na^+-dependent class of nucleoside transporters. We first discuss the functional characteristics and subtypes of nucleoside transport processes in mammalian cells. A discussion of the molecular characteristics and tissue specific functions of the cloned, concentrative nucleoside transporters follows. Particular emphasis is placed on expression systems in mammalian cells that have been developed to study the cloned transporters. In addition, new data from this laboratory are presented on the functional expression of cloned nucleoside transporters in both *X. laevis* oocytes and in transfected mammalian cells. The interactions of clinically used nucleoside analogs with the cloned transporters are described. Finally, a large section is devoted to future directions in nucleoside transporter research.

SUBTYPES OF NUCLEOSIDE TRANSPORTERS IN MAMMALIAN CELLS

The equilibrative nucleoside transporters mediate the facilitated diffusion of nucleosides across plasma membranes. This class of transporters is broadly-selective, accepting both purine and pyrimidine nucleosides (Fig. 1) and is further divided into

Table I. Clinically Used Nucleosides and Nucleoside Analogs

Acycloguanosine (acyclovir)
Ganciclovir
Azidothymidine (AZT, zidovudine)
2′,3′-Dideoxyinosine (ddI)
2′,3′-Dideoxycytidine (ddC)
Cytarabine (AraC)
2-Chloro-2′-deoxyadenosine(2CdA, Cladribine)
Lamivudine (3TC)
5-Fluorouridine (5FUrd)
Floxidine (5-Fluoro-2′-deoxyuridine)
Adenosine

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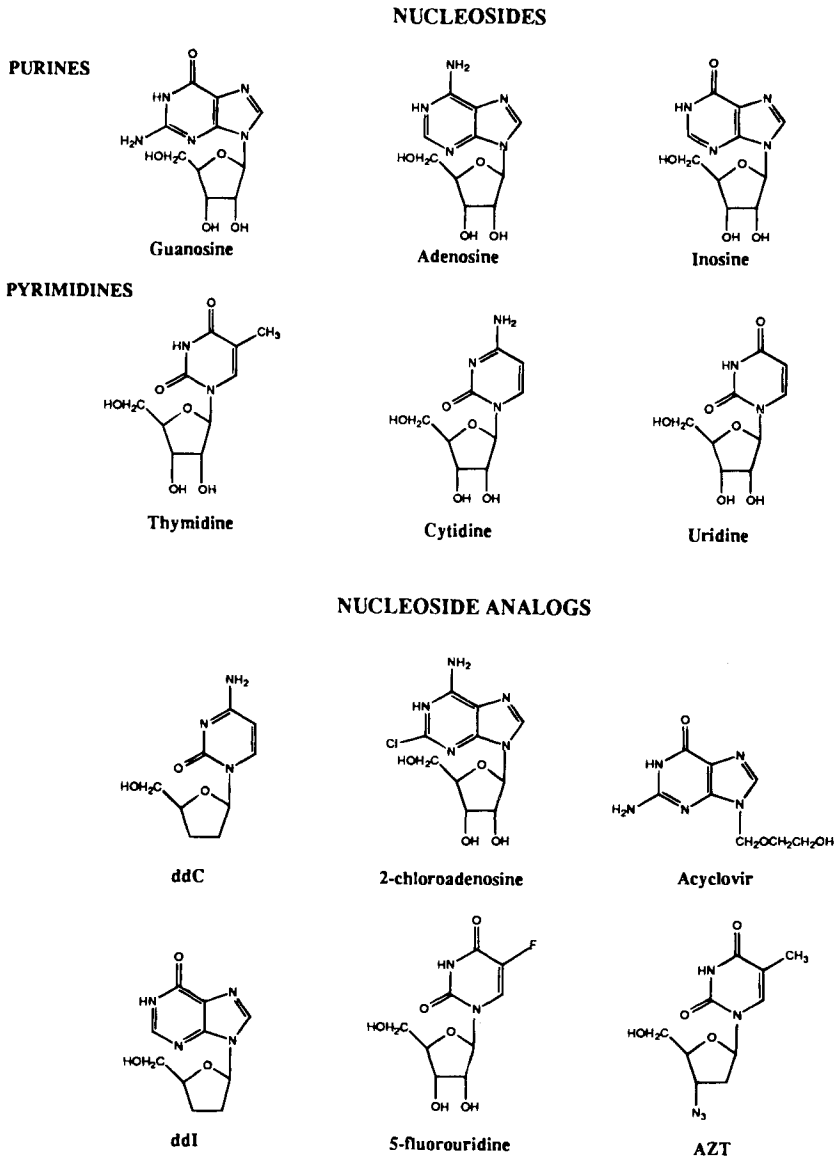

Fig. 1. Structures of nucleosides and nucleoside analogs.

the *es* (s = sensitive to nitrobenzylthioinosine (NBMPR)) and *ei* (i = insensitive to NBMPR) subtypes (Table II). In mammalian cells, equilibrative nucleoside transporters are ubiquitous and function bidirectionally in the transmembrane flux of nucleosides in accordance with the concentration gradient. Recently, an *es* type transporter was cloned from human placenta (10). For a recent review of equilibrative nucleoside transporters, see Griffith and Jarvis (18).

Table II. Characterized Nucleoside Transport Systems

Transporter	Substrate selectivity	Sensitivity to NBMPR	Na^+ dependency
N1	purine nucleosides, uridine	−	+
N2	pyrimidine nucleosides, adenosine	−	+
N3	purine and pyrimidine nucleosides	−	+
N4	pyrimidine nucleosides, guanosine, adenosine	−	+
N5	[a]2CdA, formycin B	+	+
es	purine and pyrimidine nucleosides	+	−
ei	purine and pyrimidine nucleosides	−	−

[a] Limited data available.

In contrast to the equilibrative transporters which mediate the bidirectional and downhill flux of nucleosides, the concentrative nucleoside transporters generally mediate the uphill transport of nucleosides into cells by coupling to the Na^+-gradient (18,19). These transporters are secondary active and use the physiologic Na^+-gradient (extracellular to intracellular Na^+-concentration is approximately 140 mM to 5–10 mM) generated by the ubiquitous Na^+-K^+-ATPase to move nucleosides intracellularly against their concentration gradient (18,19). Based on functional studies, five major subtypes (N1–N5) of Na^+-dependent nucleoside transporters have been characterized (Table II) (18,19). The N1 subtype is mainly purine-selective, but also accepts uridine whereas the N2 subtype is pyrimidine-selective, but also accepts adenosine. N1 and N2 subtypes have been characterized in a variety of tissues including intestine,

kidney, liver and several mammalian cell lines (14,16,20–23). The other Na^+-dependent subtypes (N3–N5) (15,17,19) are more broadly-selective and classification is based on functional studies in isolated cells and tissues (Table II). N3 has been characterized in rabbit choroid plexus as well as in mRNA expression studies from rabbit ileum and rat jejunum (24–26). N4 was characterized in human renal brush border membrane vesicles (27) and N5 was characterized in human leukemic cells (19).

Many cell types express several subtypes of nucleoside transporters (14,16,23) which precludes definitive identification of specific substrate selectivities due to substrate overlap within the system being studied. With the recent cloning of both Na^+-dependent (28–31)and equilibrative (10) nucleoside transporters, it is now possible to gain a more detailed knowledge of the different subtypes by studying cloned nucleoside transporters in isolation. With the development of specific antibodies against these transporters it will be possible to address questions about the localization and regulation of the various nucleoside transporter subtypes.

MOLECULAR CHARACTERISTICS OF CLONED Na^+-DEPENDENT NUCLEOSIDE TRANSPORTERS

The Na^+-dependent nucleoside transport proteins are minor membrane components which have not been purified or directly sequenced. Functional expression in *Xenopus laevis* oocytes, an approach based on transport activity which has been used previously in cloning a number of membrane transporters (32,33), was employed by several laboratories to isolate cDNAs encoding the nucleoside transporters. Using this approach, Huang *et al.* first succeeded in cloning an N2 subtype transporter rCNT1 in 1994 and a year later, Che *et al.* cloned an N1 subtype transporter, SPNT (28,29). Recently, using homology based methods, the human N2 homolog of rCNT1, hCNT1, was cloned by Ritzel *et al.* (30) and the human N1 homolog of rat SPNT, namely hSPNT1, was cloned in our laboratory (31).

With the cloning of these Na^+-dependent nucleoside transporters, it is now possible to study the different subtypes of cloned nucleoside transporters in isolation, generally by expressing the clones in heterologous expression systems. A suitable expression system should possess the following characteristics. First, the transporter should be expressed with proper post-translational modifications necessary for function in the expression system. Second, endogenous Na^+-dependent nucleoside transport activity should be negligible, and other Na^+-independent nucleoside transport activity should be low or controlled by specific inhibitors. Third, nucleoside metabolism in the system should be low or inhibitable to allow the separation of metabolism kinetics from transport kinetics. Earlier studies in cultured cells have demonstrated that nucleoside transport kinetics are greatly influenced by the cellular metabolism, mostly phosphorylation, of transported nucleosides by cellular kinases (for a review, see ref. (34)). Therefore, it is important to realize that kinetic measurements should be performed under conditions least affected by metabolism. Previous studies have shown that the use of very early time points to assess transport will minimize problems associated with metabolism (34).

Two major heterologous expression systems have been used to study cloned nucleoside transporters, *Xenopus laevis* oocytes and mammalian expression systems. *Xenopus laevis* oocytes are effective in translating exogenous mRNA and are capable of post-translational protein modifications (35). To express nucleoside transporters in oocytes, cRNA encoding the nucleoside transporters is introduced into the oocytes by microinjection. After a few days, the expressed transport activity will reach a maximum, and functional studies can be carried out by incubating the oocytes with radio-labeled nucleosides. In general, uptake studies are used to determine the kinetics and characteristics of transport. Uptake is defined as the amount of compound taken up by the cell and includes compound which is bound (and not removed by washing) as well as unbound compound. Experiments focused on transport should include procedures to delineate between bound compound and compound present in the cytosol as well as to account for metabolism (e.g., a specific analytical procedure).

The use of *Xenopus laevis* oocytes to express transporters is advantageous for the following reasons. First, *Xenopus laevis* oocytes express low levels of endogenous nucleoside transport activity and non-specific binding of radio-labeled ligands to oocytes can be estimated by measuring the radioactivity associated with water-injected or un-injected oocytes. Second, earlier studies with mRNA-injected oocytes established that uridine and thymidine are metabolized slowly in the oocytes (24,36). Third, oocytes are large cells (1–1.3 mm in diameter) with an intracellular volume of ~0.5 ul; therefore the approach to equilibrium takes a longer time than that in smaller cells. Thus, significant backflux is minimized at early times.

Due to seasonal variability and technical difficulties in micro-injection, *Xenopus laevis* oocytes are not ideal for large scale functional studies. Furthermore, the cDNA of the cloned transporters must be transcribed *in vitro* and capped and the resulting cRNA injected into oocytes. This *in vitro* processing may result in degradation of the RNA by nucleases and variability in expression. Finally, oocytes are amphibian cells and may not reflect mammalian post-translational processing of membrane proteins. Therefore, the development of mammalian expression systems for nucleoside transporters may be advantageous for the routine screening of drug interactions with the cloned transporters. Several mammalian expression systems have been developed and used to study nucleoside transporters. These will be discussed below.

The Cloned N2 Nucleoside Transporters, rCNT1 and hCNT1

rCNT1 cDNA was cloned from a rat jejunum library (28). The 2.4 kb cDNA predicts a protein of 648 amino acids (71 kDa) with 14 putative transmembrane domains. There are three potential N-linked and four potential O-linked glycosylation sites, and four protein kinase C-dependent phosphorylation sites.

Functional Characteristics of rCNT1

When expressed in *Xenopus laevis* oocytes, the recombinant rCNT1 transporter exhibits a high level of nucleoside transport activity with a typical N2 substrate selectivity for pyrimidine nucleosides and adenosine (28). Nucleoside uptake is Na^+-dependent and saturable with an apparent K_m of 37 μM for uridine and 26 μM for adenosine (28,37). However, the

adenosine V_{max} value is much lower than that of the uridine (0.070 ± 0.005 pmol/oocyte min^{-1} versus 21 pmol/oocyte min^{-1}), suggesting that this transporter kinetically favors the transport of pyrimidine nucleosides (37).

The oocyte expression system has been used to study the interactions of various nucleoside analogs with rCNT1 (37,38). Using this system, Yao *et al.* demonstrated that rCNT1 accepts the antiviral pyrimidine analogs, AZT and ddC, as permeants (K_m = 0.49 and 0.51 mM, respectively) (38). Recently, using the oocyte expression system our laboratory investigated the interaction of two nucleoside analogs, 2CdA and AraC, with rCNT1 isolated by RT-PCR from rat intestine (Fig. 2). The studies revealed that 2CdA and AraC produced a concentration-dependent inhibition of Na^+-dependent thymidine uptake in the oocytes (IC_{50} = 61 μM and 1.88 mM, respectively). Furthermore, studies with radio-labeled 2CdA and AraC demonstrated that rCNT1 also accepts these two compounds as permeants (Fig. 3). These data suggest that N2 transporters in the intestine may play an important role in the absorption of clinically used base- and ribose-modified nucleoside analogs.

For the reasons discussed above, a mammalian expression system, COS-1 cells transiently transfected with the cDNA of rCNT1, was developed to study the kinetics, substrate selectivity and interaction of nucleoside analogs with rCNT1 (39). Floxidine, 3TC, AZT, ddC, AraC, and Gemcitabine were shown to significantly inhibit ^{3}H-uridine uptake in this transfected cell system. The interaction of various nucleoside analogs was also examined in our laboratory in a human cell line, HeLa, transiently transfected with rCNT1 using methods described in a recent publication (40). Consistent with the previous studies in the transfected COS-1 cells (39) and in *Xenopus laevis* oocytes (38), AZT and ddC significantly inhibited the uptake of ^{3}H-thymidine, a pyrimidine nucleoside (Fig. 4). The K_m of thymidine in the transfected HeLa cells was 7.4 μM, which is similar to the K_m (12.5 μM) determined in COS-1 cells transfected

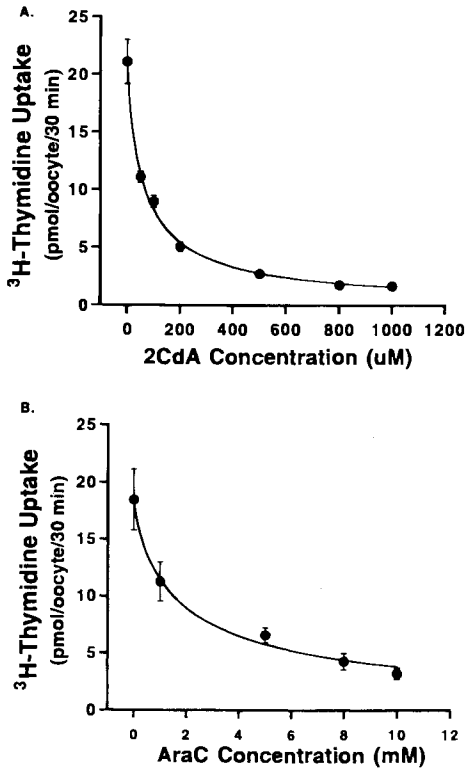

Fig. 2. Concentration-dependent inhibition of thymidine uptake by (A) 2CdA and (B) AraC in oocytes injected with rCNT1 cRNA. Uptake of ^{3}H-thymidine was measured in the presence of increasing concentrations of either unlabeled 2CdA or AraC in the presence of Na^+ (100 mM). Data are presented as mean ± SE of results from 8–10 oocytes. Oocytes were injected with 20 ng of cRNA encoding rCNT1 and uptake was carried out 2 days post-injection.

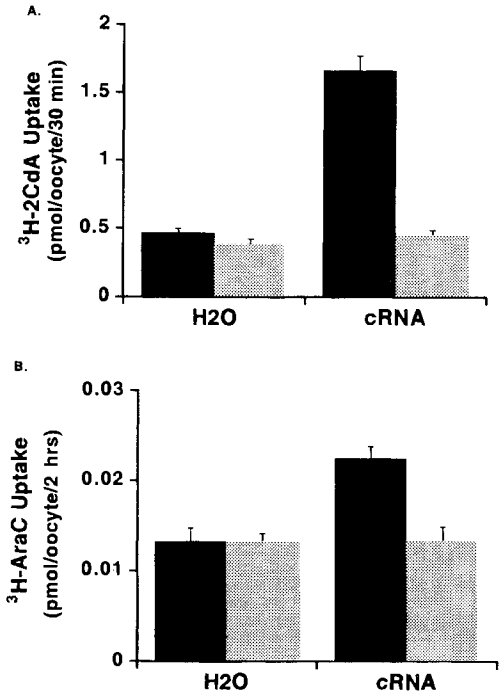

Fig. 3. Uptakes of (A) 2CdA and (B) AraC in oocytes injected with rCNT1 cRNA. Uptakes of radiolabeled compounds were measured at 25°C in the presence (solid bars) and absence of Na^+ (gray bars). Data represent the mean ± SE of results from 8–10 oocytes. Oocytes were injected with either 50 nl (20 ng) of cRNA encoding rCNT1 or 50 nl of H_2O and uptake was carried out 2 days post-injection.

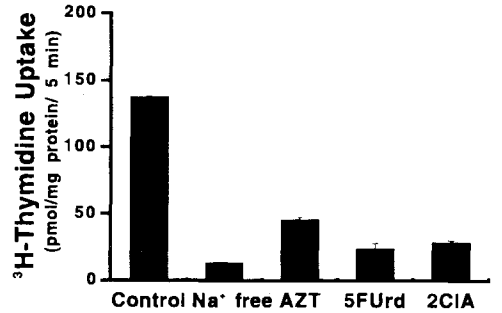

Fig. 4. Inhibition of thymidine uptake by nucleoside analogs in HeLa cells transfected with the cDNA of rCNT1. Uptake of 1 μM ^{3}H-thymidine was measured at 5 min. in the presence of 1 mM of each nucleoside analog and 10 μM of nitrobenzylthioinosine (NBMPR). Data are presented as Na^+-dependent uptake in cells transfected with rCNT1. Control cells represent Na^+ -dependent uptake without inhibitor. Data are also presented for thymidine uptake in the absence of Na^+ (Na^+ free). Data represent the mean ± SD of results from 2 to 3 wells each.

with rCNT1. Although the transfection methods differ (calcium phosphate precipitation in COS-1 cells and lipofection in HeLa cells) between the two expression systems (39,40), the data are in close agreement and suggest that either expression system would be useful in functional studies of rCNT1.

Tissue Distribution of rCNT1

The expression of the mRNA transcript of rCNT1 was detected by Northern analysis in rat intestine and kidney but not in heart, brain, spleen, lung, liver or skeletal muscle suggesting that rCNT1 may be involved in the absorption and disposition of nucleosides (28). However, by more sensitive Reverse Transcriptase-PCR (RT-PCR) analysis, Anderson *et al.* found that the mRNA transcript of rCNT1 is also present in various regions of the brain including choroid plexus, posterior hypothalamus, hippocampus, cerebral cortex, cerebellum and brain stem (41). These data suggest that a small amount of the transporter is expressed in specific regions of the brain.

Although rCNT1 was cloned from rat intestine, the distribution of this transporter along the intestine is unclear. To address this question, our laboratory employed an RT-PCR based method to detect the regional distribution of the rCNT1 mRNA. In brief, rat intestine was dissected into duodenum, jejunum, ileum, and colon. mRNA was then isolated from each segment. After quantification, the mRNA was reverse transcribed into first strand cDNA. The presence of rCNT1 cDNA was then detected by PCR amplification with rCNT1 specific primers, and the PCR product was analyzed by agarose gel electrophoresis. Using this method, we detected strong expression of rCNT1 mRNA in rat jejunum, moderate expression in duodenum and ileum, and no expression in colon (Fig. 5). These data suggest that rCNT1 mediated intestinal transport may occur primarily in the jejunum but also in duodenum and ileum. Interestingly, our results are consistent with functional studies in brush border membrane vesicles prepared from different regions of human intestine (42). The direct analysis of the regional distribution and membrane location of the rCNT1 transport protein (rather than the mRNA transcript) awaits the use of rCNT1 specific antibodies.

The role and location of rCNT1 in kidney is least understood. By RT-PCR our laboratory isolated an rCNT1 cDNA from rat kidney. Expression of this cDNA resulted in a functional transporter with properties similar to intestinal rCNT1. DNA sequencing revealed that the amino acid sequence of the kidney rCNT1 transporter is identical to that of the intestinal clone. Since, rCNT1 may play a role in the absorption of nucleoside drugs in the intestine, it is possible that the same transporter plays a role in the re-absorption of these drugs in the kidney.

Molecular Characteristics of hCNT1

The human homolog of rCNT1, hCNT1, was cloned from a human kidney cDNA library by hybridization cloning and RT-PCR strategies (30). hCNT1 is 83% identical to rCNT1 in amino acid sequence and exhibits similar transport characteristics. The transporter gene is localized to Chromosome 15. These studies indicate that the CNT family exists in human cells and may play important roles in transport of physiological and therapeutic nucleosides in humans.

The Cloned N1 Nucleoside Transporters, SPNT and hSPNT1

The N1 (purine-selective) subtype transporter, SPNT (also termed rCNT2), was cloned from a rat liver cDNA library using an expression cloning strategy in *Xenopus laevis* oocytes (29). The 2.9 kb cDNA encodes a protein of 659 amino acids (72 kDa). Hydropathy analysis revealed 14 putative membrane-spanning domains. There are five possible N-linked glycosylation sites, one ATP/GTP binding motif in the amino terminus, and several consensus sites for protein kinase A and C phosphorylation on both termini suggesting that SPNT may be regulated by mechanisms involving protein kinases, or intracellular ATP/GTP. SPNT is 64% identical to rCNT1 at the amino acid level suggesting that the two transporters belong to the same family. Functionally, SPNT exhibits typical N1 transport characteristics with a substrate selectivity for purine nucleosides and uridine. SPNT mediated adenosine uptake is Na^+-dependent and saturable with a K_m of 6 μM.

Functional Characteristics of SPNT

After SPNT was cloned from rat liver by expression cloning in oocytes, the same transporter ($SPNT_{int}$) was cloned by RT-PCR from rat intestine in our (unpublished data) and other laboratories (37). Interaction of $SPNT_{int}$ with nucleoside drugs was studied in *Xenopus laevis* oocytes. In the oocyte expression system, $SPNT_{int}$ mediated inosine uptake can be inhibited significantly by (1 mM) 2-chloroadenosine, 5-fluorouridine, ddI, 6-CL-ddP (6-chloro-2′,3′-dideoxypurine), ddA, ddU (dideoxyuridine), AraC and acyclovir (unpublished data). Although inhibition of uptake does not imply that the inhibitor is a permeant, these data may suggest that $SPNT_{int}$ plays a role in the absorption of some nucleoside drugs in the intestine.

Recently, we developed a HeLa cell expression system to study the functional characteristics of $SPNT_{int}$ (40). The nucleoside analogs, 2CdA and ddI, significantly inhibited ^{3}H-inosine uptake. The IC_{50} of 2CdA was 13 μM and that of ddI was 46 μM. 2CdA was also found to be a permeant of $SPNT_{int}$. In contrast, ddI was not a permeant of $SPNT_{int}$ (40). These data together with data shown in Fig. 4 demonstrate that the HeLa cell expression system can be used to study the functional characteristics of both SPNT and rCNT1.

Tissue Distribution of SPNT

In contrast to the limited tissue distribution of rCNT1 which is primarily localized to kidney and intestine, Northern analysis revealed that multi-transcripts of SPNT are expressed in liver, intestine, spleen, and heart (29). The relatively wide tissue distribution of SPNT may suggest that SPNT is involved in regulation of purinergic receptor-mediated nucleoside effects in addition to salvaging nucleosides (29).

Molecular Characteristics and Tissue Distribution of hSPNT1

Recently, using homology cloning strategies and reverse transcriptase polymerase reactions, we cloned the human N1 homolog, hSPNT1, from kidney (31). At the amino acid level, hSPNT1 is 81% identical to rat SPNT with the most divergent region at the N-terminus. The ATP/GTP binding motif at the

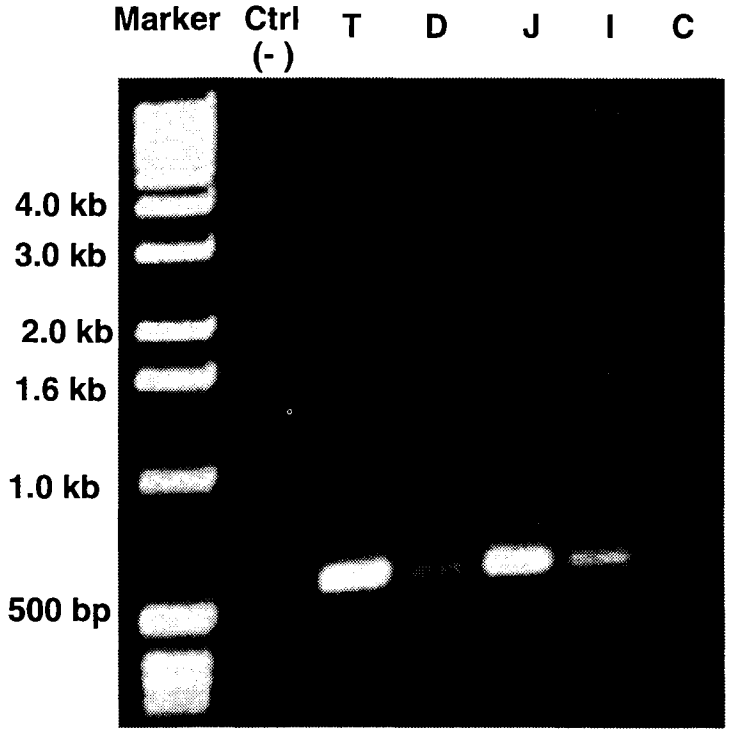

Fig. 5. RT-PCR analysis of rCNT1 mRNA transcripts in the various regions of rat intestine. The RT-PCR product was separated by electrophoresis on a 1% agarose gel. Each lane represents a DNA product resulting from RT-PCR amplification of 10 ng mRNA isolated from total rat intestine (T), duodenum (D), jejunum (J), ileum (I), and colon (C). Ctrl (−) represents negative control in which no cDNA template was added to the PCR reaction mixture.

N-terminus of SPNT is absent in hSPNT1. hSPNT1 and SPNT have the same substrate selectivity, transporting both purine nucleosides and uridine. Northern analysis revealed that multiple transcripts of hSPNT1 are widely distributed in human tissues including kidney, heart, liver, intestine, skeletal muscle, and pancreas. Interestingly, the rat SPNT transcript is absent in the kidney (29). The wide tissue distribution of hSPNT1 suggest that this transporter may play a critical role in the specific uptake and salvage of purine nucleosides in a variety of human tissues.

The molecular characteristics of rCNT1, SPNT, hCNT1, and hSPNT1 are summarized in Table III. Interaction of various nucleoside analogs with rCNT1, SPNT, and hCNT1 is listed in Table IV.

Other Nucleoside Transporters

In addition to rCNT1, SPNT, hCNT1 and hSPNT1, a putative Na+-dependent nucleoside transporter, SNST1, was cloned by Pajor and Wright by screening a rabbit kidney cDNA library with nucleotide probes derived from the Na+-glucose transporter, SGLT1, of rabbit intestine (43). SNST1 shares high homology (61% identity) with SGLT1 but no significant homology with rCNT1, SPNT, hCNT1 and hSPNT1, and is conse-

Table III. Molecular Characteristics of Clone Na+-Nucleoside Transporters

Transporter	rCNT1	SPNT	hCNT1	hSPNT1
Subtype	N2(*cit*)	N1(*cif*)	N2(*cit*)	N1(*cif*)
Cloning Source	rat jejunum	rat liver	human kidney	human kidney
Cloning Strategy	expression screening	expression screening	homology hybridization	homology RT-PCR
cDNA Length	2.4 kb	2.9 kb	2.8 kb	2.5 kb
Protein Length	648 a.a.	659 a.a.	650 a.a.	658 a.a.
Molecular Weight	71 kDa	72 kDa	71 kDa	72 kDa
Substrate Selectivity	pyrimidine, adenosine	purine, uridine	pyrimidine, adenosine	purine, uridine
Tissue Distribution	intestine, kidney	liver, jejunum, heat, spleen	not determined	kidney, intestine, heart, liver, etc.

quently considered to be a member of the SGLT transporter family. When a chimera of SNST1, cSNST1, was expressed in *Xenopus laevis* oocytes, a low (2–3 fold) level of Na^+-dependent nucleoside transport activity with broad nucleoside selectivity was observed. The mRNA transcript of SNST1 was expressed in rabbit kidney and heart, but not in liver or intestine. Although cSNST1 exhibits a substrate selectivity similar to the N3 subtype, its low transport activity, absence of mRNA transcripts in rabbit intestine (on Northern analysis), and sequence divergency from recently cloned N1 and N2 transporters suggest that this transporter may not be a major component of the well characterized N3 system reported in rabbit choroid plexus and ileum (17,26). Efforts to elucidate the physiologic role of SNST1 and cloning of nucleoside transporters from rabbit ileum or choroid plexus will provide us with a better understanding of other Na^+-dependent nucleoside transporters.

FUTURE STUDIES

With the recent cloning and characterization of rCNT1, SPNT, hCNT1, and hSPNT1, the first molecular information about the Na^+-dependent nucleoside transporters has been obtained. Sequence alignment reveals that these transporters are similar in structure with a high degree of sequence identity in the mid-region of the proteins (28–31). These findings suggest that the transporters share common structural elements which are most likely related to the underlying transport function. However, highly divergent N and C termini regions are apparent upon sequence alignment. Since amino acid termini of membrane proteins often contain information important in intracellular targeting and trafficking, it is likely that rCNT1 and SPNT are processed differently in the cell. Studies are clearly needed to identify the structural elements important in the regulation as well as in the transport function of the two proteins, particularly the elements that confer substrate recognition, Na^+-dependency and transport function. Finally, both proteins have protein kinase phosphorylation sites. The role of these sites in the function of the transporters needs to be evaluated.

A number of broadly selective Na^+-dependent nucleoside transporters (i.e., N3, N4 and N5) have been characterized in various tissues (15,17,19,25,27). However, these transporters have not been cloned and there is currently no information on the molecular structure of the transporters. SNST1, a homolog of the Na^+-glucose transporter (SGLT1), does not share a high sequence identity with the cloned Na^+-dependent nucleoside transporters (43). Nonetheless, the chimera cSNST1 has functional properties consistent with a "broadly-selective" Na^+-dependent nucleoside transporter. However because of its low transport activity and its chimeric structure, studies are needed to ascertain if the full length SNST1 is indeed a broadly-selective nucleoside transporter. The finding suggests that other gene families may encode Na^+-dependent nucleoside transporters. It is possible that Na^+-dependent nucleoside transporters are encoded by multiple gene families.

Recently, two human nucleoside transporters, hCNT1 and hSPNT1, were cloned from kidney (30). These transporters have different functional characteristics from those described for nucleoside transport in human renal brush border membrane vesicles (15,27). The data suggest that there may be other Na^+-

Table IV. Interaction of Nucleoside Analogs with Cloned Na^+-Nucleoside Transporters

Drug	Modification	rCNT1	SPNT	hCNT1	References
AZT	ribose	permeant, inhibitor K_m = 0.55 mM		permeant inhibitor	28, 30, 38 Fig. 4
Ara-C	ribose	permeant, inhibitor IC_{50} = 1.88 mM			Fig. 2b, 3b 39
Acyclovir	ribose	poor inhibitor 11% inhibition at 5 mM			39
2CdA	base	permeant, inhibitor IC_{50} = 61 μM	permeant, inhibitor IC_{50} = 13 μM		Fig. 2a, 3a 40, Fig. 4
ddA	ribose		inhibitor		40
ddI	ribose	no inhibition	not a permeant inhibitor IC_{50} = 46 μM		38, 39, 40
ddC	ribose	permeant, inhibitor K_m = 0.50 mM		inhibitor	28, 30, 38
Floxidine	base	inhibitor IC_{50} = 40–60 μM			39
Gemcitabine	ribose	inhibitor 41% inhibition at 5 mM			39
Idoxuridine	base	inhibitor IC_{50} = 40–60 μM			39
3TC	ribose	poor inhibitor 15% inhibition at 5 mM			39

dependent nucleoside transporters in human kidney that have not been cloned. Alternatively, polymorphisms in nucleoside transporters which have some differences in substrate selectivities or potencies may exist (e.g. different K_m values for a particular nucleoside). It is noteworthy that in the cloning of hCNT1, multiple related cDNAs with minor sequence differences were identified, two of which were functional (30). It is also possible that splice variants of nucleoside transporters with different substrate selectivities exist. Finally, transport properties characterized in intact tissue which express a variety of transporters with different substrate selectivities may be complex and differ from transport properties observed in an expression system in which a single cloned transporter is overexpressed.

Evidence for the existence of other nucleoside transporters also comes from studies in various animal species. The expression of the mRNA of the cloned nucleoside transporters in various tissues from rat does not uniformly coincide with expressed nucleoside activity. For example, the rat kidney appears to have a Na^+-purine transporter (44), yet Northern blotting studies and unpublished studies in our laboratory using RT-PCR revealed that SPNT is not present in rat kidney (29). These data suggest that in the rat kidney there may be other "purine-selective" Na^+-dependent nucleoside transporters.

The physiologic role of the Na^+-dependent nucleoside transporters needs further study. For example, the finding that inhibitors of nucleoside transport activity potentiate the activity of adenosine (45) may suggest that nucleoside transporters control the local concentrations of adenosine near purinergic receptors. The recent finding, using RT-PCR, of a broad distribution of rCNT1 and SPNT mRNA transcripts in various regions of the brain (41) suggests that these transporters function like neurotransmitter re-uptake transporters in the termination of purinergic activity in the brain. The possible co-regulation of nucleoside transporters and purinergic receptors or enzymes active in metabolism of nucleosides needs to be studied.

The interaction of the cloned Na^+-dependent nucleoside transporters with various nucleoside analogs including a number of dideoxynucleosides has been studied in isolated expression systems (see Figs. 3 and 4) (39,40). However, the role of these transporters in the absorption and disposition of nucleoside analogs in the intact animal has not been elucidated. In particular, mRNA transcripts of the cloned nucleoside transporters, rCNT1 (Fig. 4) and SPNT, have been found in intestine suggesting that the transporters play a role in the absorption of orally administered nucleosides or nucleoside analogs (38) Further studies localizing the cloned transporters to the brush border or basolateral membrane of various regions of the intestine are needed to determine whether the transporters play a role in intestinal secretion or absorption and whether the transporters may be involved in the regional specific absorption of nucleosides and nucleoside analogs. The development of a knockout mouse model for P-glycoprotein has led to the discovery of a variety of physiologic roles for P-glycoprotein (46). Notably, it is apparent that P-glycoprotein may constitute a major component of the "barrier" function of the blood brain barrier. The development of such knockout mouse models for nucleoside transporters may lead to an enhanced understanding of the physiologic and pharmacologic role of the transporters in the intact animal.

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