Transient Expression of a Purine-Selective Nucleoside Transporter ($SPNT_{int}$) in a Human Cell Line (HeLa)

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Received April 30, 1997; accepted July 7, 1997

Purpose. The goal of this study was to develop a mammalian expression system for the cloned rat intestinal, Na^+-dependent, purine-selective nucleoside transporter ($SPNT_{int}$) and to study the interactions of nucleosides and nucleoside analogs with this transporter.

Methods. Lipofection was used to transfect HeLa cells with a mammalian expression vector (pcDNA3) containing the cDNA insert encoding $SPNT_{int}$. Nucleoside transport activity was measured using [^{3}H]inosine, [^{3}H]uridine, [^{3}H]-dideoxyinosine (ddI), and [^{3}H]-2-chloro-2′-deoxyadenosine (2CdA) as model substrates.

Results. Expression of $SPNT_{int}$ was observed between 36 and 90 h post-transfection, with maximal expression at 66 h. At 66 h, Na^+-stimulated uptake of [^{3}H]inosine in cells transiently transfected with $SPNT_{int}$ was approximately threefold greater than that in cells transfected with empty vector ($p < 0.05$). The Na^+-stimulated uptake of both inosine and uridine was saturable (K_m = 28.1 ± 7.1 μM and 20.6 ± 5.6 μM, respectively) in the transfected cells and was significantly inhibited by the naturally occurring nucleosides (1 mM) inosine and uridine and to a lesser extent by thymidine. The nucleoside analogs ddI (IC_{50} = 46 μM) and 2CdA (IC_{50} = 13 μM) also significantly inhibited the Na^+-stimulated uptake of [^{3}H]inosine. A Na^+-stimulated uptake of [^{3}H]2CdA was observed suggesting that 2CdA is also a permeant of $SPNT_{int}$.

Conclusions. HeLa cells transiently transfected with $SPNT_{int}$ represent a useful tool to study the kinetics and interactions of drugs with $SPNT_{int}$.

KEY WORDS: nucleosides; transporters; HeLa; mammalian expression systems; nucleoside analogs.

INTRODUCTION

Nucleosides and nucleoside analogs are used clinically in the treatment of a wide array of disease states including viral infections, cardiac arrhythmias and various neoplasms. Many nucleoside analogs are poorly absorbed, polar molecules and it is becoming increasingly clear that transporters located in the intestinal brush border membrane may function in the absorption of these analogs (1–3). Two major classes of nucleoside transport processes have been described, equilibrative (4,5) and concentrative (6–9). Equilibrative nucleoside transporters are Na^+-independent, facilitative carriers and are broadly-selective for nucleosides, accepting both purines and pyrimidines. In contrast, concentrative nucleoside transporters are secondarily active, Na^+-dependent carriers and differ in their substrate selectivity. Four major concentrative processes have been described, one purine-selective (N1) (6,8,10), one pyrimidine-selective (N2) (9,11), and two broadly selective processes (N3 and N4) (7,8,12–15).

Recently, a Na^+-dependent, purine-selective transporter, SPNT, was cloned from a rat liver cDNA library by expression cloning in *Xenopus laevis* oocytes (6). Northern analysis suggested that the transporter was expressed in high levels in rat intestine. Subsequently, using RT-PCR and rat intestinal mRNA as a template, $SPNT_{int}$ was cloned (16). The cloning of the transporter from rat intestine together with the high level of expression of the mRNA transcript of SPNT in rat intestine suggests that this transporter may play a role in the intestinal absorption of nucleosides and nucleoside analogs.

The functional characteristics of SPNT have been studied in *Xenopus laevis* oocytes (6,16). However, because of seasonal variability and the complex micro-injection techniques required for protein expression, oocytes are poorly suited for routine screening of compounds for potential interactions with $SPNT_{int}$. Furthermore, for oocyte expression, cDNA has to be transcribed and capped and the resultant cRNA injected into the oocyte. This cRNA is subject to degradation by nucleases during storage (19). Therefore, the development of a mammalian heterologous expression system would be a useful model to study the interaction of drugs with $SPNT_{int}$ as well as the regulation and processing of the transport protein.

In this study, we chose HeLa cells to transfect the cDNA of $SPNT_{int}$. HeLa have been well-characterized for the equilibrative nucleoside transporter, but do not exhibit a Na^+-dependent nucleoside transport process (17). This cell line has been used previously for the expression of transport proteins (18,19). We developed an expression system for the purine-selective nucleoside transporter, $SPNT_{int}$, using HeLa cells and a lipid transfection method. This method is reproducible and allows for high levels of expression within 36 hours following transfection. HeLa cells transiently transfected with the cDNA of $SPNT_{int}$ represent a useful model in the study of the kinetics and interactions of compounds with $SPNT_{int}$.

MATERIALS AND METHODS

Materials

Zidovudine (AZT), 2′,3′-dideoxyadenosine (ddA), 2′,3′-dideoxycytidine (ddC), ddI, uridine, thymidine, inosine, 2CdA, 2-chloroadenosine, and acyclovir were purchased from Sigma. [^{3}H]inosine (sp. act. 28 Ci/mmole), [^{3}H]ddI (sp. act.38 Ci/mmole), [^{3}H]2CdA (sp. act. 3.8 Ci/mmole), and [^{3}H]uridine (sp. act. 43.8 Ci/mmole) were purchased from Moravek. The Bradford reagent was obtained from Bio-rad. DNA isolation kits were from Qiagen. LIPOFECTAMINE™, Opti-MEM™ (serum-free media), the Superscript Preamplification System, and the Trizol™ Reagent were purchased from Gibco/BRL. DME H21 medium, MEM, trypsin, phosphate buffered saline-

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ABBREVIATIONS: ddI, 2′,3′-dideoxyinosine; ddA, 2′,3′-dideoxyadenosine; ddC, 2′,3′-dideoxycytidine; 2CdA, cladribine (2-chloro-2′-deoxyadenosine); NBMPR, nitrobenzylthioinosine; AZT, zidovudine (3′-azido-3′-deoxythymidine); MEM, Modified Eagle's Medium; X-gal, 5-bromo-4-chlor-3-indoyl-D-galactopyranoside; acyclovir, acycloguanosine; FBS, fetal bovine serum.

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calcium, magnesium free (PBS), and fetal bovine serum (FBS) were purchased from the UCSF Cell Culture Facility. Corning 12-well plates were used for cell plating and Nunc flasks for cell maintenance. The pcDNA3 vector was supplied by Invitrogen and enzymes used for subcloning were from Gibco/BRL, New England Biolabs (NEB) or Boehringer Mannheim Corporation. Nu-serum was from Collaborative Biomedical Products. The pGEM-T vector was from Promega. The Maxi-Oligo(dT) Cellulose Spin Column was purchased from 5′ and 3′, Boulder, CO. IEC-6 cells and HeLa cells were obtained at the UCSF Cell Culture Facility, original stocks were from ATCC.

RT-PCR Cloning of $SPNT_{int}$

The cDNA of $SPNT_{int}$ was isolated from a rat intestinal epithelial cell line, IEC-6, by reverse transcriptase polymerase chain reaction (RT-PCR). Briefly, IEC-6 cells were cultured in DME H21 medium containing 10% Nu-serum. When the cells formed a confluent monolayer, total cellular RNA was isolated using the Trizol™ Reagent. Poly (A^+) RNA was isolated using the Maxi-Oligo(dT) Cellulose Spin Column. First strand cDNA was synthesized from 0.5 μg mRNA using the Superscript Preamplication System. Selective PCR amplification was performed using two primers (sense: 5′-CCTCCACTTCCTGCTT GTGAGAGA-3′, and antisense: 5′-CACTTTATTACAGAAA GCTTTTTAGTAATG-3′) derived from the 5′ and 3′ flanking regions of the published rat SPNT sequence. PCR was performed under the following conditions: 94°C for 1 min, 50°C for 1.5 min, 72°C for 2 min , 30 cycles followed by a final 15 min incubation at 72°C. The resulting 2.8 kb PCR product was subcloned into the pGEM-T vector to form the $pGEM.SPNT_{int}$ construct. $pGEM.SPNT_{int}$ was subjected to restriction analysis and a T7 promoter-oriented construct was selected. The sequence of the T7-oriented $pGEM.SPNT_{int}$ was determined by automated DNA sequencing (Applied Biosystems Inc.). The resulting sequence predicted a protein nearly identical to the published SPNT except the 419 alanine residue was substituted by a glycine residue. Two PCR products were sequenced and both showed the same glycine substitution at position 419.

Generation of the Expression Construct

To express rat $SPNT_{int}$ in HeLa cells, the 2.8 kb cDNA of $SPNT_{int}$ was removed from the pGEM-T vector and subcloned into the mammalian expression vector (pcDNA3). Briefly, $pGEM.SPNT_{int}$ was linearized with *SstII* and blunt-ended with T4 DNA polymerase. Subsequently, the 2.8 kb insert was removed by a *NotI* cut and ligated into pcDNA3 at the *EcoRV/NotI* sites, resulting in the $pcDNA3.SPNT_{int}$ construct. This construct was confirmed by restriction enzyme analysis.

DNA Isolation

DNA for the transfection studies was isolated with the Qiagen Endo-free DNA isolation kit. The yield from each isolation was approximately 300–700 μg DNA. Several different DNA preparations were used for this study at concentrations of 2–5 mg/ml of DNA. The DNA was resuspended in endotoxin-free TE buffer (Qiagen) and the concentration determined by spectroscopy.

Transfection

HeLa cells were grown in a 5% CO_2/95% air, humidified environment. Cells were seeded at a density of 3.6×10^5 cells/well in 12 well plates 24 hours prior to transfection. Lipid (LIPOFECTAMINE™) was used as the vehicle to deliver DNA to the cells following a modified protocol from Gibco/BRL. For each well 0.1 ml of Opti-MEM media was incubated with 2 μg of DNA and 0.1 ml of media was incubated with 6 μg of lipid. The two solutions were mixed together and then incubated for 20 minutes at room temperature. Following incubation, 0.8 ml Opti-MEM per well was added to the previous mixture and 1.0 ml of this mixture was applied to the cells in each well. Transfection time was 16 hours and this was stopped by aspiration of the transfection medium and addition of fresh MEM containing 10% FBS.

Uptake Measurements

In general, uptake studies were carried out as follows. Cells were washed twice with Na^+-free buffer. This wash solution was aspirated and the reaction mixture of either [^{3}H]inosine or [^{3}H]uridine was added to the well and incubated for the given time. Uptake of 1 μM [^{3}H]inosine (70 nM of radiolabeled inosine plus 1 μM unlabeled inosine) or 5 μM [^{3}H]uridine (50 nM of radiolabeled uridine plus 5 μM unlabeled uridine) was measured over time. All uptake assays were carried out in the presence of 10 μM nitrobenzylthioinosine (NBMPR). Uptake was measured in the presence of Na^+(128 mM NaCl, 4.73 mM KCl, 1.25 mM $CaCl_2$, 1.25 mM $MgSO_4$, 5 mM HEPES, pH 7.4) or absence of Na^+ in which Na^+ is replaced by choline (128 mM). The uptake was stopped by aspirating the reaction mixture and washing 3 times with ice-cold Na^+-free buffer. Cells were then solubilized with 1 ml 0.5% Triton-X 100 and 0.5 ml was sampled for scintillation counting.

Inhibition Studies

Inhibition experiments were carried out in duplicate or triplicate for 5 min. Data are presented as the mean ± SD. Uptake assays were stopped as stated above. All inhibition studies were carried out in the presence and absence of Na^+. Initial studies were also carried out with transfection of empty vector. The concentrations of the inhibitors are specified in the figure and table legends.

Protein Assay

For each plate used in an uptake study, 2–3 wells were reserved for protein analysis. Cells were washed twice with Na^+-free buffer and then solubilized with 0.5 ml of 1 N NaOH. The solution was neutralized by addition of 0.5 ml of 1 N HCl. A sample was taken for each protein assay. The Bradford method was used to carry out the assay using the Bio-Rad reagent. Bovine serum albumin (BSA) was used to generate the standard curve. Absorbance was read at 595 nm and the amount of protein/well was calculated from the standard curve.

Data Analysis

In general, data are expressed as mean ± standard deviation (SD). For Michaelis-Menten studies, rate of uptake was

expressed as pmol/mg protein/5 min. Data were fit to the equation $V = V_{max}[S]/(K_m + [S]) + (k_{ns}[S])$ where V is the rate of inosine or uridine uptake, [S] is the inosine or uridine concentration, k_{ns} is a constant which represents non-specific uptake expressed in pmol/mg protein/5 min in the absence of Na^+. The k_{ns} was the slope of the plot of rate of uptake versus inosine or uridine concentration in the presence of Na^+ in cells transfected with empty vector. The Kaleidagraph fitting program was used to fit the data and parameter estimates are expressed as a mean ± SE. For IC_{50} studies, data were fit to the equation $V = V_o/[1 + (I/IC_{50})^n + 3.69]$ where V is the uptake of inosine in the presence of inhibitor, V_o is inosine uptake in the absence of inhibitor, I is the inhibitor concentration, *n* is the slope, and the constant 3.69, determined experimentally, represents uptake (expressed as pmol/mg protein/5 min) in the absence of Na^+. For determination of statistical significance, a Student's unpaired *t*-test was used and $p < 0.05$ (one tail) was considered significant.

RESULTS

Initial Characterization of $SPNT_{int}$ Expression in Transfected HeLa Cells

Before transfection studies, we examined the endogenous uptake of [^{3}H]uridine in confluent HeLa cell monolayers. Our results confirmed that there was no detectable Na^+-dependent uptake of [^{3}H]uridine suggesting that HeLa would be an appropriate model for $SPNT_{int}$ transfection (data not shown). Endogenous nucleoside uptake was inhibited by NBMPR at a concentration of 10 μM; therefore, NBMPR was included in uptake studies.

Preliminary characterization of the transporter expressed in HeLa cells was necessary to determine an initial time, in the linear range, for further kinetic studies. Following incubation with [^{3}H]inosine uptake in the presence of Na^+ increased with time in the transfected cells, while that in the absence of Na^+ did not (Fig. 1). Na^+-stimulated [^{3}H]inosine uptake in HeLa cells transfected with pcDNA3 vector which did not contain the insert (empty vector) was similar to uptake in the absence of Na^+ in cells transfected with $SPNT_{int}$ (Fig. 1). Na^+-stimulated

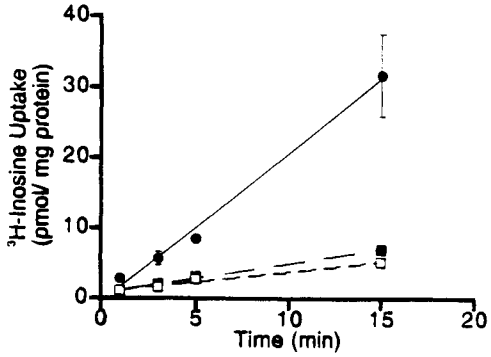

Fig. 1. Time course of 1 μM [^{3}H]inosine uptake in the presence and absence of Na^+. The uptake of [^{3}H]inosine was measured overtime in the presence (closed circles) and absence (closed squares) of Na^+ in HeLa cells transfected with $SPNT_{int}$. Control uptake of [^{3}H]inosine was carried out in cells transfected with pcDNA3 vector without the $SPNT_{int}$ insert (empty vector) in the presence (open circles) and absence (open squares) of Na^+.

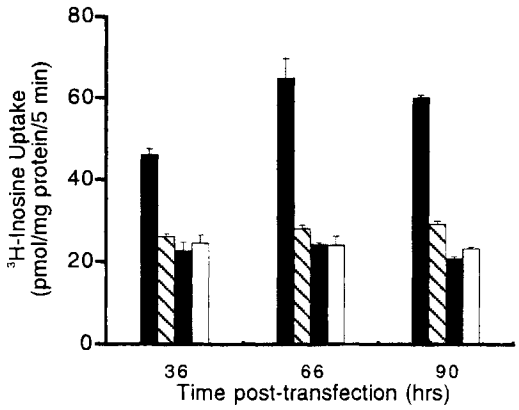

Fig. 2. Expression of transfected $SPNT_{int}$ in HeLa cells over time. Following transfection of $SPNT_{int}$ in HeLa cells, the 5 min uptake of [^{3}H]inosine was measured at 36, 66, and 90 hours post-transfection in the presence (solid bars) and absence (hatched bars) of Na^+. Control [^{3}H]inosine uptake in cells transfected with empty vector was also measured in the presence (shaded bars) and absence (open bars) of Na^+ at 5 min.

[^{3}H]inosine uptake in the transfected HeLa cells was linear at early times (1, 3 and 5 min). At later times, i.e., 30 and 60 min (data not shown) uptake began to equilibrate. Five minutes was selected for kinetic studies because it was in the linear range and allowed maximal differentiation between Na^+-dependent and independent uptake. Thin layer chromatography (TLC) demonstrated that at 5 min less than 10% of [^{3}H]inosine is metabolized in the transfected HeLa cells (data not shown).

The expression level of $SPNT_{int}$ at several times following transfection was determined. At 36 h post-transfection, Na^+-dependent [^{3}H]inosine uptake was significantly greater than uptake in transfected cells in the absence of Na^+ (46.2 ± 1.37 vs 26.2 ± 0.61 pmol/mg/5 min, $p < 0.05$; Fig. 2). Na^+-dependent [^{3}H]inosine uptake continued to increase and was enhanced approximately threefold in comparison to Na^+-independent uptake at 66 hours following transfection. Expression began to decline at 90 hours (Fig. 2) and was not present 120 hours after the initial transfection (data not shown). For further studies, uptake of the model compounds was measured between 36 and 90 h after transfection.

Michaelis-Menten Studies of ^{3}H-inosine and ^{3}H-uridine

The rate of uptake of the nucleosides, [^{3}H]inosine (Fig. 3A) and [^{3}H]uridine (Fig. 3B), was studied as a function of concentration in the range of 1 μM to 500 μM. Because the rate of uptake was linear at the higher concentrations, an equation consisting of both a saturable and a linear term was used in the fits. Data at the lower concentrations are presented in the Fig. 3 and data at all concentrations are presented as insets. Both compounds exhibited saturation kinetics with a K_m and V_{max} (mean ± SE) for inosine were 28.1 ± 7.1 μM and 343 ± 42 pmol/mg protein/5 min. For uridine, the respective values were 20.6 ± 5.6 μM and 543 ± 51.7 pmol/mg protein/5 min. The k_{ns} for inosine and uridine were 1.9 and 4.3 pmol/mg protein/5 min, respectively.

Inhibition Studies

We examined the uptake of [^{3}H]inosine in the presence of several endogenous nucleosides and nucleoside analogs each

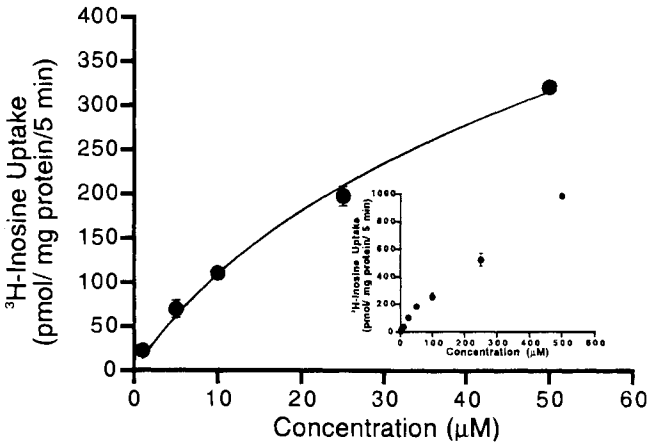

Fig. 3A. Saturability of [^{3}H]inosine uptake. Uptake of [^{3}H]inosine was measured in the presence of increasing concentrations of inosine. Uptake was measured at 5 min and data were fit to the equation $V = V_{max}[S]/(K_m + [S]) + k_{ns}[S])$. Data from Na^+-dependent uptake, adjusted for non-specific uptake with the Na^+-independent data, are presented as mean ± SE (n = 3) of a representative experiment.

at a concentration of 1 mM. Significant inhibition of [^{3}H]inosine uptake (Fig. 4A) ($p < 0.05$) was observed in the presence of the endogenous nucleosides uridine and inosine. Thymidine produced less inhibition of [^{3}H]inosine uptake. Significant inhibition ($p < 0.05$) of [^{3}H]inosine uptake was observed in the presence of ddI, ddA, acyclovir, and 2CdA (Fig. 4B). Similar results were obtained when [^{3}H]uridine was used as the model compound (Table I). Further characterization of the inhibition by ddI and 2CdA on [^{3}H]inosine uptake was carried out due to the clinical importance of these two compounds. The IC_{50}'s of ddI and 2CdA were 46 μM (Fig. 5A) and 13 μM (Fig. 5B), respectively.

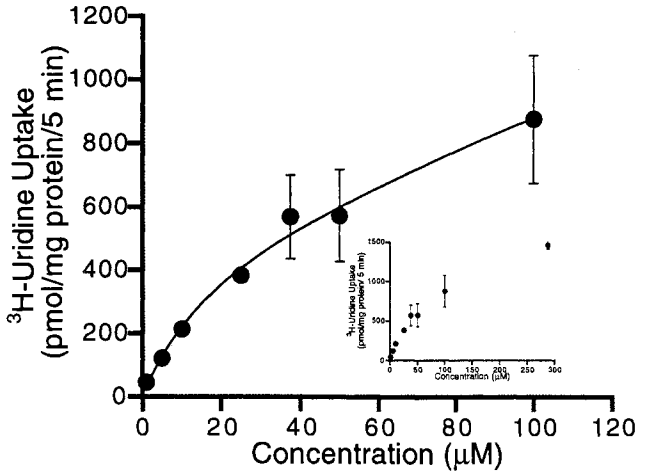

Fig. 3B. Saturability of [^{3}H]uridine uptake. Uptake of [^{3}H]uridine was measured in the presence of increasing concentrations of uridine in the presence and absence of Na^+. Uptake was measured at 5 min and data were fit to the equation $V = V_{max}[S]/(K_m + [S]) + k_{ns}[S])$. Data from Na^+-dependent uptake, adjusted for non-specific uptake with the Na^+-independent data, are presented as mean ± SE (n = 3) of a representative experiment.

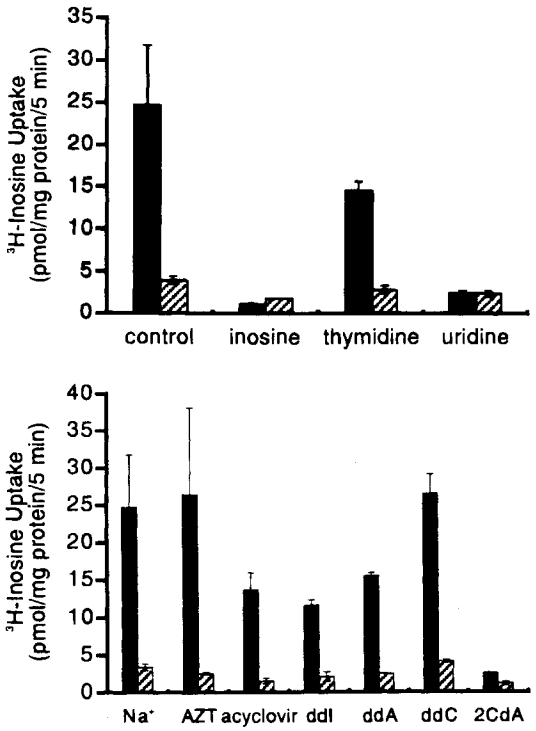

Fig. 4A and B. Inhibition of [^{3}H]inosine uptake. Uptake of 1 μM [^{3}H]inosine was measured in the presence of 1 mM of the given compound in the presence (solid bars) and absence (hatched bars) of Na^+ in HeLa cell transfected with $SPNT_{int}$. Uptake was carried out for 5 min and is presented as uptake in cells transfected with $SPNT_{int}$.

Permeant Studies

To determine whether 2CdA and ddI were also permeants of SPNT, uptake studies were performed with radiolabeled compounds. Na^+-dependent [^{3}H]2CdA uptake (at 5 min) was enhanced approximately 3-fold over uptake in the absence of Na^+ (Table II) whereas the Na^+-dependent uptake of [^{3}H]ddI

Table I. Inhibition of [^{3}H]uridine Uptake

	HeLa cells transfected with $SPNT_{int}$[b] (pmol/mg protein/5 min)	HeLa cells transfected with empty vector (pmol/mg protein/5 min)
Control	61.6 ± 0.47	17.3 ± 1.51
Inosine	19.5 ± 0.28	18.1 ± 1.40
Thymidine	41.9 ± 2.43	16.9 ± 1.87
Uridine	20.9 ± 2.49	17.3 ± 2.51
AZT	53.9 ± 1.18	18.7 ± 0.93
2-Chloroadenosine	17.9 ± 1.03	13.9 ± 0.99
Without Na^+[a]	18.7 ± 0.60	17.4 ± 0.25

Note: Uptake of 5 μM [^{3}H]uridine was measured in the presence of 1 mM of the given compound. Uptake was for 5 min and is presented as Na^+-dependent uptake in cells transfected with $SPNT_{int}$ or with empty vector. Control cells represent Na^+-dependent uptake without inhibitor. Data represent the mean ± SD of results from 2 to 3 wells each.

[a] Uptake of [^{3}H]uridine in the absence of Na^+.

[b] All values are significantly different from control ($p < 0.05$).

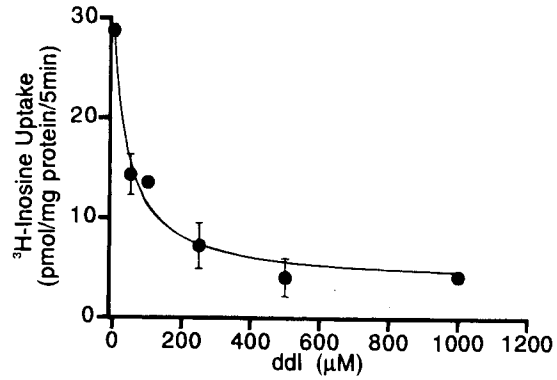

Fig. 5A. IC_{50} of ddI. Uptake of [^{3}H]inosine was measured in the presence of increasing concentrations of unlabeled ddI in the presence and absence of Na^+. Data were fit to the equation $V = V_o/[1 + (I/IC_{50})^n + 3.69]$ using the Na^+-dependent uptake data and adjusted for non-specific uptake with the Na^+-independent data. Data are presented as mean ± SE.

was not enhanced, even at later times (data not shown). These data suggest that 2CdA is a permeant of $SPNT_{int}$ whereas ddI is not. Significant inhibition of [^{3}H]2CdA uptake was observed in the presence of both unlabeled 2CdA (1 mM) and unlabeled inosine (1 mM) (Table II).

DISCUSSION

Nucleoside analogs are currently being used in the treatment of infections with viruses such as cytomegalovirus, hepatitis B virus, HIV (20,21), and herpes simplex virus. Furthermore, antineoplastic agents such as 2CdA are important in cancer chemotherapy (22). The endogenous nucleoside, adenosine, is used in the treatment of arrhythmias (23). Understanding the interaction of these analogs with the purine-selective nucleoside transporter cloned from intestine may lead to a greater understanding of the oral absorption of such compounds (24). Interactions of these compounds with nucleoside transporters may therefore be clinically important.

HeLa cells have been used for the expression of several transporters, including the human placental folate transporter (18) and a Na^+-dependent L-proline transporter cloned from rat brain (19). HeLa cells possess equilibrative nucleoside processes which have been well characterized (25) but do not exhibit Na^+-dependent nucleoside uptake. In this study, we demonstrated the functional expression, following transfection, of a saturable, Na^+-dependent, purine-selective nucleoside transporter ($SPNT_{int}$) in HeLa cells. Both uridine and inosine, known permeants of the purine-selective transporter were found to exhibit saturable and inhibitable uptake. The K_m of inosine in HeLa (28 μM) is slightly greater than that in *Xenopus laevis* oocytes (14.6 μM) (unpublished data from this laboratory). The reason for this difference is unknown, but may be due to differences in experimental conditions, membrane effects on protein tertiary structure or to differences in processing of the protein between oocytes and HeLa. Comparative data for uridine kinetics are not available.

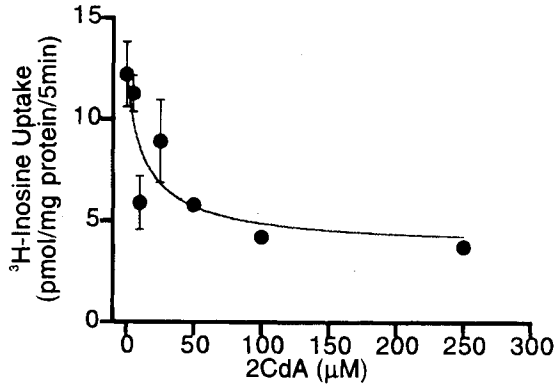

Fig. 5B. IC_{50} of 2CDA. Uptake of [^{3}H]inosine was measured at 5 min in the presence of increasing concentrations of 2CdA. Data were fit to the equation $V = V_o/[1 + (I/IC_{50})^n + 3.69]$.

Table II. [^{3}H]2CdA Uptake in HeLa Cells Transfected with $SPNT_{int}$

Control	6.85 ± 0.60
Inosine	2.91 ± 0.23[a]
Thymidine	4.69 ± 1.58
2CdA	2.23 ± 0.06[a]
Without Na^+[b]	2.99 ± 0.18[a]

Note: [^{3}H]2CdA uptake was measured at 5 min at 25°C in the presence of Na^+ (control) and 1 mM 2CdA, inosine or thymidine. Data represent the mean ± SD of results from 2 to 3 wells each and are presented as pmol/mg protein/5 min.
[a] Data are statistically different from control ($p < 0.05$).
[b] The control for this study was 8.87 ± 0.42 pmol/mg protein/5 min.

Inhibition studies confirmed the expected substrate selectivity, based on previous functional studies of SPNT in *Xenopus laevis* oocytes (6,16). Studies on the interaction of dideoxynucleoside analogs with SPNT suggest that ddI and ddA, but not AZT and ddC, significantly inhibited the uptake of inosine by $SPNT_{int}$. Because AZT and ddC are pyrimidine analogs whereas ddI and ddA are purine analogs, the data are consistent with the known purine selectivity of SPNT. Both AZT and ddC have been shown to interact with the pyrimidine-selective nucleoside transporter, cNT1 in transfected COS-1 cells (26). Since ddI is administered orally and has a low bioavailability, we further characterized its interaction with $SPNT_{int}$. Although ddI is a reasonably potent inhibitor of inosine transport by $SPNT_{int}$, it does not appear to be a permeant. These data suggest that $SPNT_{int}$ does not play a role in the intestinal absorption of ddI.

In contrast, 2CdA (Cladribine) appears to function as both an inhibitor (IC_{50} = 13.8 μM) and a permeant of $SPNT_{int}$ (Fig. 5B and Table II). 2CdA has been shown to be effective in the treatment of hairy cell leukemia (27,28). Efficacy of 2CdA has also been demonstrated in chronic lymphocytic leukemia (29), non-Hodgkin's lymphoma, and cutaneous T-cell lymphoma (27). Furthermore, although marketed as an injectable formulation, clinical studies have shown that 2CdA is orally available and well tolerated by patients (27,28). Our data suggest that $SPNT_{int}$ may play a role in the oral absorption of 2CdA. The system described here may serve as a model in which to further study the interaction of 2CdA as well as other nucleoside analogs with $SPNT_{int}$.

In summary, we have described the first transient expression system for the purine-selective nucleoside transporter, $SPNT_{int}$, in HeLa cells. We have also demonstrated for the first

time that the antineoplastic agent, 2CdA, is a substrate for $SPNT_{int}$. This expression system is simple, reliable and reproducible and may be used to study drug interactions with this carrier as well as to address questions related to the function and intracellular processing of the protein.

ACKNOWLEDGMENTS

This work was supported by NIH Grant GM42230.

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