The Broadly Selective Human $\text{Na}^+/\text{Nucleoside Cotransporter (hCNT3) Exhibits Novel Cation-coupled Nucleoside Transport Characteristics}$

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The concentrative nucleoside transporter (CNT) protein family in humans is represented by three members, hCNT1, hCNT2, and hCNT3. hCNT3, a $\text{Na}^+$/nucleoside symporter, transports a broad range of physiological purine and pyrimidine nucleosides as well as anticancer and antiviral nucleoside drugs, and belongs to a different CNT subfamily than hCNT1/2. H$^+$-dependent Escherichia coli NupC and Candida albicans CaCNT are also CNT family members. The present study utilized heterologous expression in Xenopus oocytes to investigate the specificity, mechanism, energetics, and structural basis of hCNT3 coupling. hCNT3 exhibited uniquely broad cation interactions with $\text{Na}^+$, $\text{H}^+$, and Li$^+$ not shared by $\text{Na}^+$-coupled hCNT1/2 or H$^+$-coupled NupC/CaCNT. $\text{Na}^+$ and $\text{H}^+$ activated hCNT3 through mechanisms to increase nucleoside apparent binding affinity. Direct and indirect methods demonstrated cation/nucleoside coupling stoichiometries of 2:1 in the presence of $\text{Na}^+$ and both $\text{Na}^+$ plus $\text{H}^+$, but only 1:1 in the presence of $\text{H}^+$ alone, suggesting that hCNT3 possesses two $\text{Na}^+$-binding sites, only one of which is shared by $\text{H}^+$. The H$^+$-coupled hCNT3 did not transport guanosine or 3′-azido-3′-deoxythymidine and 2′,3′-dideoxycytidine, demonstrating that $\text{Na}^+$- and $\text{H}^+$-bound versions of hCNT3 have significantly different conformations of the nucleoside binding pocket and/or translocation channel. Chimeric studies between hCNT1 and hCNT3 located hCNT3-specific cation interactions to the C-terminal half of hCNT3, setting the stage for site-directed mutagenesis experiments to identify the residues involved.

Physiological nucleosides and synthetic nucleoside analogs have important biochemical, physiological, and pharmacological activities in humans. Adenosine, for example, has purino-receptor-mediated functions in processes such as modulation of immune responses, platelet aggregation, renal function, and coronary vasodilation (1, 2). Nucleosides also provide salvage precursors for nucleic acid biosynthesis, and nucleoside drugs are commonly used in the therapy of cancer and viral infections (3, 4). Most nucleosides, including those with antineoplastic and/or antiviral activities, are hydrophilic and require specialized plasma membrane nucleoside transporter (NT) proteins for their uptake into or release from cells (5–7).

Multiple transport systems for nucleosides have been observed in human and other mammalian cells and tissues (7–9). The concentrative systems (cit, cif, and cif)$^2$ are inwardly directed $\text{Na}^+$-dependent processes present primarily in intestinal and renal epithelia and other specialized cells (7–9). The equilibrative systems (es and ei) mediate bidirectional downhill transport of nucleosides, have generally lower substrate affinities than the concentrative systems, and occur in most, possibly all, cell types (7–9). Systems cit and cif transport adenosine and uridine, but are otherwise pyrimidine and purine nucleoside-selective, respectively, whereas systems cif, es, and ei transport both pyrimidine and purine nucleosides. System es is inhibited by nanomolar concentrations of nitrobenezthioinosine (NBMPR), whereas system ei also transports nucleobases (7–10).

Molecular cloning studies have identified the human and rodent integral membrane proteins responsible for each of these nucleoside transport activities (11–18). They belong to two previously unrecognized and structurally unrelated protein families (CNT and ENT), and their relationship to the processes defined by functional studies is: CNT1 (cit), CNT2 (cif), CNT3 (cif), ENT1 (es), and ENT2 (ei) (11–18). In addition to ENT1 and ENT2, the ENT protein family also contains three further human and rodent members (ENT3, ENT4, and CLN3) (19–23). Human and other eukaryote CNTs have 13 predicted transmembrane helices (TM$s$), with an intracellular N terminus and an extracellular C terminus (24). NupC, an H$^+$-coupled CNT from Escherichia coli, has a similar predicted topological

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1 The abbreviations used are: NT, nucleoside transporter; CNT, concentrative nucleoside transporter; ENT, equilibrative nucleoside transporter; AZT, 3′-azido-3′-deoxythymidine; ddC, 2′,3′-dideoxycytidine; ddI, 2′,3′-dideoxyinosine; NBMPR, nitrobenezthioinosine (6-[(4-nitrobenzyl)thio]-9-β-D-ribofuranosyluracil); TM, putative transmembrane helix; MES, 2-(N-morpholino)ethanesulfonic acid; CCCP, carbonyl cyanide m-chlorophenylhydrazone.

2 The abbreviations used in transporter acronyms are: c, concentrative; e, equilibrative; s and i, sensitive and insensitive to inhibition by NBMPR, respectively; f, formycin B (non-metabolized purine nucleoside); t, thymidine; b, broad selectivity.
ology, but lacks TMs 1–3 (25, 26). Other CNT family members that have been functionally characterized include H\(^+\)-coupled CeCNT3 and CaCNT from Caenorhabditis elegans (27) and Candida albicans (28), respectively, and Na\(^+\)-coupled hCNT from the Pacific hagfish (Eptatretus stouti) (29, 30).

Human and mouse CNT3 (hCNT3 and mCNT3) are the most recent mammalian CNT family members to be identified (18) and, together with cib-type hagfish CNT (hCNTF) (30), form a phylogenetic CNT subfamily separate from the mammalian CNT1/2 subfamily (18). In addition to differences in substrate selectivities, members of the two subfamilies also differ in the stoichiometry of Na\(^+\)/nucleoside coupling. Contrary to a recent report in this journal (31), for example, we have shown that hCNT1 has a Na\(^+\)/nucleoside coupling ratio of 1:1 (32), whereas the coupling ratio of hCNT is 2:1 (30). Here, we extend these investigations and present a mechanistic and chimeric analysis of cation coupling in hCNT3. The results validate our previously reported differences in cation coupling between the CNT3/hCNT and CNT1/2 subfamilies and reveal additional novel features of CNT3-cation interactions.

**Experimental Procedures**

hCNT3 cDNA—cDNA encoding hCNT3 (GenBank\textsuperscript{TM} accession number AF305210) in the enhanced Xenopus plasmid expression vector pGEM-HE (33) with flanking 5' and 3'-untranslated regions from the Xenopus \(\beta\)-globin gene was obtained as previously described (18).

*In Vitro Transcription and Expression in Xenopus Oocytes—hCNT3 plasmid DNA was linearized with NheI and transcribed with T7 polymerase using the mMESSAGE mMACHINE\textsuperscript{TM} (Ambion, Austin, TX) transcription system. The remaining template was removed by digestion with RNase-free DNase I. Stages V–VI oocytes from Xenopus laevis were treated with collagenase (2 mg/ml) for 2 h, and remaining follicular layers were removed by phosphate treatment (100 mM K\(_2\)PO\(_4\)) and manual defolliculation (11). Twenty-four hours after defolliculation, oocytes were injected with either 10 nl of water containing 10 ng of RNA transcript encoding hCNT3 or 10 nl of water alone. Injected oocytes were then incubated for 4 days (radioisotope flux studies) or 4–7 days (electrophysiology) at 18 °C in modified Barth’s solution (changed daily) (85 mM NaCl, 1 mM KCl, 0.33 mM Ca\((NO_3)_2\), 0.41 mM CaCl\(_2\), 0.82 mM MgSO\(_4\), 2.4 mM NaHCO\(_3\), 10 mM Hepes, 2.5 mM sodium pyruvate, 0.05 mg/ml penicillin, and 0.1 mg/ml gentamicin sulfate, pH 7.5).

**Transport Media**—Unless otherwise stated, electrophysiological and radioisotope flux studies used Na\(^+\)-containing transport medium composed of 100 mM NaCl, 2 mM KCl, 1 mM CaCl\(_2\), 1 mM MgCl\(_2\), and 10 mM Hepes (for pH values \(\geq 6.5\)) or 10 mM MES (for pH values \(\leq 6.5\)). In experiments examining the Na\(^+\) dependence of transport (i.e., where the indicated Na\(^+\) concentration is \(<100 \text{ mM}\)) in the transport medium was replaced by equimolar choline chloride (ChCl\(_2\)) to maintain isomolarity. Proton dependence was tested in Na\(^+\)-free choline-containing transport medium (100 mM ChCl\(_2\)) at pH values ranging from 4.5 to 8.5. Experiments testing Li\(^+\) coupling of transport were performed in medium at pH 8.5 containing Li\(^+\) (100 mM LiCl) in place of Na\(^+\).

**Electrophysiological Studies**—Nucleoside-evoked membrane currents were measured in hCNT3-producing oocytes at room temperature (20 °C) using a GeneClamp 500B oocyte clamp (Axon Instruments Inc., Foster City, CA) in the two-electrode, voltage clamp mode. The GeneClamp 500B was interfaced to an IBM-compatible PC via a Digidata 1200A/D converter and controlled by pCLAMP software (version 8.0, Axon Instruments Inc.). The microelectrodes were filled with 3 M KCl and had resistances that ranged from 0.5 to 2.5 MΩ. Oocytes were penetrated with the microelectrodes, and their membrane potentials were monitored for periods of 10–15 min. Oocytes were discarded when membrane potentials were unstable, or more positive than \(-30\) mV. Unless otherwise indicated, the oocyte membrane potential was clamped at a holding potential (\(V_h\)) of \(-50\) mV, and nucleoside was added in the appropriate transport medium at a concentration of 100 \(\mu\text{M}\). Current signals were filtered at 20 Hz (four-pole Bessel filter) and sampled at a sampling interval of 20 ms. For data presentation, the signals were further filtered at 0.5 Hz by the pCLAMP program suite.

In proteophore studies, carboxyl cyanide m-chlorophenyldiamidine (CCCP) (100 \(\mu\text{M}\)) was preincubated with oocytes for 15 min prior to measuring uridine-evoked currents (100 \(\mu\text{M}\)) in Na\(^+\)-free medium (100 mM ChCl, pH 5.5). Stock solutions of CCCP were dissolved in Me\(_2\)SO. Control experiments confirmed that oocytes were unaffected by Me\(_2\)SO at its final concentration of 0.5% (v/v).

**Radioisotope Flux Studies**—Radioisotope transport assays were performed as described previously (11, 18) on groups of 10–12 oocytes at 20 °C using \(^{14}\text{C}\)-labeled nucleosides (1 \(\mu\text{Ci}/\text{ml}) in 200 \(\mu\text{l}\) of the appropriate transport medium. Unless otherwise stated, nucleoside uptake was determined at a concentration of 20 \(\mu\text{M}\). Following incubation, seven rapid washes in ice-cold choline chloride transport medium (pH 7.5) removed extracellular label, and individual oocytes were dissolved for liquid scintillation counting (LS 6000 IC, Beckman, Fullerton, CA). Uptake values represent initial rates of transport (1-min flux) and are corrected for basal non-mediated uptake in control water-injected oocytes and are means \(\pm\) S.E. of 10–12 oocytes. The experiment was performed on a single batch of oocytes used on the same day.

**Data from individual electrophysiology experiments are presented as nucleoside-evoked currents from single representative oocytes or as mean values (\(\pm\)S.E.) from four or more oocytes from the same batch of oocytes used on the same day. Each experiment was repeated at least twice on oocytes from different frogs. No nucleoside-evoked currents were detected in oocytes injected with water alone, demonstrating that currents in hCNT3-producing oocytes were transporter-specific.

**Radioisotope Flux Studies**—Radioisotope transport assays were performed as described previously (11, 18) on groups of 10–12 oocytes at 20 °C using \(^{14}\text{C}\)-labeled nucleosides (1 \(\mu\text{Ci}/\text{ml}) in 200 \(\mu\text{l}\) of the appropriate transport medium. Unless otherwise stated, nucleoside uptake was determined at a concentration of 20 \(\mu\text{M}\). Following incubation, seven rapid washes in ice-cold choline chloride transport medium (pH 7.5) removed extracellular label, and individual oocytes were dissolved in 1% (w/v) SDS for quantitation of cell-associated radioactivity by liquid scintillation counting (LS 6000 IC, Beckman, Fullerton, CA). Uptake values represent initial rates of transport (1-min flux) and are presented as means \(\pm\) S.E. of 10–12 oocytes from representative experiments. Individual experiments were performed on cells from single batches of oocytes used on the same day. Transporter-mediated fluxes were calculated as uptake in RNA transcript-injected oocytes minus uptake in control water-injected oocytes. Each experiment was repeated at least twice using oocytes from different frogs.

**Kinetic Parameters**—Kinetic parameters calculated from electrophysiological and radioisotope flux experiments were determined by least squares fits to the Michaelis-Menten and Hill equations using SigmaPlot 2000 software (Jandel Scientific Software, San Rafael, CA). Those from electrophysiological experiments were determined from fits to data from individual oocytes normalized to the \(I_{\text{max}}\) value obtained for that oocyte, and are presented as values (\(\pm\)S.E.) for single representative oocytes, or as means (\(\pm\)S.E.) of four or more cells. Those from radioisotope experiments were derived from curve fits to averaged mediated data from 10–12 oocytes, and are presented as means (\(\pm\)S.E.).

**Charge-to-Nucleoside Stoichiometry**—Na\(^+\)/nucleoside and H\(^+\)/nucleoside coupling ratios for hCNT3 were determined by simultaneously measuring Na\(^+\) or H\(^+\) currents and \(^{14}\text{C}\)-uridine (200 \(\mu\text{M}, 1 \text{min flux}) in hCNT3-producing oocytes were measured in transport media containing Na\(^+\) (black bar; 100 mM NaCl, pH 7.5), choline (open bars; 100 mM ChCl, pH 5.5–8.5), or Li\(^+\) (gray bar; 100 mM LiCl, pH 8.5). Values were corrected for basal non-mediated uptake in control water-injected oocytes and are means \(\pm\) S.E. of 10–12 oocytes. The experiment was performed on a single batch of oocytes used on the same day.

**Fig. 1. Effects of Na\(^+\), H\(^+\), and Li\(^+\) on the transport activities of oocytes expressing recombinant hCNT3.** Radioisotope fluxes of uridine (20 \(\mu\text{M}, 20^\circ\text{C}, 1\)-min flux) in hCNT3-producing oocytes were measured in transport media containing Na\(^+\) (black bar; 100 mM NaCl, pH 7.5), choline (open bars; 100 mM ChCl, pH 5.5–8.5), or Li\(^+\) (gray bar; 100 mM LiCl, pH 8.5). Values were corrected for basal non-mediated uptake in control water-injected oocytes and are means \(\pm\) S.E. of 10–12 oocytes. The experiment was performed on a single batch of oocytes used on the same day.
a 10-min period to monitor baseline currents. When the baseline was stable, the nucleoside-free medium was exchanged with medium of the same composition containing radiolabeled uridine. Current was measured for 3 min, and uptake of uridine was terminated by washing the oocyte with nucleoside-free medium until the current returned to baseline. The oocyte was then transferred to a scintillation vial and solubilized with 1% (w/v) SDS for quantitation of oocyte-associated radioactivity. Testing of each individual hCNT3-producing oocyte (from the same donor frog) under equivalent conditions yielded radiolabeled flux for each oocyte to determine the charge/uptake ratio. Basal [14C]uridine uptake was determined in control water-injected oocytes, and the bath solution was changed for 3 min to one containing 200 μM uridine in Na\textsuperscript{+}-containing transport medium (black bar; 100 mM NaCl, pH 8.5). Na\textsuperscript{+}-free transport medium (open bar; 100 mM ChCl, pH 5.5), and Na\textsuperscript{+}-free transport medium containing 100 μM CCCP (gray bar; 100 mM ChCl, pH 5.5). Currents are means ± S.E. of five different oocytes from the same batch of cells used on the same day.

RESULTS

Cation Dependence of hCNT3—We have previously used heterologous expression in Xenopus oocytes in combination with radioisotope flux assays and the two-microelectrode voltage clamp to demonstrate that recombinant hCNT3 functions as a broad specificity cib-type electrogenic Na\textsuperscript{+}/nucleoside symporter (18). The experiment of Fig. 1 extended these findings and demonstrated the effect of an imposed H\textsuperscript{+} gradient on the
initial rate of hCNT3-mediated uptake (influx) of [14C]uridine (20 μM) measured at extracellular pH values ranging from 5.5 to 8.5 under Na+-free conditions. Choline (Ch+) was substituted for Na+, and values were corrected for basal non-mediated uptake in control water-injected oocytes (<0.03 pmol/oocyte-min⁻¹ under all conditions tested). A marked pH dependence of uridine influx was apparent. In 100 mM ChCl at pH 5.5, for example, hCNT3-mediated uridine influx was 26-fold higher than at pH 8.5, and was ~60% of that obtained in the presence of 100 mM NaCl at pH 7.5. In the absence of either H⁺ or Na⁺, hCNT3 also exhibited Li⁺-dependent uridine influx (100 mM LiCl, pH 8.5, versus 100 mM ChCl, pH 8.5). In marked contrast, hCNT1 and hCNT2, the two other human CNT isoforms, showed no pH-dependent uridine uptake and exhibited very small Li⁺-mediated uridine influx (~2% of the corresponding Na⁺-mediated uridine flux; data not shown). As illustrated in Fig. 2, the novel H⁺ and Li⁺ dependence of hCNT3 were further investigated by electrophysiology. External application of 100 μM uridine to a representative hCNT3-producing oocyte clamped at ~50 mV under Na⁺-free conditions elicited inward H⁺ and Li⁺ currents that returned to baseline upon removal of substrate (Fig. 2A). As demonstrated by the mean current data in Fig. 2B, H⁺ currents were markedly inhibited by pre-treatment of oocytes with the protonophore CCCP. No currents were
observed in control water-injected oocytes (data not shown). Thus, in addition to being Na\(^+\)-dependent, hCNT3 functioned as an electrogenic H\(^+\)/nucleoside and Li\(^+\)/nucleoside symporter. These findings were also confirmed in parallel studies of mCNT3 (data not shown). Subsequent experiments focused on the mechanism(s) of Na\(^+\)/H\(^+\) and H\(^+\)/nucleoside coupling of hCNT3.

**Cation-induced Changes in hCNT3 Substrate Selectivity**—In Na\(^+\)-containing medium at physiological pH 7.5, hCNT3 transports different radiolabeled physiological pyrimidine and purine nucleosides with similar apparent \(K_m\) values and \(V_{max} / K_m\) ratios (18). As illustrated by the mean current data in Fig. 3A, a panel of pyrimidine (uridine, thymidine, and cytidine) and purine (adenosine, inosine, and guanosine) nucleosides elicited similar large inward Na\(^+\) currents when applied at 100 \(\mu\)M to hCNT3-producing oocytes in NaCl transport medium at pH 7.5. The corresponding inward currents generated by the same panel of nucleosides under Na\(^+\)-free conditions were measured in ChCl transport medium of increasing acidity (pH 8.5, 7.5, 6.5, and 5.5). For clarity of presentation, only values obtained at pH 5.5 and 8.5 are shown in Fig. 3B. Similar to the trend seen for uridine in Fig. 1, inward pH-dependent nucleoside-evoked currents were evident for uridine, thymidine, and adenine, were less marked for cytidine and inosine, and were absent for guanosine. A corresponding selectivity profile for hCNT3 H\(^+\)/nucleoside cotransport was obtained in radioisotope nucleoside influx assays (Fig. 3C). Those nucleosides in Fig. 3 (B and C) that exhibited the lowest transport activity in Na\(^+\)-free acidified medium (cytidine, inosine, and guanosine) were also tested by electrophysiology at a higher substrate concentration of 1 mM (Fig. 3D). Substrate-induced H\(^+\) currents were confirmed for cytidine and inosine, whereas guanosine-evoked currents remained low and pH-independent. For all nucleosides and conditions tested, no currents were evident (data not shown) and basal non-mediated radioisotope fluxes were <0.07 pmol/oocyte-min \(^{-1}\) in control water-injected oocytes. Na\(^+\)- and H\(^+\)-coupled hCNT3 therefore exhibited markedly different se-
lectivity profiles for physiological nucleosides.

This difference in substrate selectivity between Na⁺- and H⁺-coupled hCNT3 extended to interactions with therapeutic nucleoside analogs. In Na⁺-containing medium at physiological pH 7.5, hCNT3 efficiently transports the anticancer nucleoside drug gemcitabine, and mediates lower, but still significant, fluxes of the antiviral nucleoside drugs AZT, ddC, and ddI (18). As shown by the traces from a single representative oocyte in Fig. 4A and by the mean current data in Fig. 4B, gemcitabine (100 μM) elicited inward Na⁺ and H⁺ currents that returned to baseline upon removal of substrate, whereas ddC and AZT (1 mM) produced inward currents only in the presence of Na⁺. No currents were detected in control water-injected oocytes (data not shown). The inability of H⁺-coupled hCNT3 to support ddC and AZT transport was confirmed in parallel radioisotope flux experiments (data not shown).

Voltage Dependence of hCNT3 Transport Kinetics—The effects of membrane potential on hCNT3 transport kinetics in the presence of external Na⁺ were examined in detail by measuring the apparent affinities for uridine (Kₘ) and Na⁺ (Kₘ). As shown in Fig. 6A, the uridine-evoked maximum current (Iₘₐₓ) was determined in transport medium containing either 10 or 100 mM NaCl (pH 8.5), and mean values plotted as a function of Vₑ. As representative examples of the kinetic data used to generate Kₚ values, Fig. 6, A and B, show uridine concentration dependence curves recorded from individual oocytes at a membrane potential of −10 mV and external Na⁺ concentrations of 10 and 100 mM, respectively. Kₚ was determined at an external uridine concentration of 100 μM (pH 8.5), and mean values plotted as a function of Vₑ summarized in Fig. 6E. The representative single oocyte Na⁺-activation curve shown in Fig. 6D was measured at a holding potential of −10 mV. Iₘₐₓ values were determined independently at a saturating external uridine concentration of 100 μM and a Na⁺ concentration of 100 mM (pH 8.5) (Fig. 6E).

As demonstrated in Fig. 6C, Kₚ was unaffected by membrane potential at 100 mM external Na⁺, but was voltage-dependent at 10 mM Na⁺, decreasing from 24 to 7.3 μM as the membrane potential was made more negative. At high negative potentials, the Kₚ value at 100 mM NaCl approached that observed at 100 mM external Na⁺, suggesting that the effect of membrane potential on Kₚ was predominantly the result of voltage dependence of Na⁺ binding (35, 36). Consistent with this, we found that the Na⁺−Kₚ value decreased from 5.3 μM at −10 mV to 2.1 μM at −70 mV (Fig. 6E). As shown in Fig. 6F, Iₘₐₓ also increased as the membrane potential was made more negative. At a holding potential of −10 mV (100 mM NaCl), the...
mean uridine-evoked inward current was 37 nA. This increased to 97 nA at −70 mV, a trend that was similar to the I-V relationship shown in Fig. 5B. Calculated over the same limited V_m range (−10 to −70 mV), I-V curves for five oocytes gave an e-fold change (±S.E.) in current per 77 ± 4 mV, compared with 67 ± 11 mV for the data in Fig. 6F. I_max reflects movement of the loaded and empty carrier (36). Similar to the Na+/glucose cotransporter SGLT1, therefore, membrane potential influenced both ion binding and carrier translocation (35, 36).

**Cation Coupling of hCNT3**

![Graphs showing I-V curves](Image)

**Fig. 6. Voltage dependence of the transport kinetics of hCNT3.** A, uridine concentration-response curve measured in a single representative hCNT3-producing oocyte at an external Na⁺ concentration of 10 mM (pH 8.5) and a membrane potential of −10 mV. Currents at each uridine concentration were normalized to the fitted I_max value for that oocyte (±S.E.) of 52 ± 1 nA. The K_m value was 32 ± 3 μM. B, recordings from a single representative hCNT3-producing oocyte demonstrating a corresponding experiment at the same membrane potential (−10 mV) in the presence of 100 mM external Na⁺ (pH 8.5). Currents were normalized to the fitted I_max value of 55 ± 3 nA. The K_m was 7.6 ± 1.1 μM. C, the effect of membrane potential on uridine K_m was determined at external Na⁺ concentrations of 10 mM (open circles) and 100 mM (solid circles) (pH 8.5) and holding potentials of −10, −30, −50, and −70 mV. K_m values at each membrane potential were obtained from fits to data from individual oocytes normalized to the I_max value obtained for that cell and are presented as means ± S.E. of 4–8 oocytes. D, Na⁺ concentration-response curve (pH 8.5) measured in a single representative hCNT3-producing oocyte at a membrane potential of −10 mV (100 μM uridine). Currents at each Na⁺ concentration were normalized to the fitted I_max value of 43 ± 2 nA. The Na⁺ K_50 was 5.5 ± 0.5 mM. E, the effect of membrane potential on Na⁺ K_50 was determined at holding potentials of −10, −30, −50, and −70 mV (100 μM uridine, pH 8.5). K_50 values at each membrane potential were obtained from fits to data from individual oocytes normalized to the I_max value obtained for that cell and are presented as means ± S.E. of 5–7 oocytes. F, the effect of membrane potential on maximum current (I_max) was determined at holding potentials of −10, −30, −50, and −70 mV in the presence of 100 mM external Na⁺ (pH 8.5) and a saturating concentration of uridine (100 μM). Each value is the mean ± S.E. of 3–4 oocytes from the same batch of cells used on the same day. No currents were observed in control water-injected oocytes. Note: to more accurately determine K_m and K_50 values (panels A–E), kinetic experiments at low membrane potentials were performed on preselected oocytes with maximal currents ≥ 40 nA. The effect of membrane potential on I_max (panel F) was measured independently in a separate experiment.
measured in both hCNT3-producing and control water-injected oocytes. Kinetic parameters (K_m and V_max) derived from these data for the hCNT3-mediated component of influx (uptake in RNA transcript-injected oocytes minus uptake in water-injected oocytes) are presented in Table I. Removal of extracellular Na^+ at pH 7.5 led to a greater than 30-fold increase in the K_m value for uridine influx from 17 to 580 μM that was partially offset by a small (1.6-fold) increase in V_max (Fig. 7, A and B). As shown in Fig. 7D, the decrease in uridine apparent affinity was substantially reversed by acidification of the transport medium to pH 5.5 (uridine K_m 110 μM). In contrast, acidification of the transport medium in the presence of Na^+/H^+ had only modest effects on uridine transport kinetics (Fig. 7, A and C).

V_max: K_m ratios, a measure of transporter efficiency, were as follows: 2.0 in the presence of Na^+ (NaCl, pH 7.5), 0.09 in the absence of Na^+ (ChCl, pH 7.5), 0.58 in the presence of H^+ (ChCl, pH 5.5), and 1.7 with both cations (Na^+ plus H^+) present (NaCl, pH 5.5). Therefore, Na^+ and H^+ activated hCNT3 through mechanisms resulting in increased uridine apparent binding affinity. Relative to Na^+ alone, Na^+ plus H^+ elicited no further shift in uridine K_m, suggesting that the two cations exert their effects by binding to a common or overlapping site(s).

**Na^+ and H^+ Activation Kinetics**—The relationship between hCNT3-mediated uridine-evoked current and Na^+ concentration (pH 8.5) was measured in oocytes clamped at −50 mV at three different uridine concentrations (5, 25, and 100 μM). Kinetic parameters derived from these experiments are presented in Table II. As reported previously for hCNT3 (and mCNT3) based on [14C]uridine influx experiments (18), and as illustrated in Fig. 8A for a single representative oocyte measured at a uridine concentration of 5 μM, the Na^+ activation curve was sigmoidal with a Hill coefficient (n) consistent with an apparent Na^+/nucleoside coupling stoichiometry of 2:1 (see also Fig. 6D). Both the apparent affinity for Na^+ (K_{50}) and the maximal current (I_{max}) increased as the external concentration of uridine was raised (Table II). This pattern resembled that found for hCNT1 (32) and was consistent with a sequential mechanism of transport in which Na^+ binds to the transporter first, increasing its affinity for nucleoside, which then binds second (37–40). Parallel flux experiments with [14C]uridine produced similar findings (data not shown).

The relationship between hCNT3 current evoked by 100 μM uridine and external pH in the absence of Na^+ (ChCl, pH 8.5–4.5) was also investigated (Table II). As illustrated for the representative oocyte in Fig. 8B, and in contrast to the sigmoidal activation curve observed for Na^+, a plot of current versus H^+ concentration was hyperbolic with a Hill coefficient (n) consistent with a 1:H^+/nucleoside coupling stoichiometry of 1:1. Parallel [14C]uridine influx experiments revealed similar hyperbolic H^+ activation kinetics (data not shown). Apparent K_{50}
individual oocytes normalized to the fitted representative experiment). Values were obtained from fits to data from uridine-evoked charge influx (picomoles) and 22Na.

**Table I**

| Apparent $K_{m}$ and $V_{max}$ values for uridine transport by hCNT3 |
|-----------------|-----------------|-----------------|
| $K_{m}$ | $V_{max}$ | $K_{m}$ |
| pH 7.5 | NaCl | 17 ± 1 | 34 ± 1 | 2.0 |
|       | ChCl | 580 ± 70 | 53 ± 3 | 0.09 |
| pH 5.5 | NaCl | 25 ± 3 | 43 ± 1 | 1.7 |
|       | ChCl | 110 ± 10 | 64 ± 1 | 0.58 |

* From Fig. 7 (A–D).

**Table II**

| Na$^{+}$ and H$^{+}$ activation kinetics of hCNT3 |
|-----------------|-----------------|-----------------|
| Hill coefficients (n) and apparent affinities ($K_{m}$) for Na$^{+}$ were determined from Na$^{+}$ concentration response curves (0–100 mM NaCl, pH 8.5) in hCNT3-producing oocytes measured at uridine concentrations of 5, 25, and 100 μM (see Fig. 8A for a representative experiment at 5 μM uridine). Those for H$^{+}$ were determined from H$^{+}$ concentration response curves (pH 8.5–4.5) measured in Na$^{+}$-free transport medium (100 mM ChCl) at a uridine concentration of 100 μM (see Fig. 8B for a representative experiment). Values were obtained from fits to data from individual oocytes normalized to the fitted $I_{max}$ value obtained for that cell and are presented as means ± S.E. $I_{max}$ values (nA) (± S.E.) in the presence of Na$^{+}$ were determined separately at a saturating concentration of uridine (100 μM) with 100 mM external Na$^{+}$ (pH 8.5) in oocytes from a single batch of cells used on the same day. The numbers in parentheses denote the number of oocytes. The membrane potential was −50 mV.

| Uridine concentration | μM | n | Apparent $K_{m}$ | $I_{max}$ |
|-----------------|-----|---|-----------------|-----------|
| Na$^{+}$ | 5 | 1.7 ± 0.15(5) | 1.0 ± 0.3(5) | 22 ± 2 (6) |
|       | 25 | 1.8 ± 0.1(4) | 3.1 ± 0.1 (4) | 43 ± 2 (6) |
|       | 100 | 1.7 ± 0.15(5) | 2.4 ± 0.4(5) | 56 ± 4 (6) |
| H$^{+}$ | 100 | 0.66 ± 0.02 (6) | 480 ± 70 (6) |

* The apparent $K_{m}$ for Na$^{+}$ is in millimolar, whereas that for H$^{+}$ is in nanomolar.

values for H$^{+}$ and Na$^{+}$ differed by four orders of magnitude (480 nm and 2.4–5.9 μm, respectively) (Table II).

**Na$^{+}$/Nucleoside and H$^{+}$/Nucleoside Coupling Ratios—**The Na$^{+}$/uridine and H$^{+}$/uridine stoichiometries of hCNT3 were directly determined by simultaneously measuring uridine-evoked currents and [14C]uridine uptake under voltage clamp conditions, as described previously for SGLT1 (41), the rat kidney dicarboxylate transporter SDCT1 (42) and, most recently, for hCNT1 (32), hCNT3 (30), and CaCNT (28). Each data point in Fig. 9 (A–F) represents a single oocyte, and the Na$^{+}$/uridine or H$^{+}$/uridine coupling ratio is given by the slope of the linear fit of charge (picomoles) versus uptake (picomoles) (Table III).

The first series of experiments was performed in Na$^{+}$-containing transport medium at pH 8.5, and at holding potentials of −30, −50, and −90 mV to determine the Na$^{+}$/nucleoside coupling ratio and its voltage dependence. At a holding potential of −30 mV, the linear correlation between uridine-dependent charge and uridine accumulation gave a stoichiometry of 1.4 (Fig. 9A) (Table III). This increased to 1.6 at −50 mV and 1.9 at −90 mV (Fig. 9B and C) (Table III). In marked contrast, parallel experiments performed in Na$^{+}$-free ChCl transport medium at pH 5.5 to determine the H$^{+}$/nucleoside coupling ratio at $V_{h}$ 30, −50, and −90 mV found voltage-independent stoichiometries in the range 0.92–1.1 (Fig. 9, D–F) (Table III). The same analysis was also performed in the presence of Na$^{+}$ and H$^{+}$ (NaCl, pH 5.5). As summarized in Table III, coupling ratios were voltage-independent and in the range 1.9–2.0.

**Charge-to-Na$^{+}$ Stoichiometry—**The relationship between uridine-evoked charge influx (picomoles) and 22Na$^{+}$ uptake (picomoles) was measured in five oocytes clamped at −90 mV (Fig. 10). A linear fit of the data gave a regression line with a slope of 0.97, indicating that one net inward positive charge was transported for every Na$^{+}$ ion cotransported with uridine into the cell (Table III).

**Characterization of hCNT3/hCNT1 Chimeras—**hCNT3 and hCNT1 are 48% identical and 57% similar in predicted amino acid sequence, with the strongest residue conservation in TMs of the C-terminal halves of the proteins (Fig. 11A). The major differences lie in the putative N- and C-terminal tails and in the first three TMs. To localize domains involved in cation recognition, a chimera (hCNT3/1), in which the C-terminal half of hCNT3 (incorporating TMs 7–13) was replaced with that of hCNT1, was constructed. The splice site between the two proteins following hCNT3 residue Lys314 was engineered at the beginning of the putative extramembranous loop prior to TM 7 to divide the proteins into two approximately equal halves.
and to minimize disruption of native TMs and loops. The resulting hCNT3/1 chimeric protein was functional when produced in Xenopus oocytes and exhibited hCNT1-like substrate selectivity for influx of \( ^{3}H \) radiolabeled pyrimidine and purine nucleosides (NaCl, pH 7.5): uridine, thymidine, cytidine, adenosine, and no detectable transport of guanosine or inosine (Fig. 11B). The reciprocal chimera (hCNT1/3), representing a 50:50 construct incorporating the N-terminal half of hCNT1 and the C-terminal half of hCNT3, was non-functional and not studied further. As shown in Fig. 11C, hCNT3/1 was Na\(^{+}\)-dependent, but H\(^{+}\)-independent, demonstrating that the structural features determining H\(^{+}\) coupling reside in the C-terminal half of the protein. Similar to hCNT1 (14, 32), the relationship between hCNT3/1-mediated \( ^{14}C \) uridine influx and Na\(^{+}\) concentration at pH 7.5 was hyperbolic with a Hill coefficient (\( n \)) of 1.0 ± 0.1 (Fig. 11D), a value consistent with a Na\(^{+}\)/nucleoside coupling stoichiometry of 1:1.

**DISCUSSION**

The CNT protein family in humans is represented by three members, hCNT1, hCNT2, and hCNT3, corresponding to concentrative nucleoside transport processes cit, cif, and cib, respectively. hCNT3 is a transcriptionally regulated electrogenic transport protein that, unlike hCNT1 and hCNT2, transports a broad range of pyrimidine and purine nucleosides and nucleoside drugs (18). hCNT3 and its mouse ortholog mCNT3 are
more closely related in sequence to the prevertebrate hagfish transporter hCNT3 (30) than to mammalian CNT1/2, and thus form a separate CNT subfamily. The present study utilized heterologous expression in Xenopus oocytes in combination with electrophysiological, radioisotope flux, and chimeric experiments to characterize the selectivity, mechanism, energetics, and structural basis of hCNT3 cation coupling. Parallel experiments with mCNT3 confirmed the general applicability of the reported findings.

Unlike hCNT1 and hCNT2, which are largely Na\(^+\)-specific, hCNT3 exhibited Na\(^+\), H\(^+\), and Li\(^+\) dependences, all three cations supporting electrogenic uridine influx. In this regard, hCNT3 resembles the mammalian concentrative glucose transporters SGLT1 and SGLT3, the bacterial MelB melibiose transporter, and the mammalian Na\(^+\)/dicarboxylate cotransporter SDC1/NaDC-1, all of which can also utilize Na\(^+\), H\(^+\), or Li\(^+\) to drive cellular accumulation of substrate (42–46). Consistent with an intracellular oocyte pH of 7.3–7.6 (47), nucleoside-evoked H\(^+\) currents were minimal at external pH values of 7.5 or higher. Use of CCCP to dissipate the imposed H\(^+\)/H\(_{11011}\) chemical gradient across the cell membrane (48, 49) decreased the uridine-evoked current in acidified Na\(^+\)/H\(_{11011}\) and Na\(^+\)/Li\(^+\) symport and hCNT3 coupling within the CNT3/hfCNT subfamily is unique to h/mCNT3 and is not shared by hfCNT (30). In contrast, some other CNTs function exclusively as H\(^+\)/nucleoside symporters, including NupC from E. coli, CeCNT3 from C. elegans, and CaCNT from C. albicans (26–28).

Na\(^+\)/nucleoside and H\(^+\)/nucleoside symport by hCNT3 exhibited markedly different selectivity characteristics for physiological nucleosides and therapeutic nucleoside drugs, suggesting that Na\(^+\) and H\(^+\) binding induce cation-specific conformational changes in the hCNT3 substrate-binding pocket and/or translocation channel. For H\(^+\)-coupled hCNT3, this was reflected in markedly reduced transport activity for thymidine, cytidine, adenosine, and inosine, and inability to transport guanosine, AZT, and ddC. In a possibly related phenomenon, functional studies with microglia have shown that an inwardly directed H\(^+\) gradient can inhibit AZT uptake (50). Microglia have cib-type activity as a major component of their nucleoside transport machinery (51).

Consistent with results of recent studies of hCNT1 (32), the proton/myo-inositol cotransporter (38), and SGLT1 (39), hCNT3 kinetic experiments revealed an ordered binding mechanism. Na\(^+\) removal increased the transporter’s \(K_a\) for uridine by more than 30-fold, this being accompanied by a smaller (1.6-fold) increase in \(V_{\text{max}}\). Limiting the concentration of Na\(^+\) can therefore be overcome by increasing the concentration of uridine to reach similar maximal rates of transport (37–40). In contrast, limiting the concentration of uridine reduced both the apparent affinity of hCNT3 for Na\(^+\) and the maximal current. Na\(^+\) therefore binds to hCNT3 first, followed by nucleoside. Like SGLT1 (44), the apparent affinity of hCNT3 for H\(^+\) was four orders of magnitude higher than for Na\(^+\). H\(^+\) and Na\(^+\) binding to SGLT1 also lead to cation-specific conformational changes, which, like hCNT3, were reflected in a decrease in sugar-binding affinity and transport efficiency of the H\(^+\)/nucleoside symport (45). In the case of hCNT3, substitution of H\(^+\) for Na\(^+\) resulted in a 6-fold change in uridine apparent affinity, and a decrease of \(-70\%\) in \(V_{\text{max}}/K_m\) ratio (Table I). Unlike hCNT3, however, H\(^+\)-coupled SGLT1 exhibited only modest changes in sugar specificity compared with the Na\(^+\)-coupled transporter (45). Similar to SGLT1, hCNT3-mediated Na\(^+\)/nucleoside and H\(^+\)/nucleoside symport were voltage-dependent (35, 36). The apparent affinity of hCNT3 for uridine was voltage-insensitive at high external Na\(^+\) concentrations, but voltage-dependent when the concentration of Na\(^+\) was reduced, suggesting that the voltage dependence of the transporters apparent affinity for uridine may be due to the voltage dependence of Na\(^+\) binding (35, 36). This was supported by experiments showing a similar voltage dependence of the apparent affinity of hCNT3 for Na\(^+\). As in the case of SGLT1, these results are indicative of the presence of an ion well effect (35, 36), with pre-steady-state electrophysiological studies suggesting that Na\(^+\) binds to hCNT3 \(-40\%\) within the membrane electric field (52). Na\(^+\)/nucleoside and H\(^+\)/nucleoside IV curves and the effect of membrane potential on \(I_{\text{max}}\) values suggest that membrane potential also influences carrier translocation. This is consistent with the fact that the driving force for an electrogenic transporter is dependent on not only the gradients of substrate and cotransported ion, but also on the membrane potential.

Based on indirect Hill-type analyses of the relationship between nucleoside influx and Na\(^+\) concentration, Na\(^+\)/nucleo-

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**Table III: Stoichiometry of hCNT3**

| \(V_h\) | \(\text{Na}^{+}\) (100 mM NaCl, pH 8.5) | \([^{14}\text{C}]\)Uridine stoichiometry | \(\text{Na}^{+}\) plus H\(^+\) (100 mM NaCl, pH 5.5) | \(\text{H}^{+}\) (100 mM ChCl, pH 5.5) | \(\text{Na}^{+}\) stoichiometry |
|------|-----------------|----------------|-----------------|----------------|----------------|
| −30  | 1.4 ± 0.1 (9)*   | 2.0 ± 0.1 (9)  | 0.92 ± 0.05 (9) | 1.1 ± 0.1 (9)  | 0.97 ± 0.04 (5) |
| −50  | 1.6 ± 0.1 (9)   | 1.9 ± 0.1 (8)  |                |                |                |
| −90  | 1.9 ± 0.1 (10)  | 2.0 ± 0.1 (10) |                |                |                |

*From Fig. 9.

† From Fig. 10.

The numbers in parentheses denote the number of oocytes.
FIG. 11. Transport properties of chimera hCNT3/1. A, topographical model of hCNT3 and hCNT1. Potential membrane-spanning α-helices are numbered, and putative glycosylation sites in predicted extracellular domains in hCNT3 and hCNT1 are indicated by solid and open stars, respectively. Residues identical in the two proteins are shown as solid circles. Residues corresponding to insertions in the sequence of hCNT3 or hCNT1 are indicated by circles containing “+” and “−” signs, respectively. The arrow represents the splice site used for construction of the chimera. B, uptake of radiolabeled nucleosides by chimera hCNT3/1. Nucleoside influx (20 μM, 20 °C, 1-min flux) was measured in transport medium containing 100 mM NaCl at pH 7.5. Mediated transport was calculated as uptake in RNA transcript-injected oocytes minus uptake in control water-injected oocytes. C, radiolabeled uridine influx (20 μM, 20 °C, and 1-min flux) by hCNT1, hCNT3, and hCNT3/1 was measured in transport medium containing 100 mM NaCl at pH 7.5 (black bars) or in Na+−free transport medium (100 mM ChCl) at both pH 5.5 (gray bars) and 7.5 (open bars). Mediated transport was calculated as uptake in RNA-injected oocytes minus uptake in control water-injected oocytes. D, influx of [14C]uridine (20 μM, 20 °C, and 1-min flux) measured as a function of Na+ concentration at pH 7.5 using choline as Na+ substitute in oocytes injected with water alone (open circles) or with water containing hCNT3 RNA transcripts (solid circles). The inset in B is a Hill plot of the mediated data (Hill coefficient (n) and Na+ apparent affinity (K_{50}) presented in the text). Values in B–D are means ± S.E. of 10–12 oocytes. Each experiment in B–D was performed on cells from single batches of oocytes used on the same day.
side stoichiometries of 1:1 have been described for cit and cif transport activities in different mammalian cells and tissues (7–9) and for recombinant rCNT1 and hCNT1 produced in Xenopus oocytes (32, 53). Although Larraya et al. (31) have reported a hCNT1 Na+/nucleoside coupling ratio of 2:1 based on results of direct charge versus [3H]uridine and 22Na+ uptake studies, we have recently confirmed that the stoichiometry of hCNT1 is 1:1 (32). H+-dependent CaCNT also exhibits a coupling ratio of 1:1 (28). In marked contrast, there is evidence from Na+-activation studies of mammalian cib transporters (14, 18, 54) and from charge versus uridine uptake experiments with hagfish hfCNT (30) that members of the CNT3/hfCNT subfamily have a coupling ratio of 2:1. The charge versus uridine and charge versus Na+ uptake experiments reported here for hCNT3 confirm this stoichiometry. The Hill coefficient for Na+ activation of hCNT3 is close to 2, implying strong cooperation between the two Na+-binding sites (55, 56).

Similar to SGLT1 (39, 57, 59), but unique among the other CNTs that have been examined to date (hCNT1, hCNT2, and CaCNT) (28, 30, 32), the experimentally determined Na+/nucleoside coupling ratio of hCNT3 was voltage-dependent, increasing progressively to its maximum value of 2:1 as the membrane potential became more negative. As in the case of SGLT1 (59), this may reflect an effect of membrane potential on Na+ dissociation from the cytoplasmic face of the transporter, with a consequent reduction in Na+ recycling back to the external surface of the membrane. Other transporter families also have individual members with different cation coupling ratios. For example, members of the SGLT family have been described with 1:1 and 2:1 Na+/glucose coupling ratios (1:1 for SGLT2 and 2:1 for SGLT1/3) (39, 57, 59, 60). Similarly, the PepT1 and PepT2 proton-linked peptide transporters have 1:1 and 2:1 H+-peptide coupling ratios, respectively (61). Mechanistically, the cation-to-nucleoside coupling ratio determines the energetic cost of transport and sets the thermodynamic limit to the transmembrane nucleoside gradients that can be achieved. The concentrative capacity of hCNT3 is therefore greater than that of either hCNT1 or hCNT2.

In marked contrast to SGLT1, where both Na+ and H+ have the same 2:1 cation/sugar stoichiometries (44, 59, 62), charge/uptake analyses revealed an hCNT3 Na+/nucleoside coupling ratio of 2:1 versus 1:1 for H+. Unlike Na+, the H+ coupling ratio was membrane potential-independent. Charge/uptake experiments with both Na+ and H+ present together (Na+-containing transport medium at pH 5.5) revealed features intermediate between Na+ or H+ alone (2:1 coupling ratio and voltage-independent), suggesting that both cations contributed to the driving force under these conditions. The 2:1 charge/uptake coupling ratio under these conditions implies that one of the two Na+-binding sites is shared by H+. Because proton-activation experiments gave a Hill coefficient consistent with a 1:1 H+/nucleoside coupling ratio, it is unlikely that there exists a second (recycled) H+ bound to hCNT3. The hCNT3 cation binding site shared by Na+ and H+ is likely to be the same as that responsible for single-site cation coupling in CNTs that are either exclusively H+-dependent (CaCNT, NupC) (26, 28) or Na+-dependent (hCNT1/2) (7–9, 32, 53).

We interpret our results for cation coupling of hCNT3 in terms of the conformational equilibrium model of secondary active transport developed by Krupka (63, 64). This modified ordered binding model of secondary active transport alleviates the stringent sequential carrier states of earlier models and instead allows for flexible cation interactions such as those observed for Na+ and H+ coupling of hCNT3. Because the transporter can accept two different solutes, cation (A) and nucleoside (S), it is proposed to exist in two inwardly facing or outwardly facing conformational states: one that binds cation only (T′a and T′b) and one that binds cation and nucleoside (T′a and T′b). Normally, the equilibrium between the two outwardly facing carrier states overwhelmingly favors the T′a form and requires the addition of cation (two Na+ ions or one H+) in the case of hCNT3 to “unlock” or open the nucleoside binding site (T′a · A ↔ T′a · A), thereby promoting active transport. Both T′a · S and T′b · AS are considered mobile. The finding that a binding of an H+ to one of the two Na+-binding sites is sufficient to activate nucleoside transport presents an experimental paradigm to enable mutagenic dissection of amino acid residues contributing to each of the sites. Our 50:50 hCNT3/1 chimera demonstrated that the structural determinants of cation/nucleoside stoichiometry and H+ dependence reside in the C-terminal half of the protein. Hill-type analysis of Na+/nucleoside coupling in a corresponding 50:50 chimera between hfCNT and hCNT1 yielded similar results (30). The present finding that determinants of hCNT1 versus hCNT3 nucleoside selectivity also reside in the C-terminal half of the protein is consistent with previous mutagenesis experiments that identified residues in TMs 7 and 8 of hCNT1 that, when sequentially mutated to the corresponding residues in hCNT2, progressively changed the selectivity of the transporter from cit to cib to cif (29).

In conclusion, hCNT3 exhibited unique cation interactions with Na+, H+, and Li+ that are not shared by other members of the CNT protein family. Both indirect and direct methods indicated 2:1 and 1:1 cation/nucleoside stoichiometries for Na+ and H+, respectively, and Na+ and H+-coupled hCNT3 exhibited markedly different selectivities for nucleoside and nucleoside drug transport. Location of hCNT3-specific cation interactions to the C-terminal half of the protein sets the stage for site-directed mutagenesis experiments to identify the residues involved. The ability of hCNT3 to couple nucleoside and nucleoside drug accumulation to H+ as well as Na+ cotransport may have physiological and pharmacological relevance in the duodenum and proximal jejunum where the pH of luminal contents can be relatively acidic. As well, there is a reported acidic microenvironment present on the surface of the intestinal epithelium (58).

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The Broadly Selective Human Na⁺/Nucleoside Cotransporter (hCNT3) Exhibits Novel Cation-coupled Nucleoside Transport Characteristics

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