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Rapid Communication

Molecular Cloning of a Na^+-Dependent Nucleoside Transporter[1] from Rabbit Intestine

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Purpose. Substantial species differences in the transport kinetics of nucleosides and therapeutic nucleoside analogs have been observed in various experimental systems. To explain these differences at a molecular level, it is necessary to clone the relevant transporters and examine their functional characteristics in heterologous expression systems. The goal of the present study was to clone the nucleoside tranporters present in rabbit, an important preclinical animal model, and to functionally characterize the clone(s).
Methods. A Polymerase Chain Reaction (PCR)-based homology cloning approach in conjuction with Rapid Amplification of cDNA Ends (RACE) was used to isolate a full-length cDNA. Characterization of this transporter was accomplished through heterologous expression in *Xenopus laevis* oocytes.
Results. A full-length cDNA encoding a purine-selective, Na^+-dependent nucleoside transporter, rbSPNT1, was isolated from rabbit small intestine. The encoded protein is 658 amino acid residues in length. Hydropathy analysis suggests that rbSPNT1 has 11 to 14 transmembrane domains. In *Xenopus laevis* oocytes expressing rbSPNT1, the uptake of uridine and inosine was enhanced significantly; uridine transport was inhibited by purine, but not pyrimidine nucleosides. mRNA transcripts for rbSPNT1 were detected primarily in intestine, lung, and kidney and at lower levels in liver, brain, and heart.
Conclusions. A full-length functional nucleoside transporter was cloned. Sequence analysis and functional characterization suggest that rbSPNT1 is the rabbit homolog of the purine-selective nucleoside transporter, N1. The cloned rbSPNT1 can be used to understand the molecular mechanisms responsible for the observed species differences in the transport of nucleosides and therapeutic nucleoside analogs.

KEY WORDS: adenosine; uridine; inosine; purine; concentrative transporter.

INTRODUCTION

Membrane transporters in the epithelial lining of the intestinal lumen play an important role in the salvage of endogenous nucleosides and may be critical in the absorption of therapeutically important nucleoside analog drugs. Concentrative, or Na^+-dependent, nucleoside transporters have been characterized in intestinal epithelia from several species (1–3). Recently, purine- and pyrimidine-selective Na^+-dependent nucleoside transporters (termed SPNT (cNT2) and cNT1, respectively) were cloned from rat, human and pig (4–8).

Functional studies indicate that there are significant interspecies differences in the kinetic parameters of various nucleosides in interacting with cloned transporters from rat and human (4,7,9,10). In contrast, studies in isolated rabbit tissues suggest that concentrative nucleoside transporters from rabbit may be more similar kinetically to human transporters (3,11). To study the molecular mechanisms responsible for interspecies differences in nucleoside transport, it is necessary to clone the relevant transporters and examine their kinetic characteristics in heterologous expression systems. Using homology cloning methods, we isolated a purine-selective nucleoside transporter, termed rbSPNT1, from rabbit intestine. This study reports its cloning and initial functional characterization.

METHODS

A full-length cDNA was obtained from rabbit small intestine using a PCR-based homology cloning method. Total RNA was isolated from various tissues of New Zealand white rabbits using the TRIzol reagent (Gibco BRL). First-strand cDNA synthesis was performed using the SUPERSCRIPT Preamplification System for First Strand cDNA Synthesis (Gibco BRL) with total RNA from rabbit ileum as template. Primers were designed to the consensus regions (amino acid residues 272–282 and amino acid residues 534–544 of the rSPNT protein sequence). These primers were used in PCR amplification with ileum cDNA as the template under the following conditions: 94°C for 1 minute; 50°C for 2 minutes; 72°C for 2.5 minutes for 30 cycles. A single fragment of approximately 0.8 kb was obtained. Gene-specific primers were designed to regions of this fragment for 5′ and 3′ RACE (Rapid Amplification of cDNA Ends).

[1] The nucleotide sequence reported in this study has been submitted to GenBank with accession number AF161716.

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First-strand cDNA synthesis from ileum total RNA was performed using the 3′RACE System (Gibco BRL) per the manufacturer's protocol. The resulting cDNA was amplified with the first gene-specific primer (5′-TAAAACTGCCTCGTGGGAAGGAGAG-3′) and the Abridged Universal Amplification Primer (AUAP) under the following conditions: 94°C for 1 minute; 50°C for 2 minutes; 72°C for 1.5 minutes for 30 cycles. A nested PCR was carried out using the product from the first amplification (diluted 1:40 with water) as the template with the AUAP primer and the second gene-specific primer (5′-GCTATAGCTCTTGTTGCGAATGTCG-3′) under the following conditions: 94°C for 1 minute; 55°C for 2 minutes; 72°C for 3 minutes for 35 cycles. The resulting 1.1 kb product was subcloned and sequenced. The sequence of this fragment contained a poly-adenylation signal (AATAAA), a poly-A tail, and a region which overlapped with the initial 0.8 kb fragment, suggesting that the entire 3′ end of the same clone had been isolated. The 5′ end of this cDNA was then obtained using the 5′RACE System (Gibco BRL). First-strand cDNA synthesis was carried out using total RNA from rabbit jejunum and ileum with the spnt1 primer (5′-CCCTATGGAAGTAAGATTGGCAAATCCACAGAG-3′). PCR was performed using a gene-specific anti-sense primer (5′-GATGAGCAGAGGTGCCTCTGTCATA-3′) and the Anchor Primer under the following conditions: 94°C for 1 minute; 50°C for 2 minutes; 72°C for 2 minutes for 30 cycles. A nested PCR was performed using a second gene-specific primer (5′-ACGCATAGTGACTTGCAAAAACCAGG-3′) and the AUAP primer under the same conditions. Two bands of approximately 1.0 and 1.1 kb resulted from the nested PCR derived from the jejunum cDNA. Both fragments were subcloned and sequenced; the longer of the two appeared to be a splice variant and will be investigated further in the future. A start codon and Kozak consensus sequence were found in the longest open reading frame. Alignment with SPNT and hSPNT1 suggested that the entire 5′ end of the coding region had been obtained. Primers were then designed to amplify the full-length cDNA. A sense primer (5′-TCCTTCCCTGAGGAGCCATTAGGAATC-3′) was designed to bases 1–27 of the 5′RACE product. An anti-sense primer (5′-CTAAATGCTCACAGCAATTCCTACCC-3′) was designed to the 3′RACE product within the untranslated region, upstream of the poly-adenylation signal. PCR was carried out using these primers and jejunum cDNA under the following conditions: 94°C for 1 minute; 55°C for 2 minutes; 72°C for 3 minutes for 35 cycles. The resulting 2.2 kb cDNA was subcloned into the pGEM-T vector (Promega) and sequenced.

The tissue distribution of rbSPNT1 mRNA was determined by RT-PCR. Gene-specific primers were designed to amplify a 276 bp fragment of rbSPNT1. Total RNA or mRNA was isolated from various tissues (including kidney, intestine, liver, heart, brain, spleen and lung), and first-strand cDNA was synthesized using the SUPERSCRIPT Preamplification System for First-Strand cDNA Synthesis. cDNA was synthesized using approximately 0.5 μg mRNA from each tissue with the exception of spleen and liver for which total RNA was used (approximately 1 μg and 2.2 μg, respectively). PCR was then performed using the gene-specific primers and the resulting products were separated on a 1% agarose gel.

Functional studies were carried out using the *Xenopus laevis* oocyte expression system. Defolliculated oocytes were injected with approximately 50 ng of cRNA which had been transcribed from the cDNA *in vitro* as previously described (12). Uptake assays were performed with radio-labeled and unlabeled nucleosides 2 days post cRNA injection (13).

RESULTS AND DISCUSSION

The cDNA sequence and deduced amino acid sequence are shown in Fig. 1A. The complete 2302 bp cDNA contains an ORF of 1977 bp which encodes a 658 amino acid protein. Hydropathy analysis of the deduced protein sequence revealed 11–14 potential transmembrane domains (Fig. 1B), suggesting a topology similar to other members of this family of transporters (14). Further sequence analysis using the Genetics Computer Group (GCG) software (Wisconsin Package, versions 9 and 10) identified four potential N-linked glycosylation sites on asparagine residues 538, 605, 624, and 653. Based on a 13 transmembrane model (transmembrane domains shown in Fig. 1A), only asparagine residues 605, 624, and 653 are extracellular and thus, potentially glycosylated. Six potential PKC phosphorylation sites were identified on serine residues 5, 198, and 522 and on threonine residues 36, 295, and 604. Based on a 13 transmembrane model, all but serine-522 and threonine-295 are predicted to be intracellular and thus, are potential substrates for PKC. Only the putative N-linked glycosylation sites predicted to be extracellular and PKC phosphorylation sites predicted to be intracellular are indicated in Fig. 1A.

Alignment of the protein sequence with those of previously cloned concentrative nucleoside transporters using the Bestfit program of the GCG software showed that rbSPNT1 is 84% identical to hSPNT1 and 82% identical to rSPNT. It is less identical to the CNT1 (N2) clones (65% identical to hCNT1a and 65% identical to rCNT1), suggesting that rbSPNT1 is the rabbit homolog of human and rat SPNT. Furthermore, an alignment of amino acids 296 to 358 from this protein sequence with the corresponding region of the other cloned transporters shows that the residues determined to be critical for substrate discrimination (13,15) are identical to those residues conserved across all SPNT sequences (Fig. 2). This observation further supports the identification of rbSPNT1 as the rabbit N1 homolog.

Oocytes injected with rbSPNT1 cRNA exhibited an enhanced Na^+-dependent uptake of [^{3}H]-uridine and [^{3}H]-inosine over uninjected oocytes (controls) (Fig. 3A). [^{3}H]-Uridine (11 μM) uptake in the presence of Na^+ in cRNA-injected oocytes was 0.35 ± 0.042 pmol/oocyte/30 minutes, whereas the uptake of [^{3}H]-uridine was 0.08 ± 0.015 pmol/oocyte/30 minutes in uninjected oocytes, representing an enhancement in uptake of approximately 4-fold. [^{3}H]-Inosine (11 μM) uptake in the presence of Na^+ was enhanced approximately 16-fold in cRNA-injected oocytes compared to uninjected oocytes (1.32 ± 0.456 pmol/oocyte/30 minutes versus 0.08 ± 0.006 pmol/oocyte/30 minutes). [^{3}H]-Uridine (11 μM) uptake in the presence of Na^+ was measured in the presence of each of the endogenous nucleosides at a concentration of 1 mM. Uridine uptake was significantly inhibited ($P < 0.01$) by adenosine, guanosine, inosine and uridine but not thymidine or cytidine (Fig. 3B). This inhibition profile is consistent with an N1 or purine-selective transporter. Interestingly, cytidine and thymidine do not seem to inhibit uptake of nucleosides by rbSPNT1 (no inhibition of uptake at 1 mM concentrations) whereas they weakly inhibit uptake by the rat and human homologs (4,7).

A

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1     TTCTTCCCTGAGGAGCCATTAGGAATCCCACAAGCTGGGTTCAGAAGAGAGGATGGAGAAAGCCAGTGGAAGAAAGTCTCTTGCTCTGTCCACAGCGGAG   100
1                                                  M  E  K  A  S  G  R  K  S  L  A  L  S  T  A  E    16
                                                                    ‡
101   AACGGCATAGAGAACGCAGGGCTGGAGCTCACGGAAGAAGGGATAAATTCTGAGCAAACCAGGAGGATGGAAGTGCAAGGACACAGTCTCAGCGATGATG   200
17    N  G  I  E  N  A  G  L  E  L  T  E  E  G  I  N  S  E  Q  T  R  R  M  E  V  Q  G  H  S  L  S  D  D  V    50
                                                                 ‡
201   TGAGGCCTGCCACTCACCAAAGGAGTTATCTACAGCCTCTCACCAAGGCAAGAACTTTCTGCCAAAGACATGCAAGCTTGTTCAAGAAGATCCTGTTGGG   300
51      R  P  A  T  H  Q  R  S  Y  L  Q  P  L  T  K  A  R  T  F  C  Q  R  H  A  S  L  F  K  K  I  L  L  G    83

301   CCTGTTGTGTCTGGCGTATGCTGCCTATTTCTTGGCAGCTTGCATCTTGGATTTCCAGAGGGCACTGGCCTTATTTGTCATCACCTGTTTGGTGATCTTG   400
84     L  L  C  L  A  Y  A  A  Y  F  L  A  A  C  I  L  D  F  Q  R  A  L  A  L  F  V  I  T  C  L  V  I  L   116

401   GTCCTGCTTCTCCACTTTCTGAAAAAGTTCTTGGGTAAAAAATTAACAAGATGCCTGAAGCCCTTTAAAAATTCCCAGCTGAGACTTTGGATAAAACGGG   500
117   V  L  L  L  H  F  L  K  K  F  L  G  K  K  L  T  R  C  L  K  P  F  K  N  S  Q  L  R  L  W  I  K  R  V   150

501   TGTTCGCAGGAGTCTCCTTGGTTGGCCTTATCCTGTGGCTGGCACTAGACACAGCTCAAAGGCCAGAGCAGCTGATCTCCTTTGCAGGAATCTGCATGTT   600
151     F  A  G  V  S  L  V  G  L  I  L  W  L  A  L  D  T  A  Q  R  P  E  Q  L  I  S  F  A  G  I  C  M  F   183

601   CGTCCTCATCCTCTTTGCGTGCTCCAAACACCACAGTGCAGTGTCCTGGAGGACAGTGTTTTGGGGCCTGGGTCTTCAGTTTGTCTTTGGATTATTGGTC   700
184    V  L  I  L  F  A  C  S  K  H  H  S  A  V  S  W  R  T  V  F  W  G  L  G  L  Q  F  V  F  G  L  L  V   216
                                                    ‡
701   ATCAGAACGGATCCTGGATTTATTGCATTTCAGTGGCTGGGAGATCAGGTCCAGATTTTCCTGGCCTACACCGTCGCTGGCTCCAGTTTTGTCCTTGGGG   800
217   I  R  T  D  P  G  F  I  A  F  Q  W  L  G  D  Q  V  Q  I  F  L  A  Y  T  V  A  G  S  S  F  V  L  G  D   250

801   ATACCCTGGTCAATGATGTCTTTGCTTTTCAGTCGTTACCAATCATCATTTTCTTTGGATGCGTGATGTCCATTCTCTACTACCTGGGCCTTGTGCAGTG   900
251     T  L  V  N  D  V  F  A  F  Q  S  L  P  I  I  I  F  F  G  C  V  M  S  I  L  Y  Y  L  G  L  V  Q  W   283

901   GGTTGTTCAGAAGATCGCCTGGTTTTTGCAAGTCACTATGCGTACCACTGCCACAGAGACCCTAGCTGTAGCAGGAAACATCTTTGTGGGTATGACAGAG   1000
284    V  V  Q  K  I  A  W  F  L  Q  V  T  M  R  T  T  A  T  E  T  L  A  V  A  G  N  I  F  V  G  M  T  E   316
                                       ‡
1001  GCACCTCTGCTCATCCGCCCCTACCTTGCAGACTTGACCCTCTCTGAAATCCATGCAGTGATGACTTCAGGGTTTGCCACCATTTCAGGCACTGTGTTGG   1100
317   A  P  L  L  I  R  P  Y  L  A  D  L  T  L  S  E  I  H  A  V  M  T  S  G  F  A  T  I  S  G  T  V  L  G   350

1101  GAGCCTTCATATCCTTTGGGATTGATGCATCATCCTTGATTTCTGCTTCTGTGATGGGTGCCCCTTGTGCACTTGCCTTGTCAAAGCTGGTATATCCAGA   1200
351     A  F  I  S  F  G  I  D  A  S  S  L  I  S  A  S  V  M  G  A  P  C  A  L  A  L  S  K  L  V  Y  P  E   383

1201  AGAGGAGGAGTCCAAGTTCAAGAGTAAAGAGGGGGTAAAACTGCCTCGTGGGAAGGAGAGCAATGTCCTAGAAGCTGCCAGCAATGGAGCGACAGATGCT   1300
384    E  E  E  S  K  F  K  S  K  E  G  V  K  L  P  R  G  K  E  S  N  V  L  E  A  A  S  N  G  A  T  D  A   416

1301  ATAGCTCTTGTTGCGAATGTCGCAGCCAACCTGGTTGCCTTTCTGGCCGTGTTGGCCTTCATCAATGCTGCCCTTTCCTGGTTGGGGGAACTGGTGGACA   1400
417   I  A  L  V  A  N  V  A  A  N  L  V  A  F  L  A  V  L  A  F  I  N  A  A  L  S  W  L  G  E  L  V  D  I   450

1401  TACAGGGGCTCACTTTCCAGGTCATCTGCTCCTATATCCTAAGGCCCATGGTTTACATGATGGGTGTAGAGTGGACAGACTGTCCAATGGTGGCTGAGAT   1500
451     Q  G  L  T  F  Q  V  I  C  S  Y  I  L  R  P  M  V  Y  M  M  G  V  E  W  T  D  C  P  M  V  A  E  M   483

1501  GGTGGGAATCAAGTTCTTCACAAATGAGTTCGTGGCCTATCAGCAACTGTCTCAATACAAGAAAAAACGTCTCTCTGGAATGGAAGAATGGATTGATGGC   1600
484    V  G  I  K  F  F  T  N  E  F  V  A  Y  Q  Q  L  S  Q  Y  K  K  K  R  L  S  G  M  E  E  W  I  D  G   516

1601  CAGAAACAGTGGATTTCTGTGAGAGCTGAAGTCATTACAACATTTTCACTCTGTGGATTTGCCAATCTTAGTTCCATAGGAATAACACTGGGAGGCTTGA   1700
517   Q  K  Q  W  I  S  V  R  A  E  V  I  T  T  F  S  L  C  G  F  A  N  L  S  S  I  G  I  T  L  G  G  L  T   550

1701  CTTCAATGGTGCCCCACCGAAAAAGTGATCTGTCCAAAGTTGTGATCCGGGCCCTCTTCACAGGGTCCTGTGTATCCTTTATCAGTGCCTGTGTGGCAGG   1800
551     S  M  V  P  H  R  K  S  D  L  S  K  V  V  I  R  A  L  F  T  G  S  C  V  S  F  I  S  A  C  V  A  G   583

1801  AATCCTCTATGTTCCCCGGGGAGCTGAAACTGACTGTGTCTCCTTCCTAAGCACAAGTTTCACCAACAGAAGCTATGAGACCTATGTGTGCTGCAGGGAG   1900
584    I  L  Y  V  P  R  G  A  E  T  D  C  V  S  F  L  S  T  S  F  T  N  R  S  Y  E  T  Y  V  C  C  R  E   616
                                                                 ∇
1901  CTCTTTCAGAATACTTATCTGAATGGCACCAATCCACCTTCTTTTTCTGGTGCCTGGGAAGACAAGGCATTCAGTGCCATGGCCCTTGCTAACTGCTGTG   2000
617   L  F  Q  N  T  Y  L  N  G  T  N  P  P  S  F  S  G  A  W  E  D  K  A  F  S  A  M  A  L  A  N  C  C  G   650
                           ∇
2001  GATTCTACAACAATACTGTCTGTGCCTAAGGATGGTTGGTCCATTTCTACCACAGTTTTAATCTTAACAGCTTTTTGGTGCAAAGGTGTTTGTTTACTCA   2100
651     F  Y  N  N  T  V  C  A  *                                                                          659
          ∇
2101  GGATTCCAACCCTTTAACCAACTCTCCAAGATCCTTAACAGTAAATGTAAAAGATTCATTTGGTTCAGTGCATTCCACTAATGAAAATTAGCATTCATCA   2200
2201  TTCCACTATTTCACTGAACCATAAAGGTACTCATGGGTAGGAATTGCTGTGAGCATTTAGTTGAAAAAATAAATTTTATAATATCAAAAAAAAAAAAAAA   2300
2301  AA                                                                                                  2302
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B

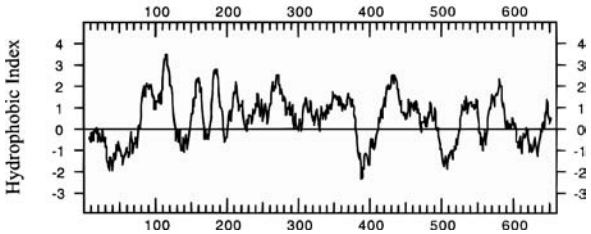

Fig. 1. (A) Nucleotide and deduced amino acid sequence of rbSPNT1. The complete cDNA (2302 bp) containing a 1977 bp ORF is predicted to encode a protein of 658 amino acids. Thirteen putative transmembrane domains are underlined. Intracellular putative PKC phosphorylation sites (‡) and extracellular putative N-linked glycosylation sites (∇) are indicated. (B) Hydropathy analysis of rbSPNT1. The Kyte–Doolittle method was used with a window of 13 residues.

Purine-selective Transporters

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 aa #   296                                                          358

rbSPNT1  MRTTATE TLAVAGNIFV GMTEAPLLIR PYLADLTLSE IHAVMTSGFA TIAGTVLGAF ISFGID
 hSPNT1  MGTTATE TLAVAGNIFV GMTEAPLLIR PYLGDMTLSE IHAVMTGGFA TISGTVLGAF IAFGVD
   SPNT  MGTTAAE TLAVAGNIFV GMTEAPLLIR PYLADMTLSE IHAVMTGGFA TIAGTVLGAF ISFGID
  mCNT2  MGTTAAE TLAVAGNIFV GMTEAPLLIR PYLADMTISE IHTVMTGGFA TIAGTVLGAF ISFGID
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Pyrimidine-selective Transporters

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 aa #   305                                                          367

 hCNT1  MGTTATE TLSVAGNIFV SQTEAPLLIR PYLADMTLSE VHVVMTGGYA TIAGSLLGAY ISFGID
  cNT1  MGTSATE TLSVAGNIFV SQTEAPLLIR PYLADMTLSE VHVVMTGGYA TIAGSLLGAY ISFGID
 pkCNT1 MGTTATE TLSVAGNIFV SQTEAPLLIR PYLADMTLSE IHVVMTGGYA TIAGSLLGAY ISFGID
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Fig. 2. Multiple sequence alignment of putative TMD 7 and 8 region. Amino acids of the purine-selective transporters, corresponding to residues 296–358 of rbSPNT1, were aligned with the corresponding amino acids of the pyrimidine-selective transporters (amino acids 305 to 367 of hCNT1). Conserved differences in amino acid residues between purine- and pyrimidine-selective transporters shown in bold.

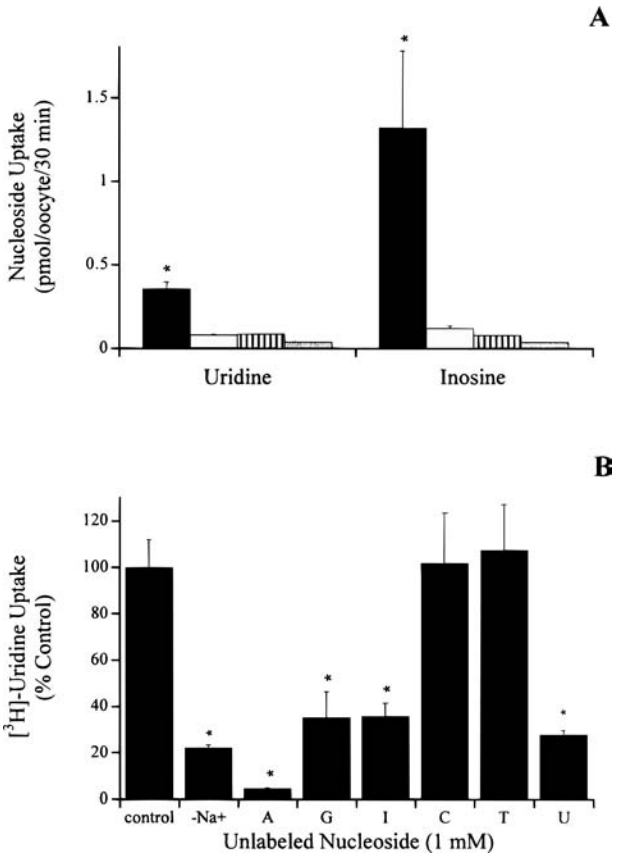

Fig. 3. (A) Nucleoside uptake in *Xenopus laevis* oocytes. Uptake of [^{3}H]-uridine (11 μM) or [^{3}H]-inosine (11 μM) in rbSPNT1 cRNA injected oocytes in the presence (solid bars) and absence (open bars) of Na^+ and uninjected control oocytes in the presence (striped bars) and absence (stippled bars) of Na^+. Asterisks indicate significant difference from control (uninjected oocytes in the presence of Na^+) ($P < 0.05$). (B) Inhibition of uridine uptake by endogenous nucleosides in *Xenopus laevis* oocytes. Uptake of [^{3}H]-uridine (11 μM) in rbSPNT1 cRNA injected oocytes in the presence of Na^+ (except where noted) in the presence of 1 mM unlabeled nucleosides abbreviated as follows: A, adenosine; G, guanosine; I, inosine; C, cytidine; T, thymidine; U, uridine. Asterisks indicate significant difference from control ($P < 0.01$).

This may suggest that rbSPNT1 is more purine-selective (i.e., less sensitive to even weak inhibition by pyrimidine nucleosides) than the other species homologs. Although detailed kinetic studies have not been performed, initial concentration-dependence studies of adenosine and inosine in inhibiting ^{3}H-uridine uptake suggest IC_{50} values in the low micromolar range (data not shown).

Bands corresponding to an amplified 276bp fragment of rbSPNT1 RNA were observed in all tissues tested except spleen (Fig. 4). The rbSPNT1 transcript appears to be most abundant in jejunum, ileum, total small intestine and lung. Moderate amounts of rbSPNT1 transcript were found in kidney medulla and kidney cortex and lesser amounts in heart, brain, and liver. rbSPNT1 appears to have a broader tissue distribution than its rat homolog; the rbSPNT1 transcript is present in kidney, lung and brain whereas the rSPNT transcript was not detected in these tissues by Northern analysis. However, the rbSPNT1 transcript seems to be present in lower abundance in liver and not at all in spleen. This is in contrast to the rSPNT transcript which is present in both of these tissues by Northern analysis and is abundant in liver (16). The distribution of the rbSPNT1 transcript appears to be more similar to that of the hSPNT1 transcript with the notable exception of the presence of the rbSPNT1 transcript in rabbit lung in abundance whereas the hSPNT1 transcript was not detected in human lung by Northern analysis (7). Bands of approximately 0.7 and 0.8 kb were seen in many of the samples. Control samples in which no reverse transcriptase was added exhibited the 0.7 and 0.8 kb bands but not the 276 bp band, suggesting that these higher molecular weight bands were due to genomic contamination. In addition, primers to glyceraldehyde-3-phosphate dehydrogenase (G3PDH) were used to amplify a fragment of G3PDH of approximately 1.0kb to verify the quality and quantity of cDNA for each tissue. Bands of approximately 1.0 kb were observed in PCR products from all tissues (data not shown).

CONCLUSIONS

A Na^+-dependent nucleoside transporter from rabbit small intestine, rbSPNT1, has been cloned. RT-PCR analysis suggests that the rbSPNT1 transcript is expressed in numerous tissues. rbSPNT1 transported uridine and inosine; [^{3}H]-uridine transport was inhibited by purine but not pyrimidine nucleosides. This inhibition profile is consistent with a purine-selective transport

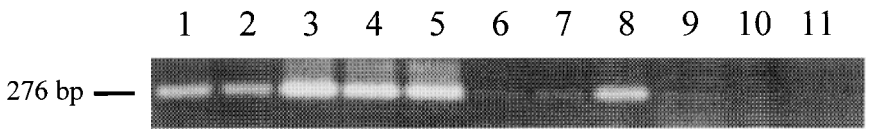

Fig. 4. Tissue distribution of rbSPNT1 mRNA transcript by RT-PCR. Total RNA isolated from rabbit tissues was used as a template to synthesize cDNA. Gene-specific primers were used to amplify a 276 bp fragment. The resulting products were analyzed on a 1% agarose gel as follows: kidney medulla (lane 1), kidney cortex (lane 2), intestine (lane 3), jejunum (lane 4), ileum (lane 5), heart (lane 6), brain (lane 7), lung (lane 8), liver (lane 9), spleen (lane 10), and water control (lane 11).

process. Further characterization of this and other rabbit nucleoside transporters in comparison to rat and human cloned transporters will provide a better understanding of the interspecies differences in the intestinal uptake of nucleosides and nucleoside analogs which may be drugs or drug candidates. Such an understanding is important in pre-clinical drug evaluation in animal models.

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