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Review

Recent advances in studies on biochemical and structural properties of equilibrative and concentrative nucleoside transporters

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Nucleoside transporters (NT) facilitate the movement of nucleosides and nucleobases across cell membranes. NT-mediated transport is vital for the synthesis of nucleic acids in cells that lack *de novo* purine synthesis. Some nucleosides display biological activity and act as signalling molecules. For example, adenosine exerts a potent action on many physiological processes including vasodilatation, hormone and neurotransmitter release, platelet aggregation, and lipolysis. Therefore, carrier-mediated transport of this nucleoside plays an important role in modulating cell function, because the efficiency of the transport processes determines adenosine availability to its receptors or to metabolizing enzymes. Nucleoside transporters are also key elements in anticancer and antiviral therapy with the use of nucleoside analogues. Mammalian cells possess two major nucleoside transporter families: equilibrative (ENT) and concentrative (CNT) Na^+-dependent ones. This review characterizes gene loci, substrate specificity, tissue distribution, membrane topology and structure of ENT and CNT proteins. Regulation of nucleoside transporters by various factors is also presented.

Keywords: nucleoside transporters, gene locus, tissue distribution, substrate specificity, structure, adenosine

Nucleosides and nucleobases play important functions in the physiology of several organs. Adenosine, an endogenous nucleoside, regulates, among other processes, blood flow, myocardial slow action potentials, lymphocyte function, glucose metabolism, and neurotransmission (Mubagwa & Flameng, 2001; Dunwiddie & Masino, 2001; Ackley *et al.*, 2003; Rosales *et al.*, 2004). Therefore, processes that modify adenosine concentration may alter its action on several tissues.

The effect of adenosine is mediated by specific cell-surface receptors A1, A2a, A2b and A3 (Olah & Stiles, 2000). The affinity of these receptors for adenosine varies. Thus their activation depends on adenosine concentration. Metabolism and transport across the plasma membrane are the main factors influencing the adenosine level. Most nucleosides and their analogues such as anticancer and antiviral nucleoside drugs are hydrophilic molecules and require specific transport proteins to be transported in and out of the cell. Two major classes of nucleoside transport systems have been described in mammalian cells, the equilibrative transport system and the concentrative one (Baldwin *et al.*, 2004; Gray *et al.*, 2004; Kong *et al.*, 2004). Nucleosides, nucleobases and their analogues are also substrates for other car-

Abbreviations: ara-A, adenine 9-β-D-arabinofuranoside (vidarabine); ara-C, cytosine 1-β-D-arabinofuranoside (cytarabine); AZT, 3′-azido-3′-deoxythymidine (zidovudine); CdA, 2-chloro-2′-deoxyadenosine (cladribine); *cib*, *c*oncentrative nucleoside transport *i*nsensitive to NBTI accepting a *b*road range of permeants; *cif*, purine-preferring *c*oncentrative nucleoside transport *i*nsensitive to NBTI accepting *f*ormicin B as a permeant; *cit*, pyrimidine-preferring *c*oncentrative nucleoside transport *i*nsensitive to NBTI accepting *t*hymidine as a permeant; *cs*, *c*oncentrative nucleoside transport *s*ensitive to NBTI; *csg*, *c*oncentrative nucleoside transport *s*ensitive to NBTI accepting *g*uanosine; CNT, concentrative nucleoside transporter; ddA, 2′,3′-dideoxyadenosine; ddC, 2′,3′-dideoxycytidine (zalcitabine); ddI, 2′,3′-dideoxyinosine (didanosine); dFdC, 2′,2′-difluorodeoxycytidine (gemcitabine); *ei*, *e*quilibrative nucleoside transport *i*nsensitive to inhibition by NBTI; ENT, equilibrative nucleoside transporter; *es*, *e*quilibrative nucleoside transport *s*ensitive to inhibition by NBTI; FdU, 5-fluoro-2′-deoxyuridine; h, human; IdU, 5-iodo-2′-deoxyuridine; IFN-γ, interferon-γ; LPS, lipopolysaccharide; m, murine; M-CSF, macrophage-colony stimulating factor; NBTI, nitrobenzylthioinosine; NT, nucleoside transporters; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; r, rat; T3, triiodo-L-thyronine; TM, transmembrane; TNF-α, tumor necrosis factor α.

rier proteins such as organic anion and cation transporters and ABC transporter proteins that mostly function as efflux transporters (Pastor-Anglada *et al.*, 2005). In our review we focus on equilibrative (ENT) and concentrative (CNT) nucleoside transporters.

The equilibrative transport system mediates nucleoside transport in both directions depending on the nucleoside concentration gradient across the plasma membrane (Fig. 1). This class of nucleoside transport system is subdivided in two types (*es* and *ei*) based on their sensitivity to nitrobenzylthioinosine (NBTI). The *es* transport system is highly sensitive to inhibition by NBTI (K_i = 0.1 to 10 nM). In contrast, the *ei* transport system is resistant to NBTI up to 1 μM (Griffith & Jarvis, 1996; Kiss *et al.*, 2000; Leung *et al.*, 2001). Currently four transporters (ENT1, ENT2, ENT3 and ENT4) belonging to the equilibrative transport system have been identified and cloned (Table 1). ENT1 and ENT3 are susceptible to NBTI, whereas this compound does not inhibit ENT2. ENT4 has been identified only recently and its transporting characteristics have not been fully determined. These four members of the equilibrative transport system are widely distributed among various cell types, but the number of molecules of each nucleoside transporter depends on the cell and tissue type. All transporters are able to transport adenosine, but they have different capacities to transport other nucleosides and nucleobases (Baldwin *et al.*, 2004; Kong *et al.*, 2004).

The concentrative transport system is Na^+-dependent and the movement of the nucleoside regardless of its concentration gradient is coupled to that of the sodium ion (Fig. 1). Six functionally different concentrative nucleoside transport activities have been described in human cells and they have been named in two different ways (Table 1). The first nomenclature is numerical and follows the order of discovery (N1–N6), and the second one is based on the substrate specificity (*cit, cif, cib, cit-like, cs, csg*). The **N1-*cif*** activity is purine-selective, the **N2-*cit*** transport system accepts pyrimidine nucleosides, the **N3-*cib*** system transports both purine and pyrimidine nucleosides, the **N4-*cit-like*** system is selective for pyrimidine nucleosides but also accepts adenosine and guanosine, the **N5-*cs*** system transports adenosine and its analogues, and the **N6-*csg*** activity is guanosine-selective (Thorn & Jarvis, 1996; Flanagan & Meckling-Gill, 1997; Cass *et al.*, 1998; Baldwin *et al.*, 1999; Cabrita *et al.*, 2002). To date three different proteins responsible for the *cit* (CNT1), *cif* (CNT2), and *cib* (CNT3) activities have been identified (Gray *et al.*, 2004; Kong *et al.*, 2004). Proteins that demonstrate the **N4-*cit-like***, **N5-*cs*** or **N6-*csg*** activities have not been identified yet.

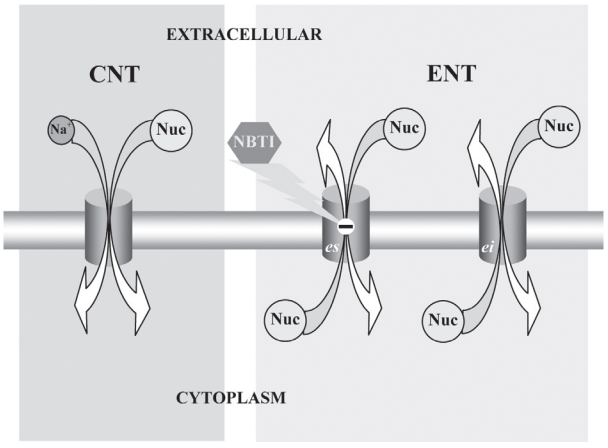

Figure 1. A model of nucleoside flux mediated by Na^+-dependent nucleoside transporters (CNT) and equilibrative nucleoside transporters (ENT).
Nuc, nucleoside, NBTI, nitrobenzylthioinosine.

THE EQUILIBRATIVE NUCLEOSIDE TRANSPORTERS FAMILY

ENT1

The human gene encoding the hENT1 protein has been localized to region p21.1-21.2 on chromosome 6 (Baldwin *et al.*, 2004). The mRNA for hENT1 is widely distributed in different tissues, including erythrocytes, liver, heart, spleen, kidney, lung, intestine, and brain (Table 2) (Griffith & Jarvis, 1996; Lum *et al.*, 2000; Pennycooke *et al.*, 2001).

Human ENT1 protein consists of 456 amino acids and its sequence displays about 78% identity to the rat (rENT1) and mouse (mENT1) homologues. Splice variants of ENT1 have been found only in the mouse. The mRNA for the shorter form of mENT1 termed mENT1.2 (458 aa) is produced by alternative splicing at the end of exon 7. Mouse ENT1.2 protein was shown to be commonly co-expressed with mENT1.1 (460 aa) and the highest level was found in the liver, heart and testis (Handa *et al.*, 2001). The two forms of mENT1 protein appear to be functionally identical, although mENT1.2 lacks the potential casein kinase II phosphorylation site.

Both rat and human ENT1 proteins display broad substrate specificity for pyrimidine and purine nucleosides, but are unable to transport the pyrimidine base uracil (Yao *et al.*, 1997). Human ENT1 has been reported to transport the nucleoside analogues used in the treatment of cancers, including CdA and ara-C. On the other hand, hENT1 only weakly transports the antiviral nucleoside analogues such as ddC and does not accept AZT (Griffiths *et al.*, 1997; Mackey *et al.*, 1998). Among the ENT1 proteins expressed in various species there are some differences in susceptibility to inhibition by specific compounds. Human ENT1, which is sensitive to NBTI, is also in-

Table 1. Nucleoside transport systems and their specificity

Nucleoside transport system	Protein	Substrate selectivity
es (equilibrative, sensitive to NBTI)	ENT1	purine and pyrimidine nucleosides
ei (equilibrative, insensitive to NBTI)	ENT2	purine and pyrimidine nucleosides and some nucleobases
es (equilibrative, sensitive to NBTI)	ENT3	purine and pyrimidine nucleosides and nucleobases
(equilibrative)	ENT4	adenosine
N1/*cif* (concentrative, insensitive to NBTI)	CNT2 (SPNT)	purine nucleosides, uridine, formycin B
N2/*cit* (concentrative, insensitive to NBTI)	CNT1	pyrimidine nucleosides, adenosine
N3/*cib* (concentrative, insensitive to NBTI)	CNT3	purine and pyrimidine nucleosides
N4/*cit*-like (concentrative)	unknown	pyrimidine nucleosides, adenosine and guanosine
N5/*cs* (concentrative, sensitive to NBTI)	unknown	adenosine analogues (formycin B, fludarabine, cladribine)
N6/*csg* (concentrative, sensitive to NBTI)	unknown	guanosine

hibited by the coronary vasodilators dipyridamole, dilazep, and draflazine. In contrast, rat ENT1 that is also inhibited by NBTI appears to be resistant to those compounds (Yao *et al.,* 1997; Sundaram *et al.,* 1998; Kiss *et al.,* 2000; Ward *et al.,* 2000; Baldwin *et al.,* 2004).

ENT2

Human ENT2 (hENT2) protein that is responsible for the *ei* type nucleoside transport is encoded by a gene localized at position 13q on chromosome 11 (Table 2). The mRNA for ENT2 was reported to be present in several tissues including heart, kidney, brain, placenta, thymus, pancreas, intestine, and prostate, but the highest expression level was observed in skeletal muscle (Griffiths *et al*., 1997; Crawford *et al*., 1998; Lum *et al*., 2000; Pennycooke *et al*., 2001).

Human ENT2 consists of 456 amino acids and their sequence displays 88% identity to mouse (mENT2) and rat (rENT2) homologues. In human, beside the 456-amino acid ENT2 protein, there exist at least two shorter forms of ENT2 generated from mRNA splice variants. The 326 aa protein termed hHNP36 lacks the first three transmembrane domains and is inactive as a nucleoside transporter. Inactive is also the second splice variant, a 301-amino acid protein named hENT2A that lacks the C-terminal domain (Crawford *et al.,* 1998).

The ENT2 protein accepts a broad range of substrates, including purine and pyrimidine nucleosides and nucleobases. It has been postulated that human ENT2 plays a role in the efflux and reuptake of inosine and hypoxanthine generated from adenosine during and after strenuous physical exercise (Griffiths *et al.,* 1997). ENT2 (both rat and human) is much less susceptible to inhibition by NBTI and the coronary vasodilators dipyridamole and draflazine than ENT1 (Yao *et al.,* 1997; Sundaram *et al.,* 1998; Ward *et al.,* 2000; Baldwin *et al.,* 2004). In contrast to hENT1 protein, hENT2 can transport the antiviral nucleoside analogue AZT and exhibits a greater affinity for ddC (Griffiths *et al.,* 1997; Crawford *et al.,* 1998; Yao *et al*., 2001).

ENT3

The gene encoding the human ENT3 (hENT3) protein is located at position q22.1 on chromosome

Table 2. Characteristics of human nucleoside transporters

Name of gene/ protein	Chromosomal location of gene	Amino-acid residues	Tissue distribution
SLC29A1/hENT1	6p21.1 – p21.2	465	ubiquitous, placenta, liver, heart, spleen, kidney, lung, colon and brain
SLC29A2/hENT2	11q13	465	ubiquitous, predominantly abundant in skeletal muscle
SLC29A3/hENT3	10q22.1	475	ubiquitous, intracellular membranes
SLC29A4/hENT4	7p22.1	530	ubiquitous
SLC28A1/hCNT1	15q25 – q26	650	jejunum, kidney, liver, small intestine, brain
SLC28A2/hCNT2	15q15	658	kidney, liver, small intestine, jejunum, colon, rectum, heart, brain, placenta, pancreas, spleen, skeletal muscle
SLC28A3/hCNT3	9q22.2	691	bone marrow, pancreas, trachea, mammary gland, placenta, intestine, lung, kidney, liver, prostate, testis

10 (Table 2). The mRNA for ENT3 has been detected in a variety of mouse and human tissues, including brain, kidney, colon, testis, liver, spleen, placenta (highest level), and in a number of neoplastic tissues (Hyde *et al.,* 2001; Baldwin *et al.,* 2004; 2005). Human ENT3 is a 475-residue protein displaying 73% identity to the mouse homologue (mENT3) (Baldwin *et al.,* 2004; 2005; Kong *et al.,* 2004). ENT3 has a characteristic, long (51 aa) hydrophilic N-terminal region preceding the first transmembrane (TM1) domain. The N-terminal region of ENT3 consists of two dileucine motifs characteristic for endosomal, lysosomal targeting motifs. This architectural design distinguishes the ENT3 protein from other members of the equilibrative transporters family. Indeed, it was demonstrated that hENT3 protein is predominantly localized intracellularly and that mutation of the dileucine motif to alanine triggers the relocation of ENT3 protein to the cell surface (Baldwin *et al.,* 2004; 2005).

In comparison with ENT1, the ENT3 protein is much less susceptible to inhibition by NBTI and coronary vasodilatory drugs like dipyridamole and dilazep. Human ENT3 demonstrates a broad selectivity for nucleosides, but does not transport hypoxanthine. The hENT3 protein facilitates transport of several adenosine analogues like CdA and cordycepin (3′-deoxyadenosine). The antiviral purine and pyrimidine nucleoside analogues ddI, ddC and in particular AZT are efficiently transported by this protein (Baldwin *et al*., 2004; 2005).

ENT4

The gene encoding the human ENT4 protein is located on chromosome 7 at position p22.1. The mRNA for hENT4 was detected in several human tissues (Table 2). Human ENT4 is a 530-residue protein displaying 86% identity to its 528-residue mouse homologue (Baldwin *et al.,* 2004). The substrate specificity of hENT4 has not been determined in detail, but among the ENT proteins hENT4 has the lowest affinity for adenosine (Kong *et al.,* 2004).

THE CONCENTRATIVE NUCLEOSIDE TRANSPORTERS FAMILY

CNT1

The mRNA for rCNT1 was detected in several tissues including jejunum, kidney, liver, small intestine, and in many regions of the brain (Huang *et al.,* 1993; Anderson *et al.,* 1996; Pennycooke *et al.,* 2001). The gene encoding human CNT1 protein is located at position q25-26 on chromosome 15 (Table 2). Human CNT1 is a 650-amino acid protein displaying 83% identity to its rat 648-residue homologue (Ritzel *et al.,* 1997). Rat CNT1 protein appears to be a typical *cit* type transporter (N2 subtype) dependent upon sodium and preferentially transporting pyrimidine nucleosides (Huang *et al.,* 1994; Ritzel *et al.,* 1997; Wang *et al.,* 1997). This protein also binds adenosine, but this purine is weakly transported. Rat CNT1 transports several antiviral nucleoside analogues, including AZT and ddC. Nucleoside analogues such as ara-C and dFdC that are used in anticancer therapy are also substrates for hCNT1 (Yao *et al.,* 1997).

CNT2

The protein responsible for the *cif* type process (N1) that was identified by molecular cloning in the rat liver was named sodium-dependent purine nucleoside transporter (SPNT). The concentrative transporter cloned later from rat intestine was called rCNT2 (Huang *et al.,* 1993; Wang *et al.,* 1997). These two transporters are almost identical proteins that differ in a conservative substitution of glycine for alanine and valine for isoleucine at positions 419 and 522, respectively (Che *et al.,* 1995; Yao *et al.,* 1997; Cass *et al.,* 1998).

The gene encoding human CNT2 (hCNT2) protein is located at position q15 on chromosome 15 (Table 2) (Ritzel *et al.,* 1998). The mRNA for hCNT2 has been detected in a wide range of tissues including liver, kidney, jejunum, spleen, heart, brain, placenta, pancreas, colon, rectum, small intestine and skeletal muscle (Che *et al.,* 1995; Wang *et al.,* 1997; Pennycooke *et al.,* 2001).

Human and rat CNT2 proteins are 81% identical in respect to amino-acid sequences, but have different substrate specificities and regulation (Che *et al.,* 1995; Wang *et al.,* 1997). Both, rat and human CNT2 proteins transport purine nucleosides, including formycin B and uridine (Che *et al.,* 1995; Wang & Giacomini, 1997). Human CNT2 has also been reported to facilitate the uptake of antiviral compounds such as ddI and ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) used in the treatment of patients with HIV and hepatitis C, respectively (Ritzel *et al.,* 1997; Gerstin *et al.,* 2002). However, hCNT2 does not transport the antiviral drugs like ddA, ara-A, FdU and IdU (Patil *et al.,* 1998; Ritzel *et al.,* 1998; Gerstin *et al.,* 2002).

CNT3

The gene for human CNT3 protein is located on chromosome 9 at position q22. The mRNA for hCNT3 has been detected in pancreas, trachea, bone marrow, and mammary gland (Table 2). This protein exhibits a *cib* activity (N3 subtype) and transports both purine and pyrimidine nucleosides in a sodium

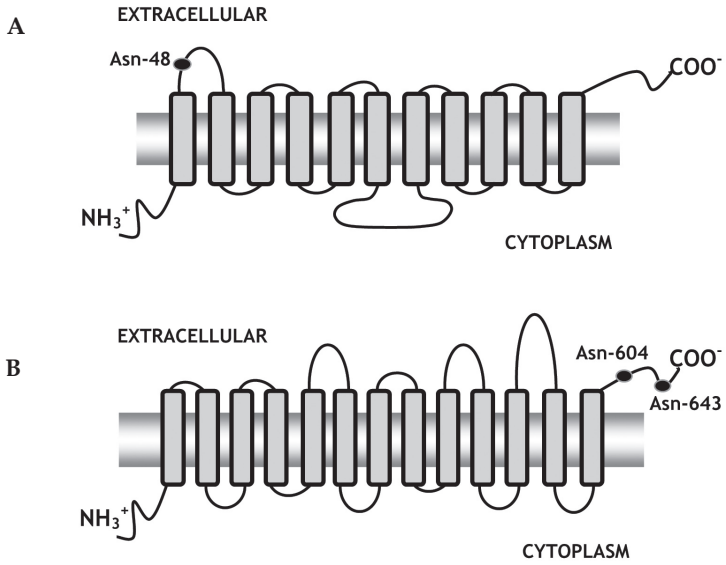

Figure 2. Topology of hENT1 (A) and hCNT1 (B).
Grey rectangles indicate transmembrane α-helices. Black circles indicate glycosylation sites in hENT1 (Vickers *et al.,* 1999) and hCNT1 (Loewen *et al.,* 1999).

dependent manner (Ritzel *et al.,* 2001). The hCNT3 protein exhibits a 2:1 stoichiometry for cotransport of Na^+ and nucleoside (Ritzel *et al.,* 2001) in contrast to the 1:1 ratio employed by CNT1 and CNT2 (Plagemann & Aran, 1990). The CNT3 protein facilitates transport of several purine and pyrimidine analogues such as CdA, dFdC and FdU that are used in anticancer therapy (Ritzel *et al.,* 2001; Zhang *et al.,* 2003; Badagnani *et al.,* 2005).

TOPOLOGY AND FUNCTION OF THE ENT AND CNT PROTEINS

Each member of the ENT family consists of 11 hydrophobic transmembrane (TM) α-helices arranged in the plasma membrane by such way that the N-terminus is cytoplasmic while the C-terminus is located in the extracellular space (Sundaram *et al.,* 2001a). These transporters are posttranslationally modified by glycosylation. The sites of glycosylation in hENT1 and hENT2 are located in the large extracellular loop between TM helices 1 and 2 (Fig. 2). Human ENT1 contains one such site, whereas hENT2 has two (Griffiths *et al.,* 1997; Vickers *et al.,* 1999; Sundaram *et al.,* 2001a). Experiments with cells grown in a medium containing a drug that prevents glycosylation (tunicamycin) showed that glycosylation of the ENT1 protein is not essential for its *es* activity (Hogue *et al.,* 1990). However, a study on a glycosylation-defective mutant of hENT1 has demonstrated that glycosylation of hENT1 modulates its sensitivity to inhibition by NBTI and coronary vasodilators dipyridamole and dilazep (Vickers *et al.,* 1999). In contrast, N-glycosylation of hENT2 has no effect on its nucleoside transport kinetics, but is required for efficient targeting of this carrier protein to the plasma membrane (Ward *et al.,* 2003). Human and mouse ENT3 are also glycosylated within the 1-2 TM loop, whereas the exact location of the glycosylation site in the C-terminal tail of ENT4 remains unknown. Another feature of the mammalian ENT proteins is a large hydrophilic loop between helices 6 and 7 at the cytoplasmic side (Baldwin *et al.,* 2004).

The structural determinants for substrate recognition and discrimination by ENT have been recently uncovered based on experiments with recombinant proteins and site-directed mutagenesis. The results of these experiments indicated that the transmembrane region of ENT2 encompassing helices 1–6 is responsible for transport of 3′-deoxynucleosides (Yao *et al.,* 2001), while the TM helices 5–6 of this transporter appear to recognize nucleobases (Yao *et al.,* 2002). Mutation of glycine to serine at position 154 in hENT1 resulted in a loss of its ability to bind NBTI and decreased the affinity for cytidine and adenosine (Hyde *et al.,* 2001; SenGupta & Unadkat, 2004). Another glycine residue at position 184 in hENT1 was shown to be important for targeting of the transporter to the plasma membrane or/and for the correct folding (SenGupta *et al.,* 2002).

Work on chimeric rat and human ENT proteins indicated that TM domains 3–6 contain residues responsible for the sensitivity to coronary vasodilators and to NBTI (Sundaram *et al.,* 1998; 2001b). The amino acid at position 33 in hENT1 (Met) and hENT2 (Ile) was shown to be responsible for the sensitivity of these transporters to coronary vasodilators such as dilazep, draflazine, and dipyridamole (Visser *et al.,* 2002).

The topological model of hCNT proteins consists of 13 TM helices with the N-terminal region located at the cytoplasmic side (Wang & Giacomini, 1997; Ritzel *et al.,* 1998; Hamilton *et al.,* 2001). In hCNT the C-terminal hydrophilic part positioned in the extracellular space is glycosylated (Fig. 2) (Hamilton *et al.,* 2001). Experiments on chimeras constructed from rat CNT1 and CNT2 proteins indicated that region encompassing TM 7

and 9 is responsible for substrate selectivity (Wang & Giacomini, 1997; Hamilton *et al.*, 2001). Those studies showed that replacing four residues (Ser^{319}, Gln^{320}, Ser^{353}, Leu^{354}) in the TM 7–9 region of hCNT1 to the corresponding residues in hCNT2 (Gly^{313}, Met^{314}, Thr^{347}, Val^{348}) resulted in a change of the substrate specificity of the transporter from the *cit* (pyrimidine specific) to the *cif* (purine specific) type. Further work indicated that even a single mutation of any of these residues changed the transporter specificity and/or affinity (Loewen *et al.*, 1999). Mutation of serine 319 to glycine in TM7 of hCNT1 produced a transporter able to transport both purines and pyrimidines (*cib* type), whereas additional mutation of glutamine 320 to methionine increased the activity of the transporter (Loewen *et al.*, 1999). Mutational studies performed on rat CNT1 yielded similar results (Wang & Giacomini, 1999). Those studies indicate that serine 319/318 (human/rat) is responsible for pyrimidine selectivity of CNT1, whereas the adjacent glutamine (320 and 319 in human and rat, respectively) is important for the modulation of the apparent affinity for nucleosides.

REGULATION OF NUCLEOSIDE TRANSPORTERS

Our understanding of the mechanisms that regulate the nucleoside transporters is relatively weak comparing to knowledge on their structure and kinetics. Data gathered so far indicate that the expression level of nucleoside transporters depends on the kind of tissue and its physiological state. Down-regulation and recycling is a relatively well-described phenomenon among the regulatory mechanisms of nucleoside transporters. A study performed on adrenal chromaffin cells, a cellular model with a high similarity to neuronal cells, showed that NBTI binding caused quick internalization of the transporter protein. As a result, 50–60% of the transporter protein was destroyed, but the remaining 40–50% returned to the plasma membrane (Torres *et al.*, 1992). Other studies demonstrated the presence of a cytoplasmic pool of nucleoside transporters in mammalian reticulocytes (Blostein & Grafova, 1987; Liang & Johnstone, 1992).

A study performed on starved rats has demonstrated that food supply modulates the expression level of CNT1 in rat small intestine. It was showed that nucleotide-deficient diets increased the amount of CNT1 protein in jejunum brush border membranes. On the other hand, nucleotide-supplemented diet increased the CNT1 level in the liver but decreased it in the jejunum. These findings suggest that the supply of nucleotides modulates CNT1 expression in a tissue-specific manner (Lopez-Navarro *et al.*, 1996; Mercader *et al.*, 1996; Patil & Unadkat, 1997; Valdes *et al.*, 2000).

Hormonal regulation of nucleoside transport activity has been reported in several tissues and cell types. Exposition of cultured chromaffin cells to T3 resulted in an increase in the number of nucleoside transporters and stimulation of adenosine transport (Fideu & Miras-Portugal, 1992). In thyroidectomized rats the NBTI-sensitive adenosine transport was shown to be reduced by 45% in brain stem synaptosomal preparations (Fideu *et al.*, 1994). A study on rat liver documented up-regulation of nucleoside transport by glucagon through a mechanism involving membrane hyperpolarization (Gomez-Angelats *et al.*, 1996). Insulin was also able to stimulate Na^+-dependent uridine uptake by a process consistent with *de novo* synthesis of the carrier protein (Gomez-Angelats *et al.*, 1996; Pastor-Anglada *et al.*, 1998). The important role of insulin in the regulation of nucleoside transporters supports the multiple observations from experimental and human diabetes. Decreased nucleoside uptake was reported in hippocampal slices from diabetic rats (Morrison *et al.*, 1992; Cassar *et al.*, 1998). It was also reported that the NBTI-sensitive adenosine transport is reduced in endothelial cells isolated from human diabetic umbilical vein (Sobrevia *et al.*, 1994; Vasquez *et al.*, 2004). On the other hand, in human cells isolated from diabetic umbilical artery smooth muscle adenosine transport was significantly raised (Aguayo *et al.*, 2001). A study performed on rats showed that the mRNA levels of rENT1, rENT2, rCNT1, and CNT2 were significantly altered in diabetic heart, liver, and kidney (Pawelczyk *et al.*, 2003). Experiments performed on cultured rat T and B lymphocytes demonstrated that nucleoside transporters expression levels were independently and differentially regulated by glucose and insulin (Sakowicz *et al.*, 2004; 2005). It appears that in rat lymphocytes the expression level of rENT2 and rCNT2 highly depends on insulin but is not affected by changes in extracellular glucose level, whereas the expression level of rENT1 is sensitive to extracellular glucose level but not to insulin. Experiments conducted on rat B lymphocytes have showed that insulin utilizes different signalling pathways to regulate expression of CNT2 and ENT2 transporters (Sakowicz *et al.*, 2005). It was demonstrated that insulin up-regulated the level of ENT2 by signalling through PI3K with no involvement of MAK kinases. On the other hand, insulin-induced suppression of CNT2 expression was totally blocked by an inhibitor of MEK-1 (PD98059), indicating that insulin controls CNT2 level by signalling through the MAPK pathway. An elevated level of glucose suppressed expression of ENT1 transporter in rat B lymphocytes by signalling through the MAP kinase pathway (Sakowicz *et al.*, 2005). The ability of elevated glucose to regulate nucleoside transporters by signalling through the MAPK pathway was also reported for human umbilical vein endothelial cells

and human aortic smooth muscle cells (Montecinos *et al.,* 2000; Leung *et al.,* 2001). In cultured human aortic smooth muscle cells, an increase of extracellular glucose concentration induced expression of ENT1 (Leung *et al.,* 2001; 2005). On the other hand, in human umbilical vein endothelium (HUVEC) the expression level of ENT1 and its transport activity were shown to be reduced by elevated level of glucose (Aguayo *et al*., 2005). In HUVEC, elevated glucose level affected adenosine transport by a mechanism that involved endothelial NO synthase, PKC and MAP kinases (Montecinos *et al*., 2000; Aguayo *et al*., 2005). An involvement of P_{2Y2} purinoceptors in the effect of elevated glucose on the NBTI-sensitive adenosine transport in HUVEC was also reported (Parodi *et al*., 2002). A modulatory role of ATP on the NBTI-sensitive nucleoside transporter in bovine chromaffin cells was also described (Casillas *et al*., 1993; Delicado *et al*., 1994).

The data gathered so far indicate that the functioning of nucleoside transporters depends also on cell cycle progression and cell differentiation. Studies performed on LA-N-2 human neuroblastoma cells documented an increased expression of NBTI-insensitive equilibrative nucleoside transporter and enhanced uptake of formycin B during cell differentiation (Jones *et al.,* 1994). Studies conducted on hepatocytes have indicated that hepatocarcinogenesis leads to a selective loss of the concentrative nucleoside transporter CNT2 and an increased expression of nucleoside transporter ENT2 (Plagemann & Wohlhueter, 1985; del Santo *et al.,* 1998; Dragan *et al.,* 2000; Pastor-Anglada *et al.,* 2001). On the other hand, synchronized hepatoma cell line FAO that had been induced to proliferate displayed an increase in ENT2, CNT1 and CNT2 mRNA levels just before the peak of thymidine incorporation into DNA. In those cells the mRNA level of ENT1 did not change during proliferation. This suggests that ENT2, CNT1 and CNT2 expression appears to be cell cycle-dependent, but ENT1 is constitutively expressed (del Santo *et al.,* 1998; Valdes *et al.,* 2002). It has been demonstrated that in rat during liver regeneration after partial hepatectomy, the activity of sodium-dependent transporters increase concurrently with elevated mRNA and protein levels of CNT1 and CNT2 (Ruiz-Montasell *et al.,* 1993; Felipe *et al.,* 1997; Pastor-Anglada *et al.,* 1998; del Santo *et al.,* 1998). An involvement of growth factors in the regulation of expression of nucleoside transporters was demonstrated in murine bone marrow-derived macrophages. These cells express both the equilibrative (ENT1, ENT2) and concentrative (CNT1, CNT2) nucleoside transporters. A study with the use of isoform-specific probes and antibodies showed that M-CSF-induction of cultured macrophages to proliferate specifically up-regulated only ENT1 at both the mRNA and protein levels. In contrast, exposure of these cells to INF-γ increased the expression of CNT1 and CNT2 by a STAT1-independent pathway. INF-γ also down-regulated the M-CSF-induced expression of ENT1 (Soler *et al.,* 2001a; 2003). Exposition of rat foetal hepatocytes to growth hormones such as dexamethasone or T3 induced selective up-regulation of CNT2 protein and mRNA level and suppressed ENT1 and ENT2 expression (del Santo *et al.,* 2001). Studies performed on human B cell lines have shown that differentiation factors such as phorbol esters (PMA) and LPS up-regulate concentrative transport systems, but down-regulate the equilibrative transporters in a PKC-dependent manner (Soler *et al.,* 1998). PMA and LPS induce also expression of TNF-α, but TNF-α alone alters the expression level of equilibrative nucleoside transporters in a PKC-independent manner. On the other hand, it has been demonstrated that activation of concentrative transporters by TNF-α is a process dependent on PKC (Soler *et al.,* 1998; 2001b). Another player involved in PMA-induced alteration of nucleoside transporters is NO, which is required in the regulation of the concentrative transporters but not of the equilibrative ones (Soler *et al.,* 2000).

CONCLUSIONS

Over the past two decades important advances in the understanding of nucleoside transporter functioning have been achieved. Identification and molecular cloning of the ENT and CNT families from mammals and protozoan parasites have provided detailed information about the structure, function, regulation, and tissue and cellular localization. Structure–function analyses of various nucleoside transporter chimeras and mutants revealed important elements involved in substrate and inhibitor recognition and binding. However, the mechanisms that regulate nucleoside transporters in various tissues and cell types are just beginning to be understood. Because of the ability of these transporters to handle nucleoside analogues used in treatment of patients with cancer and viral diseases, the ongoing research allow the designing of more specifically targeted new compounds or improving the existing drugs. New nucleoside-based drugs are welcome not only in the treatment of cancer and viral diseases, but also in cardiovascular disorders and parasitic infections.

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