Physiological Roles of Vascular Nucleoside Transporters


Abstract—Nucleoside transporters (NTs) comprise 2 widely expressed families, the equilibrative nucleoside transporters (diffusion-limited channels) and concentrative nucleoside transporters (sodium-dependent transporters). Because of their anatomic position at the blood–tissue interface, vascular NTs are in an ideal position to influence vascular nucleoside levels, particularly adenosine, which among others plays an important role in tissue protection during acute injury. For example, endothelial NTs contribute to preserving the vascular integrity during conditions of limited oxygen availability (hypoxia). Indeed, hypoxia-inducible factor-1–dependent repression of NTs results in enhanced extracellular adenosine signaling and thus attenuates hypoxia-associated increases in vascular leakage. In addition, vascular NTs also contribute to cardiac ischemic preconditioning, coronary vasodilation, and inhibition of platelet aggregation. Moreover, vascular nucleoside uptake via NTs is important for nucleoside recovery, particularly in cells lacking de novo nucleotide synthesis pathways (erythrocytes, leukocytes). Taken together, vascular NTs are critical in modulating adenosine-mediated responses during conditions such as inflammation or hypoxia. (Arterioscler Thromb Vasc Biol. 2007;27:1004-1013.)

Key Words: adenosine signaling ■ concentrative nucleoside transporter ■ endothelial barrier ■ equilibrative nucleoside transporter ■ hypoxia ■ inflammation ■ ischemia ■ preconditioning

Two different families of nucleoside transporters (NTs) have been characterized, the equilibrative nucleoside transporter (ENT) family (SLC29), responsible for passive transport, and the concentrative nucleoside transporter (CNT) family (SLC28), an active transport system.1–4 They are involved in transmembrane transport of nucleosides. A nucleoside can be derived via phosphohydrolysis of a nucleotide, which is defined as an organic molecule consisting of a nitrogenous heterocyclic base (a purine or a pyrimidine), a pentose sugar (deoxyribose or ribose), and a phosphate or polyphosphate group. A nucleoside is similar, except that it contains only the sugar and base, without a phosphate. The first members of the NT family were identified in human cells at the molecular level less than one decade ago, and since then family members have been detected in most eukaryotic tissues.1,2

The human SLC29 family of proteins contains 4 members, designated ENTs (ENT1–4), because they transport nucleosides, synthetic nucleoside analogs, and nucleobases as diffusion-limited channels.5 The human SLC28 family consists of 3 subtypes of sodium-dependent CNTs (CNT1–3) that transport both naturally occurring nucleosides or synthetic nucleoside analogs.4

Nucleoside transporters play important roles in many aspects of eukaryotic physiology. They are involved in nucleoside salvage pathways, where they mediate the first step of nucleotide biosynthesis.2–4 Similarly, NTs are involved in nucleoside reuptake in the kidneys (Figure 1). In addition, NTs play an important role in cancer treatment, because they are involved in the transmembrane transport of nucleoside analog anti-cancer drugs.4 Moreover, NTs modulate extracellular signaling effects of nucleosides, particularly of the anti-inflammatory molecule adenosine.2–6 Extracellular adenosine is mainly derived from adenine nucleotides (particularly adenosine triphosphate [ATP]), which are released by different cell types (eg, platelets or neutrophils; Figure 2).7,8 Extracellular ATP is readily converted on the endothelial surface to adenosine, attributable to the enzymatic activity of the ecto-apyrase (NTPDase 1, CD39, ATP conversion to adenosine monophosphate [AMP]) and the ecto-5’-nucleotidase (CD73, AMP conversion to adenosine).9–13 Acting on 4 different types of adenosine receptors (ARs) (A1AR, A2AAR, A2BAR, and A3AR), adenosine has important physiological roles as a signaling molecule, particularly as an anti-inflammatory or during tissue protection.14–20 For example, studies of acute inflammation provide genetic and pharmacological evidence for A3AR signaling as a mechanism for limiting inflammatory responses in vitro and in vivo.5,6,20 Similarly, in vitro studies have indicated an additional role of signaling through vascular A3AR in re-sealing of endothelia during transendothelial migration of neutrophils,14 particularly during conditions of limited oxygen availability.13 Moreover, pharmacological inhibition of the A3AR during hypoxia exposure is associated with increased pulmonary

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edema and vascular leakage. It is well-established that during hypoxia, extracellular adenosine levels increase and AR-dependent signaling contributes to tissue protection. However, adenosine can also be rapidly cleared from the extracellular space through passive or active uptake by NTs. Thus, increases or decreases of NT function or expression represent an innate cellular strategy to modulate extracellular adenosine signaling.

In addition, members of the SLC28 and SLC29 families also play critical roles in retrieval of nucleosides for nucleotide biosynthesis. They have been found to contribute to ischemic preconditioning of the myocardium, and are pharmacologically significant as transporters of anticancer or antiviral drugs.

Adenosine Transporters

Equilibrative Transporter Family

The SLC29 family, characterized by passive transport of nucleosides and nucleobases, contains 4 members termed ENT 1 to 4. All 4 transport adenosine but differ in their abilities to transport other nucleosides or nucleobases. The best-characterized of these isoforms, ENT1 and ENT2, are broad-selectivity ENTs that have been classified on the basis of their sensitivity to inhibition by nitrobenzylthioinosine (also known as nitrobenzylmercaptopurine riboside), as “es” (equilibrative-sensitive, ENT1), or “ei” (equilibrative-insensitive, ENT2). The 2 human isoforms also differ in their sensitivities to inhibition by coronary vasodilators such as dipyridamole, dilazep, and drafazine, with hENT1 being 100- to 1000-fold more sensitive than hENT2.

The originally proposed 11 transmembrane domain (TM) structure with a cytoplasmic N-terminus and an extracellular C-terminus of hENT1 has been confirmed with glycosylation scanning mutagenesis and the use of antibodies as topological probes. Studies of different human and rat ENTs show that the region encompassing TM3–6 contains residues responsible for both sensitivity or resistance to nitrobenzylthioinosine, as well as coronary vasodilators. The region from

Figure 1. Proposed role of nucleoside transporters in nucleoside recovery in the kidney. In the renal tubular epithelia, nucleoside transporters are expressed differentially, with ENTs (particularly ENT1) expressed mainly in the basolateral membrane and concentrative nucleoside transporters (particularly CNT1) at the apical surface. This anatomic distribution suggests a coordinated transport, with an active, sodium-dependent component from the tubulus lumen via CNT1 and a passive, diffusion-dependent component through the basolateral membrane via ENT1. Taken together, this transepithelial nucleoside flux is important for nucleoside recovery from the urine.

Figure 2. Model of adenosine uptake via nucleoside transporters. Different cell types can release ATP extracellularly, e.g., activated neutrophils (polymorphonuclear granulocytes) via connexin 43 hemichannels or platelets via granular release. The ecto-apyrase CD39 and ecto-5′-nucleotidase CD73 convert ATP to AMP, and AMP to adenosine, respectively. Once generated into the extracellular space, adenosine can activate endothelial ARs or is taken up by passive (ENTs) or active nucleoside transporters (adenosine-sodium co-transporters, CNTs). These nucleoside transports act to terminate adenosine signaling, thereby modulating vascular adenosine effects.
TM1 to TM6 contributes to the ability of ENT2 to transport 3-deoxynucleosides\textsuperscript{34} and the TM5–6 region to the transport of nucleobases by ENT2.\textsuperscript{35}

**ENT1 (SLC29A1)**

The human gene encoding hENT1 has been localized in the p21.1 to 21.2 region of chromosome 6.\textsuperscript{36} Historically, the "es" facilitated diffusion system has been studied in human erythrocytes since the late 1980s.\textsuperscript{37} However, it was only in 1997 that Griffiths et al.\textsuperscript{18} isolated and cloned the cDNA for the first ENT, ENT1, from human placenta. It is homologous to several proteins of unknown function in yeast, nematodes, plants, and mammals.\textsuperscript{38} Both human and rodent ENT1 transport a wide range of purine and pyrimidine nucleosides but are unable to transport the pyrimidine base uracil or nucleotides, such as ATP.\textsuperscript{3} Human ENT1 is a low-affinity transporter with \( K_m \) values for adenosine \( \approx 50 \) \( \mu \)mol/L and for cytidine \( 680 \) \( \mu \)mol/L.\textsuperscript{38}

ENT1 is ubiquitously distributed in human and rodent tissues, but its expression varies between different tissues.\textsuperscript{3} In the rodent kidney, \( r\)ENT1 is located on the basolateral surface of tubular epithelial cells as opposed to \( r\)CNT1, which is located apically.\textsuperscript{3,39} suggesting that ENT1 and CNT1 may operate in tandem to establish transepithelial nucleoside flux (Figure 1).\textsuperscript{3} ENT1 and ENT2 are the predominant NTs of the vascular endothelium.\textsuperscript{40} ENT1 is expressed at approximately twice the level as ENT2 and is most likely to control adenosine signaling in conditions of hypoxia and ischemia.\textsuperscript{15} Moreover, the ENT1 promoter activity was measured in conditions of hypoxia and normoxia and showed hypoxia-dependent repression of activity through the transcriptional factor HIF-1\textalpha\textsuperscript{15} (for more detail see section Nucleoside Transporters in Hypoxia).\textsuperscript{15}

Choi et al produced mice gene targeted for ENT1 by deleting 425 base pairs in the protein coding region of the gene.\textsuperscript{41} These mice were viable, phenotypically normal, and had normal reproductive behavior. Spontaneous mortality in these mice was not significantly different from controls, suggesting that the animals had normal immune function, despite the fact that ENT1 has been implied to be important for nucleoside salvage in lymphocytes and erythrocytes.\textsuperscript{42} Interestingly, the ENT1-null mice showed decreased hypnotic and ataxic responses to ethanol and were prone for greater alcohol consumption than littermate controls.\textsuperscript{41}

**ENT2 (SLC29A2)**

ENT2, previously known as "ei" transporter, is less sensitive to the cardiac vasodilators dipyridamole, dilazep, or drafazine. The human ENT2 gene is localized at position 13q on chromosome 11.\textsuperscript{43} ENT2 is expressed in a wide range of tissues, including the vascular endothelium, heart, brain, placenta, thymus, pancreas, prostate, and kidney, with particularly high expression in skeletal muscle.\textsuperscript{3,44,45} Human ENT2 also transports cytidine and guanosine with \( K_m \) values of 5 and 3 mmol/L, respectively.\textsuperscript{2}

In addition to the mentioned amino bases, human and rodent ENT2 transport a broad range of purine and pyrimidine nucleosides but with a lower affinity than ENT1.\textsuperscript{3,44,45} ENT2 has a 2.8-fold lower affinity for adenosine than ENT1,\textsuperscript{46} which might explain the observation that ENT1 is the dominant NT regulating adenosine signaling in vascular endothelial cells.\textsuperscript{15} However, ENT2 also transports inosine and different nucleobases, with the exception of cytosine.\textsuperscript{35,44}

The ability of ENT2 to transport hypoxanthine, its higher affinity for inosine, and its abundance in skeletal muscle cells suggest that it may have a role in nucleoside uptake during muscle exercise and recovery.\textsuperscript{44} Tissues expressing ENT2 also express ENT1, and thus to clone ENT2 an "es"-deficient cell line was devised.\textsuperscript{44} DUP-785, an inhibitor of de novo uridine synthesis, in combination with uridine, was used to select for cells expressing nucleoside transport, and the addition of nitrobenzylthioinosine (an ENT1 inhibitor) allowed for the identification of cells that possessed "ei" transporters only.\textsuperscript{44}

**ENT3 (SLC29A3)**

ENT3 and ENT4 have been recently identified and isolated as a result of the completion of the human genome project.\textsuperscript{3} The gene encoding for the human ENT3 is located at position 22.1 of chromosome 10.\textsuperscript{43} Human ENT3 is widely distributed in different tissues with particular abundance in the placenta, from which it was originally cloned.\textsuperscript{2,3}

In contrast to ENT1 and ENT2, the endogenous and green fluorescent protein-tagged forms of the full-length hENT3 protein were found to be predominantly intracellular proteins that co-localized, in part, with lysosomal markers in cultured human cells. Transport activity was relatively insensitive to the classical nucleoside transport inhibitors nitrobenzylthioinosine, dipyridamole, and dilazep, and is sodium ion-independent.\textsuperscript{47}

**ENT4 (SLC29A4)**

The gene encoding for hENT4 is located at position 22.1 on chromosome 7.\textsuperscript{43} Some studies suggest the ubiquitous distribution of this NT.\textsuperscript{3,43} It has been recently confirmed that ENT4 is abundantly expressed in the brain, skeletal muscle, and the heart, and there are substantial amounts of the transporter in the intestine, pancreas, kidney, liver, bone marrow, and lymph node.\textsuperscript{48}

A recent study by Barnes et al indicates that ENT4 is abundant in the heart, especially in ventricular myocytes and vascular endothelial cells and is absent in the SA and AV nodes. It is known that under physiological conditions, ENT4 has lower affinity for adenosine than the other EN Ts,\textsuperscript{3,26} and also efficiently transports serotonin, which is important for cardiac development, structure, and function.\textsuperscript{48} However, ENT4 exhibits optimal transport activity for adenosine only between pH 5.5 and 6.5, with virtually no activity at pH 7.4.\textsuperscript{48} The \( K_m \) value of human ENT4 for adenosine at pH 5.5 is 0.78 mmol/L.\textsuperscript{48} Because it is rather unlikely to experience such low pH values under physiological conditions, the physiological roles of hENT4 remain unclear. ENT4 is only weakly inhibited by the classical ENT1 inhibitors dipyridamole, dilazep, and nitrobenzylthioinosine.\textsuperscript{48}

**CNT Family**

Active sodium-dependent nucleoside transport is mediated by members of the concentrative NT family, also classified as
the SLC28 family. Active transport is found particularly in specialized epithelial tissues such as small intestine, kidney, and liver.\textsuperscript{29} CNTs have also been identified in endothelial cells, although they have a minimal contribution to nucleoside transport compared with ENTs.\textsuperscript{40,49} These transporters differ in their substrate selectivity: hCNT1 (SLC28A1) transports pyrimidine nucleosides and adenosine, hCNT2 (SLC28A2) transports purine nucleosides and uridine, and hCNT3 (SLC28A3) transports both pyridimine and purine nucleosides.\textsuperscript{50–52} CNT1 and CNT2 use a 1:1 sodium-to-nucleoside ratio for transport, whereas the sodium-to-nucleoside ratio of CNT3 is 2:1.\textsuperscript{4} In general, the sodium concentration gradient across mammalian cell membranes favors movement of sodium and nucleoside into the cell. Therefore, the individual contribution of ENT transport versus CNT transport can be studied by adding or removing sodium to the experimental buffer, because CNT function is terminated in the absence of sodium.\textsuperscript{53} The current model of hCNT structure proposes 13 TM domains with a cytoplasmatic N-terminus and a glycosylated extracellular C-terminus.\textsuperscript{43,53} Studies indicate that the region between TM7 and TM9 is responsible for substrate specificity.\textsuperscript{39,43}

**CNT1 (SLC28A1)**

The gene encoding the human CNT1 protein is located at position q25–26 on chromosome 15.\textsuperscript{4,43} CNT is primarily expressed in epithelial tissues, such as small intestine, kidney, and liver, and in many regions of the brain.\textsuperscript{4,43,54} CNT1 transports pyrimidine nucleosides as well as adenosine, the latter in a high-affinity, low-capacity manner.\textsuperscript{4,43,50,55} Having similar kinetic properties to hCNT1, the Km value for rCNT1 for adenosine is 26 \(\mu\)mol/L, compared with 37 \(\mu\)mol/L for uridine, and the \(V_{max}\) is lower for adenosine, showing that the transporter favors pyrimidines.\textsuperscript{4,43,50,55}

Recently, new roles of CNTs beyond salvage have been proposed. It has been shown that CNT1 and CNT2 expression in hepatocytes are associated with differentiation and poorly differentiated hepatoma cells show low expression of CNTs.\textsuperscript{53,56,57} In addition, studies in macrophages support the hypothesis that CNT upregulation is not involved in cell proliferation but rather in cell activation and apoptosis.\textsuperscript{58,59} In a study by Soler et al,\textsuperscript{59} macrophage colony stimulating factor induced macrophage proliferation, whereas interferon gamma led to activation and blocked proliferation. Treatment with interferon gamma only induced CNT1 and CNT2, whereas macrophage colony stimulating factor only upregulated ENT1.\textsuperscript{59} Thus, opposite cell responses seem to be accompanied by the upregulation of specific NTs. Also, ENTs and CNTs can be regulated in an opposite fashion. For example, in diabetic cardiac myocytes, an increase in extracellular adenosine is correlated with a decrease in ENT transport, but at the same time an increase in CNT1 and CNT2 expression was observed.\textsuperscript{60}

**CNT2 (SLC28A2)**

The human gene locus for CNT2 is 15q13–14.\textsuperscript{4,43} This NT has been detected in a wide range of human tissues such as the heart, liver, kidney, brain, placenta, pancreas, skeletal muscle, colon, and the small intestine.\textsuperscript{4,61} Human and rodent CNT2 transport purine nucleosides and uridine.\textsuperscript{4,43} The Km value of hCNT2 for uridine is between 37 and 45 \(\mu\)mol/L.\textsuperscript{51} Recently, CNT2 has been implicated in the control of adenosine signaling, because A1R agonist activation in hepatocytes and hepatoma cells increases CNT2 transport activity.\textsuperscript{62} A recent study in hepatocytes provides the first indication of posttranscriptional regulation of CNT2, showing that bile acids stimulate translocation of CNT2 to the plasma membrane, possibly fine-tuning energy metabolism in hepatocytes.\textsuperscript{63}

**CNT3 (SLC28A3)**

The human gene encoding CNT3 is located at position q22.2 on chromosome 9.\textsuperscript{4,43} and has been recently cloned and characterized.\textsuperscript{50} Human CNT3 is localized in the trachea, pancreas, bone marrow, and mammary gland, as well as in small concentrations in the intestine, lung, placenta, prostate, testis, and liver.\textsuperscript{50} This sodium-dependent NT has a broad selectivity for both pyrimidine and purine nucleosides, as well as for a number of anticancer and antiviral agents.\textsuperscript{50} The Km values of hCNT3 vary between 15 and 53 \(\mu\)mol/L (cytidine, adenosine<uridine, thymidine<guanosine, inosine).\textsuperscript{50} Recently, reduction of CNT3 and ENT2 transport has been shown to be involved in the resistance of T-lymphoblastic cell lines to thiopurines.\textsuperscript{64}

**Nucleoside Transporters Regulate Adenosine Signaling**

**Nucleoside Transport During Hypoxia and Inflammation: The Endothelial Barrier**

Recent reports suggest that hypoxia contributes to a broad range of diseases, and that a number of parallels exist between tissue responses to hypoxia and to acute inflammation.\textsuperscript{65} Emigration of polymorphonuclear granulocytes through the endothelial and epithelial barrier in hypoxic conditions may lead to a disruption of tissue barriers with the potential of extravascular fluid leakage and subsequent edema formation.\textsuperscript{66–71} It has been shown that extracellular adenine nucleotide metabolites, namely adenosine, may function as an endogenous protective mechanism during hypoxia and inflammation.\textsuperscript{8,72,73} Several studies show that elevations in cAMP through the activation of AR promotes “resealing” of endothelial monolayers, and thus may function as an endogenous pathway to dampen permeability changes during leukocyte-endothelial interactions.\textsuperscript{13,14,74}

As outlined earlier in the text, hypoxia coordinates increases in extracellular adenosine production and signaling (Figure 3). Therefore, during hypoxia adenosine flux is directed predominantly from the extracellular to the intracellular space\textsuperscript{23} (Figure 3). This puts NTs in an ideal position to control extracellular adenosine half-life, along with extracellular adenosine deaminase, which converts adenosine to inosine.\textsuperscript{75} In fact, studies in cardiac myocytes suggested that ENT1 transcriptional repression as a possible way to elevate extracellular adenosine levels during hypoxia.\textsuperscript{76} The predominant NTs of the vascular endothelium are ENT1 and ENT2, with a minimal contribution of CNTs.\textsuperscript{40} It may appear counterintuitive to many readers that a passive transporter...
such as ENT1 can have a greater physiological role than an active transporter. This is only possible when the extracellular adenosine concentration is much higher than the intracellular, for example during conditions of hypoxia. In addition, vascular ENTs are expressed to a much higher degree than CNTs.

Studies in vascular endothelia confirmed that NTs are involved in the regulation of adenosine signaling during adverse conditions such as hypoxia and inflammation. Examinations of expression levels of ENT1 and ENT2 revealed a transcriptionally dependent decrease in mRNA, protein, and function of ENT1 and ENT2 in vascular endothelia during hypoxia, resulting in increases of vascular adenosine half-life (Figure 4). Moreover, studies of the ENT1 promoter revealed a putative binding site for hypoxia-inducible factor (HIF)-1, the key regulator of hypoxia regulated gene expression (Figure 4). HIF-1 is a heterodimeric transcription factor, the activation of which is dependent on stabilization of an O$_2$-dependent degradation domain. While HIF-1α is rapidly degraded during normoxia, this subunit forms a stable heterodimer with the HIF-1β subunit during hypoxic conditions, translocates to the nucleus, and binds to the promoter of hypoxia-responsive genes. It has been recently shown that HIF can function both as a transcriptional activator as well as a repressor. Promoter constructs and site-directed mutagenesis confirmed HIF-1α–dependent repression of the ENT1 promoter (Figure 4). Moreover, in vivo studies of ambient hypoxia in mice with tissue-targeted deletion of HIF-1α confirmed hypoxia-dependence of ENT1 expression. Additional in vitro and in vivo studies of adenosine signaling demonstrated that decreased adenosine uptake caused by transcriptional repression of ENTs promotes vascular barrier and dampens neutrophil tissue accumulation during hypoxia. In summary, these studies reveal ENT repression as an innate transcriptional adaptation of the vasculature to limit excessive inflammatory responses and for...
maintaining the integrity of the vascular barrier during hypoxia by enhancing extracellular adenosine signaling.

Despite the central role of adenosine in adaptation to hypoxia and innate inflammatory responses, chronically increased levels of adenosine may be detrimental. For example, levels of adenosine are increased in the lungs of asthmatic subjects, in whom elevations correlate with the degree of inflammatory insult, suggesting a provocative role of adenosine in asthma or chronic obstructive pulmonary disease. Moreover, studies in adenosine deaminase (ADA)-deficient mice showed that A2AR antagonist treatment was associated with anti-inflammatory properties in the lung. Other studies have found increases in vascular endothelial growth factor expression by A3AR in endothelia, which could theoretically promote vascular leakage. Given the biological necessity to balance extracellular adenosine levels with potential chronic toxicity, mechanisms to modulate extracellular nucleoside levels (eg, via extracellular deamination or NTs) have become central for a variety of physiological systems.

Adaptive responses during acute hypoxia and inflammation include increased adenosine production, as well as increased adenosine signaling caused by transcriptional induction at the receptor level. Moreover, hypoxia-induced repression of NTs contributes to increasing vascular adenosine levels and signaling effects. These adaptive responses represent innate metabolic and transcriptional pathways to reduce vascular leakage associated with hypoxia and to attenuate the inflammatory response.

Cardiovascular Effects of NTs
Ischemic Preconditioning
It is well-documented that cardiomyocytes are less prone to ischemia-induced tissue damage if they have been previously exposed to short periods of ischemia, a phenomenon known as ischemic preconditioning. It has been long-known that the infarct size limiting effect of ischemic preconditioning is dependent, at least in part, on extracellular adenosine production and signaling via ARs. In fact, a recent study provided pharmacological and genetic evidence of the importance of cd73 and the A3AR in ischemic preconditioning. More specifically, cd73 or A3AR gene deletion abolished the infarct size-limiting effect of preconditioning in vivo. In addition, 5'-nucleotidases or A3AR agonist treatment attenuated infarct sizes in wild-type animals. Based on such studies showing adenosine-dependence of cardioprotection by ischemic preconditioning ischemic preconditioning, cardiac NTs are in an ideal position to modulate such responses. In fact, cardiomyocytes express high levels of all 4 ENTs. In contrast, CNT2 is the only concentrative NT expressed in myocytes. The ENTs expressed by cardiomyocytes are responsible for adenosine efflux during short time periods of hypoxia. More recent studies indicate that the expression of ENT1 in cardiomyocytes may be transcriptionally regulated by hypoxia, thereby functioning to fine-tune extracellular levels of adenosine. A transient ENT1 induction through protein kinase C seems to be responsible for fast release and subsequent adenosine uptake during acute hypoxia. However, ENT1 mRNA is decreased during longer time periods of hypoxia, possibly as a response to conserve intracellular adenine nucleotide pools or alternatively preventing termination of adenosine signaling. Thus, chronically, ENT1 levels seem to be reduced, but ENT1 induction in acute hypoxia seems to aid in ischemic preconditioning by temporarily increasing the extracellular adenosine pool and activating A3ARs.

In diabetic cardiomyocytes, however, there is significantly reduced bidirectional adenosine transport and chronically increased unidirectional Na-dependent uptake of adenosine. Such changes in NT expression indicate that the ability of diabetic myocytes to release adenosine in acute ischemic attacks is reduced, because adenosine can only leave the cardiomyocyte via ENTs; and at the same time, extracellular adenosine signaling is terminated by Na-dependent uptake. This may result in reduced potential of adenosine efflux in conditions of ischemia or hypoxia, thereby altering the ability of diabetic myocardium to precondition, contributing to an increase in cardiac complications observed in diabetic patients.

Vascular Effects
Ischemia impairs endothelial function. However, all 4 ARs are expressed on endothelial cells, and A1ARs and A2ARs are involved in vasodilation and vascular proliferation. Also, endogenous activation of A1ARs or exogenous A1AR activation may limit vascular injury. The extracellular adenosine (Ado) concentration can be increased by ENT inhibitors, and these can thus potentiate adenosine signaling and coronary vasodilation. For example, drafalazine, an adenosine uptake blocker, significantly increases coronary conductance.

Platelet Aggregation
Adenosine inhibits platelet aggregation as it stimulates the production of cAMP through A2AARs. Similarly, gentargeted mice for the A3AR have more efficient aggregation and the anti-aggregation properties of the nonselective AR agonist NECA (5'-ethyl-carboxamidoadenosine) are abolished. Thus, metabolism of ATP and ADP to AMP and adenosine inhibits platelet aggregation by increasing the extracellular adenosine concentration. As a result, the presence of endothelial CD39 and plasma ectonucleotidase, which converts ATP directly to AMP, seems to be important for normal hemostasis and prevention of excessive platelet aggregation. However, the plasma ectonucleotidases seem to be negligible compared with endothelial CD39, because the ATP half-life in the lung microvasculature is only ∼1 second, compared with a half-life of 30 minutes in whole plasma. Because platelet activation and aggregation are important changes of atherosclerosis, the loss of vascular ATPDase (cd39, NTPDase1) can cause vascular injury. However, erythrocytes take up extracellular adenosine via their NTs, thereby removing a potent inhibitor of platelet function. Dipyrindamole is used as a platelet aggregation inhibitor, and the mechanism by which it performs its function is by blocking the uptake of extracellular adenosine.
Pharmacological Relevance of Vascular NTs as Cardiovascular Drugs

Inhibition of adenosine flux is the primary mechanism by which dilapect (N,N'-bis-(3,4,5-trimethoxybenzoyloxy) propyl)-homopiperazine, drafazine (2-(aminocarbonyl)-4-amino-2,6-dichlorophenyl)-4-[5,5-bis(4-fluorophenyl)-pentyl]-1-piperazineacetamide 2HCl) and dipyridamole (2,6-bis(diethanolamino)-4,8-dipiperidinopyrimido-[5,4-d]pyrimidine) produce their clinically relevant vasodilatory effects in the coronary vasculature. Dipyridamole is used in nuclear imaging techniques, as well as to detect coronary artery disease through echocardiography. In the presence of coronary atherosclerosis, dipyridamole causes greater increase in flow in normal vessels compared with diseased vessels, because the latter exhibit less vasodilation by adenosine. In addition to causing vasodilation, dipyridamole is also known to inhibit platelet aggregation and proliferation of vascular smooth muscle cells, and to prevent reactive oxygen species generation by neutrophils. These effects can be attributed to the inhibition of adenosine reuptake into cells and thus increased adenosine signaling. As mentioned, adenosine acts as a vasodilator through the activation of A2AAdoR or A2BAdoR. Previous studies suggest that dipyridamole can be used to decrease mean arterial blood pressure, although it is not clear whether this is only dependent on adenosine uptake inhibition or on increased cAMP levels attributable to dipyridamole. The fact that decrease in blood pressure is not a clinical observation in dipyridamole-treated patients is probably caused by the short effect of the drug on increasing the plasma adenosine concentration. Mean arterial pressure is the decrease ∼10 minutes after drug administration and returns to baselines within 40 minutes.

More recently, possible new vasodilative drugs have been identified. Thiazolidinediones are a new class of anti-diabetic agents that also have vasodilatory and anti-proliferative effects on vascular smooth muscle cells. As a competitive inhibitor of ENT1, troglitazone may enhance the vasodilatory effect of adenosine by inhibiting ENT1.

Retrieval of Nucleosides for Nucleotide Synthesis: The Nucleotide Salvage Pathway

A major physiological role of nucleosides is as precursors to nucleotides, which are necessary for the formation of DNA and RNA, as well as supplying ATP for energy-dependent cellular activities. Most cells are capable of de novo synthesis of nucleosides and nucleotides, with de novo synthesis in the liver being crucial for whole-body nucleoside and nucleotide homeostasis. However, such synthesis is energetically costly, and thus the recycling of nucleosides known as salvage is often more favorable. In previous studies, DUP-785, an inhibitor of de novo uridine synthesis, in combination with uridine was used to select for cells expressing nucleoside transport, showing that nucleoside transport is necessary for salvage. Some cells in the intravascular compartment, such as erythrocytes and leukocytes, are deficient in de novo biosynthetic pathways and are thus dependent on NTs for the replenishment of the cellular nucleotide pool. The nucleoside transport system of human erythrocytes is expressed to such a high degree that the adenosine half-life in human blood is only ∼10 seconds, and the rate-limiting effect of uptake is not transport by erythrocytes, but by adenosine breakdown. Although there is no evidence for a role of the CNT system in human erythrocytes, there may be indications for other concentrative transport mechanisms. However, gene-targeted mice for ENT1 are viable and their spontaneous mortality is not significantly different from controls, challenging the view that ENT1 is mandatory for salvage in erythrocytes and immune cells. Huang et al however, identified novel inhibitors of ENT1 and ENT2 transport in human erythroleukemia cells, namely MAPK inhibitors. These compounds completely prevented the salvage of pyrimidine nucleosides. Also, macrophage colony stimulation factor is responsible for macrophage proliferation and upregulates ENT1 3- to 4-fold, suggesting that this transporter is responsible for nucleoside salvage in macrophages. However, it is not clear why deletion of the ENT1 gene does not have any immunosuppressive effect, possibly because of compensation through the ENT2 or CNT systems. Further research is needed to identify the role and dispensability of each of those systems for the salvage pathway of human blood cells.

Conclusion

Although they have only recently been identified, NTs are now known to have multiple functions in human physiology. In the vasculature, changes in NT expression or function can result in changes of extracellular nucleoside levels, thereby directly modulating vascular adenosine signaling. Thus, vascular NTs contribute to a broad range of physiological functions, including innate adaptive pathways to modulate vascular barrier function, acute inflammation, vascular tone, ischemic preconditioning, or platelet aggregation. Although recent progress has unrevealed multiple aspects of NT physiology, there is still much research necessary to understand such mechanisms in a more detailed way. It is our conviction that from a long-term perspective, molecular studies on vascular NT biology will further contribute to the treatment of vascular disorders involving acute inflammation, vascular leakage, myocardial ischemia, or platelet function.

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Disclosures

None.

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