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Review

Adenosine as a metabolic regulator of tissue function: production of adenosine by cytoplasmic 5′-nucleotidases

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Adenosine is a product of complete dephosphorylation of adenine nucleotides which takes place in various compartments of the cell. This nucleoside is a significant signal molecule engaged in regulation of physiology and modulation of the function of numerous cell types (i.e. neurons, platelets, neutrophils, mast cells and smooth muscle cells in bronchi and vasculature, myocytes etc.). As part a of purinergic signaling system, adenosine mediates neurotransmission, conduction, secretion, vasodilation, proliferation and cell death. Most of the effects of adenosine help to protect cells and tissues during stress conditions such as ischemia or anoxia. Adenosine receptors and nucleoside transporters are targets for potential drugs in many pathophysiological situations. The adenosine-producing system in vertebrates involves a cascade dephosphorylating ATP and ending with 5′-nucleotidase (EC 3.1.3.5) localized either on the membrane or inside the cell. In this paper the cytoplasmic variants of 5′-nucleotidase are broadly characterized as well as their clinical relevance. The role of AMP-selective 5′-nucleotidase (cN-I) in the heart, skeletal muscle and brain is highlighted. cN-I action is crucial during ischemia and important for the efficacy of some nucleoside-based drugs and in the regulation of the substrate pool for nucleic acids synthesis. Inhibitors used in studying the roles of cytoplasmic and membrane-bound 5′-nucleotidases are also described.

ADENOSINE — A REGULATORY NUCLEOSIDE

Adenosine (Ado) is a signaling molecule working in an autocrine or paracrine way. The potent effect of adenylic compounds in the heart and blood vessels was first described by Drury and Szent-Györgi in 1929. The large amounts of adenosine produced by a cascade hydrolysis of ATP can affect the homeostasis of high-energy compounds inside the cell in a feed-back way. This was the reason to name adenosine "a retaliatory metabolite" that is able to counteract the action of the enzymes raising

cAMP level and triggering an energy burst. In such a way adenosine plays an important role in balancing energy supply with demand in the tissues where the energy output is high (Newby, 1984; Downey & Forman, 1993).

The metabolism of adenosine is illustrated in Fig. 1. This metabolite is generated mainly by two enzymatic systems: intra- (cN-I) or extracellularly localized 5′-nucleotidases (e5′-NT) and cytoplasmic *S*-adenosylhomocysteine hydrolase (SAHH; hydrolysis of SAH is associated with transmethylation reactions). The processes of adenosine elimination in the
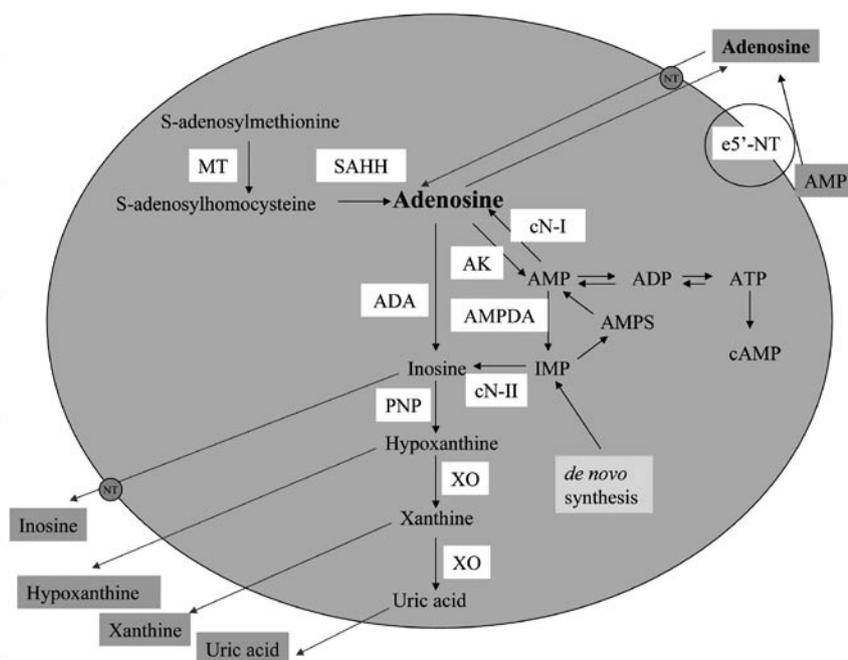

Figure 1. Adenosine metabolism in the cell.
cN-I, cytoplasmic 5'-nucleotidase-I (AMP selective); cN-II, cytoplasmic 5'-nucleotidase-II (IMP selective or IMP/GMP selective); ADA, adenosine deaminase; AK, adenosine kinase; AMPDA, AMP deaminase; e5'-NT, ecto-5'-nucleotidase; MT, methyltransferase; NT, nucleoside transporter; PNP, purine nucleoside phosphorylase; SAHH, *S*-adenosylhomocysteine hydrolase; XO, xanthine oxidase.

cell involve reactions catalyzed by adenosine deaminase and adenosine kinase (Shryock & Belardinelli, 1997) yielding inosine or 5'-AMP, respectively. The reaction of phosphorylation predominates when adenosine occurs at a low physiological concentration (< 1 μM) whereas adenosine deaminase is activated at higher concentrations of the substrate (> 10 μM). Purine nucleoside phosphorylase has only a negligible activity towards adenosine and degrades mainly inosine and guanosine. Some cells may carry out purine conversion down to uric acid as the ultimate product, others release less oxidized purines.

5'-Nucleotidases (EC 3.1.3.6) have a regulatory impact on purine metabolism and can influence the cellular level of (deoxy)adenylates and other nucleotides. Dephosphorylation of different nucleoside monophosphates is performed by distinct isoforms although they are generally only regiospecific towards the 5'-position in ribose or deoxyribose. Seven mammalian 5'-nucleotidases have been cloned until now (for a review see Bianchi & Spychala, 2003). Four isoforms are ubiquitous (eN, cN-II, cdN, mdN) whereas the others display more tissue-specific expression (cN-IA, cN-IB, cN-III).

The physiological effect of adenosine is mediated *via* specific receptors. They are classified as purinergic P1-type and situated in the plasma membrane. These G protein-coupled receptors have seven trans-membrane protein domains. Up to now four types of adenosine receptors (A_1, A_{2A}, A_{2B} and A_3) have been recognized and cloned from many mammalian and non-mammalian species (for a recent review see Yaar *et al.*, 2005). The subclassification of adenosine receptors is based on the effects which they cause over changing cAMP concentration in the cell and their affinity for the ligand (see Table 1). Within any particular subtype, the receptors from various mammalian tissues are highly similar to each other. Adenosine is their only endogenous agonist, however, A_3 receptor can be activated also by inosine (Fredholm *et al.*, 2001). The distribution of the subtypes varies greatly. For example, those localized in the heart are classified as A_1 (present only on atrial cells), A_{2A} (of intermediate abundance) and A_3 (of low abundance) (Fredholm *et al.*, 2000). The A_1-, A_{2A}- and A_{2B}-type receptors are present on skeletal muscle filaments (Fredholm *et al.*, 2000; Radegran & Hellsten, 2000). The genomic structure appears to be similar for all human adenosine receptor genes and all four types are asparagine-linked glycoproteins (Fredholm *et al.*, 2000). The A_{2A}- and A_{2B}-type preferably interact with the G_s protein and the A_1- and A_3-type act *via* the $G_{i/o}$ protein.

Adenosine exerts diverse physiological effects in various tissues (Imai & Nakazawa, 1991). The effects vary greatly depending on the population of the receptor subtype in the particular tissue (Table 2). Generally, this nucleoside is an inhibitory signaling molecule. It protects the myocardial and cerebrovascular systems against ischemia-induced damage (Ely & Berne, 1992; Obata, 2002) by slowing cardiac output. It also limits the degree of vascular

Table 1. Distribution of adenosine receptors and effects of their activation (from Fredholm *et al.*, 2000)

Type	A_1	A_{2A} (low affinity)	A_{2B} (high affinity)	A_3
Species of cloning	man, rat, mouse, dog, cow, rabbit, guinea pig, chicken	man, rat, mouse, dog, guinea pig	man, rat, mouse, chicken	man, rat, mouse, dog, sheep, chicken
Organs with high expression	brain, spinal cord, eye, adrenal gland, atria	spleen, thymus, leukocytes, blood platelets, striatopallidal GABA-nergic neurons, olfactory bulb	caecum, colon, bladder	testis (rat), mast cells (rat)
Effects of G-protein coupling	$\downarrow$ cAMP $\uparrow$ IP$_3$/DAG (PLC) $\uparrow$ arachidonate (PLA$_2$) $\uparrow$ choline (PLD)	$\uparrow$ cAMP $\uparrow$ IP$_3$	$\uparrow$ cAMP $\uparrow$ IP$_3$/DAG (PLC)	$\downarrow$ cAMP $\uparrow$ IP$_3$/DAG (PLC)

Explanation: $\downarrow$, decrease; and $\uparrow$, increase of concentration.

injury after ischemia and reperfusion by reducing reactive oxygen species release and inhibiting platelet aggregation (Ely & Berne, 1992). Adenosine is implicated in neuromodulatory (Dunwiddie & Haas, 1985; Lu *et al.*, 2004) and anti-inflammatory mechanisms (Cronstein, 1994). It has also analgesic properties (Sawynok & Sweeney, 1989), may influence proliferation, survival or apoptosis of many different cells (Jacobson *et al.*, 1999) and work as a potent suppressor in the immune system (Spychala *et al.*, 1997).

Adenosine receptors are considered as putative targets for specific drug development. The adenosine receptors-based therapy concerns Parkinson's disease, hypoxia/ischemia, epilepsy, kidney disease and asthma (Fredholm, 2003).

duction and antagonizing adrenergic stimulation. All of them bring the myocardial oxygen supply-to-demand ratio back to normal (Ballardinelli *et al.*, 1989).

The adenosine production in the myocardium is dependent on both cytoplasmic and extracellular 5'-nucleotidases. However, only cN-I is the isoform that is sensitive to the level of energy compounds inside the cell. Decrease of ATP and increase of ADP is a primary stimulus for cN-I to work (Skladanowski & Newby, 1990; Darvish *et al.*, 1996). Adenosine circulating in coronary vessels originates both from myocytes (Rubio *et al.*, 1973) and endothelial cells (Deussen *et al.*, 1986). This has led to a theory that not only myocardial but also endothelially-produced adenosine has a protective role.

ROLE OF ADENOSINE IN THE HEART

The physiological function of adenosine in the cardiovascular and central nervous systems is well recognized (Berne *et al.*, 1983). When a stress situation occurs such as ischemia, hypoxia or increased workload, the myocardial metabolism is adapted to reverse the pathological consequences (Rubio *et al.*, 1973). If the rate of energy consumption exceeds the rate of energy production, the net breakdown of ATP results in an increased production of adenosine. Elimination of the stress takes place by restoring the balance between ATP generation and utilization. The actions exerted then are: coronary vasodilation, inhibition of the sinoatrial and atrioventricular con-

ROLE OF ADENOSINE IN NERVOUS SYSTEM

As mentioned in Table 2 adenosine is a neuronal signaling substance which plays a significant role in the central and peripheral nervous systems (Zimmermann, 1994). In CNS adenosine targets are neurons, glia and cerebral blood vessels; in the periphery it regulates neurons, muscles and endothelial cells. Adenosine can modulate neural functions in many ways. It inhibits the release of most transmitters *via* A_1 receptors, presumably by inhibition of presynaptic Ca^{2+}-channels. The neuroprotective role of adenosine concerns also the control of the release of ATP from purinergic neurons. Adenosine may play a specific role in the development and regener-

Table 2. Summary of physiological effects of the activation of adenosine receptors (according to Relavic & Burnstock, 1998)

A_1	Inhibition of neurotransmission; negative chronotropy, dromotropy and inotropy; up-regulation of insulin secretion and control of lipolysis; ischemic preconditioning of heart; modulation of nociception
A_{2A}	Facilitation of neurotransmitter release; negative interaction with A_1 and A_3 types and other receptors; vasodilation of capillary blood vessels; stimulation of growth of endothelial cells and angiogenesis
A_{2B}	Degranulation of mastocytoma cells; possible role in allergic and inflammatory disorders; vasodilation of capillary blood vessels; possible tropic role in release of other signaling substances
A_3	Possible role in inflammation; proapoptotic effects; cardioprotective effects (the same as A_1)

ation of the nervous system (Zimmermann & Braun, 1996). In the salvage system, adenosine may serve as a substrate for restoration of adenylates after re-uptake *via* nucleoside transporters (Thorn & Jarvis, 1996).

ROLE OF ADENOSINE IN THE SKELETAL MUSCLE

The role of adenosine in the skeletal muscle is less well elucidated. As a vasoactive substance, it is a candidate regulatory compound of blood flow (Radegran & Hellsten, 2000). A more important role probably concerns the connections between muscle and nerves. Adenosine-induced apoptosis may represent a pathogenic pathway in muscular dystrophy (Jacobson et al., 1999). Adenosine exerts effects in both neuromuscular junctions and contractile myofibrils. It is known that adenosine derived from newly released ATP by cascadic degradation is the cause of skeletal neuromuscular depression (Silinsky, 1975). In a negative feedback the secretion of acetylcholine and ATP is inhibited by extracellularly produced adenosine.

Adenosine takes part in regulation of energy metabolism of a working muscle. Two functional proteins: tropomyosin– and troponin–complex, present in thin filaments, regulate the process of contraction. The allosteric mechanism of this control is dependent on Ca^{2+} concentration. A nervous impulse causes an increase of cytoplasmic calcium. Following that, Ca^{2+} binds to troponin and induces conformational changes of the muscle proteins that allow contraction to occur. The release of adenosine has been found to parallel the cytoplasmic Ca^{2+} pulse in the contracting animal muscle. Also adenosine formed outside the cell was elevated in electrically stimulated mammalian skeletal muscle cells (Hellsten, 1999). The role of adenosine in the skeletal muscle can be regarded from various aspects. The most important are the physiological regulation of blood flow in capillary vessels surrounding muscle bundles and the metabolic control of glucose utilization as a fuel (Dobson et al., 1971; Han et al., 1998). It has been found that adenosine may accumulate in the muscle tissue and unchanged can be released during contraction (Ballard, 1991; Radegran & Hellsten, 2000). Its sources might be the contracting myocytes, vascular endothelial cells or nerve endings (Radegran & Hellsten, 2000).

Adenosine is an autocrine or paracrine regulator in skeletal muscles as well as in the heart. In the myocytic plasma membrane, two types of adenosine receptors (A_{2A} and A_{2B}) are present. Three receptors (A_1, A_{2A} and A_{2B}) are found on vascular endothelial and smooth muscle cells (Radegran &

Hellsten, 2000). Adenosine is known to be released from endothelial cells during systemic hypoxia and then stimulates A_1 receptors (Bryan & Marshall, 1999a). Hypoxia-induced vasodilation is initiated by stimulation of A_1 receptor followed by K_{ATP}-channel opening and evoking the synthesis of nitric oxide (Bryan & Marshall, 1999b). Hellsten and Frandsen (1997) confirmed that adenosine is released from the skeletal muscle mainly under conditions of an insufficient oxygen supply. The decrease of oxygen level in the working muscle however do not affect the rate of adenosine production (Ballard, 1991; Cheng et al., 2000). The adenosine efflux can be rather stimulated by decrease of intracellular pH, resulted from lactate accumulation during hypoxic exercise. This is driven mainly by increased extracellular AMP concentration and an increased affinity of membrane-bound e5′-NT for AMP (Cheng et al., 2000). It was observed in that paper, that putative cytoplasmic 5′-nucleotidase in the skeletal muscle has too low an activity for effective dephosphorylation of AMP at a micromolar concentration.

Despite that, it seems that the amount of adenosine in the contracting muscle cannot be supplied only by ecto-5′-nucleotidase (Rubio et al., 1973). A recent report confirmed the existence of a cytoplasmic AMP-specific form of 5′-nucleotidase (cN-I) closely resembling the heart isoform. The purified enzyme processed AMP at a submillimolar concentration and was allosterically activated by ADP. This observation gives more credibility to the role of intracellularly formed adenosine in physiological regulation of skeletal muscle function (Tkacz-Stachowska et al., 2005).

ROLE OF ADENOSINE IN TUMOR PROMOTION

Some of the adenosine functions have recently been implicated in the promotion of tumorigenesis. It acts as a very potent cytoprotective and immunosuppressive agent and also has an ability to inhibit cytokine synthesis and adhesion of cells to the endothelium as well as to influence the function of lymphocytes T, macrophages and natural killer cells. Moreover, adenosine takes part in cell induction and proliferation, also in relation to cancer cells (Spychala, 2000). However, more results are needed to verify this new aspect of adenosine as a signaling molecule.

THE FAMILY OF 5′-NUCLEOTIDASES

5′-Nucleotidases hydrolyze non-cyclic nucleoside monophosphates or deoxynucleoside monophosphates to (deoxy)nucleosides and inorganic

phosphate. Some variants can transfer inorganic phosphate from a nucleoside monophosphate onto another nucleoside. The group of 5′-nucleotidases together with nucleoside kinases regulate nucleotide levels in proliferating cells (Bianchi *et al.*, 1986). The nomenclature of 5′-NTs is based mainly on their subcellular compartmentation. Five are localized in the cytosol, one is in the mitochondrial matrix and one is attached to the outer side of the plasma membrane. All cytoplasmic forms are Mg^{2+}-dependent. They vary in substrate specificity, molecular and kinetic properties. Their common feature is subunit complexity and allosteric regulation. The enzymes are filogenetically distant and there are no significant similarities between the primary structures of these proteins. For details see the recent review by Bianchi and Spychala (2003).

DIFFERENT ROLES OF CYTOPLASMIC 5′-NUCLEOTIDASES IN TISSUE-SPECIFIC METABOLISM

cN-I

5′-Nucleotidase-I is highly expressed in skeletal and heart muscles (Sala-Newby *et al.*, 1999; Sala-Newby *et al.*, 2000). Until now cN-I has been purified and characterized from the heart of many species including rabbit (Yamazaki *et al.*, 1991), pigeon (Sala-Newby *et al.*, 2000), rat (Truong *et al.*, 1988), dog (Darvish & Metting, 1993), and man (Skladanowski *et al.*, 1996). cN-I has been cloned from pigeon (Sala-Newby *et al.*, 2000), mouse (Sala-Newby & Newby, 2001) and human (Hunsucker *et al.*, 2001). Heart is the richest source of cN-I in pigeon but this form is also abundant in the brain and breast muscle. In human tissues, there are two genes (*NT5C1A* and *NT5C1B*) giving closely related products – cN-IA and cN-IB, respectively. cN-IA mRNA expression is highest in skeletal and heart muscle, a moderate level of the enzyme appears in the pancreas and brain and a low level is present in the kidney, testes and uterus (Hunsucker *et al.*, 2001).

Cytoplasmic 5′-nucleotidase-I has a high affinity for both nucleoside and deoxynucleoside monophosphates (see Table 3). The latter, however, are converted with a much lower rate. The preferred substrate for cN-I is AMP but also various pyrimidine deoxyribonucleoside monophosphates (Garvey *et al.*, 1998; Hunsucker *et al.*, 2001; Tkacz-Stachowska *et al.*, 2005). The K_m for AMP in partially purified human heart enzyme was 1.46 mM (Skladanowski *et al.*, 1996), similarly to the purified recombinant human heart enzyme where it was 1.9 mM (Hunsucker *et al.*, 2001).

cN-I is strongly activated by ADP (Skladanowski & Newby, 1990). Depending on the substrate concentration and the source of enzyme, half-maximal activation of cN-I appears to vary between 10 and 89 µM ADP (Skladanowski & Newby, 1990; Yamazaki *et al.*, 1991; Garvey *et al.*, 1998; Hunsucker *et al.*, 2001). The impulse from ADP increase seems to be the main trigger to activate cN-I. To a lesser degree the enzyme is activated by GTP (Hunsucker *et al.*, 2001). The activity of cN-I is influenced by pH (Newby, 1988; Yamazaki *et al.*, 1991; Skladanowski *et al.*, 1996) and different divalent cations: Mg^{2+}, Mn^{2+} and Co^{2+} (Skladanowski & Newby, 1990; Yamazaki *et al.*, 1991; Darvish & Metting, 1993; Skladanowski *et al.*, 1996). A selective inhibitor of cN-I is 5-ethynyl-dideoxyuridine (5-EddU) (Garvey *et al.*, 1998). An acknowledged role of the enzyme in humans is delivering adenosine during ischemic or hypoxic condition. This is crucial for prevention of heart damage (Cross *et al.*, 2002). Also its role in modulation of the pharmacological effects of nucleoside-based drugs is considered (Hunsucker *et al.*, 2005).

Murine cN-I has been cloned from the heart and testes (Sala-Newby & Newby, 2001). It appeared then that the enzyme was a product of a distinct gene. The sequence was closely related to human cN-IB but not to cN-IA. Among highly homologous genes one worth noting is a gene that encodes putative human autoimmune infertility protein (AIRP) but the exact relation of both genes calls further investigations. Human cN-IB is highly expressed in testes; the lowest level of expression estimated by RT-PCR analysis was observed in the brain and skeletal muscle. Full substrate specificity pattern of cN-IB is still unknown, but the initial enzyme characteristic appears similar to that of cN-IA. Overexpression of cloned murine cN-IB in COS-7 cells showed that the enzyme preferably hydrolyzes AMP and is allosterically activated by ADP, both characteristic also to cN-IA (Sala-Newby & Newby, 2001).

cN-II

Cytoplasmic 5′-nucleotidase-II (cN-II) is also known as high-K_m 5′-NT, purine 5′-NT or IMP/GMP selective 5′-NT. It is a 6-hydroxypurine nucleoside monophosphate-specific enzyme (Itoh, 1993; see also Table 3). It is not involved in the regulation of the deoxyribonucleotide pool but affects IMP and GTP concentration (Gazziola *et al.*, 2001). cN-II has a phosphotransferase activity and the formation of a covalently-bound phosphoenzyme intermediate has been found (Allegrini *et al.*, 2001). The phosphate transfer onto a purine nucleoside acceptor is dependent on the local concentration of nucleosides. In its absence, the phosphate moiety is removed by hydrolysis. Under physiological conditions, the phos-

phate can be transferred onto inosine and guanosine and also onto nucleoside analogs. The enzyme is widely expressed and its sequence is remarkably conserved among various vertebrates. Human cN-II was cloned in 1994 by Oka and coworkers. Highly elevated activity of a soluble 5'-nucleotidase has been associated with developmental neurological disorders and Lesh-Nyhan syndrome (Page *et al.*, 1997; Pesi *et al.*, 2000). Judging by its substrate preferences the enzyme should be classed as cN-II.

cN-III

Cytoplasmic 5'-nucleotidase-III (cN-III) is also known as PN-I, P5'N-1 or UMPH-1 (uridine monophosphate hydrolase-1). Its enzymatic activity (see also Table 3) is inhibited by exposition to heavy metals and thiol-reactive compounds (Amici & Magni, 2002). The sequence of cN-III is identical with that of another protein termed p36, an interferon α-induced protein of unknown function (Amici *et al.*, 2000). cN-III catalyzes dephosphorylation of pyrimidine nucleoside monophosphates to the corresponding nucleosides. It is much less active with purine nucleotides. The enzyme is highly expressed in red blood cells where it participates in the degradation of RNA during erythrocyte maturation. A cN-III deficiency is associated with inherited hemolytic anemia (Rees *et al.*, 2003).

MOLECULAR GENETICS OF ISOFORM cN-I

The first successful cloning of a full length vertebrate 5'-nucleotidase-I was done by Sala-Newby and coworkers in 1999. They utilized PCR-based screening of pigeon ventricular library with a degenerated primer derived from the peptide sequence of the heart isoform. The longest among the clones obtained encoded an open reading frame of 358 amino acids with a predicted molecular mass of 39 472 Da. At that time, no homologs of the enzyme existed in the SWISS PROT database. The cloned cN-I molecule was not significantly similar to cN-II or to e5'-NT. Therefore it was classified as the first member of a new family of 5'-nucleotidases. A detailed sequence analysis, based on a motifs prediction software tool (PSORT program) revealed a putative nuclear localization sequence (NLS) at the N terminus. Expression studies, carried out by Northern blotting technique, found one major transcript of approx. 2.3 kb in several pigeon tissues, including brain, skeletal muscle and heart. In spite of the NLS presence, when exogenously expressed in green monkey kidney cell line (COS-7), cN-I was localized to the cytoplasm, as judged by immunochemistry studies with enzyme-specific antibodies. This result was in line with the previous data obtained by electron microscopic immunogold labeling of AMP-selective 5'-nucleotidase, as it was then named, in sections from dog heart (Darvish & Metting, 1993). This pointed to a lack of functional consequences of NLS presence. One could speculate on another possible explanation of the observed discrepancy between the predicted and actual localization of the enzyme, involving conditional targeting of cN-I to the nucleus upon an as yet unidentified stimulus. On the other hand, the PSORT program used in those studies distinguished three types of NLS sequence, and used the heuristic that nuclear proteins are usually enriched in basic residues which comprise more than 20% of the molecule, resulting in a prediction accuracy of 57% as estimated for yeast proteins. Until now there is no evidence in the literature that AMP-selective cytoplasmic 5'-nucleotidase of any studied species localizes to the nucleus.

Table 3. **Summary of properties of cytoplasmic 5'-nucleotidases**

Isoform	cN-I		cN-II	cN-III
Gene loci products	cN-IA	cN-IB		
Nucleotides preferentially hydrolyzed	AMP dCMP, dUMP, dTMP	AMP	IMP, dIMP, GMP, dGMP, XMP	CMP, UMP, dUMP, dCMP, dTMP
Ability to transfer phosphate to other nucleoside	no	not determined	yes	yes
Activators	ADP, GTP, dNDP	ADP	dATP, ATP, GTP, ADP, Ap4A, 2,3-bisphosphoglycerate	not known
Mg^{2+} dependence	K_a 3.5–10 mM		K_a 100–200 mM	yes
Highly expressed in	skeletal muscle, heart, brain	testis	pancreas, skeletal muscle, heart	erythrocytes, lymphoid cells
Selective inhibitors	5-ethynyl-dideoxyuridine	not known	5'-deoxyisobuthylthio-adenosine (IBTA)	not known
Enzyme deficiency	not known	not known	not known	hemolytic anemia

Other members of the cN-I family were cloned from mouse and human. Unlikely the pigeon, both organisms have two isoforms of cN-I. The human gene coding for cN-IA is located on chromosome 1, in the region 1p34.3-p33. It comprises six exons and gives rise to a protein of 369 amino acids. No alternative splicing products have been observed for this gene. The other gene coding for cN-IB is located on chromosome 2 and it maps to the region 2p24.2. The protein product of this gene is 550 amino acids long. A comparison of the sequences of those two genes reveals that cN-IB includes an additional exon, which adds 181 amino acids to the N-terminus of the protein. Both cN-I-encoding alleles contain the so-called "5-nucleotidase signature" composed of an ATP-binding motif and a Mg^{2+}-binding site. All intracellular nucleotidases including also the 3′,5′-specific variant cdN and the mitochondrial variant mdN (Rinaldo-Matthis *et al.*, 2002) contain three common motifs: I, II, III, being conserved catalytic sequences and S motif involved in substrate recognition.

PURINE STRUCTURAL ANALOGS AS INHIBITORS AND SUBSTRATES OF VARIOUS 5′-NUCLEOTIDASES

It is almost canonical that high and stable ATP level ensures survival of the cell. On the other hand, adenosine coming from degradation of ATP has numerous positive effects on tissues. As cN-II has been found as a rate-limiting enzyme in ATP degradation, many studies used cN-II inhibitors to check how it influences the whole adenine nucleotide metabolism. Nucleoside analogs based on thioalkylation of the 5′-position in the ribose moiety in adenosine and inosine able to block the activity of cN-II and to some extent cN-I have been described earlier (Meghji *et al.*, 1993; Skladanowski *et al.*, 1998). Later, the cN-I selective compound — 5-ethynyl-2′,3′-dideoxyuridine inhibiting the hydrolysis of AMP to adenosine in a concentration-dependent manner with an IC_{50} value of 20 μM was reported (Garvey & Prus, 1999). These studies were performed, however, only *in vitro*. ADP analog, AOPCP (α,β-methylene-adenosine diphosphate), has been frequently used to block ecto-5′-nucleotidase when studying its role in organ physiology. When AOPCP was infused to the whole animal heart, the effect of a lowered adenosine level on ischemic preconditioning was visible (Kitakaze *et al.*, 1994). Other e5′-NT inhibitors like pentoxifillin or 6-thiopurine enhanced the protective action of cardioplegic fluid during full ischemia (Bolling *et al.*, 1992). The differences in the substrate binding site in cN-I and cN-II were studied and a cru-

cial role of the dipole moment of the base in substrate analogs was reported (Skladanowski *et al.*, 1996). These studies might have significance when the role of various cytoplasmic 5′-nucleotidases is considered in the metabolism of anti-cancer and anti-viral nucleoside analogs (for a review see Hunsucker *et al.*, 2005). The nucleotide forms of those analogs were found to be deactivated back to the corresponding nucleoside by cytoplasmic 5′-nucleotidases. On the other hand, cN-II has been shown to introduce a phosphate moiety to nucleoside prodrugs like deoxycoformycin forming then an active drug (Pesi *et al.*, 1994).

CLINICAL RELEVANCE OF CYTOPLASMIC 5′-NUCLEOTIDASES

The great variety of actions of adenosine emphasizes the importance of the enzymes involved in its production. The only described genetic disorder related to 5′-nucleotidase deficiency, hereditary hemolytic anemia, is caused by a mutation in erythrocyte-specific cN-III. Elevated levels of cN-II have been found in neurological defects and in patients with Lesh-Nyhan syndrome (Pesi *et al.*, 2000; Allegrini *et al.*, 2001). Several studies have shown increased levels of cN-II mRNA in some cancers such as acute myeloid leukemia that correlated with more aggressive leukemic phenotype and poor clinical outcome due to resistance to cytarabine therapy (Galmarini *et al.*, 2001).

In the past decades, investigations were focused mostly on cN-IA and its role in hypoxia and ischemia. Nowadays the clinical potential of soluble 5′-nucleotidases has been linked to the resistance to anti-cancer and anti-viral drugs basing on their ability to metabolize several analogue monophosphates. Up to date, cN-II has been studied the most extensively in this aspect with the most promising results both *in vivo* and *in vitro*. The drug resistance was found to be closely related to the level of the enzyme in various cell lines and with different analogues. There is some evidence that the increased levels of cN-II are accompanied by a decrease in dCK and therefore the ratio of both enzymes might be an important determining factor whether the cell would respond positively to the therapy. Creating a compound that could modulate this ratio could be beneficial for the therapy (Hunsucker *et al.*, 2005).

The family of soluble 5′-nucleotidases seems to have an increasing clinical potential, but there is still much investigation to be done before specific 5′-NT variants of relevance are identified. Development of their inhibitors or compounds that would either overcome the drug resistance or be poorer substrates could move the research forward.

TRANSPORT OF ADENOSINE *VIA* CELL MEMBRANE

Adenosine can cross the cell membrane through influx or efflux. Like other purine or pyrimidine nucleosides, it is transported by systems classified into two classes: equilibrative and concentrative. Adenosine transport during the influx has been more thoroughly studied and now it is well established that targeting the transporter protein by inhibitors (also drugs) has a consequence in the elevation of the extracellular concentration of adenosine required for the activation of P1-type receptors (Paterson *et al.*, 1977; Klabunde, 1983; Zhang & Lautt, 1991). This might be of pharmacological importance when adenosine is formed extracellularly. During intracellular adenosine formation and subsequent release from the cells, inhibition of the equilibrative transport system can elevate the cytosolic concentration of the metabolite. In such a case, counteracting nucleoside escape, for example during ischemia or washout during reperfusion, may be beneficial for regeneration of the nucleotide pool after a period of ATP breakdown. It has also been suggested that inhibitors of nucleoside transport may decrease the loss of chemotherapeutically active nucleoside-based drugs from the cells. For example, millimolar concentrations of dipyridamole prevented excretion of inosine from T cell mutants overproducing purine nucleosides (Ullman & Kaur, 1983). Regulation of adenosine transport in mammalian heart during periods of ischemia or hypoxia have been considered as an important modulator of the heart function strongly dependent on the level of this metabolite. Nucleoside transporters are also under tight regulation by various metabolic and physiological factors (for a review see Podgorska *et al.*, 2005).

CONCLUDING REMARKS

The aim of this review was to highlight the signaling role of adenosine, a molecule formed by ATP metabolism. Several adenosine effects attract the attention of clinicians. In the heart, adenosine acts as a feed-back regulator of ATP metabolism and provides the fine tuning of the balance between ATP production and utilization. In addition it can convey also a cytoprotective effect against oxidative stress. Clinical studies proved that it might have some beneficial role during coronary reperfusion therapy and minimize the ischemia/reperfusion injury. Activated adenosine receptors in CNS and peripheral tissues have been found as part of a mechanism for modulation of pain transmission. The role in skeletal muscles has not been elaborated yet in details but there are some proofs that a substantial amount produced

in this tissue may have an impact on other tissues in the body.

There is a variety of enzymes which can be responsible for adenosine production. Apart from SAHH of little activity and connected to the transmethylation pathway, there are AMP hydrolases or ADP apyrases linked to ATP degradation. They are ubiquitously localized inside and outside the cell. Both adenosine producing enzymes, nucleoside membrane transporters and adenosine receptors offer themselves as molecular targets for developing a whole range of pharmaceutical agents as receptor ligands, modulators or enzyme inhibitors.

The simplest conclusion is that after more than 50 years of studies on adenosine and its mechanisms of action we still have more questions than answers. This makes adenosine related research still exciting and promising to the clinical field.

Acknowledgements

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