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REVIEW

The Purine Metabolism of Human Erythrocytes

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Abstract—This review summarizes currently available information about a crucial part of erythrocyte metabolism, that is, purine nucleotide conversions and their relationships with other conversion pathways. We describe the cellular resynthesis, interconversion, and degradation of purine compounds, and also the regulatory mechanisms in the conversion pathways. We also mention purine metabolism disorders and their clinical consequences. The literature is fragmentary because studies have concentrated only on selected aspects of purine metabolism; hence the need for a synthetic approach.

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Key words: erythrocytes, purine metabolism, purine bases, nucleotides, *salvage* pathway, catabolic pathway

Over the last ten years or so, many new interesting details concerning purine metabolism have been revealed. However, the regulation of the purine conversion pathways is still a subject of discussion and research.

Abbreviations: ADA) adenosine deaminase (EC 3.5.4.4); Ade) adenine; Ado) adenosine; AdoK) adenosine kinase (EC 2.7.1.20); AEC) adenylate energy charge; AK) adenylate kinase (EC 2.7.4.3); AMP-D) AMP deaminase (EC 3.5.4.6); APRT) adenine phosphoribosyltransferase (EC 2.4.2.7); 2,3-BPG) 2,3-bisphosphoglycerate; cN-I) cytosolic AMP-specific 5′-nucleotidase (EC 3.2.3.5); cN-II) cytosolic IMP- and GMP-specific 5′-nucleotidase (EC 3.2.3.5); GEC) guanylate energy charge; Gua) guanine; Guo) guanosine; GuoK) guanosine kinase (EC 2.7.1.73); HGPRT) hypoxanthine-guanine phosphoribosyltransferase (EC 2.4.2.8); HK) hexokinase (EC 2.7.1.1); Hyp) hypoxanthine; Ino) inosine; InoK) inosine kinase (EC 2.7.1.73); MTA) methylthioadenosine; 5′-NT) 5′-nucleotidase (EC 3.1.3.5); PFK) phosphofructokinase (EC 2.7.1.11); PGK) phosphoglycerate kinase (EC 2.7.2.3); P_i) orthophosphate; PP_i) pyrophosphate; PK) pyruvate kinase (EC 2.7.1.40); PNP) purine nucleoside phosphorylase (EC 2.4.2.1); PRPP) 5′-phosphoribosyl 1-pyrophosphate; Rib-1-P) ribose-1-phosphate; Rib-5-P) ribose-5-phosphate; SAH) S-adenosylhomocysteine; SAHH) S-adenosylhomocysteine hydrolase (EC 3.3.1.1); SAM) S-adenosylmethionine; TA) transaldolase (EC 2.2.1.2); TAN) total adenine nucleotides; TGN) total guanine nucleotides; TK) transketolase (EC 2.2.1.1); XMP) xanthosine-5′-monophosphate.

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Purine metabolism involves synthesis and degradation of purine nucleotides and determines the value of the adenylate and guanylate pool; in this way, it is responsible for the absolute value of intracellular ATP and GTP concentrations. The major site of purine synthesis is in the liver. There purine nucleotides are synthesized *de novo* as mononucleotides: IMP, AMP, and GMP, where IMP is the precursor yielding AMP and GMP. The biosynthesis of IMP is a multi-stage process, which leads to the formation of the first fully formed nucleotide from 5′-phosphoribosyl 1-pyrophosphate (PRPP) through a series of reactions utilizing ATP, tetrahydrofolate derivatives, glutamine, glycine, and aspartate.

The unique erythrocyte structure and metabolism is due to, among other reasons, a deficiency of glutamine PRPP amidotransferase (EC 2.4.2.14) and its inability to synthesize purine nucleotides in the *de novo* pathway. Hence, in erythrocytes, these compounds are produced in *salvage* reactions, which means that already existing purine bases (adenine (Ade), hypoxanthine (Hyp), and guanine (Gua)) and nucleosides (adenosine (Ado), inosine (Ino), and guanosine (Guo)) are reutilized. Reutilization of nucleosides and nitrogenous bases is a source of erythrocyte purine mononucleotides. The metabolic purpose of these reactions is to introduce these compounds into the high energy purine nucleotide synthesis pathways (ATP, ADP, GTP, and GDP). The nucleosides, as products of mononucleotide dephosphorylation (AMP →

Ado, IMP → Ino, GMP → Guo), undergo deamination (Ado → Ino) and phosphorolysis (Ino → Hyp, Guo → Gua), releasing Hyp and Gua as the final products of the purine nucleotide catabolism in erythrocytes.

Therefore, the purine nucleotide metabolism in human erythrocytes is based on two fundamental pathways: the *salvage* pathway and the catabolic pathway.

BIOSYNTHETIC PATHWAY – *SALVAGE* PATHWAY

PRPP: a central metabolite in the *salvage* pathway. 5-Phosphoribosyl-1-pyrophosphate (PRPP) is an essential substrate for the synthesis of purine and pyrimidine nucleotides. Hence, the availability of PRPP plays an important role in erythrocyte metabolism.

PRPP is formed through the action of PRPP synthetase (5-phosphoribosyl-1-pyrophosphatase, EC 2.7.6.1), which activates carbon 1 of ribose-5-phosphate (Rib-5-P) by transferring to it the pyrophosphate moiety of ATP. In erythrocytes, Rib-5-P is generated in the oxidative pentose phosphate pathway as well as in reactions catalyzed by transketolase (TK, EC 2.2.1.1) and transaldolase (TA, EC 2.2.1.2). This pathway has two main functions. First, it provides reducing equivalents in the form of NADPH, which can be used for the conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH). This is important for detoxification of reactive oxygen species. Second, Rib-5-P is generated, which is then transformed into PRPP. This is an essential precursor substance for the salvage of the purine bases Ade, Hyp, and Gua to form AMP, IMP, and GMP, respectively.

In erythrocytes, Ino and Guo can also be the source of Rib-5-P; they are converted into Hyp and Gua with participation of purine nucleoside phosphorylase (PNP). Phosphorolysis of the *N*-ribosidic bonds of purine nucleosides results in a release of ribose-1-phosphate (Rib-1-P), which in a reaction catalyzed by ribosylphosphate mutase (EC 5.4.2.7) is converted to Rib-5-P.

The rate of PRPP synthesis depends both on Rib-5-P availability and PRPP synthetase activity, which is partially inhibited in the presence of high concentrations of purine nucleotides and the reaction products. Since PRPP is also involved in pyrimidine nucleotide and NAD^+ syntheses, the synthesis of this compound is subjected to cumulated feedback inhibition by metabolites produced by various conversion pathways using PRPP [1, 2].

The regulation of PRPP synthesis by P_i is an important regulation mechanism in purine metabolism in erythrocytes, because PRPP synthesis and PRPP synthetase are sensitive to even very small differences in cellular P_i concentration [3, 4]. Moreover, in erythrocytes with a deficit of pyruvate kinase (PK, EC 2.7.1.40), an increase in 2,3-bisphosphoglycerate (2,3-BPG) concentration results in the inhibition of hexokinase activity (HK, EC 2.7.1.1), glucose-6-phosphate dehydrogenase (G-6-PD, EC 1.1.1.49), 6-phosphogluconate dehydrogenase (6-PGD, EC 3.1.1.31), and also transaldolase (TA, EC 2.2.1.2) and transketolase (TK, EC 2.2.1.1) [5, 6].

A decrease in pentose phosphate pathway enzyme activity can limit the amount of erythrocyte PRPP and in consequence affect the ATP concentration in the cell [7]. Moreover, it has been reported that 2,3-BPG can lead to dissociation of PRPP synthetase into smaller aggregates, or its monomeric forms. This could cause a decrease in the enzyme activity and lower erythrocyte PRPP concentration [8].

The conversion of purine bases (Ade, Hyp, and Gua) into mononucleotides (AMP, IMP, and GMP) requires PRPP as a substrate for reactions catalyzed by phosphoribosyltransferases—enzymes that catalyze the recovery of preformed bases for use in cellular metabolism. As a result of reutilization, the phosphoribosyl group from PRPP is transported to the suitable purine base. The reactions are catalyzed by adenine phosphoribosyltransferase (Ade → AMP) and hypoxanthine-guanine phosphoribosyltransferase (Hyp → IMP, Gua → GMP). For these enzymes, the forward reaction appears to be ordered and sequential with PRPP binding first followed by the purine base. After catalysis, pyrophosphate (PP_i) is released before the nucleotide. The reaction chemistry has been reported to proceed via either a dissociative (S_n1) or an associative (S_n2) type mechanism. Based on their similarity to other enzymes, purine phosphoribosyltransferases have been proposed to catalyze the S_n1 type reaction with the formation of an unstable ribooxocarbenium ion intermediate [9].

AMP resynthesis. Adenine phosphoribosyltransferase (APRT, EC 2.4.2.7) is a non-abundant intracellular enzyme that contributes to the salvage of purine bases by catalyzing the phosphoribosylation of Ade to form AMP. This reaction seems to be the only mechanism through which free Ade is incorporated into its corresponding nucleotide in humans.

Unlike Gua and Hyp, Ade is not produced as a result of PNP activity; an enzyme catalyzing phosphorolytic breakdown of nucleosides (it does not react with Ado) into purine bases and ribose-1-phosphate (Rib-1-P). Therefore, the polyamine biosynthetic pathway is a basic source of Ade in erythrocytes. Methylthioadenosine (MTA) is a naturally occurring sulfur-containing nucleoside present in all mammalian tissues. MTA is produced from S-adenosylmethionine (SAM) mainly through the polyamine biosynthetic pathway, where it behaves as a powerful inhibitory product. This compound is metabolized solely by MTA-phosphorylase (EC 2.4.2.28) to yield 5-methylthioribose-1-phosphate and Ade, a crucial step in the methionine and purine salvage pathways, respectively [10]. The same enzyme (MTA-phosphorylase) can also release Ade and Rib-1-P from Ado under high Ado concentrations, e.g., during tissue ischemia [11].

Some other derivatives of Ade have been also shown to increase the adenylate pool. S-Adenosylmethionine (SAM), 5′-iodo-5′-deoxyadenosine, neplanocin A, and 2′-deoxyadenosine were found to increase the ATP concentration in human erythrocytes [12-14]. This process was independent of adenosine kinase (AdoK) and did not operate in erythrocytes lacking APRT, showing that the purine base must be released in this process before incorporation into ATP. The studies on purified S-adenosylhomocysteine hydrolase (SAHH) showed that in the reaction catalyzed by this enzyme, reversible S-adenosylhomocysteine (SAH) hydrolysis into Ado and L-homocysteine, an unstable indirect metabolite is produced—3-ketoadenosine, whose glycoside bond undergoes spontaneous non-enzymatic hydrolysis accompanied by the release of Ade [14]. In this way, erythrocyte Ade can be released when nucleosides bind with SAHH, and can take part in the purine nucleotide synthesis due to the APRT-catalyzed reaction [14].

APRT catalyzes AMP and PP_i formation using Ade and PRPP as substrates. The PP_i is degraded to P_i through inorganic pyrophosphatase, which moves the reaction equilibrium to the right, thus making it irreversible. The erythrocyte enzyme has been purified by affinity chromatography [15], and the amino acid sequence has been determined. The molecular weight was calculated to be 19 kD, and the enzyme is formed of two apparently identical subunits [16, 17].

APRT is activated by divalent cations, the most effective of which is Mg^{2+}, and is strongly inhibited by the products of the reaction and by ADP and ATP (competitive inhibitors). The inhibition by AMP is competitive with respect to PRPP and non-competitive to Ade, whereas the inhibition by PP_i is not competitive with either of the substrates. GMP is an allosteric inhibitor, although it is not certain if it plays any regulatory role *in vivo* due to its low intracellular concentration (table) [11, 18].

The most crucial parameter influencing the rate of reaction at a stable Ade concentration is the PRPP/AMP ratio, because the reaction is feedback inhibited by AMP on the basis of mutual exclusiveness of the AMP and PRPP bond by APRT [11, 22].

APRT is widely distributed through various tissues, especially in the liver. However, for clinical purposes, its activity (24 ± 4.8 nmol/h per mg hemoglobin) is determined in erythrocytes.

APRT deficiency is inherited through a recessive autosome, the gene being located in the long arm of chromosome 16 [23]. Clinical symptoms only appear with the formation of kidney crystals or stones composed of 2,8-dihydroxyadenine [24]. The erythrocyte activity of APRT increases in PNP deficiencies [25] and in most patients with total or partial hypoxanthine-guanine phosphoribosyltransferase (HGPRT) deficiency [26].

The source of purine nucleotides in erythrocytes is also nucleosides (Ado, Ino, Guo). In the presence of ATP and kinases with various substrate specificities, the nucleosides can be phosphorylated to mononucleotides.

Adenosine kinase (AdoK) catalyzes the phosphorylation of Ado into AMP using ATP or GTP as substrates. The physiological function of AdoK is associated with the regulation of extracellular Ado levels and the preservation of intracellular adenylate pools. The relationship between energy metabolism and metabolism of Ado is presented in Fig. 1.

It has been reported that the majority of basal Ado production during normoxia is derived from the action of SAHH (reversible hydrolysis of SAH into Ado and L-homocysteine) [27]. However, during times of ischemia or cellular stress, increased amounts of Ado are formed almost exclusively from AMP by means of 5′-nucleotidase (5′-NT) [28, 29]. In the cell, generated Ado can be deaminated to Ino by adenosine deaminase (ADA), phosphorylated to AMP by AdoK, or transported into extracellular fluid. Purine nucleosides including Ado are rapidly transported across the human red blood cell membrane by a carrier-mediated process [30-34], which influences the extracellular Ado concentration. The short plasma half-life (~1 sec) and extracellular levels of Ado (in normal human plasma varying from 0.02-0.2 μM) are regulated by an efficient uptake and by metabolism in endothelial cells and erythrocytes [19, 31].

At low erythrocyte Ado concentration, the phosphorylation reaction is predominantly catalyzed by AdoK, which has a much lower Michaelis constant (K_m) for Ado than ADA [35]. In contrast, at high concentrations, Ado becomes effectively deaminated by ADA with a high V_{max} and also inhibits AdoK activity [36, 37]. Increased uptake of Ado by red blood cells may lead to an increase in total adenine nucleotides (TAN), which was confirmed by *in vitro* studies [31, 38, 39]. It was found that AdoK but not

Mean purine nucleotide concentrations (μM) in human erythrocytes

ATP	ADP	AMP	GTP	GDP	GMP
1519 [19]	171 [19]	12 [19]	86 [19]	14 [19]	17 [21]
1625 [19]	191 [19]	17 [19]	64 [19]	16 [19]	
1570 [20]	137 [20]		66 [20]	17 [20]	

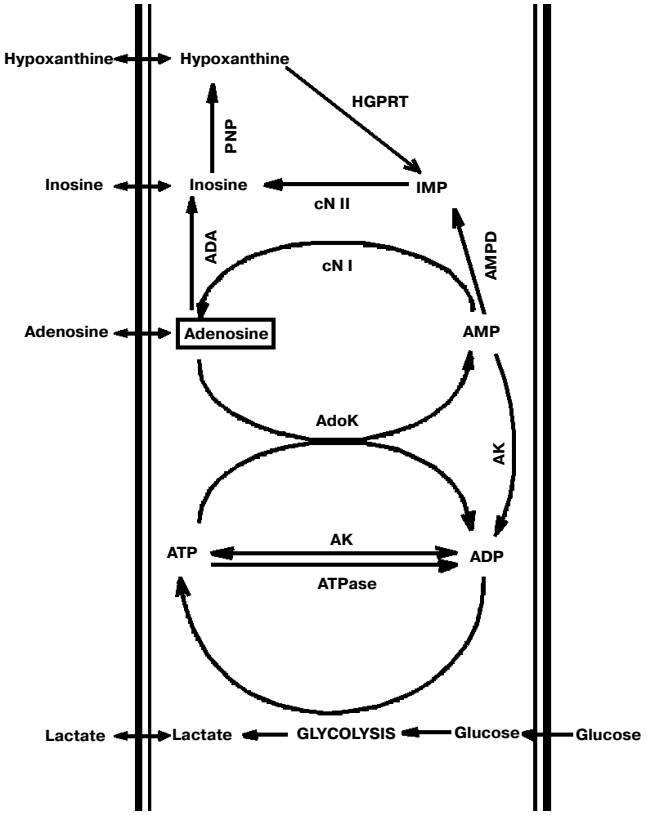

Fig. 1. Relationship between energy metabolism and metabolism of Ado (based on [38]).

ADA was stimulated in cells having a high P_i content [31, 38]. The reaction catalyzed by AdoK prevents excessive AMP catabolism, and thus saves the intracellular adenylate pool [14, 38].

Ade and Ado reutilization reactions are the source of erythrocyte adenine nucleotides (ATP, ADP, and AMP). The metabolic goal of Ade phosphoribosylation and Ado phosphorylation is the introduction of these compounds into the high energy adenine nucleotide (ATP and ADP) pathway.

Therefore, the resynthesis of adenine nucleotides (which constitute 85% of all free erythrocyte nucleotides) can take place both on the PRPP-dependent pathway (requiring APRT activity and Ade as a substrate) and the adenosine pathway (requiring AdoK activity and Ado as a substrate). The APRT K_m value for Ade is 1-5 μM [40], and the AdoK K_m value for Ado is 2 μM. The determined Ade and Ado concentrations in erythrocytes are 13 and 1 μM, respectively [35].

Experiments carried out on the glycolysis model in human erythrocytes proved that the lower PK activity may result in a 50% decrease in ATP concentration, which is not accompanied by an increase in ADP and AMP [41]. These changes can be explained by disorders in the resynthesis pathway of these nucleosides; it could be inferred from the fact that in erythrocytes with a deficit of that enzyme, PRPP concentration is lower (PRPP is a direct substrate in reactions catalyzed by APRT and HGPRT) and the adenosine pathway is impaired at the AdoK stage. A decrease in PRPP results in lower adenine nucleotide concentration, lower NAD^+ concentration, the inhibition of pyruvate kinase activity, and the inhibition of 3-phosphoglyceraldehyde dehydrogenase and, as a consequence, of glycolysis [42]. The drastic ATP decrease can lead to disorders in the active transport of K^+ into the erythrocyte. Electrolyte deficit leads to the loss of osmotic water, erythrocyte plasticity, and their premature destruction in the spleen [43].

IMP and GMP resynthesis. HGPRT is a purine salvage enzyme that catalyze the Mg^{2+}-dependent transfer of a phosphoribosyl group from PRPP to the N9 position of 6-oxopurines (Hyp and Gua) to form the corresponding nucleoside 5′-monophosphates (IMP, GMP) and inorganic pyrophosphate (PP_i) [26]. These are critical reactions in purine salvage pathways.

HGPRT erythrocyte activities in relation to Hyp and Gua are 2.14 ± 0.15 and 2.49 ± 0.24 μmol/min per mg of protein, respectively.

In erythrocytes, HGPRT is 217 residues long with molecular weight 24 kD [44]. In its native state, as a tetramer of enzyme subunits, HGPRT is coded for by a single genetic locus [45]. Wilson et al. [44-46] have described multiple isoelectric forms of the enzyme subunits in erythrocytes. This heterogeneity appears to be due to a series of post-translational modifications in erythrocytes: acetylation of the NH_2-terminal alanine and partial deamidation of asparagine 106. HGPRT is a cytoplasmic enzyme that is active from pH 7.1 to 9.1. Kinetic studies on the influence of Mg^{2+} suggests that Mg^{2+} activates the synthase by bonding with PRPP. PRPP, in the form of dimagnesium salt, is the most favorable substrate for HGPRT [47, 48].

A complete lack of HGPRT activity in humans causes Lesch–Nyhan syndrome [49], characterized by hyperuricemia and neural disorders including mental retardation and compulsive self-mutilation behavior [49], whereas a partial deficiency of HGPRT leads to gouty arthritis [50].

The source of purine nucleotides (IMP and GMP) in erythrocytes is also nucleosides (Ino, Guo). In the presence of ATP and kinases with various substrate specificities, the nucleosides can be phosphorylated to mononucleotides. Inosine kinase (InoK) and guanosine kinase (GuoK) catalyze the reactions of Ino and Guo phosphorylation into IMP and GMP.

MONONUCLEOTIDE CONVERSIONS

It is most likely that in mature erythrocytes IMP is not a significant source of AMP, because erythrocytes do not show any adenylosuccinate synthase activity (EC

6.3.4.4), a key enzyme in the purine nucleotide cycle [12, 51]. Hence, the erythrocyte reaction leading to irreversible hydrolytic deamination of AMP into IMP would be strictly controlled to protect the cellular ATP.

AMP produced in erythrocytes is phosphorylated to ADP in a reaction catalyzed by adenylate kinase (AK, EC 2.7.4.3) [52]. The resynthesis of ATP from ADP takes place in substrate phosphorylation reactions involving phosphoglycerate kinase (PGK, EC 2.7.2.3) and pyruvate kinase (PK, EC 2.7.1.40). Those reactions are the only source of ATP in erythrocytes.

GMP in reaction catalyzed by guanylate kinases, nucleoside monophosphate kinase (EC 2.7.4.8) and nucleoside diphosphate kinase (EC 2.7.4.6), is phosphorylated to GDP and GTP, respectively [53]. The source of GMP in erythrocytes can be also IMP, which with the participation of IMP dehydrogenase (EC 1.2.1.14) and NAD^+ is oxidized to xanthosine-5′-monophosphate (XMP). XMP, after the addition of glutamine NH_2 group in the presence of ATP, finally becomes GMP. The latter reaction is catalyzed by GMP synthase (EC 6.3.5.2) cooperating with ATP [20].

Monophosphate nucleoside kinase activity in human erythrocytes is 0.35 U/ml, which is only one fourth of the AK activity. Nucleoside diphosphate kinase activity (76.0 U/ml) is 60 times higher than AK activity and 200 times higher than GMP kinase [54].

Total adenine nucleotides (TAN = [ATP] + [ADP] + [AMP]) and total guanine nucleotides (TGN = [GTP] + [GDP] + [GMP]) in normal conditions are maintained at a stable level. Maintaining the ratios of concentrations is possible due to constant conversions of purine nucleotides. The ratio of particular nucleotide concentrations within these pools is maintained mainly by the activity of adenylate kinase, guanylate kinase, and the enzymes connected with the catabolism of these compounds.

Adenylate kinase acts as a buffer system when adenine nucleotide concentrations change. At the same time, it regulates all the reactions involving adenine nucleotides acting as activators or inhibitors [55, 56].

The relationship between ATP, ADP, and AMP concentrations in a cell is determined by the state of balance in reactions catalyzed by adenylate kinase: 2 ADP $\leftrightarrow$ ATP + AMP. These reactions allow the use of the richly energetic phosphate in ADP for ATP synthesis, AMP rephosphorylation, and also for AMP concentration increase when ATP concentration decreases. AMP, as an allosteric effector, leads to the enhancement of catabolic reactions resulting in ATP concentration increases in a cell. Thus, the elements of TAN are mutually transformed in AK-catalyzed reactions.

Under physiological conditions, the total adenine nucleotide concentration does not change significantly, whereas the content of the nucleotide pool does change (depending on the activity of various metabolic pathways).

In the 1960s, Atkinson set forth a hypothesis that TAN reflects the cell energy level and shapes the direction of enzymatic reactions [57].

Maintaining high phosphorylation potential is a prerequisite for using the energy accumulated in ATP and GTP [44]. It is expressed either as a ratio of ATP (GTP) concentration to the product of ADP (GDP) and P_i concentrations, or with Atkinson's formula describing the adenylate energy charge (AEC) and guanylate energy charge (GEC):

$$AEC = \frac{[ATP] + 0.5[ADP]}{[ATP] + [ADP] + [AMP]},$$

$$GEC = \frac{[GTP] + 0.5[GDP]}{[GTP] + [GDP] + [GMP]}.$$

Theoretical values of AEC and GEC are between 0 (when the system contains only AMP or GMP) and 1 (when the system contains only ATP or GTP). In homeostasis, erythrocyte AEC and GEC are between 0.85-0.95 [58-60].

The adenylate energy charge value reflects in a general way the ability of cells to carry out ATP-dependent anabolic processes. The ratios of adenine nucleotide concentrations expressed by these formulas are regulated directly by the balance of AK-catalyzed reactions.

Because the AEC value depends on the balance between the ATP-consuming reaction and ATP-producing metabolic pathways, the activities of particular metabolic pathways is regulated by the AEC value. Cells stabilize the AEC value by adjusting the speed of reactions utilizing ATP to the state of the energy demand. Decreases in AEC values lead to an increase in ATP. Therefore, if the processes connected with the energy consumption are activated to an extent where AEC values decrease, ATP production is rapidly accelerated to cover the demand for the utilized energy [55].

In erythrocytes, the speed of glycolysis (the only source of ATP) is controlled by phosphofructokinase (PFK). Under physiological conditions, at the correct ATP and fructose-6-phosphate concentration, the enzyme shows only 0.1% of its activity. The activity of erythrocyte PFK is regulated by effectors, of which ATP is the most important inhibitor, and AMP and P_i are the strongest activators [61]. ATP increase lowers the affinity of PFK to the substrate [62]. The inhibiting effect of ATP is neutralized by AMP, and therefore PFK activity increases the moment ATP/AMP ratio decreases and P_i concentration increases. Thus, cellular ATP concentration decrease results in PFK activity increase. Simultaneously, due to the AK catalyzed reaction, AMP concentration increases (AMP serving as a PFK activator).

PURINE DEGRADATION

Mononucleotide catabolism. The catabolic pathways of purine nucleotides can take different courses in the first stages and begin with mononucleotide dephosphorylation (Ado, Ino, and Guo). Hydrolytic splitting of the phosphate group from the 5′-nucleotide position involves intracellular 5′-NT (unlike ecto-5′-NT, connected to the extracellular area of the membrane). Erythrocyte and cytosolic 5′-NT occurs as several isoenzymes, which can be differentiated on the basis of substrate preference [63]. The differences in the catalyzed reactions can be confirmed by kinetic studies. The AMP-selective cytosolic 5′-NT (cN-I) is responsible for the release of Ado from ATP, while the IMP- and GMP-selective cytosolic 5′-NT (cN-II) release Ino and Guo from IMP and GMP, respectively [64, 65].

Studies on purified erythrocyte 5′-NT showed that this is a protein with a molecular weight 36 kD and is a monomeric protein at pH = p*I*. The activity is dependent on Mg^{2+}. Its inhibitors are heavy metal ions and thiol-reactive reagents, and the activator is ADP but not ATP [66]. It has been shown that erythrocyte 5′-NT (cN-I) is active at increased AMP concentration. This enzyme activity does not depend on the P_i concentration [63, 65].

Erythrocyte cN-II is responsible for Ino release from IMP and Guo from GMP. It is an enzymatic protein with molecular weight 65 kD [67], activated by ATP in the presence of IMP as a substrate, and inhibited by P_i [64, 68]. Therefore, the activation of that enzyme by ATP depends on the substrate and P_i concentrations. It has been shown that cN-II is activated by 2,3-BPG [64, 69]. Moreover, it has been reported that ADP and 2,3-BPG can act synergistically. Together they stimulate the enzyme four times as much as the case of mere additive activity of these compounds [69].

Under physiological conditions, AMP catabolism in erythrocytes takes place mainly on the path of deamination to IMP and NH_3 in a reaction catalyzed by AMP deaminase (AMP-D)—a cytoplasmic enzyme with high substrate specificity in relation to AMP [63]. *In vitro* studies show that erythrocytes incubated in Ringer–Krebs bicarbonate (5 mM glucose, 1.2 mM P_i, pH 7.7) maintain constant concentrations of adenine nucleotides for several hours. Under those conditions, the speed of Hyp production as a final product of adenine nucleotide catabolism in erythrocytes is about 4 mmol/h per ml of packed cells and does not change after adding ADA and AdoK inhibitors. The results of that experiment indicate the involvement of AMP deamination reaction and then IMP dephosphorylation in Hyp production under physiological conditions [63].

AMP-D occurs as several isoenzymes. The largest amount can be found in liver (isoenzyme L), skeleton muscles (isoenzyme M), and erythrocytes (isoenzyme E) [70]. The erythrocyte isoenzyme occurs in two molecular forms (E1 and E2) with different kinetic properties; they are post-transcriptionally modified products of the same gene *AMPD3*. The gene codes a 285-kD protein [62]. The p*I* for that enzyme has been determined at pH 5.0 and the optimal pH value is 7.0 [71].

Red blood cells show a high AMP-D activity (V_{max} 200 mmol/h per liter cells) and hence the effective control of AMP deamination plays a significant role in adenine nucleotide concentration regulation in cells (especially ATP). It has been shown that AMP-D has the properties of an allosteric protein. The main so far recognized activators of the erythrocyte deaminase are metal ions (K^+, Na^+, and Ca^{2+}) and ATP and ADP. P_i, IMP, and 2,3-BPG inhibit the enzyme activity [72]. IMP has also been reported to activate the enzyme activity. Activation of the enzyme by IMP is due to prevention of the inhibitory effect of P_i. Addition of 2-5 mM IMP to hemolysates of red blood cells containing 1 mM ATP and 3 mM 2,3-BPG lead to threefold increase in AMP deamination rate [73]. Under physiological conditions, a low Ca^{2+} concentration and high 2,3-BPG result in low AMP-D activity in red blood cells [64, 72, 73]. K^+ at 100-150 mM, a concentration close to the physiological one in cells, stabilizes the AMP-D molecule in such a conformation that it is able to bind its physiological ligands—AMP, ATP, and P_i. The enzyme is activated by the substrate and inhibited by the product of the reaction. It has been shown that in the presence of physiological concentrations of effectors (100 mM KCl, 1 mM ATP, 3 mM 2,3-BPG, and 1 mM P_i) erythrocytes incubated in Ringer–Krebs bicarbonate (pH 7.4) containing 5 mM glucose and 5-10 μM AMP have low AMP-D activity. Increase in AMP concentration leads to 15-fold increase in the enzyme activity [63].

TAN in a cell significantly depends on a reaction catalyzed by AMP-D. The significance of that reaction is connected with the regulation of TAN component concentrations and buffering changes in AEC value [55].

Increase in AMP concentration in a cell leads to a decrease in AEC. AMP-D influenced AMP deamination moves the balance of the AK-catalyzed reaction to ATP synthesis, which prevents drastic decrease in AEC value. ATP concentration increase is a signal for enhancing anabolic conversions, which in turn result in a release of significant amounts of P_i, inhibition of AMP-D activity, and decrease in ATP absolute amount. Therefore, P_i and ATP activity at low concentrations inhibits AMP-D activity, preventing excessive decrease in TAN [63]. The activity of the mechanism stabilizing AEC is especially important when the cell demand for energy is higher than the supply [55]. It has been shown that the activity stabilizing the AEC value occurs at the expense of a decrease in total adenine nucleotide concentration and total ATP concentration. Experimental studies have shown that AEC decrease in erythrocyte is accompanied by a decrease in TAN [55, 58, 74].

Nucleoside catabolism. The short plasma half-life of Ado is a consequence of its rapid catabolism into Ino and Hyp in endothelial cells and erythrocytes, which possess high activities of ADA and PNP.

A key role in the breakdown of Ado is played by the nucleoside transporter, which facilitates the uptake of Ado. It is located on endothelial cells and on erythrocytes [32-34]. Extracellular Ado can be either phosphorylated or transported into the erythrocytes where a subsequent intracellular deamination will take place. The kinetic parameters show that Ado will be preferentially phosphorylated at physiological concentrations. However, at higher extracellular concentrations of Ado, AdoK will be inhibited and practically all Ado will enter erythrocytes and will be deaminated. Of the two ADA isoenzymes, ADA_1 occurs intracellularly (e.g., in erythrocytes). The K_m for ADA_1 is $5.2 \cdot 10^{-5}$ M, optimum pH between 7.0-7.7. Due to its activity these cells take up Ado and 2′-deoxyadenosine efficiently, deaminating substrates to Ino and 2′-deoxyinosine, respectively, releasing ammonia. In this way, they protect cells from an excessive exogenous 2′-deoxyadenosine [32]. The catalytically active protein of the enzyme is a metalloenzyme (1 mol Zn^{2+} per mol protein [75]). ADA_1 can function in a monomeric form with molecular weight 30-47 kD and a dimeric form of two ADA_1 molecules connected by glycoprotein forming a compound with molecular weight 230-440 kD [76].

A genetic defect of the ADA gene has been correlated with the lack of ADA enzymes giving rise to severe combined immunodeficiency disorder, SCID [77, 78], where both T- and B-lymphocytes are unable to proliferate and mount antigenic challenges. Immunodeficiency results from the toxic effects of ADA substrates, including apoptosis induced by deoxyadenosine triphosphate pool expansion (deoxyadenosine is phosphorylated to dATP) [79] and inhibition of transmethylation reactions caused by impaired catabolism of S-adenosylhomocysteine [77].

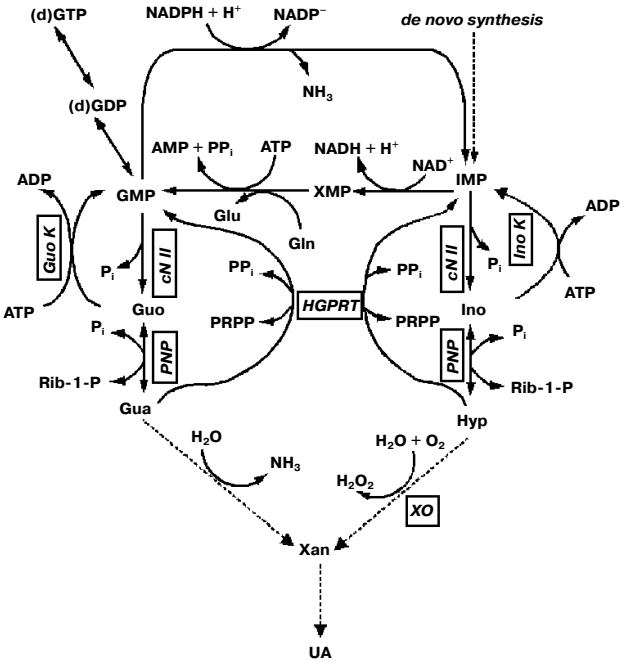

Fig. 3. Overview of guanine nucleotides metabolism in human erythrocytes (solid lines) and out of them (dotted lines). Based on [14, 20].

In degradative pathways, Ado is deaminated by ADA to give Ino. Ino, a naturally occurring purine, was long considered to be an inactive metabolite of Ado. However, recently Ino has been shown to be an immunomodulator and anti-inflammatory agent [80].

Ino and Guo glycoside bonds can be phosphorylated in a reaction catalyzed by PNP. Ino is then converted to Hyp and Rib-1-P, and Guo into Gua and Rib-1-P. The K_m values for Ino and phosphate were found to be 60 and 667 μM, respectively [81]. Studies on PNP activity in human erythrocytes showed cases of a deficit of that enzyme leading to an increase in PRPP, Ino, and NAD^+. Moreover, they showed a relation between the PNP activity and 2,3-BPG concentration in red blood cells. With a PNP activity increase (increase in Hyp and Rib-1-P concentrations), 2,3-BPG increases.

The PNP activity of erythrocytes is about four times higher than that of ADA, so Hyp will be a more prominent product than Ino [82]. Hyp reaches the extracellular space by diffusion and is not transported by the nucleoside transporter. Because erythrocytes do not show xan-

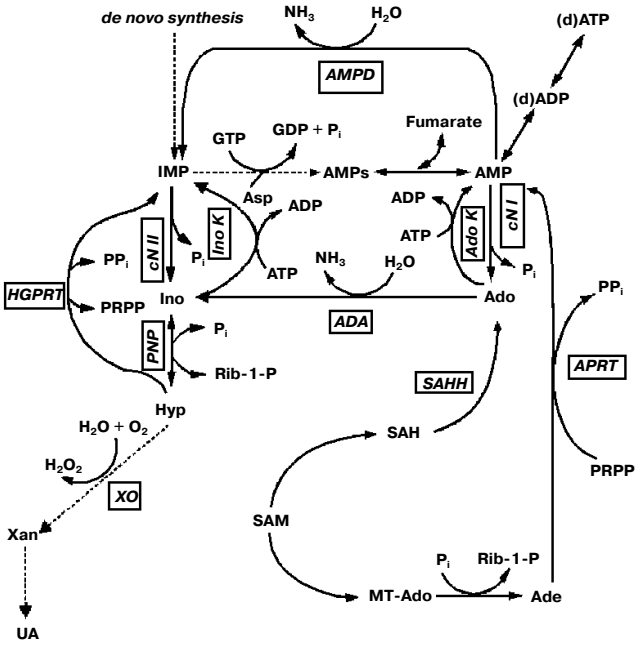

Fig. 2. Overview of adenine nucleotide metabolism in human erythrocytes (solid lines) and out of them (dotted lines). Based on [14, 20].

thine oxidase activity (EC 1.15.1.1) (Hyp → Xan) or guanase activity (EC 3.5.4.3) (Gua → Xan), Hyp and Gua are the final products of purine nucleotide metabolism. These bases can be included in the conversion pathways as substrates for HGPRT (reutilization reactions). Rib-1-P, after isomerization to Rib-5-P, can become a substrate in PRPP synthesis, or can be included into conversions in the pentose phosphate pathway, supplying indirect products for glycolysis (glyceraldehyde-3-phosphate and fructose-6-phosphate).

Adenine nucleotide metabolism pathways are presented in Fig. 2. Guanine nucleotide metabolism pathways are presented in Fig. 3.

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