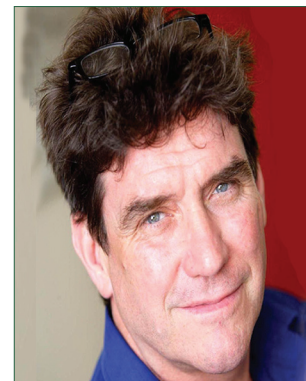


ATP, an Extracellular Signaling Molecule in Red Blood Cells: A Messenger for Malaria?

Ghania Ramdani, Gordon Langsley

Adenosine 5' triphosphate (ATP), discovered in 1929 by Karl Lohmannest, is described as an essential energy source for cells. In the biochemistry of all living organisms, ATP hydrolysis provides the energy required for the chemical reactions of metabolism. It is the precursor of a number of essential enzyme cofactors, such as nicotinamide adenine dinucleotide (NAD⁺) and coenzyme A [NAD⁺, flavin adenine dinucleotide (FAD), and is ATP coenzyme A are all formed from ATP] and is the source of the phosphoryl group in most kinase-mediated phosphorylation reactions. Another essential, but less known function is that ATP plays a very important role as an extracellular signaling molecule, allowing cells and tissues to communicate. ATP is converted into cAMP, a major second messenger involved in many cellular processes, by adenylyl cyclase, a membrane-associated enzyme. In this review, we describe the role of ATP as a beneficial extracellular molecule released by healthy red blood cells (RBCs) in response to hypoxia to mediate a vasodilator signal, by oxidatively stressed RBCs, and by *Plasmodium falciparum*-infected RBCs (iRBCs), and its similarity with released ATP that by the combined action of the ectonucleotidases CD39 and CD73 is converted to adenosine that mediates sickling in sickle cell disease (SCD). (*Biomed J* 2014;37:284-292)



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Key words: adenosine, adenosine 5' triphosphate, CD39, CD73, malaria, *Plasmodium*, red blood cell

Adenosine 5' triphosphate released from RBCs

Adenosine 5' triphosphate as a vasodilator signal

RBCs mainly facilitate oxygen transport in the body. However, this is not the only function of these abundant enucleated cells, whose cytoplasm is rich in hemoglobin and poor in organelles; they are also redoubtable sensors of oxygen allowing local vascular control.^[1]

In 1992, Bergfeld and Forrester reported that human RBCs release adenosine 5' triphosphate (ATP) in response to the combined effects of hypoxia and hypercapnia (abnormally elevated levels of blood carbon dioxide).^[2] More recent studies show that exposure to reduced oxygen tension (~35 mm Hg) is sufficient to stimulate ATP release from the RBCs of hamsters,^[3] rabbits,^[4] rats,^[5] and humans.^[6] Indeed, ATP is released from RBCs in blood samples subjected to hypoxia.^[7]

In addition to RBCs, it is known that endothelial cells also release ATP when they are exposed to a high shear stress.^[8]

Decrease in oxygen pressure (pO₂: partial pressure of oxygen, that is the amount of oxygen in the blood, normally referred to as dissolved oxygen in blood) is caused by an increase of cellular oxygen consumption or a decrease in oxygen supply or systemic hypoxia, all of which can trigger RBC vasodilator signals to compensate for the mismatch between the supply and demand of oxygen.^[7] Lack of O₂ triggers RBC deoxygenation and induces a cascade of events including nitric oxide (NO) production by deoxyhemoglobin, mediated by nitrite reduction, i.e. the reaction between nitrite and deoxygenated hemoglobin provides a mechanism by which NO is synthesized *in vivo*.^[9] This leads to the possible formation of S-nitrosohemoglobin (SNO-Hb).^[7] SNO-Hb is the bioactive form of NO in blood and plays an important role in regulating the blood flow.^[10] ATP is

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released and contributes to the regulation of blood flow and O₂ delivery to tissues to satisfy the metabolic needs of cells.^[5,10] ATP is released upon activation of a signaling pathway that is triggered when RBCs enter an area in which the oxygen demands of tissues exceed supply. Thus ATP acts in a paracrine manner to increase vascular caliber leading to an increase in oxygen supply.

How RBCs release ATP

ATP efflux is linearly related to hemoglobin saturation with oxygen;^[5] the more saturated the hemoglobin is, the less ATP is released, suggesting that the conformational change in hemoglobin and its desaturation when the oxygen levels drop causes ATP release. Thus, physiological and pharmacological stimuli induce ATP release from RBCs. Physiologically, RBCs release ATP in response to mechanical deformation^[11] that occurs as RBCs flow throughout the body, from arteries to veins and capillaries, as well as passing through the slits in the spleen,^[12] and in response to reduced oxygen tension.^[2] Further, RBCs can release ATP in a dose-dependent manner following activation by β-adrenergic receptors present at the RBCs' surface or via prostacyclin receptors.^[13]

In recent years, Sprague's group described a pathway for ATP release from RBCs. This pathway includes the heterotrimeric G proteins Gs and Gi,^[13-15] adenylyl cyclase (AC),^[16] protein kinase A,^[16] and cystic fibrosis transmembrane conductance regulator (CFTR)^[17] [Figure 1]. Studies have shown that activation of Gs-coupled receptors, β-adrenergic and prostacyclin (also called prostaglandin I₂ or PGI₂) receptors on the RBCs' surface leads to increased cAMP levels and ATP release.^[11,13] Gs is the AC-activating pro-

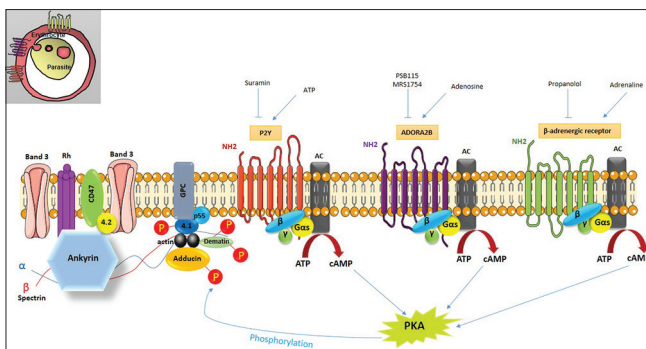


Figure 1: *P. falciparum*-infected RBCs secrete metabolite(s) that contribute to parasite infectivity and changes in RBC deformability. The metabolite can be ATP, which signals via P2Y receptors expressed at RBCs' surface, or an ATP-derived metabolite such as adenosine that signals via ADORA2B receptors. β-adrenergic receptors are also implicated in parasite infectivity. All these receptors are coupled to G proteins and are responsible for cAMP production via activating adenylyl cyclases, which convert ATP to cAMP. cAMP activates PKA that phosphorylates RBC cytoskeletal proteins resulting in a change plasma membrane properties.

tein, while Gi is traditionally described as inhibiting the activity of certain isoforms of AC,^[18-22] but it also activates isoforms II, IV, and VII of AC that are expressed on RBCs' surface.^[19,22,23] For example, direct activation of Gi induces an increase in cAMP and ATP release by rabbit and human RBCs.^[13,14] In addition, direct activation of Gi with wasp venom extract (mastoparan) stimulates cAMP levels and, thus, ATP release from rabbit RBCs.^[14,24]

How ATP triggers vasodilation

The balance between the supply and demand of O₂ in metabolically active tissues is a fundamental physiological process, and RBCs play a key role in tissue oxygen supply by the controlled release of ATP in areas where O₂ needs increase, by balancing microvascular O₂ supply with demand in local tissues.

Indeed, RBCs contain millimolar (mM) amounts of ATP produced mainly by glycolysis.^[1] This ATP is released in response to hypoxia to trigger vasodilator signals.^[2] When ATP is released into the vascular lumen, it can interact with P2-type purinergic receptors present on the endothelium, which elicits an endothelium-dependent vasoactive response. There are two different classes of P2 receptors, P2X and P2Y, and in mammals, there is a family of seven P2X (P2X1-7) receptors and eight P2Y (P2Y1, P2Y2, P2Y4, P2Y6, P2Y11-14) receptors.^[25,26]

Activation of purinergic receptors stimulates the synthesis and release of several vasodilators such as NO and arachidonic acid from the endothelial cells. In cerebral circulation, ATP and its related degradation products, including adenosine, are vasoactive mediators.^[27,28] For example, Forrester *et al.* noted that interluminal ATP was a potent vasodilator in baboon cerebral circulation.^[29] It is likely that ATP is the primary agonist and at equimolar concentrations, ADP is slightly less efficient than ATP in causing local dilatory responses and conductive responses.^[28]

How ATP release by RBCs is regulated

The release of ATP by RBCs is controlled by a negative feedback mechanism. In an intact microcirculation, when RBCs are in contact with an NO-rich environment, NO may act as a negative feedback to inhibit release of more ATP. Incubation of human^[11] and rabbit RBCs^[24,30] with an NO donor such as spermine NONOate (used to produce a controlled release of NO in solution) prevents ATP release in response to reduced O₂ tension. In addition to stimulating soluble guanylate cyclase, NO can also target either the adenylyl cyclase itself to inhibit enzyme activation^[31,32] or Gi subtypes of G proteins,^[33] both components of signal transduction pathways used for ATP release by RBCs. Thus, NO is capable of inhibiting ATP release in response to exposure to reduced oxygen tension through Gi inactivation.

Dynamics of ATP release from RBCs

The function of extracellular ATP is very well described for RBCs that regulate blood pressure through ATP release, which acts as signaling molecule in a vasodilatory response. We have described above that RBCs can release ATP following mechanical deformation. We know now that retraction of the actin-spectrin cytoskeleton network triggers mechanosensitive ATP release and shear-dependent membrane viscosity controls the release rate.^[34]

RBC deformation is an important trigger of ATP release. Adding chemicals to stiffen RBC cytoskeleton (e.g. diamide) decreases the amount of ATP released, suggesting that cell deformation is a necessary trigger element.^[35] RBCs that play the role of oxygen carriers are susceptible to deform. To avoid abusive ATP release, the mechano-dependent ATP release mechanism should be highly regulated, and these mechanisms of RBCs mechanosensing are still controversial. Regulation occurs between the initiation of the increase in shear stress and ATP release. Even if RBCs deform significantly by short-term constrictions, no measurable ATP is released,^[34] and the amount of released ATP is dependent on the duration of the shear stress. Using microfluidic^[35,36] and microbore tubing channels,^[37,38] it was shown that the amount of ATP released is also controlled by shear stress amplitude. In summary, a large deformation of RBCs is, in itself, insufficient to trigger a significant amount of ATP release. The required condition is that RBC deformations must occur in a well-defined time scale and should be of relatively long duration to induce significant ATP release.

ATP release from oxidative stressed RBCs

Oxidative stress reflects an imbalance between production of reactive oxygen species (ROS) and the ability of biological systems to detoxify or to repair the damage caused by these intermediates. Oxidative stress is associated with increased production of oxidized species, or a significant decrease in the effectiveness of the antioxidant defense systems such as glutathione.^[39] Disruption of normal cell redox state can cause toxic effects through production of peroxides and free radicals that cause damage to all cellular components, including proteins, lipids, and DNA. In addition, some ROS act as messengers in cellular redox signaling. Thus, oxidative stress can cause disruptions in normal cell signaling. In humans, oxidative stress may be involved in cancers, Parkinson's disease, Alzheimer's disease, and when it affects RBCs it can be responsible for sickle cell disease (SCD).^[40]

ATP output coming from RBC deformation is clearly linked to the antioxidant defense status of cells. First, intracellular ATP levels are decreased by hydrogen peroxide (H₂O₂).^[41] In addition to oxidative stress-caused damage and loss of glutathione, ATP levels drop due to inhibition of glyceraldehyde-3-phosphate dehydrogenase.^[41] This ATP

depletion prevents controlled cell death by apoptosis and causes the cell to simply fall apart.^[41,42] So, cellular ATP is an important determinant of cell death; cells remain alive when a certain level of ATP is maintained, but when ATP falls below this level, apoptosis is activated and a severe drop in cellular ATP causes cell necrosis.^[41]

In addition to the decrease of intracellular ATP levels, oxidative stress is directly correlated to ATP release. Lower levels of oxidative stress generated by treatment with dehydroepiandrosterone (DHEA, an antioxidant) lead to a decrease in nicotinamide adenine dinucleotide phosphate (NADPH) levels and decreased ATP release.^[43] NADPH acts as a glutathione reductase (GSHred) substrate in order to maintain antioxidant GSH in its reduced form, and decreased NADPH levels directly correlate with GSH production. In its reduced form, this small tripeptide is the main non-enzymatic antioxidant in RBCs.^[44] GSH is oxidized to its dimeric form GSSG to protect the cells against oxidative attack. GSH is generated at the expense of NADPH. To generate NADPH, unlike other cells that have several methods to produce it, RBCs rely solely on glucose-6-phosphate dehydrogenase (G6PD), a key enzyme of pentose phosphate pathway that oxidizes NADPH and converts glucose-6-phosphate (G6P) to 6-phosphogluconate (6GP). G6PD also serves to determine RBC age^[45,46] and, in addition to GSH, is decisive in the antioxidant status of RBCs.^[47] G6PD-deficient individuals are subjected to high oxidative stress due to a lack in NADPH production, and thus have a weakened antioxidant defense system.^[43] For example, G6PD activity is low in the RBCs of diabetic patients compared to healthy individuals,^[48] leading to decreased NADPH levels in these patients, making their RBCs less deformable than healthy subjects.^[49] In addition, mutations in G6PD afford protection against malaria, and for this reason, hereditary G6PD deficiency is frequent in malaria-endemic areas.^[50] The protection afforded by mutant G6PD can be due to reduced parasite invasion and/or poor parasite replication within G6PD RBCs.^[51] So, there is a direct relationship between NADPH concentrations and ATP release resulting from RBC deformation. Correlating with a decrease in NADPH concentrations, there is a decrease in ATP release stemming from RBC deformation. RBCs obtained from patients with type 2 diabetes show the same pattern,^[43] confirming that by affecting deformability of RBCs, oxidative stress will lead to a decrease in the level of ATP release.

ATP release following Plasmodium falciparum infection

Role of new permeation pathways – NPP

The clinical signs of malaria are the result of the invasion and development of *P. falciparum* within RBCs. Infection is accompanied by major changes in the host red

blood cell^[52] that affect lipid composition, deformability, and also its osmotic and transport properties. This results in sequestration of *P. falciparum*-infected RBCs (iRBCs), and rigidity of the host RBC leads to cerebral malaria, the most severe complication of malaria.^[52,53]

During its intracellular development, *P. falciparum* makes the RBC appear older; iRBCs become less dense and less deformable. iRBCs lose their typical biconcave shape, take on a more spherical shape, become covered with dense microscopic projections (knobs), and expose more phosphatidylserines (PS) at their surface.^[54] iRBCs become osmotically fragile and more permeable to a wide variety of molecules through the appearance of NPP.^[54] iRBCs lose several cellular components such as sialic acid, reduced glutathione, and especially ATP [Figure 1]. The decreased levels of antioxidants and ATP have several consequences, such as clustering of the band-3 anion transporter, as well as exposure of neo-antigens that contribute to antigenic variation and increased iRBC adhesion via PS and band-3 that allows the parasitized RBC to escape destruction by spleen.^[54]

Of all the leached molecules, we will focus on ATP. Both iRBCs and, as we described above, damaged cells or cells responding to a hypoxic signal can release ATP, and its concentration is therefore increased in the culture medium.^[55] The released ATP appears to play a key role at several levels. It plays a role in infection, as parasites are unable to infect RBCs that have been depleted of their ATP.^[55] ATP also acts as an important extracellular signal to modulate the interaction between RBCs and *Plasmodium*, and it has been also reported to increase the intracellular Ca²⁺ levels within *P. falciparum*, *P. berghei*, and *P. yoelii* parasites, suggesting the potential presence of purinergic receptors on the surface of the parasite.^[56] ATP is also involved in the growth of *P. falciparum* and *P. berghei*, and treatment with suramin, a specific purinergic receptor antagonist, reduces parasite growth *in vitro* and *in vivo*, respectively.^[57] Finally, ATP is involved in endothelial cell inflammation induced following infection with *Plasmodium*.^[58] Like oxidative stress, RBC infection by *P. falciparum* induces increased permeability of anions and organic osmolytes. Induction of osmolyte permeability in iRBCs involves autocrine purino-receptor signaling, as extracellular ATP does not induce permeability in uninfected or unoxidized RBCs.^[57]

RBCs express P2Y1 receptors, and RBCs of P2Y1-deficient mice show a delayed onset of osmolyte permeability following infection by *P. berghei* or following oxidation, when compared to wild-type RBCs.^[59] As stated, iRBCs have NPP,^[59,60] and NPP first appear between 10 and 20 h and are maximal around 40 h post-invasion.^[57] At the trophozoite stage (circa 20 h post-infection), the iRBC membrane becomes permeable to substances such as sorbitol and glucose,^[61] and a wide variety of solutes including anions,

carbohydrates, amino acids, nucleosides, and organic or inorganic cations.^[59]

NPP provide the parasite with the nutrients required for rapid growth and allows the parasite to eliminate metabolic waste products. Pharmacological inhibition of NPP leads to death of parasites cultured *in vitro*. Thus, the parasite must obtain purine bases and pantothenic acid from the extracellular medium,^[62] and release amino acids stemming essentially from hemoglobin degradation^[63] and excrete lactic acid produced by parasite glycolysis, whose activity is 100 times more intensive than RBC glycolysis.^[64] NPP are believed to consist of two types of channel: The first is present in small numbers and is load- and size-selective, while the other one is about 100 times more abundant and is anion selective, but does not admit non-electrolytes except perhaps nucleosides.^[60]

Role of cAMP/protein kinase A pathway

ATP plays an important role in *P. falciparum* infection. RBCs release ATP in response to parasitic infection.^[65] In a model of mouse malaria using *P. chabaudi*-infected RBCs, the released ATP acts via an autocrine loop to stimulate parasite growth, since it can bind to P2Y receptors on the surface of the parasite to maintain the infection process^[66] [Figure 1]. Then the released ATP has a deleterious effect on the host, as it augments infection via its binding to P2Y expressed either on the RBCs or on the surface of the parasite.^[66]

We have described above the involvement of NPP in the release of ATP by iRBCs. Also, the contribution of hypoxia is not excluded, since iRBC adhesion to vascular endothelium creates hypoxia, which can induce ATP release by RBCs. Another hypothesis is that oxidative stress induced by *P. falciparum* infection can promote ATP release from RBCs.

In the next section, we will describe the involvement of a cAMP-dependent protein kinase A (PKA) pathway in ATP release from RBCs, which therefore implies the involvement of Gs signaling to activate AC, a key enzyme in cAMP production. Indeed, it has been shown that receptor-mediated activation of the heterotrimeric G protein Gs results in ATP release from RBCs.^[13]

Role of CFTR

CFTR is an ion channel of ATP-binding cassette (ABC) transporter class, encoded by the *CFTR* gene. In epithelial cells, it carries chloride^[67] and thiocyanate^[68] ions across the plasma membrane. Mutations in the *CFTR* gene affect the chloride ion channel transport in cell membranes and leads to cystic fibrosis.

The involvement of CFTR in ATP release from RBCs has already been demonstrated. Indeed, activation of CFTR is involved in ATP release induced by mechanical deformation of rabbit and human RBCs.^[13] A cAMP-dependent

PKA pathway plays an essential role in CFTR activation, as increases in cAMP generated by AC,^[69] whose activity is regulated by heterotrimeric G proteins,^[21] induces channel activation via cAMP-dependent PKA-mediated phosphorylation of the R domain of CFTR.^[69]

Role of β -adrenergic receptors

β -adrenergic receptor and adenosine receptor agonists (isoproterenol and 5'-N-ethylcarboxamidoadenosine, respectively) stimulated parasite entry into RBCs, while the respective antagonists (propranolol and 8-(*p*-sulfophenyl) theophylline) inhibited entry.^[70] Heterotrimeric G proteins are involved in the signal transduction pathway that leads to ATP release from the RBCs, as it has been shown that the β -adrenergic receptors coupled to G proteins play an essential role in the release process.^[13] Indeed, incubation of RBCs with epinephrine or iloprost, which activate stimulatory Gs subunits via a β -adrenergic-mediated mechanism, stimulates ATP release, whereas pre-treatment with propranolol, a receptor antagonist, inhibits ATP release.^[13] The ATP released by iRBCs that enhances parasite infectivity may be derived from the activation of the β -adrenergic receptors, as it has been demonstrated that signaling via the β 2-adrenergic receptor provokes G protein-regulated entry of the human malaria parasite.^[71] Also, agonists that stimulate cAMP production led to an increase in infection that could be blocked by specific receptor antagonists.^[13] Moreover, peptides that inhibit G protein function reduced parasite growth *in vitro*,^[71] and β -antagonists also reduced *P. berghei* growth *in vivo*. Thus, signaling via the RBC β 2-adrenergic receptor and G α s may regulate infection across different plasmodial species. As RBC G protein signaling also contributes to parasite proliferation, it may present a novel antimalarial target.^[70]

Modulation of membrane properties by PKA

It has been shown previously that mechanical deformation of RBCs could stimulate the activity of the heterotrimeric G protein Gs, initiating a signal transduction pathway that mediates ATP release from RBCs.^[13] Also, Zhang *et al.* have shown that in sickle cell RBCs, adenosine signals through ADORA2B to increase the levels of cAMP and activate PKA.^[72] Activation of the PKA-signaling pathway leads to induction of extracellular signal-regulated kinase (ERK) 1/2 and their combined phosphorylation of adducins, dematins, and the intercellular adhesion molecule 4 (ICAM-4)^[73] [Figure 1]. Indeed, in mature RBCs, phosphorylation plays an important role in the regulation of membrane functions in response to stress and other physiological stimuli. Spectrin, actin, band 4.1, adducins, and dematin can be all phosphorylated.^[74] Phosphorylation of band 4.1, combined with PKA-mediated phosphorylation of S726 and S713 in adducins results in a marked reduction in membrane stabil-

ity.^[75] Both observations demonstrate, albeit by two different and distinct mechanisms, involvement of PKA in the process of ATP release by RBCs. Thus, activated PKA plays a key role in RBC plasma membrane stability via phosphorylation of the erythrocyte cytoskeleton.

We have discussed above that RBC deformability increases ATP release, and PKA phosphorylation of cytoskeletal proteins increases erythrocyte plasma membrane deformability, which leads to ATP release. Post-transcriptional modifications of RBC cytoskeletal proteins, such as phosphorylation, play a key role in regulating membrane functions, due to the lack of regulation of gene transcription in enucleate red blood cells.^[76]

The RBC membrane skeleton is classically described as a structure formed by junctional complexes linked to flexible spectrin molecules. Spectrins, actin, protein 4.1, adducin, dematin, tropomyosin, and tropomodulin represent the most abundant constituents of the cytoskeleton and can all be phosphorylated by different kinases including PKA, protein kinase C (PKC), and casein kinases. PKA and PKC can phosphorylate adducins on Ser726 and Ser713, but only the phosphorylation by PKA reduces adducin's affinity for spectrin-F-actin complexes.^[76] Phosphorylation of band 4.1 also results in a marked decrease in membrane mechanical stability as a result of its decreased ability to bind both actin and spectrin.^[75]

RBCs infected with *P. falciparum* release ATP. We have discussed above that activation of β -adrenergic receptors appears to be involved in this phenomenon, and they likely activate cAMP-dependent PKA that leads to phosphorylation of erythrocyte cytoskeletal proteins. Indeed, it has been demonstrated that *P. falciparum* infection induces post-transcriptional modification of RBC cytoskeleton^[77] and the RBC plasma membrane is extensively modified during the intracellular development of *P. falciparum*.^[78] RBC serine/threonine kinases such as casein kinases, PKC, and PKA have been reported to be activated in parasitized erythrocytes.^[76] Also, activation of endogenous PKA by cAMP decreases the mechanical stability of the membrane. This effect is mediated primarily by phosphorylation of dematin, which facilitates interaction between spectrin and F-actin, and phosphorylation of dematin by PKA markedly diminishes this affinity.^[79] These findings imply that dematin also contributes to the maintenance of mechanical stability of RBC plasma membrane by facilitating spectrin-actin interactions and that phosphorylation of dematin by PKA can modulate these effects.^[79]

ATP release in sickle cell disease

SCD is a hereditary disease characterized by a valine for glutamic acid substitution at position six in β -globin, which creates a hydrophobic patch in HbS tetramer, resulting

in a tendency to polymerize and form long polymers that distort the shape of RBCs.^[80] Sickle RBCs are abnormally rigid, have reduced deformability, and are easily destroyed. The lifespan of RBCs of SCD patients is shortened, leading to a chronic extravascular and intravascular hemolysis and chronic hemolytic anemia. Furthermore, SCD RBCs have damaged surfaces that increase their adhesion to the vascular endothelium, a process that contributes to acute vaso-occlusion. Adenosine is the nucleotide that is highly elevated in the plasma of human patients and SCD transgenic mice.^[72] SCD patients are exposed to hypoxia, and hypoxia-induced increases in adenosine contribute to fatal complications and multiple sickling, tissue damage, pulmonary dysfunction, and priapism.^[81] Adenosine, a derivative of ATP, is not only readily released by SCD erythrocytes, but also by RBCs experiencing hypoxia, or following oxidative stress or infection by *Plasmodium*, as discussed below.

The ATP concentration inside RBCs is high (~5 mM) and a rise in plasma adenosine in response to hypoxia is likely due to ATP release and its conversion to adenosine. For example, ATP concentrations in the plasma of SCD transgenic mice are significantly higher compared to those observed in wild-type mice plasma.^[72] Ectonucleotidases such as CD39 on the RBC surface hydrolyze ATP and ADP to AMP.^[81] Then, AMP undergoes dephosphorylation by the ectonucleotidase (CD73) that is also present on RBC surface to produce adenosine [Figure 2]. Adenosine contributes to the pathogenesis of SCD, and in fact, it increases directly 2,3-biphosphoglycerate (2,3-BPG) levels in the RBCs,

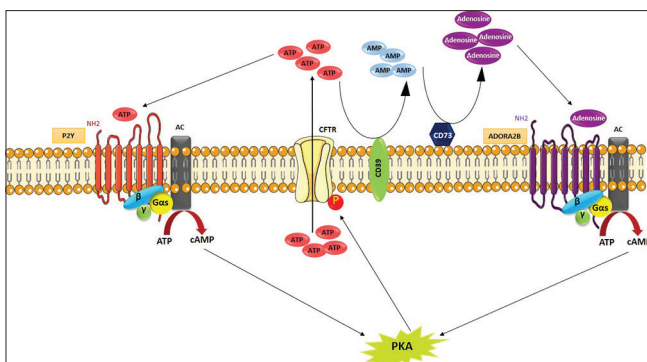


Figure 2: RBCs release ATP following *P. falciparum* infection. CFTR activation by PKA-mediated phosphorylation contributes to ATP release. Released ATP can signal directly via binding to RBC P2Y receptors or following conversion to AMP and adenosine via the successive action of two ectonucleotidases, CD39 and CD73. ATP-derived adenosine can then signal via the ADORA2B receptor. Both ADORA2B and P2Y receptors are coupled to G proteins, and their activation ultimately leads to cAMP production and PKA activation that will play different roles in maintaining the infection process, such as CFTR phosphorylation, to sustain an ATP-release loop. Several additional players can act to regulate these pathways, such as for example, phosphodiesterases that function to degrade cAMP, thus ending the signal.

which contribute to O₂ being released from hemoglobin to induce sickling.^[81]

It has been shown that 2,3-BPG is an RBC-specific metabolite that decreases the binding affinity of oxygen for hemoglobin, and its induction is mediated by the A2B receptor for adenosine (A2BR).^[81] Thus, excessive adenosine signaling by A2BR has a pathological role in SCD.^[72,81] Extracellular adenosine affects the physiological and pathological processes on target cells by signaling through different receptors including ADORA1, ADORA2A, ADORA2B, and ADORA3. All these receptors are G protein coupled and each has a distinct affinity for adenosine and a distinct cellular and tissue distribution. A2A and A2B couple to the stimulatory G protein, G_s, to activate AC that results in increased cAMP levels.^[82]

Conclusions

ATP is a nucleotide principally known as a cellular energy source, but it can also act as a potent extracellular signaling molecule. ATP can be rapidly converted to adenosine by the combined actions of the ecto-5'-nucleotidases CD39 and CD73 expressed on the RBC surface [Figure 2]. In this review, we have summarized the role of ATP as an extracellular signaling molecule in a particular cell model, RBCs, and in the context of erythrocyte infection by malaria parasites.

Intracellular ATP concentration of RBCs can reach amounts of up to 5mM. All this ATP present in RBCs allows them to play a role over and above just oxygen transport in blood, i.e. also as an oxygen sensor to mediate local vascular control. Indeed, following hypoxia, RBC hemoglobin undergoes deoxygenation, which initiates a cascade of events including ATP release. ATP binds to purinergic P2 subtype receptors present on the vascular endothelium and stimulates NO synthesis and its release as a potent vasodilator.

Adenosine resulting from ATP conversion is also a vasoactive mediator. It plays a crucial role in SCD; but unlike its beneficial role in healthy individuals, it plays a detrimental role in SCD patients. Increased amounts of ATP in the circulation derived from chronic sickle red cell hemolysis and tissue damage from vaso-occlusion is rapidly converted to adenosine by the combined actions of CD39 and CD73 [Figure 2].

ATP is also released by iRBCs, and this can occur in different ways. First and probably in response to hypoxia, sequestration of iRBCs causes hypoxia by obstruction of postcapillary venules.^[83] Also, iRBCs become osmotically fragile and, due to NPP, become more permeable to a wide variety of molecules. As stated above, intracellular ATP levels are altered by H₂O₂ and oxidative stress is directly correlated to ATP release. This may be another mechanism to explain

ATP release by iRBCs. Especially, as infection with *Plasmodium* makes iRBCs appear older, less dense, less deformable, and more oxidatively stressed.^[84] The decreased levels of antioxidants and ATP have several consequences in malaria, such as clustering of the band-3 anion transporter, as well as exposure of neo-antigens that contribute to antigenic variation and increased iRBC adhesion, which allows them to escape destruction by spleen. β -adrenergic receptors and/or prostacyclin receptors are also implicated in ATP release by iRBCs, as they stimulate parasite entry into RBCs and augment intraerythrocytic growth. Adenosine receptors can also be implicated in addition to β -adrenergic receptors, suggesting that ATP is not the only molecule secreted upon *P. falciparum* infection of erythrocytes that could contribute to parasite growth.

iRBCs, damaged cells, or cells responding to a hypoxic signal can release ATP, which is an important determinant for the increased RBC invasiveness of *P. falciparum*, but ATP is probably not the only molecule involved in *Plasmodium* infectivity. Increased adenosine release upon *P. falciparum* infection may also signal via the adenosine receptor expressed on the surface of iRBCs to maintain the infection process. An involvement of β -adrenergic receptors is not to be excluded, but parasite-derived agonists responsible for their activation have not been described. However, the possibility that RBCs may function as a reservoir for nor-adrenaline and adrenaline had been suggested. So, adrenaline might be released in a controlled manner by iRBCs or released into plasma upon iRBC lysis.^[85]

Summary

ATP release from RBCs is increased upon *Plasmodium* infection, where it contributes to RBC invasion via signaling through P2Y receptors. As RBC ATP levels are high, when the RBC plasma membrane is damaged, lysed, or traversed by *Plasmodium* parasites during invasion, extracellular ATP levels can increase substantially. On the RBC surface, CD39 can hydrolyze ATP and ADP to AMP and CD73 can dephosphorylate AMP to adenosine. Conditioned media from cultured iRBCs, when added to uninfected RBCs, alter their plasma membrane deformability implying that iRBCs secrete into the media a metabolite(s) that has both autocrine and *in trans* effects on RBC plasma membrane deformability.^[86] Within a few hours of incubation of normal RBCs in spent medium from iRBC cultures, a change in the plasma membrane properties of uninfected RBCs occurred, as within 24 h, they showed equivalent deformability changes to iRBCs.^[86] Clearly, iRBCs secrete an exo-chemical compound responsible for the change in deformability of non-infected RBCs and the “bystander effect” might be due to ATP release, or its metabolites adenosine and inosine. These secreted metabolites can act *in cis* on iRBCs and *in trans* on surrounding healthy RBCs.

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