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Purinoceptor signaling in malaria-infected erythrocytes

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Abstract

Human erythrocytes are endowed with ATP release pathways and metabotropic and ionotropic purinoceptors. This review summarizes the pivotal function of purinergic signaling in erythrocyte control of vascular tone, in hemolytic septicemia, and in malaria. In malaria, the intraerythrocytic parasite exploits the purinergic signaling of its host to adapt the erythrocyte to its requirements.

Keywords: Malaria; Plasmodium falciparum; New permeability pathways; Purinergic signaling; ATP release; Patch-clamp recording

1. Introduction

Mature human erythrocytes travel about 100 miles and circulate more than 100.000 times through the body during their normal life span of 120 ± 4 days. The most obvious task thereby is the transport of blood oxygen and carbon dioxide as well as buffering of the pH in the blood. These functions depend on carboanhydrase, hemoglobin and band 3 anion exchanger. The latter two are the most abundant proteins in the erythrocyte cytosol and membrane, respectively. Because of this high abundance of hemoglobin which accounts for 98% of the cytosol protein content and because of the substantial absence of intracellular organelles, mature human erythrocytes are commonly simplified to hemoglobin-containing sacks.

However, increasing numbers of proteins are identified in mature human erythrocytes. Among those are proteins that build up signaling cascades. Outside-in signaling via membrane receptors such as purinergic receptors [1,2], as well as inside-out signaling via release of, e.g., ATP has been reported [3]. Moreover, intracellular signaling molecules such as protein kinases [4], have been unequivocally demonstrated to be functional suggesting that mature human erythrocytes are endowed with complex signaling similar to nucleated cells. Furthermore, mature human erythrocytes functionally express an unexpected diversity of ion channels that endows these small enucleated cells with a toolkit for electrosignaling. This toolkit enables erythrocytes to quickly respond to internal or external stimuli with changes in cytosolic free Ca^{2+}, de- or hyperpolarization of the membrane, cell swelling or shrinkage, and release or uptake of channel-permeable solutes. Moreover, the ion channels are integral modules of complex programs such as oxygen-regulated ATP release [3], or stress-induced programmed erythrocyte death [5].

Being largely silent under resting conditions, erythrocyte channels may build up membrane conductances in the nS range upon various signals. A strong stimulator of erythrocyte ion channel activity is the intraerythrocytic amplification of the malaria parasite *Plasmodium falciparum* [6–18]. The altered membrane permeabilities of parasitized erythrocytes were first described almost three decades ago [19]. Meanwhile, these so called *New Permeability Pathways* have been characterized by tracer flux, isosmotic hemolysis experiments and patch-clamp recording as organic osmolyte and anion-selective channels. They play a pivotal role for parasite development by supplying the parasite with nutrients, disposing of metabolic waste and organic osmolytes, adapting the host's electrolyte composition to the parasite's needs, and lowering the colloid osmotic pressure of the host erythrocyte [19,20]. The latter function has been postulated to avoid premature hemolysis of the host cell [21]. The number of

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pathways (and their nature - erythrocyte- or parasite-derived) that form the altered permeabilities of infected erythrocytes is highly controversially debated [22–24]. Parasite-encoded proteins have been demonstrated to contribute to the *New Permeability Pathways* [25]. On the other hand, overwhelming evidence indicates that intrinsic erythrocyte channels are generating a principal fraction of the *New Permeability Pathways* [6,13,14,16,17,24].

Invasion of the *Plasmodium* merozoite requires specific molecular interactions between host receptors and parasite ligands which trigger downstream signaling cascades regulating the rhoptry discharge and the actomyosin motor complex of the parasite [26–28]. Inside its host cell, *Plasmodium* interferes with several erythrocyte signaling pathways that target the erythrocyte membrane in order to adapt the erythrocyte cytosol to its needs. By trafficking *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) into the host erythrocyte membrane the parasite increases the cytoadherance of late stage-parasitized erythrocytes to the microvasculature [29]. By conferring oxidative stress [30], the parasite affects the host's redox state resulting in the induction of organic osmolyte and anion channel activity [14,31] and in the activation of ClC-2 Cl^- channels. The activity of ClC-2 channels has been demonstrated to counteract the volume expansion of the parasitized cell [6,13,14,16,17,24]. In addition, the parasite has been demonstrated to trigger the erythrocyte death program which increases the erythrocyte Ca^{2+} permeability [32] probably as a prerequisite of the parasite's Ca^{2+} signaling [33] and the interconnected cGMP signaling [34]. At the same time, the parasite prevents the execution of the erythrocyte death program as deduced from the comparison between parasitized sickle cell trait and normal erythrocytes [32].

Finally, the parasite employs the erythrocytic autocrine purinergic signaling [17] possibly by modulating the protein kinase A pathway [4,35–37]. Protein kinase A and autocrine purinergic signaling has been shown to contribute to the formation of the organic osmolyte and anion permeability in the host membrane. Experimental interference with the autocrine purinergic signaling strongly impairs the parasite development *in vitro* and in a malaria mouse model *in vivo* [17] indicating the significance of this pathway for the malaria blood stage. The present review article, therefore, focuses on the autocrine purinergic signaling in parasitized erythrocytes. In addition, it briefly summarizes the physiological and pathophysiological function of purinergic signaling in uninfected erythrocytes.

2. Erythrocytes in the control of vascular tone

Various stress stimuli such as hypoxia/hypercapnia [38], mechanical deformation [39–41], reduced pH [42], hypotonic swelling [43] ligation of the prostacyclin- [44] or beta adrenergic receptor [45,46], and extracellular ADP/ATP [47] stimulate the release of ATP by human and other mammalian erythrocytes. The ATP release is regulated by heterotrimeric Gs [48] and Gi G proteins [49,50] and requires cyclic AMP formation by adenylyl cyclase [45,51] protein kinase A [52] and CFTR activity [39,53]. Cytosolic cyclic AMP concentrations and ATP release are under the tight control of phosphodiesterases which in turn are activated by protein kinases A and C [52,54]. Activation of the latter kinase has been demonstrated to be stimulated by exchange factors directly activated by cyclic AMP (EPAC) [46]. Insulin has been shown to inhibit cyclic AMP accumulation and ATP release via erythrocyte insulin receptor, phosphoinositide 3-kinase and phosphodiesterase [55].

Erythrocyte-derived ATP induces relaxation of blood vessels via the formation of NO by endothelial cells [38,56–58]. NO in turn inhibits ATP release from erythrocytes [59] via inactivation of the heterotrimeric G protein Gi [50]. By those mechanisms, erythrocytes sense the oxygen tension, regulate the vascular resistance of exercising skeletal muscular or pulmonary vessels, and ultimately determine the O_2 supply to the tissue [60]. Erythrocytes from patients with primary pulmonary hypertension have an impaired ATP release [61] highlighting the importance of erythrocyte ATP release for arterial smooth muscle control. In erythrocytes from patients with type II diabetes, G_i expression and associated ATP release is reduced [62]. In addition, the type II diabetes-associated increase in circulating insulin further inhibits the low oxygen-induced ATP release, which might contribute to insufficient oxygen delivery to the tissue [55,63]. In summary, ATP release by erythrocytes is regulated in a highly complex manner employing several surface receptors (prostacyclin, beta adrenergic, insulin, and purinergic receptors) and intracellular signaling cascades (protein kinase A, protein kinase C, phosphoinositide 3-kinase pathways). In addition, erythrocytes must be endowed with mechanosensation of membrane-deformation or cell swelling as well as chemosensation of O_2, CO_2, NO and pH and downstream signal transduction that cross-talks with the receptor-triggered ATP release machinery.

3. ATP release pathways of erythrocytes

Liberation of cytosolic ATP into the blood or interstitial space can occur via necrotic cell death, via exocytosis or via membrane transport mediated by ATP-permeable channels or nucleotide transporters. Human erythrocytes functionally express pannexin-1 protein in the plasma membrane [43]. Pannexin-1 is a nonselective channel with a high conductance, which forms either gap junctions at cell–cell contacts or hemi channels in nucleated cells. Pannexin-1 has been demonstrated to conduct ATP. In human erythrocytes, bulk of ATP release induced by hypotonic swelling [43] or low oxygen tension [64] is mediated by pannexin-1. Albeit attenuated, pannexin-1-deficient mouse erythrocytes still release ATP [65], indicating further ATP exit routes. This pannexin-1-independent pathway of human erythrocytes is stimulated by prostacyclins [65,66] and blocked by Bcl-xL BH44-23 and TRO19622, two unrelated inhibitors of the voltage-dependent anion channels (VDAC) [66]. VDAC, which permeabilizes the outer mitochondrial membrane for organic molecules, may be trafficked to the plasma membrane and has been found in mature human erythrocytes [6,66]. Hence, pannexin-1 and plasmalemmal