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REVIEWS

Erythrocytes as Regulators of Blood Vessel Tone

O. G. Luneva[a], S. V. Sidorenko[a], G. V. Maksimov[a], R. Grygorczyk[b], and S. N. Orlov[a, c]

[a] *Faculty of Biology, Moscow Lomonosov State University, Leninskie Gory, 1, build. 12, Moscow, 119991 Russia*
e-mail: sergeinorlov@yandex.ru

[b] *Department of Medicine, University of Montreal, 900 rue St-Denis, Montreal (Quebec) H2X0A9, Canada*

[c] *Tomsk State University, pr. Lenina, 36, Tomsk, 634050 Russia*

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Abstract—A drop in oxygen partial pressure results in elevation of blood vessel diameter. It has been demonstrated that isolated vessels exhibit this unique feature only when they are perfused in the presence of erythrocytes. More recently, it was shown that haemoglobin plays a key role in oxygen sensing. Its deoxygenated form interacts with band 3 protein, triggering the cascade of non-identified intracellular signals involved in nitric oxide production and release of ATP interacting with P2Y purinergic receptors in endothelial cells. In this review, we summarize the data on mechanisms of ATP release from erythrocytes, as well as on its physiological and pathophysiological implications.

Keywords: erythrocytes, haemolysis, oxygen sensor, purinergic signalling system, blood vessels

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INTRODUCTION

The regulation of blood vessel tone maintains the tissue supply of oxygen, glucose, and other substances required for the synthesis of macroenergetic compounds which is balanced with energy demands. At the level of the organism, blood vessel tone is under the control of blood pressure in systemic and pulmonary circulation. In addition, several organs exhibit systems involved in the regulation of local blood flow, such as myogenic response, whose mechanism is investigated in more detail. Myogenic response or myogenic tone is an intrinsic property of small (<100–200 mm) blood vessels to decrease their diameter in response to elevation of pressure of the luminal fluid. Myogenic tone detected in skeletal, mesenteric, renal, brain, and coronary vessels plays a key role in the maintenance of constant blood flow in spite of variation of systemic blood pressure. Local blood flow is the major determinant of regulation of metabolic activity of the tissue, and resistance to blood flow is determined as $R_{bf} \sim 1/d^4$, where d is the inner vessel diameter [1]. The role of myogenic tone as a naturally created instrument defending target organs from elevation of systemic blood pressure was intensively investigated by numerous research teams (for review, see [2]). For our review, it is important to underline that myogenic tone is an intrinsic property of smooth muscle cells (SMC) preserved in the absence of endothelium and blood cells [3–6]. The elevation of small vessel diameter in response to attenuation of partial oxygen pressure (pO_2) is another important system controlling local blood flow [7]. We focus our mini-review on the molecular mechanisms of this phenomenon with an emphasis on the role of erythrocytes and purinergic signalling.

MECHANISMS OF THE INVOLVEMENT OF ERYTHROCYTES IN BLOOD VESSEL TONE REGULATION

In 2000, Dietrich and co-workers demonstrated that the presence of erythrocytes in the luminal fluid is obligatory for the elevation of brain arteriole diameter in response to attenuation of pO_2 [8]. Because the elevation of perfusate viscosity with dextran did not have the same action, they proposed that attenuation of the blood vessel tone in hypoxic conditions is caused by the presence in erythrocytes of vasodilator, rather than by the inhibition of myogenic tone. Later on, this phenomenon was reproduced in several laboratories (for review, see [9, 10]). It should be noted that during severe hypoxia, the dilation of blood vessels possessing myogenic response may occur in the absence of erythrocytes. Typically, this phenomenon is explained by a drastic decrease of the ATP content in SMC, which, in turn, leads to opening of ATP-sensitive K^+ channels (K_{ATP}) and sarcolemma hypopolarization [11]. In the case of rat coronary artery, the dilatation evoked by the drop of pO_2 was preserved in the presence of the K_{ATP} inhibitors but was partially suppressed by inhibitors of NO synthesis [12]. Factors determining blood vessel bed-specific character of erythrocyte-independent regulation of blood flow in hypoxic conditions remain unknown. Possible roles of erythrocytes in blood tone regulation are considered below.

Gasotransmitters

Nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H_2S) are considered as natural gasotransmitters, which form during catabolism of amino acids. Unlike canonical hormones and neurotransmitters, they fluently penetrate into a cell and trigger diverse signalling cascades and cellular responses without binding with specific receptors exposed to extracellular milieu [13, 14]. In cardiovascular systems, endothelium is a major source of NO and CO [13, 15] whereas H_2S is produced by SMC, as well as by adipocytes and erythrocytes [16, 17]. NO produced by endothelial synthase (eNOS) binds in SMC with heme containing soluble guanylate cyclase, which leads to activation of this enzyme, accumulation of cGMP, and dilatation of blood vessels. The same mechanism determines dilatory action of CO, but the efficiency of this gasotransmitter is less than that of NO [15, 18].

It was shown that NO produced by endothelium also binds with haemoglobin, which can release NO in hypoxic conditions [19]. It should be noted, however, that because of limited diffusion, this mechanism may contribute to regulation of vessels whose diameter is less than 25 μm [20]. It was proposed that erythrocytes may generate NO by its release from S-nitrosohaemoglobin (SNO-Hb), which is formed upon binding with cysteine β93 of the β-chain of the oxygenated protein [21, 22]. This hypothesis, however, contradicts the data indicating the lack of essential differences in the content of SNO-Hb in human arterial and venous blood [23]. Moreover, Isbell and co-workers demonstrated that substitution of cysteine β93 for alanine lacking the NO-binding ability does not affect the systemic and pulmonary haemodynamics in mice, and elevation of the pulmonary artery diameter evoked by hypoxia is the same under its perfusion with erythrocytes from wild-type and genetically manipulated mice [24]. In addition, erythrocytes may generate NO by its formation from nitrate (NO_2^-) in reaction $Hb(Fe^{2+}) + NO_2^- = Hb(Fe^{3+}) + NO + OH^-$ [25] occurring in the presence of deoxygenated haemoglobin [26]. Indeed, both in vivo and in vitro experiments showed a vasodilatory action of nitrites [25]. However, these effects of nitrites were preserved in normoxic conditions, as well as in the absence of erythrocytes [27].

Garlic-derived polysulfides are considered as a major source of exogenous H_2S produced in the presence of erythrocytes [28] that probably underlies antiatherosclerotic action of garlic extracts. In contrast to NO and CO, there are no reports showing any significant action of H_2S and its donor NaHS on the cGMP system [29, 30]. Considering these negative results it was assumed that H_2S-induced vasorelaxation is mediated by activation of K^+ via its interaction with cysteine residues. This conclusion is mainly based on the suppression of dilatory actions of NaHS by glibenclamide and other K_{ATP} inhibitors [15, 31]. To the best of our knowledge, the direct evidence for the action of this gasotransmitter on the activity of K_{ATP} channels is limited to a few publications [32, 33].

Purinergic Signalling System

Soon after the pioneering paper of Burnstock and Kennedy [34], several laboratories reported that application of ATP inside the vessel lumen results in relaxation of different vascular beds. This action of ATP is mediated by endothelium-derived NO, and in endothelium denuded vessels, ATP evokes NO-independent vasoconstriction (for review, see [35]). It was also shown that vasodilatation is caused by interaction of ATP with G-protein coupled P2Y purinergic receptors of endothelial cells, whereas vasoconstriction is mediated by its interaction with P2X receptors in SMC, which operate as nonselective cation channels [36] (Fig. 1). Side-by-side with the NO production, activation of P2Y receptors triggers catabolism of arachidonic acid and accumulation of prostacylins PGI_1 and PGI_2, which also leads to vasorelaxation via activation of cAMP-mediated signalling and/or opening of K^+ channels [10]. The presence of highly active endonucleases discovered by V.A. Engelhardt and co-workers [37], suggests rapid degradation of ATP and accumulation of adenosine (Ado) activating P1 receptors. The contradictory data on the regulation of vascular tone by adenosine in hypoxic condition [12, 38] are outside the scope of this review.

The search for the major source of intravascular ATP performed by Bergfeld and Forrester resulted in the discovery of the ATP release by isolated human erythrocytes exposed to a decreased pO_2 [39]. Later on, this finding was reproduced in erythrocytes of other mammals [8, 40]. In the overwhelming number of investigations, ATP release from hypoxic erythrocytes led to a 2–3-fold elevation of extracellular ATP content estimated by luciferase luminescence. For example, during perfusion of isolated arterioles from rat brain, 5–10-fold attenuation of pO_2 resulted in elevation of the extracellular concentration of ATP from 8 to 14 μM [8]. This observation is consistent with the data showing that in the absence of any additional stimuli the concentration of ATP in venous blood as compared with arterial is increased by 20–40% [41]. Based on these data, a hypothesis was put forward suggesting that erythrocyte is not just an oxygen carrier but is also a pO_2-dependent regulator of its delivery [40]. In this connection it is noteworthy that, along with hypoxia, ATP release from erythrocytes can also be invoked by their mechanical deformation during passage through vessels with inner diameter comparable with erythrocyte size [42], as well as by acidification of the extracellular milieu [40], elevation of the CO_2 content in the blood [39], blood flow turbulence [43], and modest temperature elevation [44]; combination of these stimuli may significantly potentiate the

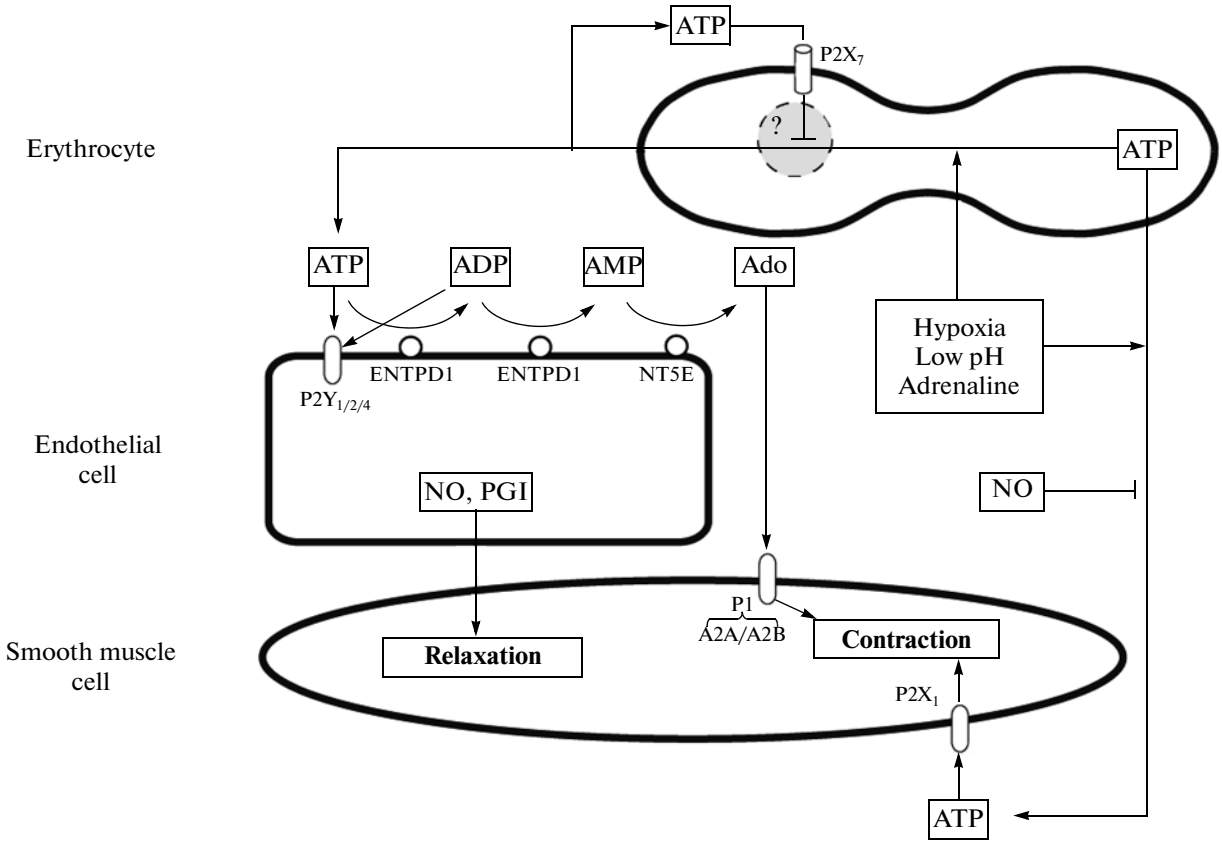

Fig. 1. Scheme illustrating interaction of erythrocytes, endothelial and smooth muscle cells in regulation of blood vessel tone via purinergic receptor and NO-mediated signaling system (→, activation and ⊣, inhibition). ENTPD1 and NT5E are ectonucleotidases involved in catabolism of ATP; $P2X_1$, $P2Y_{1/2/4}$, A2A and A2B are major purinergic receptors involved in regulation of vascular smooth muscle and endothelial cells. For more details, see text.

ATP release under hypoxic conditions. It should also be noted that because of the well-developed network of cell-to-cell contacts (Fig. 1), the local excitation of endothelial P2Y receptors will spread along the vascular bed with a speed of 50 μm/s [45].

In 1972, Parker and Snow reported that 40-min incubation of canine erythrocytes lacking Na^+,K^+-ATPase in the presence of 0.5 mM ATP abolished the transmembrane gradients of Na^+ and K^+ [46]. This action of ATP sharply diminished in the presence of Mg^{2+}, thus indicating an involvement of non-selective cation channels activated by ATP^{4-}. Indeed, much later $P2X_7$ receptors were identified in human erythrocytes and their activation increased the rate of transmembrane Na^+ and K^+ fluxes 5–10-fold [47]. It is well documented that activation of $P2X_7$ receptors causes the death of leucocytes, macrophages, and cells of some other types [48]. Sluyter and co-workers demonstrated that prolonged incubation of human erythrocytes with ATP increased the content of phosphatidylserine in the outer surface of the membranes [49], which is known as a marker of apoptosis. The death of the cells of the immune system and of the erythrocytes caused by activation of $P2X_7$ receptors was observed in high-sodium medium, and was not affected by the presence of Ca^{2+} [48, 49]. Activation of $P2X_7$ receptors may affect the functional state of nucleated cells through the activation of the Na_i^+/K_i^+-sensitive, Ca_i^{2+}-independent regulation mechanism of gene transcription discovered in our laboratory [50]. Mechanisms of the involvement of $P2X_7$ receptors in the function of nucleus-free erythrocytes remain unknown.

IDENTIFICATION OF THE pO_2 SENSOR

In accordance with the initial hypothesis, the O_2 sensor responsible for the regulation of vascular tone should be located in the vessels themselves or in the neighbouring parenchyma [51, 52]. The data considered above suggest that the O_2 sensor is located in erythrocytes. In this connection it can be assumed that the haemoglobin tetramer known as the oxygen sensor contributes to the sensing of pO_2 via interaction of its deoxygenated tense form (T-Hb) with an unknown adaptor protein, triggering a downstream signalling cascade terminated by the ATP release (Fig. 2). This hypothesis is consistent with the negative correlation of ATP release with the content of the oxygenated

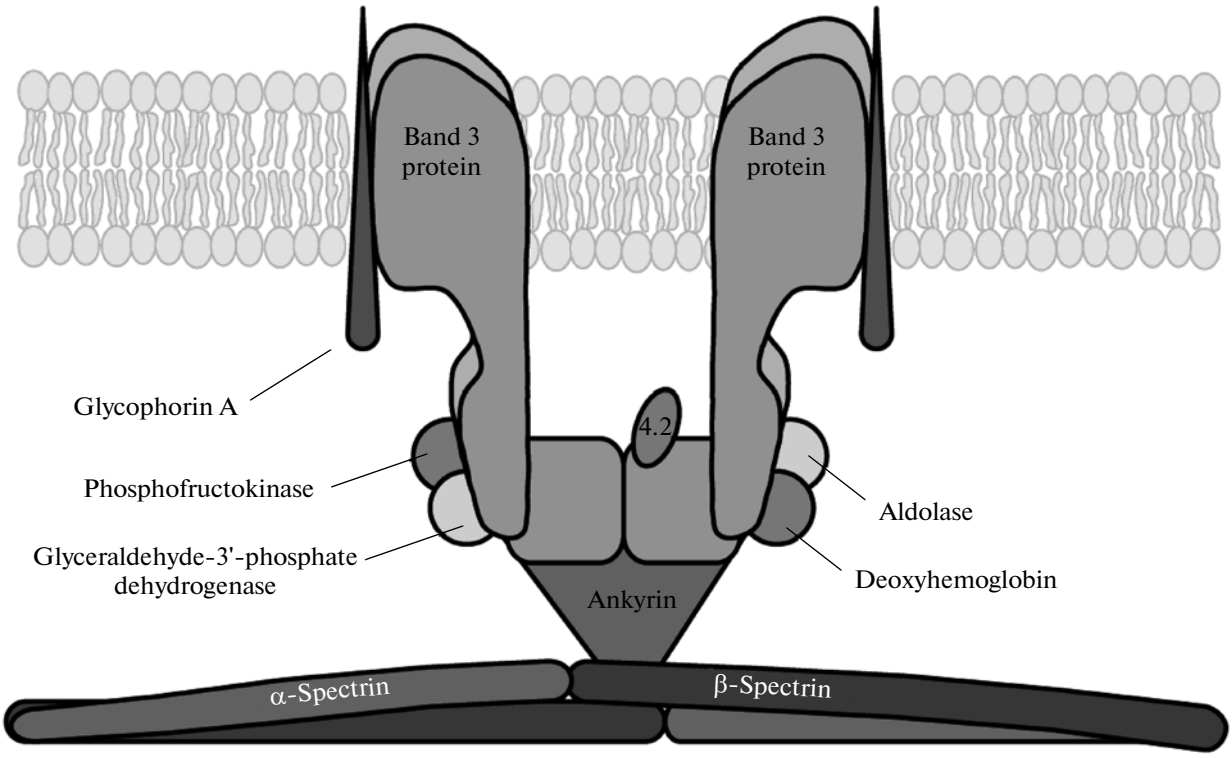

Fig. 2. Organization of band 3 multiprotein complex and its role in the regulation of erythrocyte function by hypoxia (modified from [115]). For explanation, see text.

relaxed form of haemoglobin (R-Hb), as well as with suppression of hypoxia-induced ATP release by CO [41]. It is well documented that the affinity of haemoglobin to CO is 2–3 orders of magnitude higher than to O_2 and that CO completely blocks the O_2-induced transition to T-Hb.

In cell-free model experiments it was shown that haemoglobin binds to the cytoplasmic domain of band 3 protein, the major integral protein of the erythrocyte membrane, playing a key role in the organization of the membrane cytoskeleton as well as in anion transport (anion exchanger 1, AE1) [53]. It was also shown that both in humans and mice, the affinity of this band 3 fragment with M_r of 43 kDa (CDB3) to T-Hb is much higher than to the oxygenated haemoglobin [54, 55]. It was also shown that the same band 3 fragment interacts with glyceraldehide-3-phosphate, aldolase, pyruvate kinase, and other key enzymes of glycolysis and pentose-phosphate pathways [56, 57], and the binding to deoxyhaemoglobin leads to release of this enzymes and activation of glycolysis [58]. Taken together, these data suggest that band 3–T-Hb interaction triggers an elevation of the local concentration of ATP and its release from erythrocytes [41] (Fig. 2). This hypothesis is currently being examined in our laboratory.

MECHANISM OF ATP RELEASE FROM ERYTHROCYTES

In spite of numerous studies on ATP release, the mechanism of this phenomenon as well as its regulation of pO_2 and other above-listed stimuli remains poorly investigated. Indeed, nucleus-free mammalian erythrocytes lack the endoplasmic reticulum providing endocytosis, the major mechanism of ATP release in cells of other types studied so far [59, 60]. In this connection, several research teams carried out screening of compounds involved in the regulation of transport across the plasma membrane. It was found that ATP release from human erythrocytes is increased in the presence of nitrites [61], as well as upon addition of cell-permeant cAMP analogues and activators of cAMP-mediated signalling, such as agonists of β-adrenergic receptors (adrenalin and isoproterenol), PGI_2 and its stable analogue iloprost, activator of adenylate cyclase forskolin, and phosphodiesterase inhibitor papaverin [62–64]. It was also shown that ATP release is decreased in the presence of inhibitors of the cAMP-dependent protein kinase A [62–64], NO [65], insulin [66], C-peptide [67], statins [68], and amiloide peptide [69]. We found that ATP release can also be triggered by dimethyl sulfoxide, a commonly employed vehicle dissolving forskolin and other amphypatic compounds [70]. This observation should be taken into account in the analysis of these experiments.

Because the anion transport in cystic fibrosis mediated by the transmembrane conductance regulator (CFTR) is increased by cAMP, it was suggested that ATP release from erythrocytes is mediated by CFTR [71, 72]. It should be noted, however, that electrophysiological experiments failed to demonstrate any detectable permeability of CFTR for ATP [73]. Recently, it was shown that inhibitors of voltage-dependent anion channel VDAC1 suppress ATP release evoked by the activation of PGI_1 receptors [74]. Electrophysiological examination of this hypothesis has not been performed yet. In another set of experiments it was demonstrated that ATP efflux from human erythrocytes is decreases by carbenoxolone, peptide ^{10}panx1, and other inhibitors of pannexin 1 (Panx1) – a channel permeable for compounds with $M_r < 900$ kDa [62]. It was also shown that Panx1 is expressed in human erythrocytes and that ATP release is accompanied by uptake of dyes penetrating into the cells through this channel [75]. However, ATP release was also observed in erythrocytes isolated from panx1$^{-/-}$ mice [63]. In the previous section the data showing the presence of the $P2X_7$ receptors in erythrocytes were presented. The role of these receptors in the ATP release triggered by hypoxia has not been examined so far.

It is clear that along with transmembrane transport, elevation of extracellular ATP concentration may result from the disruption of single erythrocytes, i.e., the process termed haemolysis. After the discovery of the loss of the lipid bilayer asymmetry and exposure of phosphatidylserine on the outer monolayer in dying erythrocytes and of other markers of programmed cell death (apoptosis), this process, which probably precedes haemolysis, was termed eryptosis [76]. At present, the library of National Institutes of the Health of the USA counts 1874 studies on intravascular haemolysis activated in hypoxic conditions or during extensive exercise (for review, see [77–81]). However, the overwhelming number of these investigations lack the systematic comparison of accumulation of extracellular ATP and erythrocyte haemolysis. Mairbaurl and co-workers demonstrated that ATP efflux from human erythrocytes triggered by mechanical stimuli is proportional to the haemoglobin release, whereas hypoxia has no impact on haemolysis [77]. Recently, we compared ATP and haemoglobin release in human erythrocytes subjected to hypo-osmotic swelling, hypoxia, and mechanical stress. In parallel experiments we found highly significant positive correlation between the accumulation of extracellular ATP and haemoglobin. In additional experiments, we visualized erythrocytes using infrared microscopy with simultaneous registration of the ATP-dependent luminescence of luciferase. These experiments demonstrated that luminescence spikes occurred only upon cell lysis [70]. These data indicate that upon application of all stimuli listed above, haemolysis is the major mechanism of elevation of the extracellular ATP concentration.

In normal conditions, erythrocytes circulate in blood flow for 100–120 days and then, after clasterization of band 3 protein and complement C3, they are captured and digested by a system of macrophages in spleen, liver, and bone marrow [82]. In vitro experiments demonstrated that eryptosis can be triggered by elevation of the intracellular Ca^{2+} concentration, activation of Ca@2+-sensitive K^+ channels, the loss of potassium and chloride – which, in turn, leads to attenuation of the erythrocyte deformability – as well as upon dissipation of transmembrane gradient of sodium and potassium induced by the addition of valinomycin, monensin, gramicidin, or other monovalent cation-selective ionophores [83]. Elevation of haemolysis in hypoxic conditions and application of mechanical stresses may be caused by alteration of the interaction between the band 3 protein and other proteins of the cytoskeleton network [84, 85]. Additional experiments should be performed to examine this attractive hypothesis.

FEATURES REVEALED IN NUCLEATED ERYTHROCYTES

In all vertebrates studied so far, the affinity of haemoglobin to oxygen drastically decreases upon cytoplasm acidification (so-called Bohr's effect) [86]. In addition, this parameter decreases at higher haemoglobin concentration, which may be due to the loss of intracellular water and a decrease of the cell volume [87]. It should be noted, however, that because of an extremely high permeability of nuclear-free mammalian erythrocytes for anions as compared to the permeability for Na^+ and K^+, pH_i mainly depends on the extracellular concentration of protons (pH_o) and major anion chloride:

$$[H^+]_o/[H^+]_i = [HCO_3^-]_i/[HCO_3^-]_o = [Cl^-]_i/[Cl^-]_o = r,$$

It also means that pH_i is not affected by activity of other ion transporters whose activity is much lower than the activity of the anion transport by band 3 protein. The only exception is Ca^{2+}-sensitive K^+ channels (K_{Ca}); their activation leads to a drastic hyperpolarization of erythrocytes, loss of K^+ and Cl^-, cell shrinkage, and cytoplasm acidification. This process is illustrated by experiments in which the addition to rat erythrocytes of K^+ ionophore valinomycin changed the membrane potential and caused an accumulation of extracellular protons (Fig. 3a). In nucleated erythrocytes, the contribution of anion permeability into the net ion fluxes is much less than in mammals; for example, in carp erythrocytes the valinomycin-induced hyperpolarization has no impact on the transmembrane fluxes of hydroxyl anion OH^- [88]. As anticipated, these differences were abolished in the presence of protonophore CCCP (Fig. 3b), i.e., under the conditions where protons are distributed independently of the

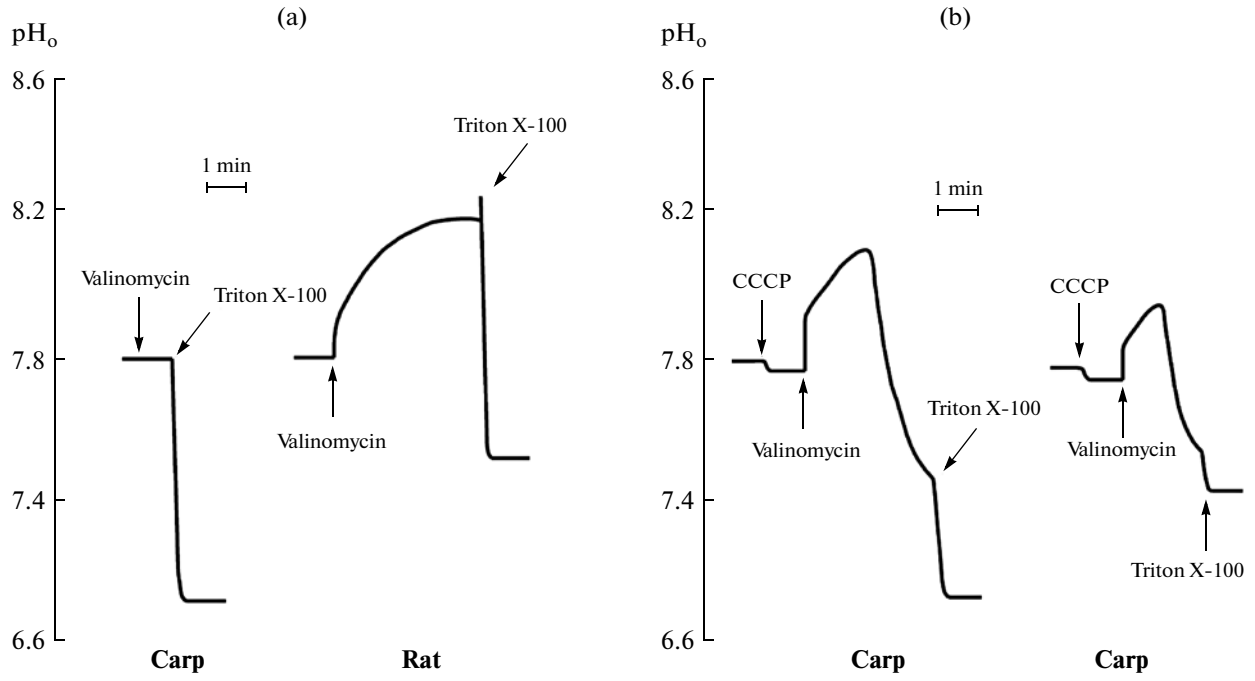

Fig. 3. Kinetics of extracellular pH (pH_o) changes triggered by K^+ ionophore valinomycin in carp and rat erythrocytes in the absence (a) and presence (b) of protonophore CCCP.

anion permeability, in accordance with the membrane electrical potential: $E_m = RT/F(pH_i - pH_o)$.

This feature suggests that in contrast to mammalians, in nucleated erythrocytes monovalent cation transporters contribute to regulation of pH_i and cell volume. This regulatory system was subjected to detailed investigation in erythrocytes of bony fishes (Teleostei). In these species, increased activity is accompanied by the accumulation of lactic acid. Importantly, because of the absence of the effective regulation of the acid–base balance in the kidney, lactic acid-induced plasma acidification is accompanied by a drop in erythrocyte pH_i. In fishes, plasma acidification via unknown mechanisms triggers a massive release of catecholamines leading to accumulation of cAMP and activation of a unique cAMP-dependent isoform of Na^+/H^+ antiporter found in these cells [89]. Activation of this carrier results in normalization of pH_i and elevation of haemoglobin affinity for O_2 (for review, see [87, 90, 91]).

In bird erythrocytes, catecholamines activate a ubiquitous isoform of $Na^+,K^+,2Cl^-$ transporter NKCC1 [92, 93]. In addition to catecholamines, this carrier is also activated by hypoxia; moreover, the action of catecholamines and hypoxia is additive [93]. This phenomenon probably underlies the increase of erythrocyte volume and affinity of haemoglobin for oxygen during long-lasting flights. In fishes, along with $Na^+,K^+,2Cl^-$ co-transport, haemoglobin deoxygenation increases the rate of Na^+/H^+ exchange and inhibits K^+,Cl^- co-transport [90, 94]. Oxygen-sensitive ion transporters were also found in several amphibian species [90, 95]. It has been proposed that in nucleated erythrocytes, pO_2 sensing is accomplished not only by haemoglobin but also by hypoxia-inducible factor (HIF-1a) [90, 91]. This hypothesis needs further substantiation. To the best of our knowledge, the data on the regulation of ATP release by hypoxia in nucleated erythrocytes is limited to the study of Jensen and co-workers [96]. They demonstrated that in contrast to mammals, hypoxia does not trigger ATP release from fish erythrocytes.

PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL IMPLICATIONS

The data considered above suggest that the role of erythrocyte as regulators of blood vessel tone is mainly manifested in vascular beds of organs characterised by sharp changes in their energy demands. Obviously, any disturbances of this regulatory system may be involved in the pathogenesis of diseases with hypoxic complications. In this section we give several illustrating examples.

Intensive Exercise

Skeletal muscles are exposed to considerable fluctuations of the oxygen consumption during changes in exercise intensity [97, 98]. It has been shown that in skeletal muscle, blood supply is under the control of NOS and cyclooxygenase (COX) [99]. It has also been demonstrated that intensive exercise is followed by ATP concentration rise in venous blood [100]. In the

case of intact endothelium, arterial injection of ATP leads to relaxation of the skeletal muscle vessels [101, 102]; this phenomenon is accompanied by an elevated production of NO [103] and arachidonic acid metabolites [104], and is suppressed by inhibitors of NOS and COX [38]. The role of local temperature elevation [44] and other factors accompanying intensive exercise in ATP release from erythrocytes remains poorly investigated. It is suggested that ATP-induced NO release from endothelial cells evoked by local hypoxia counteracts blood vessel contraction due to exercise-induced activation of the sympathetic nervous system that, in turn, activates smooth muscle β1- and β2-adrenergic receptors [105].

Less is known about the mechanism of augmented production of vasorelaxing derivatives of amino acids triggered by exercises. We assume that this phenomenon is caused by dissipation of the transmembrane gradient of monovalent cations occurring in excited skeletal muscles. Indeed, both in humans and experimental animals, long-lasting exercise was found to result in a 3–4-fold elevation of $[Na^+]_i$ and attenuation by 15–25% of $[K^+]_i$ as a consequence of permanent activation of voltage-gated K^+ and Na^+ channels and partial inactivation of the Na^+,K^+-ATPase [106, 107]. Recently, we demonstrated that both in smooth muscle and in endothelial cells, elevation of the $[Na^+]_i/[K^+]_i$ ratio due to hypoxia and energy depletion resulted in a sharp elevation of the expression of inducible cyclooxygenase 2 (COX-2), i.e., one of the key enzymes of arachidonic acid catabolism [50, 108]. The role of an elevated $[Na^+]_i/[K^+]_i$ ratio in regulation of COX-2 expression in skeletal muscle remains unknown.

Idiopathic Pulmonary Hypertension (IPH)

IPH is characterized by elevation of the resistance in pulmonary circulation that might be caused by abnormalities of NO-mediated signalling. Sprague and co-workers demonstrated that in IPH patients, ATP release triggered by the passage of erythrocytes though small blood vessels (diameter < 5 μm) is decreased [109]. This defect was probably caused by lower deformability of erythrocytes rather than by a decreased content of intracellular ATP. The authors suggested that these features of erythrocytes underlie a decreased NO production which is observed in endothelial cells of patients with IPH.

Type 2 Diabetes Mellitus

Insulin-independent type 2 diabetes mellitus is characterized by decreased delivery of O_2 to skin and skeletal muscles, which is regarded a main cause of poor wound healing and early fatigue in response to exercise [110]. It was shown that hypoxia-induced ATP release is decreased in erythrocytes of diabetic patients [111]. Does this phenomenon contribute to insufficient oxygen supply to tissues? To answer this question, changes of blood vessel diameters in skeletal muscles of a control group and patients with type 2 diabetes mellitus were compared. It was shown that, unlike the control group, hypoxia did not affect the diameter of blood vessels during their perfusion with the blood from diabetic patients [112]. It was also shown that ATP release from erythrocytes of diabetic patients is normalized in the presence of phosphodiesterase inhibitors [113], as well as in the medium containing peptide C and insulin at a control molar ratio (1 : 1) rather than after a 6-fold rise of the peptide C content, as observed in diabetic patients [114]. These data are considered as background for novel therapeutic approaches to the treatment of diabetic complications resulting in abnormal regulation of the blood vessel tone by erythrocytes [10].

Endothelium Dysfunction

In the overwhelming number of observations, endothelium dysfunction is caused by the disruption of the endothelial cell monolayer integrity. High blood pressure, increased plasma cholesterol content, diabetes mellitus, and obesity are considered as major causes of endothelium dysfunction. Endothelium dysfunction decreases NO production in response to diverse vasorelaxing stimuli and provides an access for hormones and neurotransmitters to smooth muscle cells. In the case of ATP, this results in activation of P2X receptors and a decrease of the blood vessel diameter (Fig. 1). In other words, in endothelium dysfunction combined with hypoxia and intravascular mechanical stresses, ATP release from erythrocytes will attenuate rather than increase the blood flow [35], thus worsening the disease. ATP efflux from erythrocytes is suppressed by NO [65] that partially compensates for the pathophysiological consequences of the endothelium dysfunction. The role of endothelium dysfunction in the regulation of systems providing ATP release from erythrocytes is currently under investigation.

Increased eryptosis was observed in several pathologies including sepsis, renal insufficiency, diabetes mellitus, and hyperthermia [83]. However, its relationship to the ATP-dependent regulation of vascular tone has not been examined.

UNRESOLVED ISSUES AND PERSPECTIVES OF FORTHCOMING STUDIES

Data considered in our mini-review show that side-by-side with oxygen delivery, erythrocytes are able to regulate blood vessel tone. This regulatory system include erythrocytes not only as oxygen-dependent donors and acceptors of NO, but also as a major source of ATP for activation of purinergic receptors. Increased number of indicates that this regulatory system is involved in the pathogenesis of pulmonary

hypertension and diabetes mellitus, as well as in the pathogenesis of diverse diseases linked to endothelium dysfunction. There is a body of evidence for consideration of haemoglobin as a primary oxygen sensor involved in NO evidence transport, as well in ATP release via interaction of its deoxygenated form with band 3 protein. What is the molecular origin of other proteins involved in the transduction of signals triggered by haemoglobin deoxygenation? Can we consider intravascular haemolysis as a universal mechanism of ATP release induced by hypoxia and other physiological and pathophysiological stimuli? Which mechanisms provide the pO_2-dependent disruption of the erythrocyte membrane integrity? Which erythrocyte function is affected by $P2X_7$ receptors in normal and pathophysiological conditions? We firmly believe that forthcoming studies will give answers to these questions.

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Translated by S. Orlov

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