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Erythrocyte-derived ATP and perfusion distribution: Role of intracellular and intercellular communication

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Abstract

In complex organisms, both intracellular and intercellular communication is critical for the appropriate regulation of the distribution of perfusion to assure optimal oxygen (O_2) delivery and organ function. The mobile erythrocyte is in a unique position in the circulation since it both senses and responds to a reduction in O_2 tension in its environment. When erythrocytes enter a region of the microcirculation in which O_2 tension is reduced, they release both O_2 and the vasodilator, adenosine triphosphate (ATP) via activation of a specific and dedicated signaling pathway that requires increases in cAMP, that are regulated by phosphodiesterase 3B. The ATP released initiates a conducted vasodilation that results in alterations in the distribution of perfusion to meet the tissue's metabolic needs. This delivery mechanism is modulated by both positive and negative feedback regulators. Importantly, defects in low O_2-induced ATP release from erythrocytes have been observed in several human disease states in which impaired vascular function is present. Understanding of the role of erythrocytes in controlling perfusion distribution and the signaling pathways that are responsible for ATP release from these cells makes the erythrocyte a novel therapeutic target for the development of new approaches for the treatment of vascular dysfunction.

Keywords

Red blood cells; blood flow regulation; O_2 delivery; prostacyclin; phosphodiesterase

Introduction

Intracellular communication is vital for the survival of any organism. However, in higher organisms, in addition to intracellular signaling, there must be interactions between and among cells such that a coordinated system emerges. Unless all components of such complex systems interact appropriately, homeostasis will not be maintained with pathological consequences. Classical interactions among cells occur via both positive and negative mechanisms which modulate their function. A prime example is the intracellular and intercellular mechanisms which regulate the distribution of blood flow to assure optimal oxygen (O_2) delivery and organ function.

Within the microvasculature, vessel caliber must be modulated to direct blood flow to meet, but not exceed, the metabolic and/or functional needs of tissues and organs. This tight regulation of blood flow optimizes the distribution of cardiac output thereby minimizing demands on the pump during times of increased tissue need. Although the regulation of the supply of O_2 to a tissue is a basic requirement for the circulatory system, the complexity of a

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microvasculature that responds not only to local tissue O_2 needs, but also to blood pressure, shear stress and vasoactive mediators complicates the process. In addition, O_2 transport within the microvasculature is influenced not only by the magnitude and distribution of blood flow but also by the unique rheological properties of erythrocytes in bifurcating microvascular networks and the diffusional exchange of O_2 among all vessels within the microvasculature (21–26,59,74). Thus, although alterations in total flow to a tissue such as skeletal muscle is important, it is only by directing that flow to the regions of the tissue where it is needed that O_2 delivery is effectively supplied to the tissue (67). The appropriate distribution of perfusion to meet tissue needs requires interaction and tight coordination among several components of the O_2 supply system.

In skeletal muscle the O_2 demands of the tissue vary widely. In a 1929 lecture, August Krogh stated “it seemed to me clear that there must be some mechanism regulating the conditions of supply [of oxygen]. With constant conditions the facilities for transport must be either ridiculously out of proportion to the requirements of the muscles during rest or ridiculously inadequate to meet their needs during heavy work.” (44). Although numerous theories have been advanced to explain how the microcirculation of skeletal muscle is regulated to permit perfusion to be directed to meet precisely tissue metabolic needs (17,33,38,56,58), none has provided the sensitivity required to fully explain the tight control observed *in vivo*. What has been critically lacking in most models is a mechanism by which O_2 need is sensed locally and its delivery altered to precisely meet that need.

The erythrocyte and the regulation of perfusion distribution in skeletal muscle

New insights into a potential mechanism that explains the tight regulation of flow distribution in skeletal muscle were derived from a 1993 study of O_2 transport in hamster skeletal muscle capillaries where it was suggested that, in severe hypoxia, O_2 content (O_2 saturation) was more important than O_2 tension in the maintenance of O_2 supply (75). O_2 content reflects the extent of binding of O_2 to hemoglobin within erythrocytes while O_2 tension determines the diffusive transport of O_2 from the erythrocyte to the tissue. If O_2 content rather than O_2 tension is the important factor in regulating O_2 supply, then the erythrocyte itself must be a critical component of this regulatory system since it is the only part of the O_2 transport pathway that is directly influenced by O_2 content. Erythrocytes traverse the entire vasculature and their O_2 content at a particular point in the tissue is directly linked to the level of tissue O_2 utilization. Therefore, if the erythrocyte itself were able to sense O_2 need and alter perfusion to meet that need, it would provide an efficient means of increasing blood flow wherever and whenever the need might arise eliminating the need for a diverse network of sensing sites throughout the vasculature.

The idea that the erythrocyte, the major supplier of O_2, might also serve as a sensor of O_2 requirements and affector of changes in O_2 supply has been the subject of intensive investigation over the past several years. Erythrocytes are known to contain millimolar amounts of adenosine 5-triphosphate (ATP) which is produced in the circulating erythrocyte primarily by membrane bound glycolytic pathways. It has been established that isolated healthy human erythrocytes (7,20), as well as those of rabbits (51,52), rats (14,18,20) and hamsters (20,23), release ATP when exposed to reduced O_2 tension. ATP release from human erythrocytes is detected when these cells are exposed to O_2 tensions of 23 ± 1 mmHg which is very near the point at which hemoglobin is 50% saturated (P_{50}) (Figure 1). These results are consistent with the observation of Jagger et al. that ATP release from rat erythrocytes correlates with the extent of hemoglobin desaturation (39). In humans, there is no observed gender difference in the amount of ATP released in response to this stimulus

(Figure 2). However, although not extensively studied in humans, an early study in male hamsters suggested that increasing age may moderate the response to low O_2 tension (23).

The rapid well-regulated release of ATP from erythrocytes exposed to reduced O_2 has been shown to occur via activation of a discrete intracellular signaling pathway (52,62–65,70,71). Key components of this pathway are the heterotrimeric G protein, Gi (52,63), adenylyl cyclase (62,65), increases in intracellular cAMP (62,65), protein kinase A (PKA) (65) and the cystic fibrosis transmembrane conductance regulator (CFTR) (64). Recently, the final conduit for ATP release in response to activation of the signaling pathway by reduced O_2 tension has been identified as pannexin 1 (48,71) This pathway is depicted in Figure 3.

Although the main components of the pathway for low O_2-induced ATP release have been characterized, a critical question remains. How can a decrease in O_2 tension lead to activation of Gi and, consequently, ATP release? The first insights into a possible mechanism came from the report that the conformational change in hemoglobin which occurs as it desaturates was required for low O_2 induced ATP release (39). It is well established that a significant component of erythrocyte hemoglobin is bound to the cell membrane (73). In vascular smooth muscle cells, G-proteins have been shown to be activated by mechanical stress (46). Therefore, it is reasonable to postulate that a similar stress resulting from the conformational change in the hemoglobin molecule as it releases O_2 could similarly activate Gi in the erythrocyte. In support of this hypothesis, we reported that mechanical deformation of erythrocytes in a filtrometer resulted in ATP release via a signaling pathway that is similar to that activated following exposure of erythrocytes to low O_2 (39,53,64,65,72). In addition, we found that reductions in erythrocyte membrane deformability are associated with impaired low O_2-induced ATP release (73). In the latter study neither direct activation of Gi nor ATP release in response to activation of an alternate signaling pathway were inhibited by decreased erythrocyte deformability. Taken together, these studies provide support for the hypothesis that ATP released from erythrocytes exposed to reduced O_2 is the result of desaturation of membrane bound hemoglobin resulting in mechanical activation of Gi. The exact mechanism by which Gi is activated by mechanical force remains under investigation.

Erythrocyte-released ATP initiates conducted vasodilation

If one accepts that ATP is released from erythrocytes in a controlled manner as they perfuse a region of tissue with a low O_2 tension, then this ATP must initiate a conducted vasodilation that extends beyond the site of initiation for there to be an effective increase in vascular perfusion (O_2 delivery) (45). Using arterioles in the intact hamster cheek pouch retractor muscle, McCullough et al. (49) demonstrated a concentration-dependent conducted vasodilator response to the intra-arteriolar application of ATP with the maximum dilation occurring at 10^{-6} M. Importantly, similar amounts of adenosine were ineffective in producing a conducted response in these vessels. The vasodilator response initiated by ATP was conducted as far as 1,200 μm upstream at a rate of approximately 50 μm/s. Since one would anticipate that the O_2 tension at the downstream end of the capillaries and in the venules would be most reflective of local tissue O_2 utilization, Collins et al. (11) subsequently investigated the impact of application of similar amounts of ATP into collecting venules. They observed a similar conducted vasodilation, the speed of which was influenced by the architecture of the intervening vasculature. In these studies, erythrocyte flux in the second order and terminal arterioles and associated capillaries increased by 66, 51 and 98%, respectively following the intravenular application of 10^{-6} M ATP. When this same concentration was placed into an arteriole or adjacent to a group of capillaries, tissue O_2 tension increased significantly (unpublished observation) again demonstrating an important effect on tissue O_2 supply. It is critical to note that the intraarteriolar application

of 10^{-6} M ATP, the concentration that produced the maximum conducted vasodilation, is of the same order of magnitude as would be predicted to be released from erythrocytes perfusing a microvessel within a hypoxic tissue region based on amounts of ATP released from isolated erythrocytes in vitro (7,20,23,39,49,73). Interestingly, the vasodilator response to the intravenular application of ATP occurred as a series of oscillations (11). Upon further investigation it was determined that the number of oscillations seen in a given experimental application of ATP coincided with the number of third order arterioles fed by the second order arteriole being studied (11). This result confirmed that the vascular architecture plays a role in the conducted response with signals transmitted along pathways of different lengths. In addition it established that conduction of the signal can occur through the capillary network supporting a role for the endothelium in its transmission (16,79). The physiological importance of this control mechanism was demonstrated in a study by Dietrich et al. (14) using isolated erythrocyte perfused rat cerebral arterioles. They showed that the time course for sensing of a low O_2 environment, the release of ATP from erythrocytes, and a vasodilatory response is on the order of 500 ms well within the time frame necessary for an effective perfusion control system for the regulation of O_2 delivery. This time course is consistent with in vitro studies evaluating the release of ATP from erythrocytes in response to mechanical deformation (27,78). In additional studies in which the O_2 tension on the surface of an intact muscle was lowered in a stepwise fashion using a computer-controlled gas flow chamber (19), the increases in flow that occurred within the capillary bed were likewise consistent with such a time course (personal communication from Dr. C.G. Ellis).

When ATP is released into the vessel lumen, it interacts with purinergic receptors on endothelial cells that can elicit both endothelium-dependent and smooth muscle cell-dependent vasoactive responses which are subsequently conducted along the microvessels. Several vasodilators have been reported to be synthesized subsequent to activation of purinergic receptors including nitric oxide (NO) and products of arachidonic acid metabolism (58). In skeletal muscle, McCullough and Collins (11,49) each observed that the conducted vasodilation to intraluminal ATP was eliminated or significantly diminished following systemic administration of the NO synthase inhibitor, L-NAME, implicating NO as an important vascular mediator in these vessels. The observation that at the highest concentration of ATP studied (10^{-4}M) some conducted response remained after the administration of L-NAME suggests that other factors may also be involved in the response. Although these studies demonstrate that NO is an important mediator of ATP-induced conducted vasodilation in hamster skeletal muscle, other affector molecules have been suggested to play such a role in other species and other vascular beds. For example, endothelial P_2Y_2-specific stimulation of large cerebral arterioles induced the release of NO as well as a non-NO, non-cyclooxygenase-dependent factor (81,82). However, a similar stimulation applied to smaller cerebral arterioles, resulted only in the production of the non-NO, non-cyclooxygenase-dependent factor (34). This factor is possibly a cytochrome P_{450} monooxygenase product such as epoxyeicosanoic acids (EETs) (15). EETs have been shown to activate calcium-sensitive potassium channels resulting in hyperpolarization (9,29) which can spread through gap junctions to induce upstream dilation.

Intracellular regulation of the signaling pathways for ATP release from erythrocytes

As shown in Figures 2 and 3, release of ATP from erythrocytes requires increases in cAMP. This cyclic nucleotide is a critical second messenger in multiple signaling pathways in all cells so localization of increases in cAMP within a given signaling pathway is essential to maintain discrete responses to an external stimulus (35). This compartmentalization of cAMP is accomplished largely by phosphodiesterases (PDEs) that are specific to individual

signaling pathways (2,4,5). Human erythrocytes express PDEs 2, 3A, 3B, 4 and 5, each of which has been shown to be associated with distinct signaling pathways for ATP release (2).

Extracellular regulation of signaling pathways for ATP release from erythrocytes

In addition to the activity of PDEs, erythrocyte-ATP release can be positively and negatively influenced by extracellular mediators. The binding of erythrocyte-derived ATP to purinergic receptors on endothelial cells results in the synthesis and release of vasodilators including prostacyclin (PGI_2) and NO (13,41). These vasodilators are released extraluminally where they act on vascular smooth muscle to produce vasodilation. However, PGI_2 and NO are also released into the vascular lumen where they interact with formed elements in the blood. For example, both vasodilators have been shown to inhibit the activation and/or aggregation of circulating platelets (57). In addition, we have determined that both PGI_2 and NO interact with erythrocytes serving as positive and negative feedback regulators of ATP release, respectively (53,62).

PGI_2 released into the vascular lumen can bind to specific receptors (IP receptors) present on erythrocytes (62). The IP receptor is coupled to the heterotrimeric G-protein Gs rather than Gi and stimulates ATP release from erythrocytes via a signaling pathway that also requires increases in cAMP that are regulated by an isoform of PDE3 (2,5,32,71). Although the IP receptor signaling pathway shares most components with the low O_2 pathway for ATP release, the PDE involved in this pathway is likely PDE3A while PDE3B regulates cAMP in the low O_2 pathway (Figure 4) (1,3,32). In addition, the final conduit for ATP release in the IP receptor signaling pathway is not pannexin 1 (71) but rather the voltage-dependent anion channel (VDAC) (72). Thus, PGI_2 released into the vascular lumen stimulates additional ATP release from erythrocytes in a *positive feedback* manner via activation of a signaling pathway distinct from the pathway activated by low O_2.

In contrast to the effect of PGI_2, NO released into the vascular lumen *inhibits* low O_2-induced ATP release, but not ATP release stimulated by activation of the IP receptor (53). Thus, when erythrocytes enter a tissue region where NO levels are already elevated, additional low O_2-induced ATP release would be inhibited. In this way NO interacts with erythrocytes in a *negative feedback fashion* to selectively inhibit additional ATP-induced synthesis of NO. Although the precise mechanism by which NO inhibits this signaling pathway has not been fully determined, NO appears to directly interfere with activation of Gi, a necessary event for low O_2-induced ATP release (53) (Figure 4).

In addition to feedback regulation by mediators released from the endothelium, ATP release from erythrocytes is also controlled by its effect on the tissue. In skeletal muscle, if ATP release from erythrocytes is, in large part, a consequence of exposure of the erythrocytes to reduced tissue O_2 levels, then when O_2 supply has been appropriately directed to that region by erythrocyte-released ATP, tissue PO_2 will be normalized and the stimulus for additional ATP release will be eliminated. This tight control between the sensing of O_2 need and response of the erythrocytes to that need is a classic example of a negative feedback system designed for optimization of O_2 supply in skeletal muscle.

Effect of general and directed vasodilation on the matching of O_2 delivery with need in skeletal muscle

The potential importance of erythrocyte-derived ATP as a regulator of O_2 supply to skeletal muscle is suggested by a recent study of erythrocytes from individuals with type 2 diabetes (DM2), a human condition in which skeletal muscle O_2 delivery is often significantly

compromised (12,28,50,51,76,77). In these studies, it was shown that although DM2 erythrocytes release reduced amounts of ATP in response to low O_2, ATP release in response to the prostacyclin analog, iloprost, was enhanced (68,69). Thus, although the mechanism to direct blood flow to regions of increased O_2 need in the skeletal muscle microcirculation would be impaired, general vasodilation involving both direct effects on the vascular smooth muscle and ATP release from erythrocytes via activation of IP receptors would be intact. To determine if increases in total blood flow produced by vasodilation that is not specifically directed to areas of increased O_2 need is sufficient to ameliorate defects in O_2 supply to skeletal muscle in this disease state, a computational model of O_2 transport by capillary networks, based on an array of 19 parallel capillaries was employed (67). The study demonstrated that in order for a uniform increase in total flow (general vasodilation) to eliminate low O_2 tension regions, total flow must be increased by a factor of 4. However, this global increase in perfusion resulted in an abnormally high tissue O_2 tension in some regions of the tissue. This result is in contrast to that predicted when blood flow was specifically directed to areas of need (low O_2-induced ATP release). Under the latter condition, a normal tissue O_2 tension was reestablished (67). These simulations strongly support the hypothesis that restoration of the ability of erythrocytes to release ATP in response to reduced O_2 tension would be of greater value in restoring O_2 delivery to meet metabolic need in skeletal muscle than would general vasodilators (67).

Abnormalities of ATP release from erythrocytes in human disease

If erythrocyte-released ATP is important for the regulation of vascular resistance *in vivo*, abnormalities in that release would be anticipated to be associated with human disease. Although the mechanisms responsible for defects in ATP release in all cases are not fully understood, in several human pathologies defects in erythrocyte ATP release have been identified (Table 1).

Cystic Fibrosis (CF)

In addition to ATP release in response to low O_2 or PGI_2, human erythrocytes also release ATP in response to mechanical deformation that they encounter when traversing the microcirculation (47,64). Several years ago we reported that erythrocytes of humans with CF released significantly less ATP when deformed by passage through pores of 8 or 5 μm in diameter in a filtrometer than did cells of healthy controls or humans with chronic obstructive pulmonary disease not related to CF (64). This defect in ATP release could not be attributed to decreased cellular ATP levels or altered erythrocyte deformability. Importantly, it has been shown that CF erythrocytes do not stimulate NO synthesis in the circulation of isolated rabbit lungs (47). Taken together these studies confirm the requirement for CFTR activity in ATP release pathways in human erythrocytes and demonstrate that defects in erythrocyte ATP release are associated with a human condition in which pulmonary vascular resistance is increased.

Idiopathic Pulmonary Arterial Hypertension (IPAH)

IPAH is a human condition characterized by increased pulmonary vascular resistance in the absence of severe airway disease and vascular remodeling in the lung accompanied by impaired NO synthesis and/activity (54,55). Although the mechanisms responsible for the development of IPAH remain under active investigation, we have shown that ATP release from erythrocytes of humans with IPAH is defective and could contribute to increased vascular resistance in this human condition (66). Like those of humans with CF, erythrocytes of humans with IPAH fail to release ATP when subjected to mechanical deformation (66). Again this defect could not be related to a decrease in total ATP content of the cells. However, in contrast to CF erythrocytes, cells from humans with IPAH were less

deformable than those of healthy controls (64,66). This finding is of particular interest because we have shown that decreased erythrocyte deformability is associated with impaired ATP release when erythrocytes are exposed to low O_2 but not when Gi is activated pharmacologically (73). Studies are currently underway to determine if one of the mainstays in the treatment of IPAH, PGI_2 and its analogs, stimulate ATP release from IPAH erythrocytes. Such a finding would provide a novel mechanism of action of these agents when administered therapeutically.

Type 2 Diabetes (DM2)

As noted above, individuals with DM2 demonstrate an inability to supply adequate amounts of O_2 to skin and skeletal muscle leading to delayed wound healing and claudication (8,41,50,75). Erythrocytes of humans with DM2 exhibit decreased expression of Gαi2. Since Gi is a component of the signaling pathway for low O_2-induced ATP release from erythrocytes (52,63), the consequence of this defect in Gi expression on ATP release from DM2 erythrocytes demanded to be investigated. It was determined that DM2 erythrocytes release reduced amounts of ATP in response to exposure to low O_2 tension as well as in response to direct activation of Gi (68,69). The functional consequence of impaired low O_2-induced ATP release is illustrated by studies in isolated arterioles. When 50 μm skeletal muscle arterioles perfused with healthy human erythrocytes were exposed to reduced extraluminal O_2 tension, the vessels dilated (67). However, when these same vessels were perfused with DM2 erythrocytes, the vessels failed to dilate in response to the same reduction in extraluminal O_2 tension (67). The latter finding supports the hypothesis that, in DM2, a defect in low O_2-induced ATP release from erythrocytes could contribute to the development and severity of the associated peripheral vascular disease.

As discussed above, computational modeling demonstrated that restoration of the ability of erythrocytes to release ATP in response to low O_2 tension in DM2 would be of greater value in restoring O_2 delivery to meet metabolic need in skeletal muscle than would general vasodilators (67). Therefore, there could be a significant beneficial effect on O_2 delivery to skeletal muscle in DM2 if the capacity of erythrocytes to direct flow to specific tissue regions could be restored pharmacologically. Since levels of cAMP are critical for the regulation of ATP release, one could expect that protection of any cAMP that is generated in response to exposure of DM2 erythrocytes to low O_2 would have therapeutic value (Figure 2). The phosphodiesterase (PDE) that degrades increases in cAMP in this signaling pathway has been identified as PDE3B (31). Recently we tested the hypothesis that a selective inhibitor of PDE3 activity would restore low O_2 induced ATP release from human DM2 erythrocytes. Indeed, pretreatment of these cells with cilostazol, a PDE3 inhibitor that is used clinically, restored low O_2-induced ATP release (61). However, of far greater physiological importance, it was shown that cilostazol restored the ability of DM2 erythrocytes to stimulate dilation of isolated perfused skeletal muscle arterioles exposed to reduced extraluminal O_2 (61). These results demonstrate that defects in low O_2-induced ATP release from DM2 erythrocytes can be corrected pharmacologically and suggest that erythrocytes should be viewed as a novel target for the development of new approaches for the treatment of the peripheral vascular disease.

Prediabetes

It is well recognized that impaired vascular function is often present at the time of diagnosis of DM2 suggesting that this major complication of diabetes begins during the prediabetic period (30,36,37,79). Prediabetes is a precursor to DM2 and is characterized by the presence of supra-normal circulating insulin levels that are required to maintain blood glucose within the normal range in the face of peripheral insulin resistance (6,40,60,80). Using the Zucker Diabetic Fatty rat (ZDF) model of DM2 we found that, during the prediabetic period, total

O_2 supply to the extensor digitorum longus muscle was 64% of control due to a significantly lower O_2 supply per capillary and higher O_2 extraction (18). These findings suggested that the average muscle tissue O_2 tension in the prediabetic animals was significantly lower implicating a maldistribution of microvascular perfusion, an interpretation confirmed by computational modeling (18). In these studies we determined that erythrocytes of both control animals and animals with prediabetes release ATP in response to low O_2 when washed to remove the insulin that was present. In contrast, when the same erythrocytes were subsequently incubated with insulin at concentrations present in the prediabetic rats, ATP release to low O_2 was inhibited (18). In separate studies it was shown that insulin inhibited both low O_2-induced ATP release from human erythrocytes and the ability of these cells to stimulate dilation of isolated arterioles exposed to reduced extraluminal O_2 (61). We then examined the mechanism by which insulin could exert such an effect. In adipocytes and hepatocytes insulin has been shown to activate a specific phosphodiesterase, PDE3B (43,81). Since PDE3B is equally expressed in healthy humans and DM2 erythrocytes (61) and PDE3 activity regulates increases in cAMP levels in the low O_2-induced ATP release pathway (31,32), we investigated the hypothesis that inhibitors of PDE3 could oppose the adverse effects of insulin on ATP release. We determined that pretreatment of erythrocytes with either the PDE3 inhibitor, cilostazol, or an inhibitor of phosphoinositide 3-kinase, a component of the insulin signaling pathway, prevented insulin-induced inhibition of low O_2-induced ATP release from human erythrocytes (30,31). Importantly, cilostazol also restored the ability of erythrocytes exposed to insulin to dilate arterioles exposed to reduced extraluminal O_2 (30). These studies suggest that early intervention with PDE3 inhibitors could have utility in delaying or preventing a component of the vascular disease of DM2.

Summary and future directions

Much remains to be learned regarding the contribution of low O_2-induced ATP release from erythrocytes to the regulation of the distribution of microvascular perfusion. Future studies will require a combination of new *in vitro* and *in situ* approaches coupled with detailed computational modeling. The predictions of such evidence-based modeling must then be tested and validated using *in vivo* experiments under a range of physiological and pathophysiological conditions. What is clear is that healthy erythrocytes release physiologically relevant amounts of ATP in a tightly controlled manner via activation of distinct G protein-coupled signal transduction pathways. Importantly, ATP released in the microcirculation of skeletal muscle stimulates conducted vasodilation that results in increased erythrocyte supply rate and tissue O_2 delivery.

The mechanism described here for the regulation of the distribution of perfusion in skeletal muscle involves both intracellular signaling and intercellular communication between and among various cell types and is modulated by both positive and negative feedback controllers. If this control system is important for optimizing O_2 supply under physiological conditions, then any compromise of this system would likely disrupt the homeostatic balance. The observation that reduced ATP release from erythrocytes is found in a number of human conditions associated with abnormal levels of perfusion pressure or perfusion distribution supports the hypothesis that erythrocyte-released ATP is an important component in the regulation of perfusion. If this is the case, then the erythrocyte should be considered a valid therapeutic target for the development of novel approaches for the treatment of diseases such as pulmonary hypertension and diabetes.

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References

1. Adderley SP, Dufaux EA, Sridharan M, Bowles EA, Hanson MS, Stephenson AH, Ellsworth ML, Sprague RS. Iloprost-and isoproterenol-induced increases in cAMP are regulated by different phosphodiesterases in erythrocytes of both rabbits and humans. Am J Physiol. 2009; 296:H1617–H1624.
2. Adderley SP, Sprague RS, Stephenson AH, Hanson MS. Regulation of cAMP by phosphodiesterases in erythrocytes. Pharmacol Rep. 2010; 62(3):475–482. [PubMed: 20631411]
3. Adderley SP, Sridharan M, Bowles EA, Stephenson AH, Ellsworth ML, Sprague RS. Protein Kinases A and C regulate receptor-Mediated increases in cAMP in rabbit erythrocytes. Am J Physiol. 2010; 298:587–593.
4. Adderley SP, Sridharan M, Bowles EA, Stephenson AH, Sprague RS, Ellsworth ML. Inhibition of ATP release from erythrocytes: a role for EPACs and PKC. Microcirc. 2011; 18(2):128–135.
5. Adderley SP, Thuet KM, Sridharan M, Bowles EA, Stephenson AH, Ellsworth ML, Sprague RS. Identification of cytosolic phosphodiesterases in the erythrocyte: a possible role for PDE5. Med Sci Monit. 2011; 17(5):CR241–CR247. [PubMed: 21525805]
6. Arcaro G, Cretti A, Balzano S, Lechi A, Muggeo M, Bonora E, Bonodomma RC. Insulin causes endothelial dysfunction in humans. Circulation. 2002; 105:576–582. [PubMed: 11827922]
7. Bergfeld GR, Forrester T. Release of ATP from human erythrocytes in response to a brief period of hypoxia and hypercapnia. Cardiovasc. Res. 1992; 26:40–47. [PubMed: 1325292]
8. Brand FN, Abbott RD, Kanel WB. Diabetes, intermittent claudication and risk of cardiovascular events. The Framingham Study. Diabetes. 1989; 38(4):504–509. [PubMed: 2925008]
9. Campbell WB, Gebremedhin D, Pratt PF, Harder DR. Identification of epoxyeicosatrienoic acids as endothelium derived hyperpolarizing factors. Circ Res. 1996; 78:415–423. [PubMed: 8593700]
10. Chalmers C, Bird BD, Whitwam JG. Evaluation of a new thin film tonometer. Br. J. Anaesth. 1974; 46:253–259. [PubMed: 4451597]
11. Collins DM, McCullough WT, Ellsworth ML. Conducted vascular responses: communication across the capillary bed. Microvasc Res. 1998; 56(1):43–53. [PubMed: 9683562]
12. Collison DJ, Rea R, Donnelly R. Masterclass series in peripheral arterial disease. Vasc. Med. 2004; 9:307–310. [PubMed: 15678624]
13. Dazel HH, Westfall DP. Receptors for adenine nucleotides and nucleosides: Subclassification, distribution and molecular characterization. Pharmacolog Rev. 1994; 46:449–466.
14. Dietrich HH, Ellsworth ML, Sprague RS, Dacey RG Jr. Red blood cell regulation of microvascular tone through adenosine triphosphate. Am J Physiol. 2000; 278(4):H1294–H1298.
15. Dietrich HH, Horiuchi T, Xiang C, Falck JR, Hongo K, Dacey RG Jr. Mechanism of ATP-induced local and conducted vasomotor responses in isolated rat cerebral penetrating arterioles. J Vasc Res. 2008; 46:253–264. [PubMed: 18984964]
16. Dietrich HH, Tyml K. Capillary as a communicating medium in the microvasculature. Microvasc Res. 1992; 43:87–99. [PubMed: 1318992]
17. Duling BR, Berne RM. Longitudinal gradients in periarteriolar oxygen tension. A possible mechanism for the participation of oxygen in local regulation of blood flow. Circ Res. 1970; 27:669–678. [PubMed: 5486243]
18. Ellis CG, Goldman D, Hanson MS, Stephenson AH, Milkovich S, Benlamri A, Ellsworth ML, Sprague RS. Defects in oxygen supply to skeletal muscle of prediabetic ZDF rats. Am. J. Physiol. 2010; 298:H1661–H1670.
19. Ellis CG, Milkovich S, Goldman D. Experimental protocol investigating local regulation of oxygen supply in rat skeletal muscle in vivo. J. Vasc. Res. 2006; 43(Suppl.1):45.
20. Ellsworth ML. The red blood cell as an oxygen sensor: what is the evidence? Acta Physiol Scand. 2000; 168:551–559. [PubMed: 10759592]

21. Ellsworth ML, Ellis CG, Goldman D, Stephenson AH, Dietrich HH, Sprague RS. Erythrocytes: oxygen sensors and modulators of vascular tone. Physiology (Bethesda). 2009; 24:107–116. [PubMed: 19364913]
22. Ellsworth ML, Ellis CG, Popel AS, Pittman RN. Role of microvessels in oxygen supply to tissue. NIPS. 1994; 9:119–123.
23. Ellsworth ML, Forrester T, Ellis CG, Dietrich HH. The erythrocyte as a regulator of vascular tone. Am J Physiol. 1995; 269:H2155–H2161. [PubMed: 8594927]
24. Ellsworth ML, Pittman RN. Heterogeneity of oxygen diffusion through hamster striated muscles. Am. J. Physiol. 1984; 246:H161–H167. [PubMed: 6696127]
25. Ellsworth ML, Pittman RN. Arterioles supply oxygen to capillaries by diffusion as well as by convection. Am J Physiol. 1990; 258:H1240–H1243. [PubMed: 2331012]
26. Ellsworth ML, Popel AS, Pittman RN. Assessment and impact of heterogeneities of convective oxygen transport parameters in capillaries of striated muscle: Experimental and theoretical. Microvasc. Res. 1988; 35:241–362.
27. Forsyth AM, Wan J, Owrutsky PD, Abkarian M, Stone HA. Multiscale approach to link red blood cell dynamics, shear viscosity, and ATP release. Proc Natl Acad Sci USA. 2011; 108(27):10986–10991. [PubMed: 21690355]
28. Fowkes FG. Epidemiological research on peripheral vascular disease. J. Clin. Epidemiol. 2001; 54:863–868. [PubMed: 11520644]
29. Fukao M, Mason HS, Kenyon JL, Horowitz B, Keef KD. Regulation of BK(Ca) channels expressed in human embryonic kidney 293 cells by epoxyeicosatrienoic acid. Mol Pharmacol. 2001; 59:16–23. [PubMed: 11125019]
30. Hanson MS, Ellsworth ML, Achilleus D, Stephenson AH, Bowles EA, Sridharan M, Adderley A, Sprague RS. Insulin inhibits low oxygen-induced ATP release from human erythrocytes: Implications for vascular control. Microcirc. 2009; 16:424–433.
31. Hanson MS, Stephenson AH, Bowles EA, Sprague RS. Insulin inhibits human erythrocyte cAMP accumulation and ATP release: role of phosphodiesterase 3 and phosphoinositide 3-kinase. Exp Biol & Med. 2010; 235:256–262.
32. Hanson MS, Stephenson AH, Bowles EA, Sridharan M, Adderley S, Sprague RS. Phosphodiesterase 3 is present in rabbit and human erythrocytes and its inhibition potentiates iloprost-induced increases in cAMP. Am. J. Physiol. 2008; 295(2):H786–H793.
33. Hester RL. Uptake of metabolites by postcapillary venules: mechanism for the control of arteriolar diameter. Microvasc Res. 1993; 46:254–261. [PubMed: 7504161]
34. Horiuchi T, Dietrich HH, Hongo K, Dacey RG Jr. Comparison of P_2 receptor subtypes producing dilation in rat intracerebral arterioles. Stroke. 2003; 34:1473–1478. [PubMed: 12730558]
35. Iancu RV, Jones SW, Harvey RD. Compartmentation of cAMP signaling in cardiac myocytes: a computational study. Biophys J. 2007; 92(9):3317–3331. [Erratum in: Biophys J. 5;94 (2):714-, 2008]. [PubMed: 17293406]
36. Jaap AJ, Hammersley MS, Shore AC, Tooke JE. Reduced microvascular hyperaemia in subjects at risk of developing type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia. 1994; 37:214–216. [PubMed: 8163058]
37. Jaap AJ, Shore AC, Tooke JE. Relationship of insulin resistance to microvascular dysfunction in subjects with fasting hyperglycemia. Diabetologia. 1997; 40:238–243. [PubMed: 9049487]
38. Jackson WF. Arteriolar oxygen reactivity: where is the sensor. Am J Physiol. 1987; 253(22):H1120–H1126. [PubMed: 3318502]
39. Jagger JE, Bateman RM, Ellsworth ML, Ellis CG. Role of erythrocyte in regulating local O_2 delivery mediated by hemoglobin oxygenation. Am. J. Physiol. 2001; 280:H2833–H2839.
40. Kanauchi M, Kanauchi K, Inoue T, Kimura K, Saito Y. Surrogate markers of insulin resistance in assessing individuals with new categories “prehypertension” and “prediabetes”. Clin chem. Lab Med. 2007; 45:35–39. [PubMed: 17243912]
41. Kennedy C, Delbro D, Burnstock G. P_2-purinoceptors mediate both vasodilation and vasoconstriction of the isolated rat femoral artery. Eur. J. Pharmacol. 1985; 1072:161–168. [PubMed: 2984001]

42. King L. Impaired wound healing in patients with diabetes. Nurs. Stand. 2001; 15(38):39–45. [PubMed: 12205773]
43. Kitamura T, Kitamura Y, Kuroda S, Hino Y, Ando M, Kotani K, Konishi H, Matsuzaki H, Kikkawa U, Ogawa W, Kasuga M. Insulin-induced phosphorylation and activation of cyclic nucleotide phosphodiesterase 3B by the serine-threonine kinase Akt. Mol Cell Biol. 1999; 19:6286–6296. [PubMed: 10454575]
44. Krogh, A.; Krogh, A.; Lecture, II. The Anatomy and Physiology of Capillaries. Hafner Publishing Company; 1959. The distribution and number of capillaries in selected organs; p. 231929
45. Kurjiaka DT, Segal SS. Conducted vasodilation elevates flow in arteriole networks of hamster striated muscle. Am J Physiol. 1995; 269:H1723–H1728. [PubMed: 7503270]
46. Li C, Xu Q. Mechanical stress-initiated signal transductions in vascular smooth muscle. Cellular Signaling. 2000; 12:435–445.
47. Liang G, Stephenson AH, Lonigro AJ, Sprague RS. Erythrocytes of humans with cystic fibrosis fail to stimulate nitric oxide synthesis in isolated rabbit lungs. Am. J. Physiol. 2005; 288:H1580–H1585.
48. Locovei S, Bao L, Dahl G. Pannexin 1 in erythrocytes: function without a gap. Proc Natl Acad Sci U S A. 2006; 103:7655–7659. [PubMed: 16682648]
49. McCullough WT, Collins DM, Ellsworth ML. Arteriolar responses to extracellular ATP in striated muscle. Am J Physiol. 1997; 272:H1886–H1891. [PubMed: 9139975]
50. Melher P, Jeffers B, Estacio R, Schrier R. Association of hypertension and complications in NIDDM. Am. J. Hyperten. 1997; 10:152–161.
51. Morrish NJ, Wang S-L, Stevens LK, Fuller JH. Keen H and the WHO Multinational Study Group. Mortality and causes of death in the WHO multinational study of vascular disease in diabetes. Diabetologia. 2001; 44:S14–S21. [PubMed: 11587045]
52. Olearczyk JJ, Stephenson AH, Lonigro AJ, Sprague RS. Heterotrimeric G Protein Gi is Involved in a Signal Transduction Pathway for ATP Release from Erythrocytes. Am. J. Physiol. 2004; 286:H940–H945.
53. Olearczyk JJ, Stephenson AH, Lonigro AJ, Sprague RS. NO Inhibits Signal Transduction Pathway for ATP Release from Erythrocytes via Its Action on Heterotrimeric G Protein Gi. Am. J. Physiol. 2004; 287:H748–H754.
54. Olschewski H, Olschewski A, Rose F, Schermuly R, Schutte H, Weissmann N, Seeger W, Grimminger F. Physiological basis for the treatment of pulmonary hypertension. J Lab Clin Med. 2001; 138:287–297. [PubMed: 11709653]
55. Olschewski H, Rose F, Grunig E, Ghofrani HA, Walmrath D, Schulz R, Schermuly R, Grimminger F, Seeger W. Cellular pathophysiology and therapy of pulmonary hypertension. J Lab Clin Med. 2001; 138:367–377. [PubMed: 11753283]
56. Pittman RN, Duling BR. Oxygen sensitivity of vascular smooth muscle. Microvasc Res. 1973; 6:202–211. [PubMed: 4748020]
57. Radomski MW, Palmer RM, Moncada S. Comparative pharmacology of endothelium-derived relaxing factor, nitric oxide and prostacyclin in platelets. Br J Pharmacol. 1987; 92(1):181–187. [PubMed: 3311265]
58. Saito Y, Eraslan A, Hester RL. Role of EDRFs in the control of arteriolar diameter during increased metabolism of striated muscle. Am J Physiol. 1994; 267:H195–H200. [PubMed: 8048585]
59. Secomb TW, Hsu R. Simulation of O_2 transport in skeletal muscle: diffusive exchange between arterioles and capillaries. Am J Physiol. 1994; 267:H1214–H1221. [PubMed: 8092288]
60. Segure JS, Campo C, Ruilope LM, Rodicio JL. Do we need to target ‘prediabeti’ hypertensive patients? J Hyperten. 2005; 23:2119–2125.
61. Sprague RS, Bowles EA, Achilleus D, Stephenson AH, Ellis CG, Ellsworth ML. A selective phosphodiesterase 3 inhibitor rescues low PO_2-induced ATP release from erythrocytes of humans with type 2 diabetes: implications for vascular control. Am J Physiol. 2011 Sept.Epub;
62. Sprague RS, Bowles EA, Hanson MS, DuFaux E, Sridharan M, Adderley S, Ellsworth ML, Stephenson AH. Prostacyclin analogues stimulate receptor-mediated cAMP synthesis and ATP release from rabbit and human erythrocytes. Microcirc. 2008; 15:461–471.

63. Sprague RS, Bowles EA, Olearczyk JJ, Stephenson AH, Lonigro AJ. The role of G protein β subunits in the release of ATP from human erythrocytes. J. Physiol. and Pharmacol. 2002; 53:667–674. [PubMed: 12512701]
64. Sprague RS, Ellsworth ML, Stephenson AH, Kleinhenz ME, Lonigro AJ. Deformation-induced ATP release from red blood cells requires cystic fibrosis transmembrane conductance regulator activity. Am. J. Physiol. 1998; 275:H1726–H1732. [PubMed: 9815080]
65. Sprague RS, Ellsworth ML, Stephenson AH, Lonigro AJ. Participation of cAMP in a signal-transduction pathway relating erythrocyte deformation to ATP Release. Am. J. Physiol. 2001; 281:C1158–C1164.
66. Sprague RS, Ellsworth ML, Stephenson AH, Lonigro AJ. Impaired Release of ATP from Red Blood Cells of Humans with Primary Pulmonary Hypertension. Exp. Biol. and Med. 2001; 226:434–439.
67. Sprague RS, Goldman D, Bowles EA, Achilleus D, Stephenson AH, Ellis CG, Ellsworth ML. Divergent effects of low O_2 tension and iloprost on ATP release from erythrocytes of humans with type 2 diabetes: Implications for O_2 supply to skeletal muscle. Am. J. Physiol. 2010; 299:H566–H573.
68. Sprague RS, Stephenson AH, Bowles EA, Stumpf MS, Lonigro AJ. Reduced expression of Gi in erythrocytes of humans with diabetes type 2 is associated with impairment of both cAMP generation and ATP release. Diabetes. 2006; 55:3588–3593. [PubMed: 17130508]
69. Sprague RS, Stephenson AH, Bowles EA, Stumpf MS, Ricketts G, Lonigro A. Expression of the heterotrimeric G protein Gi and ATP release are impaired in erythrocytes of humans with diabetes mellitus. Adv. Exp. Med. & Bio. 2006; 588:207–216. [PubMed: 17089891]
70. Sprague RS, Stephenson AH, Ellsworth ML. Red not dead: signaling in and from erythrocytes. Trends Endocrinol Metab. 2007; 18(9):350–355. [PubMed: 17959385]
71. Sridharan M, Adderley SP, Bowles EA, Egan TM, Stephenson AH, Ellsworth ML, Sprague RS. Pannexin 1 is the conduit for low oxygen tension-induced ATP release from human erythrocytes. Am. J. Physiol. 2010; 299:H1146–H1152.
72. Sridharan M, Bowles EA, Richards JP, Krantic M, Davis KL, Dietrich KA, Stephenson AH, Ellsworth ML, Sprague RS. Prostacyclin Receptor-Mediated ATP Release from Erythrocytes Requires the Voltage-Dependent Anion Channel (VDAC). Am J Physiol Heart Circ Physiol. 2011 Dec 9. [Epub ahead of print].
73. Sridharan M, Sprague RS, Adderley SP, Bowles EA, Ellsworth ML, Stephenson AH. Diamide decreases deformability of rabbit erythrocytes and attenuates low oxygen tension-induced ATP release. Exp Biol Med (Maywood). 2010; 235(9):1142–1148. [PubMed: 20682601]
74. Stein JC, Ellis CG, Ellsworth ML. Relationship between capillary and systemic venous PO_2 during nonhypoxic and hypoxic ventilation. Am J Physiol. 1993; 265:H537–H542. 1993. [PubMed: 8368357]
75. Stein JC, Ellsworth ML. Capillary oxygen transport during severe hypoxia: role of hemoglobin oxygen affinity. J Appl Physiol. 1993; 75(4):1601–1607. [PubMed: 8282609]
76. Stern MP. The effect off glycemic control ion the incidence of macrovascular complications of diabetes. Arch. Fam. Med. 1998; 7:155–162. [PubMed: 9519921]
77. Uusitipa M, Niskanen L, Siitonen O, Pyorala K. 5-year incidence of atherosclerotic vascular disease in relation to gender, risk factors, insulin level and abnormalities in lipoprotein composition in non-insulin dependent diabetic and non-diabetic individuals. Circulation. 1990; 82:27–36. [PubMed: 2194696]
78. Wan J, Ristenpart WD, Stone HA. Dynamics of shear-induced ATP release from red blood cells. Proc Natl Acad Sci U S A. 2008; 105(43):16432–16437. [PubMed: 18922780]
79. Welsh DG, Segal SS. Endothelial and smooth muscle cell conduction in arterioles controlling blood flow. Am. J. Physiol. 1998; 274:H178–H186. [PubMed: 9458866]
80. Wiernsperger N. Defects in microvascular haemodynamics during prediabetes: contributor or epiphenomenon? Diabetologica. 2000; 43:1439–1448.
81. Wijkander J, Landstrom TR, Manganiello V, Belfrage P, Degerman E. Insulin-induced phosphorylation and activation of phosphodiesterase 3B in rat adipocytes: possible role for protein

kinase B but not mitogen-activated protein kinase or p70 S6 kinase. Endocrinology. 1998; 139:219–227. [PubMed: 9421418]

82. You J, Johnson TD, Childres WF, Bryan RM Jr. Endothelial-mediated dilations of rat middle cerebral arteries by ATP and ADP. Am J Physiol. 1997; 273 H1472-H147.
83. You JP, Johnson TD, Marrelli SP, Bryan RM Jr. Functional heterogeneity of endothelial P_2 purinoceptors in the cerebrovascular tree of the rat. Am J Physiol. 1999; 277:H893–H900. [PubMed: 10484408]

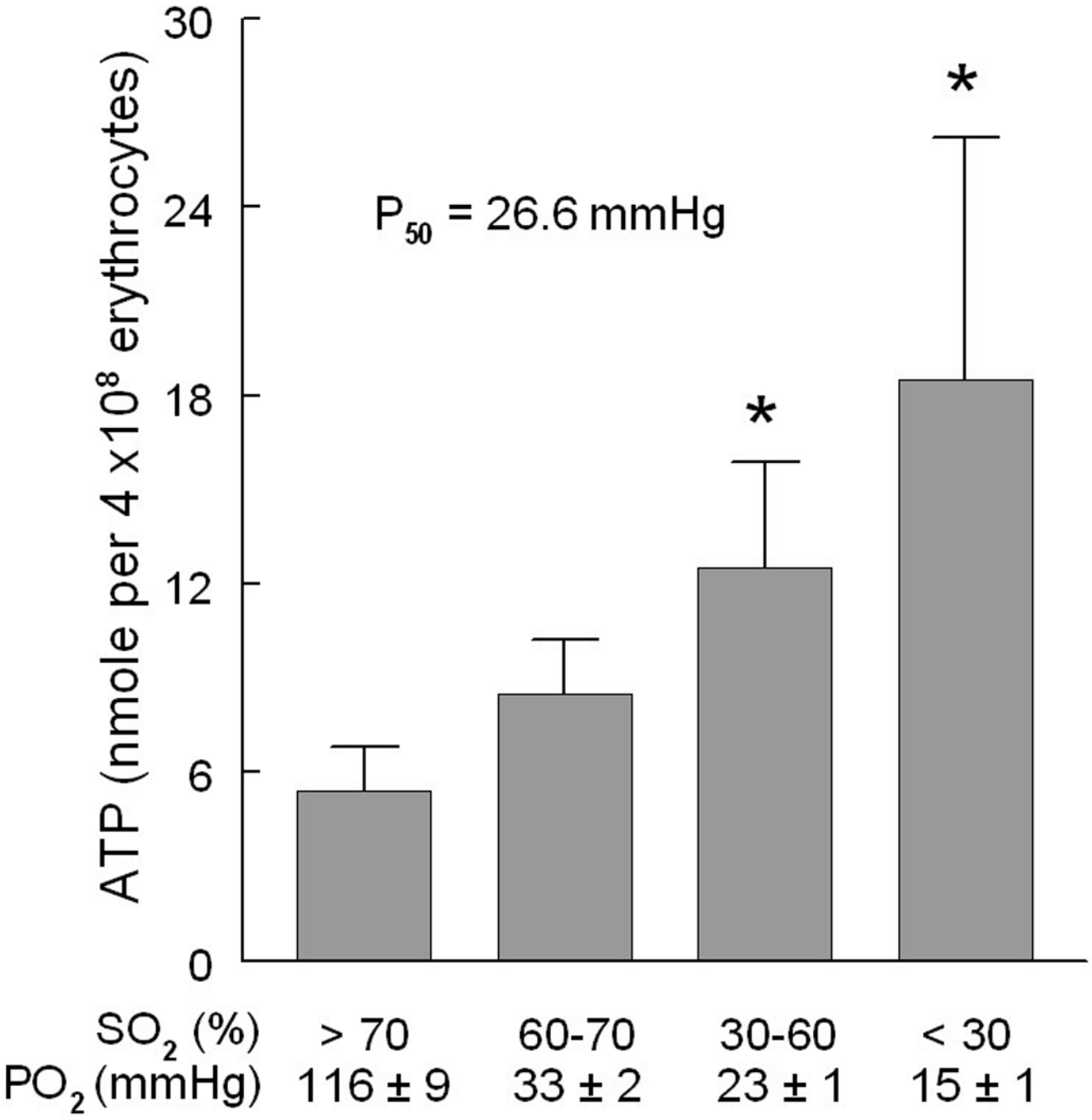

Figure 1.

Effect of exposure to reduced O_2 tension on ATP release from erythrocytes of healthy humans. In a thin-film tonometer (10), isolated erythrocytes (hematocrit 20%) were exposed to gas mixtures containing 15% O_2, 4.5% O_2, 2% O_2 or 0% O_2 in combination with 6% CO_2 and balance nitrogen. ATP release was determined after a 30 min equilibration with 15% O_2 and 10 min after exposure to the reduced O_2 levels. SO_2 = percent hemoglobin saturation. Values are the means ± SE. * = greater than value at SO_2 >70 ($p< 0.05$, ANOVA followed by pos hoc testing (LSD)).

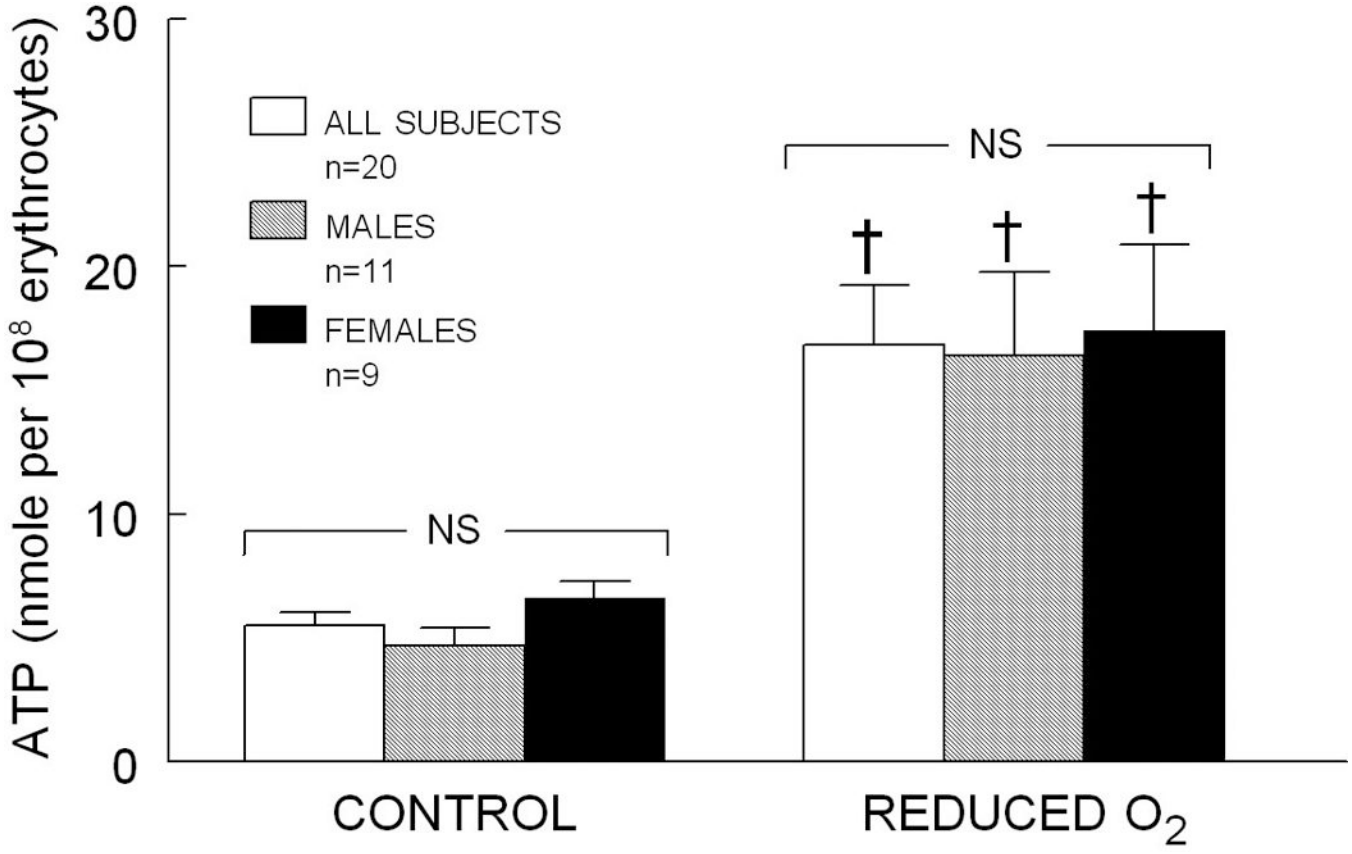

Figure 2.

Effect of exposure to reduced O_2 tension on ATP release from erythrocytes of healthy humans. In a thin-film tonometer (10), isolated erythrocytes (hematocrit 20%) were exposed to gas mixtures containing either 15% O_2, 6% CO_2, balance nitrogen (CONTROL) or 0% O_2, 6% CO_2, balance nitrogen (REDUCED O_2). ATP release was determined after a 30 min equilibration with 15% O_2 and 10 min after exposure to 0% O_2. The PO_2 values during normoxia were 116±4, 119±6 and 113±5 mm Hg for all subjects, males and females, respectively. In response to low O_2, the PO_2 values were 18±2, 19±3 and 18±2 mm Hg, for the three groups. Values are the means ± SE. † = greater than respective CONTROL value ($p < 0.01$, ANOVA followed by pos hoc testing (LSD)). NS = no significant difference.

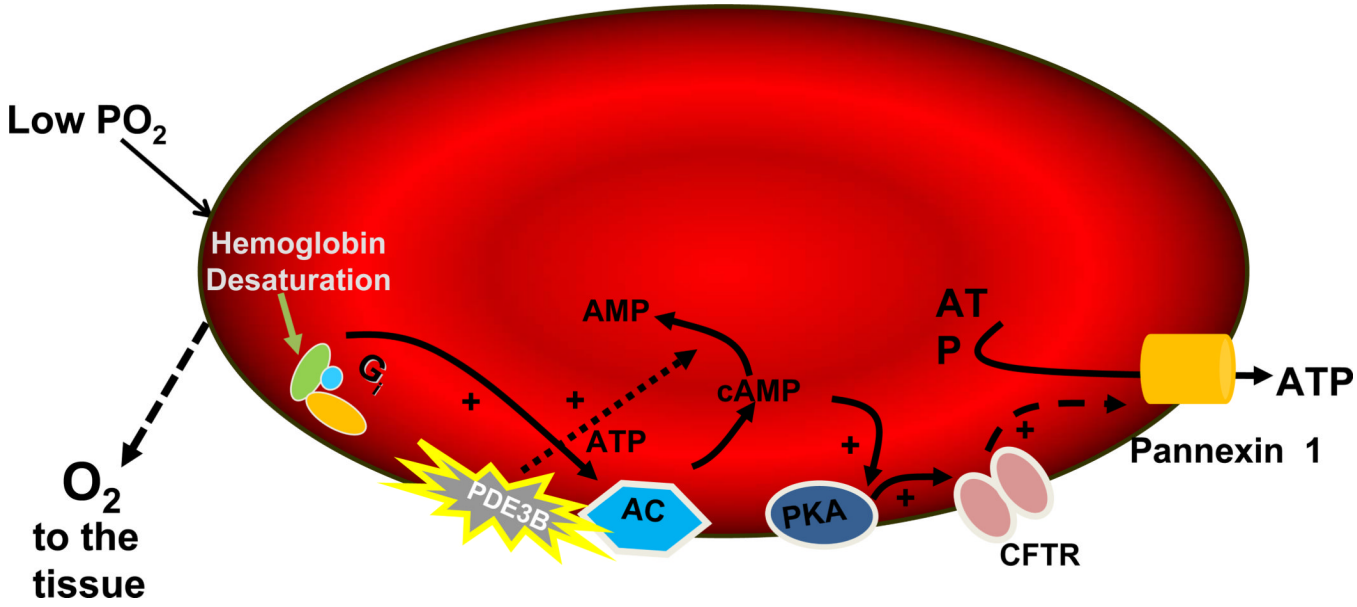

Figure 3.

Proposed signaling pathway for ATP release from erythrocytes in response to exposure to low O_2. Exposure of erythrocytes of low O_2 results in desaturation of hemoglobin and activation of Gi leading to increases in cAMP that are regulated by PDE3B activity. Increases in cAMP activate PKA and, subsequently, CFTR. The final conduit for ATP release in this pathway is pannexin 1.

Abbreviations: EC = endothelial cells, SMC = vascular smooth muscle cells; Gi = heterotrimeric G protein; PDE3B = phosphodiesterase 3B; cAMP = cyclic adenosine monophosphate; AMP = adenosine monophosphate; AC = adenylyl cyclase; PKA = protein kinase A; CFTR = cystic fibrosis transmembrane conductance regulator; ATP = adenosine triphosphate; P_{2y} = purinergic receptor of the P_{2y} group; NO = nitric oxide; PGI_2 = prostacyclin; EDHF = endothelium-derived hyperpolarizing factors; **(+)** = activation and **(−)** = inhibition.

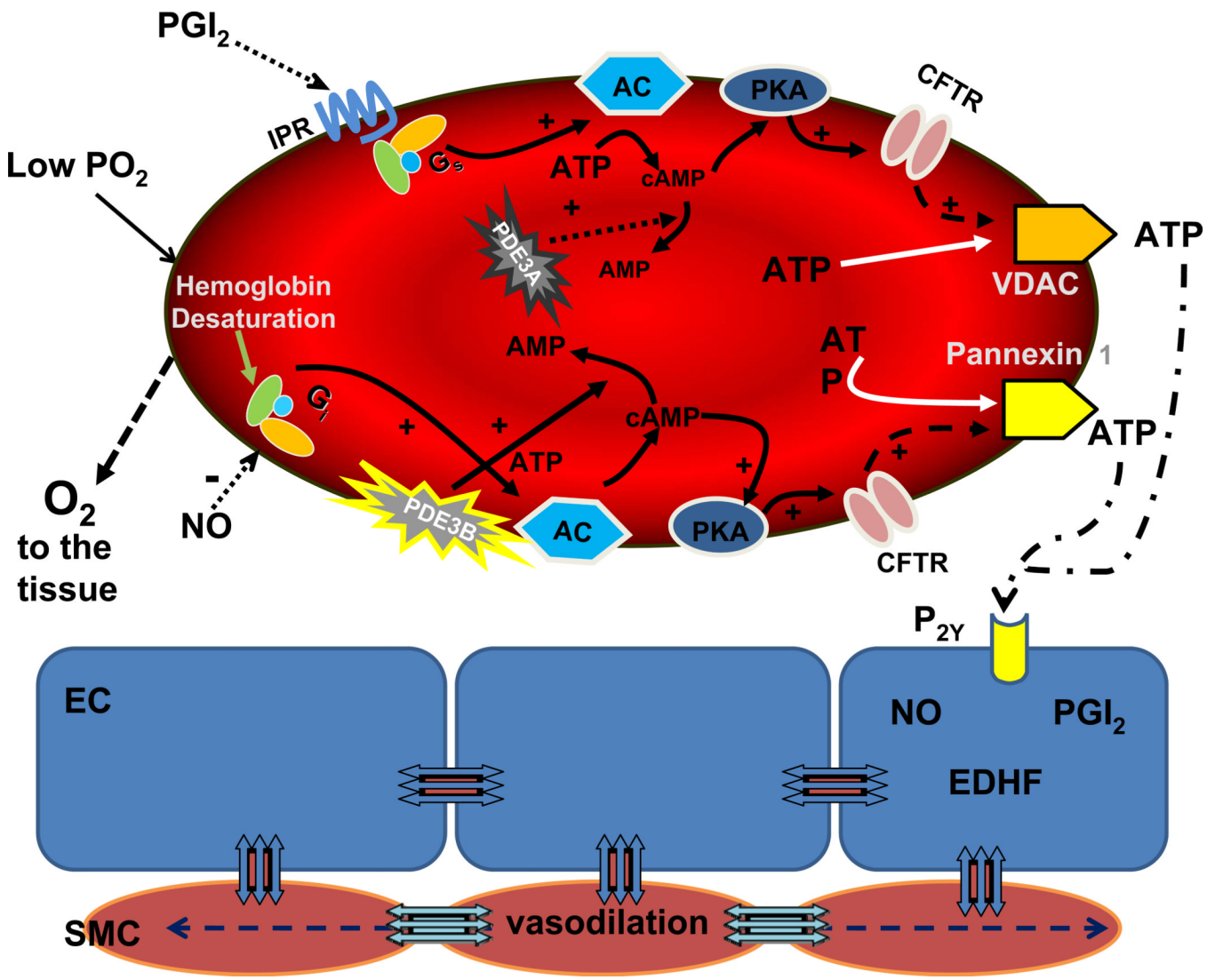

Figure 4.

Proposed signaling pathways for ATP release from erythrocytes via activation of the prostacyclin receptor (IPR) and the interaction of nitric oxide (NO) with the pathway for low O_2-induced ATP release. Exposure of erythrocytes to prostacyclin (PGI_2), or it analogs, results in activation of the Gs-coupled IPR leading to increases in cAMP that are regulated by PDE3A activity. Increases in cAMP activate PKA and, subsequently, CFTR. The final conduit for ATP release in the IPR signaling pathway is the voltage-dependent anion channel (VDAC).

Abbreviations: EC = endothelial cells, SMC = vascular smooth muscle cells; Gi = heterotrimeric G protein; Gs = heterotrimeric G protein; PDE3B = phosphodiesterase 3B; PDE3A = phosphodiesterase 3A; cAMP = cyclic adenosine monophosphate; AMP = adenosine monophosphate; AC = adenylyl cyclase; PKA = protein kinase A; CFTR = cystic fibrosis transmembrane conductance regulator; ATP = adenosine triphosphate; P_{2y} = purinergic receptor of the P_{2y} group; NO = nitric oxide; PGI_2 = prostacyclin; EDHF = endothelium-derived hyperpolarizing factors; **(+)** = activation and **(−)** = inhibition.

Table 1

Pathological Conditions Associated with Decreased ATP Release from Erythrocytes

Clinical Condition	Probable or Potential Site of Defect	Potential Remedy	References
Cystic Fibrosis	CFTR inactivity	?	47,64
Idiopathic Pulmonary Arterial Hypertension	?	PGI_2 analogs and/or PDE inhibitors	62,66
Diabetes	Decreased erythrocyte expression of Gi Decreased erythrocyte deformability	Inhibit PDE3 and/or administer PGI_2 Enhance deformability	61,67–69
Prediabetes	Elevated insulin increases activity of PDE3	Inhibit PDE3	18,30,31