Published in final edited form as:

Physiology (Bethesda). 2009 April ; 24: 107–116. doi:10.1152/physiol.00038.2008.

Erythrocytes: Oxygen Sensors and Modulators of Vascular Tone in Regions of Low PO₂

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Abstract

Through oxygen-dependent release of the vasodilator ATP, the mobile erythrocyte plays a fundamental role in matching microvascular oxygen supply with local tissue oxygen demand. Signal transduction within the erythrocyte and microvessels as well as feedback mechanisms controlling ATP release have been described. Our understanding of the impact of this novel control mechanism will rely on the integration of *in vivo* experiments and computational models.

Summary—Erythrocytes play a fundamental role in tissue oxygen supply via the controlled release of ATP in areas of increased oxygen need.

The matching of oxygen supply with oxygen demand in metabolically active tissue is a fundamental physiological process. Although a number of theories to explain this critical function have been proposed, none have been either universally accepted or fully tested in the intact microcirculation. Any attempt to comprehend the mechanism(s) by which oxygen delivery and metabolic need are matched must, by necessity, be based on the combination of experimental observations and theoretical models. In 1919, August Krogh, in conjunction with a mathematician colleague, Mr. K. Erlang, proposed the first oxygen transport model based on the assumption that each capillary is the sole supplier to a cylindrical region of tissue surrounding it (the Krogh cylinder) (55). Under this simple model, oxygen is assumed to diffuse in the radial direction with a uniform diffusivity and to be consumed in the tissue at a uniform constant rate (Figure 1A). This model permitted Krogh to propose that capillary density must be actively regulated in response to changing metabolic activity to ensure adequate tissue oxygenation. Specifically, this model predicts that in response to a fall in oxygen delivery, capillary density would increase to maintain tissue oxygenation. However, no mechanism for this alteration was proposed. Krogh's model and the interpretations derived from it have continued to influence the way most researchers and clinicians view tissue oxygenation. However, recent studies of microvascular oxygen transport have demonstrated the inadequacies of Krogh's idealized single capillary model as a comprehensive descriptor of tissue oxygenation.

Over thirty years ago, Duling and Berne (19) reported that oxygen levels in the blood diminished along the arteriolar tree with up to two thirds lost prior to entering the capillary bed. Numerous researchers, using a variety of techniques in different organs and species, have confirmed this finding (23,90,92). Thus, it is clear that there is exchange of O_2 that occurs among microvessels that is not accounted for by the Krogh model. Although it is not fully understood why there is such a large pre-capillary decrease in O_2 , Ellsworth and Pittman (26) provided evidence that some of the O_2 leaving the arterioles diffuses to erythrocytes flowing through nearby capillaries resulting in an increase in their O_2 saturation. Since O_2 is transported by diffusion from arterioles to capillaries, it is also likely that O_2 exchange occurs between capillaries with different O_2 levels (23) as proposed in theoretical models (27,37). This exchange would be consistent with quantitative studies of microvascular blood flow which have demonstrated considerable spatial heterogeneity of capillary perfusion (93) and a corresponding heterogeneity in O_2 delivery. Thus groups of capillaries, rather than the single capillary on which Krogh's model was based, need to be considered in evaluating the mechanisms responsible for maintaining tissue O_2 requirements and uniform O_2 delivery. As depicted in Figure 1B, the current view is that the microcirculation supplies oxygen to tissue using multiple vessel types that are functionally connected through both convective oxygen flow (blood flow) and diffusive oxygen transfer (23,37). Inherent in this complexity is the need for a mechanism to direct the convective oxygen flow to regions of the tissue where oxygen is required to satisfy metabolic needs. This directed distribution of peripheral blood flow is analogous to the mechanisms inherent in the pulmonary circulation which direct blood flow to those regions of the lung that are well ventilated (96).

One might expect that mechanisms associated with blood flow regulation such as wall shear stress and pressure (myogenic tone) would be sufficient to properly distribute blood flow and consequently, O_2 supply within the arteriolar tree. However, at the microvascular level, total blood flow may not accurately reflect local O_2 supply. The convective distribution of oxygen within the arteriolar tree is impacted by the diffusional loss of oxygen to nearby erythrocytes in microvessels. Most of the diffusional loss of oxygen in the arteriole is from erythrocytes near the wall of the vessel, that is, at the edge of the cell free plasma layer. Thus, these cells have a lower O_2 saturation than those nearer the center of the vessel (9,25). At vessel bifurcations, this uneven distribution affects convective oxygen supply to a greater or lesser extent depending on the relative flow in the downstream branches. For example, if blood flow into a downstream branch is very low, only plasma and the erythrocytes with lower O_2 will be skimmed into this branch (Figure 2). As flow into the side branch increases as a result of downstream vasodilation, flow into that branch will be derived from a larger cross-sectional region of the lumen of the upstream vessel, allowing blood with higher hematocrit and erythrocytes with higher O_2 saturation to enter that branch. Thus, an increase in O_2 demand in one region of tissue would induce a redistribution of erythrocytes (O_2 delivery) not solely described by the simple redistribution of blood flow. In light of these complexities, it becomes abundantly clear that the simplistic Krogh approach to oxygen delivery will not adequately explain the complex interactions apparent in the intact microvasculature. Therefore, a local control mechanism must exist that is capable of sensing O_2 need and adjusting flow within the small arterioles feeding the capillary network.

Numerous studies have focused on the blood vessels themselves or on discrete regions within the tissue as the sensor of O_2 requirements in a tissue (18,23,26,27,43,49,64,72). However, the exquisite accuracy in matching O_2 supply with tissue O_2 demand in skeletal muscle *in vivo* mandates a control system that is both more sensitive and responsive than those previously described. The system required must be able to sense localized need and to initiate an integrated response that results in appropriate increases in local oxygen supply. Could this controller be as simple as the erythrocyte itself?

A Case for the Erythrocyte as a Vascular Controller

Fundamental to any system that regulates the delivery of appropriate amounts of oxygen to meet changing tissue metabolic needs is the requirement that the need be detected, quantified and subsequently coupled to a mechanism that will appropriately alter blood flow (O_2 delivery). Such a mechanism requires interplay among tissue gas exchange, tissue metabolism and vascular smooth muscle function. Moreover, the process must be regulated within a narrow range (45). The vascular endothelium participates in controlling vascular caliber (7,32,54,94) and coordinating the response to local, diverse stimuli initiated within the tissue (13,17,32, 44,76,77,78,94). It would be reasonable to suggest that one or more components of the oxygen transport pathway communicate directly or indirectly with the endothelium to appropriately alter microvascular perfusion.

In 1993, Stein and Ellsworth suggested (88) that, in severe hypoxia, the oxygen content of the blood supplying the tissue was more important than its oxygen tension for the maintenance of oxygen supply in hamster skeletal muscle. Oxygen content (oxygen saturation), a reflection of the extent of binding of oxygen to hemoglobin within the erythrocyte, is related to oxygen tension by the characteristic oxyhemoglobin dissociation curve. Oxygen tension determines the diffusive transfer of oxygen from the erythrocyte to the tissue. Thus, if oxygen content rather than oxygen tension were the important factor in regulating oxygen delivery, then the erythrocyte itself would assume a central role in the process since it contains the only component of the oxygen transport pathway which is directly influenced by oxygen content, hemoglobin. The oxygen content of the erythrocyte as it traverses a tissue is directly linked to the level of oxygen utilization of that tissue (Figure 3). Therefore, if the erythrocyte itself were able to sense oxygen need and affect an alteration in vascular caliber leading to appropriate changes in blood flow, this property of the erythrocyte would provide an efficient means of matching oxygen delivery (blood flow) with metabolic need, eliminating the requirement for a diverse network of sensing sites throughout the vasculature. It is intriguing to think that the mobile erythrocyte, whose level of oxygen content at a particular point in a tissue is directly linked to the level of oxygen utilization by that tissue could, itself augment blood flow and oxygen delivery wherever and whenever the need might arise.

The establishment that the erythrocyte, the major supplier of oxygen, also functions as a sensor of oxygen requirements and effector of changes in oxygen supply would provide an important level of precision to local vascular control. How could such a small, anucleated bag of hemoglobin accomplish this task? Erythrocytes contain millimolar quantities of adenosine 5' triphosphate (ATP) (65) which is produced primarily by membrane bound glycolytic pathways. In 1992, Bergfeld and Forrester reported that human erythrocytes release ATP in response to the combined effects of hypoxia and hypercapnia (5). More recent studies have shown that exposure to reduced oxygen tension (~35 mmHg) alone is sufficient to stimulate ATP release from erythrocytes of hamsters (24), rabbits (85), rats (50) and humans (84). While these studies have examined ATP release in response to reductions in oxygen tension, Jagger et al (50), demonstrated that ATP efflux was linearly related to hemoglobin O_2 saturation suggesting that the conformational change of hemoglobin, as it de-saturates during a fall in oxygen levels, elicits the release of ATP. This conclusion was further supported by an observed inhibition of ATP release upon exposure of the erythrocytes to carbon monoxide which would prevent the decrease in hemoglobin oxygen saturation when oxygen tension was reduced (50). The importance of hemoglobin oxygen saturation in ATP release was later confirmed in human studies by González-Alonso and his group (38,39). If release of ATP from erythrocytes is directly linked to a physiological stimulus, such as a decrease in oxygen saturation, then there must be a signal transduction pathway connecting them.

The Signal Transduction Pathway for ATP Release from Erythrocytes

Strong evidence exists supporting the controlled release of ATP from erythrocytes in response to both physiological and pharmacological stimuli. Physiologically, erythrocytes release ATP in response to mechanical deformation (82,83,86), as would be encountered when these cells traverse the microcirculation, as well as in response to exposure to reduced oxygen tension (5,15,22,50,70). In both cases, the amount of ATP released is influenced by the magnitude of the stimulus. In addition to physiological stimuli, erythrocytes release ATP in response to receptor-mediated activation of erythrocyte membrane bound β -adrenergic receptors or prostacyclin receptors in a concentration-dependent manner (68,80).

The finding that ATP is released in a controlled fashion, suggests that erythrocytes possess a mechanism that directly links the stimuli to release of ATP. Over the past several years, Sprague's group has delineated a signaling pathway for ATP release from erythrocytes (Figure 3). This pathway includes the heterotrimeric G proteins Gs and Gi (68,69,70,81), adenylyl cyclase (AC) (86), protein kinase A (86) and the cystic fibrosis transmembrane conductance regulator (CFTR) (83). Studies have clearly shown that activation of Gs-coupled, β -adrenergic receptors and prostacyclin (IP) receptors in erythrocytes results in concentration-dependent increases in cAMP and ATP release from erythrocytes (6,68,80). However, Olearczyk et al. demonstrated that the G protein that is activated when erythrocytes are exposed to deformation or reduced oxygen tension is not Gs, but rather Gi (69,70). The direct activation of Gi with the wasp venom extract, mastoparan 7, stimulates increases in cAMP and ATP release from erythrocytes (69,70). Although the α subunit of Gi is well known to inhibit the activity of some AC isoforms, its associated β subunits, specifically subunits 1 through 4 (4,28,33,60,89,91), have been shown to activate AC isoforms II, IV and VII. Importantly, Gi α , β subunits 1 through 4 and AC II are all components of human erythrocyte membranes (81). A central role for Gi in the pathway for ATP release from erythrocytes in response to reduced oxygen tension was demonstrated by the finding that incubation of erythrocytes with pertussis toxin, which binds to Gi preventing its dissociation into the component subunits, prevented ATP release in response to this physiological stimulus (70). Although Gi activation is clearly involved in ATP release from erythrocytes exposed to reduced oxygen tension, the mechanism that directly couples the decrease in oxygen saturation of the hemoglobin molecule to the activation of Gi remains under investigation. One possibility is that the conformational change in the hemoglobin molecules bound to the erythrocyte membrane directly activate Gi or some other aspect of the release pathway (50).

Vascular Control by ATP

If we accept that ATP is released from erythrocytes in a controlled manner as they perfuse a region of tissue with a low PO_2 , then this ATP must initiate a conducted vasodilation which extends beyond the site of initiation in order for there to be an effective increase in vascular perfusion (O_2 delivery) (56) (Figure 3). Using arterioles in the intact hamster cheek pouch retractor muscle, McCullough et al (59) demonstrated a dose-dependent conducted vasodilator response to the intraluminal 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 1200 μm upstream at a rate of approximately 50 $\mu m/s$. Since one would anticipate that the oxygen tension at the downstream end of the capillaries and in the venules would be most reflective of local tissue oxygen utilization, Collins et al (10) 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. It is important to note that the intraarteriolar application of $10^{-6}M$ ATP, the concentration which 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 (24,59). Dietrich et al. (15) later established that the time course for sensing of a low oxygen environment, the release of ATP from erythrocytes and a vasodilatory response is on the order of 500 msec, supporting the potential physiological importance of this control mechanism. In recent studies in which the oxygen tension on the surface of an intact muscle was lowered in a step wise fashion using a computer controlled gas flow chamber, the increases in flow which occurred within the capillary bed were consistent with such a time course (21).

When ATP is released into the vascular lumen, it can interact with receptors present on the endothelium which can elicit both endothelium-dependent and smooth muscle cell-dependent vasoactive responses which would be conducted along the vasculature (Figure 4). Endothelial cells possess purinergic receptors that, when activated, stimulate the synthesis and release of several vasodilators. These vasodilators include nitric oxide as well as products of arachidonic acid metabolism. Although the receptor activated and the mediator released may vary in different tissues, the receptors present in the cerebral circulation have been particularly well characterized.

In the cerebral circulation, ATP and its related breakdown products, including adenosine, are vasoactive mediators. Forrester et al. found that intraluminal ATP was a potent vasodilator in the baboon cerebral circulation (29) and that topically applied ATP dilated cat pial vessels at a much lower concentration than adenosine (30). These reports lead to the hypothesis that ATP, or a closely related breakdown product rather than adenosine, is the vasoactive purine in the cerebral circulation (30). Although in the cerebral microcirculation ATP, ADP and adenosine are all potent vasodilators (14,53), only ATP and ADP consistently caused conducted vasomotor responses. (14,53). Since at equimolar concentrations ADP was slightly less potent in causing dilatory local and conducted responses (53) than ATP, it is likely that ATP may be the primary agonist. This corresponds with observations by Ikeuchi and Nishizaki where in brain artery endothelial cells ATP caused stronger potassium currents than ADP while AMP had no effect (48).

Numerous studies have attempted to link neuronal activation, local oxygen tension and cerebral blood flow. Neuronal activation can lead to a small, but significant, drop in local oxygen tension which is followed by an increase in local blood flow (2). This neuronal activation-induced decrease in oxygen tension could elicit ATP release from erythrocytes resulting in an increase in blood flow. Indeed, ATP is released from erythrocytes perfusing isolated cerebral arterioles within fractions of a second after entering the hypoxic vessel (15). Although not all studies have detected the drop in oxygen tension associated with neuronal activation (79), there is increasing evidence suggesting that, in the brain, ATP released from erythrocytes could contribute to a rapid initial increase in blood flow in response to neuronal activity.

ATP released from erythrocytes is known to activate specific P_2 purinergic receptors on the vascular endothelium. Although it is simpler to consider two distinct classes of these P_2 receptors, P_{2X} and P_{2Y} , more recent evidence indicates that the mammalian P_{2X} and P_{2Y} receptors are actually families of receptors consisting of seven P_{2X} receptors (P_{2X1-7}) and eight P_{2Y} receptors (P_{2Y1} , P_{2Y2} , P_{2Y4} , P_{2Y6} , $P_{2Y11-14}$) (1,34). Studies in cerebral microvessels have delineated the specific receptors present and the vasoactive response to their stimulation. In isolated non-pressurized pial arterioles, Lewis et al. concluded that P_{2Y2} and P_{2Y6} -like receptors are responsible for the observed vessel constriction (57). In isolated and pressurized rat penetrating arterioles, Horiuchi et al. reported that ATP constricts the vessels transiently via smooth muscular P_{2X1} -receptors and dilates the vessels via endothelial P_{2Y2} (47). Similarly, You et al. found that in larger size cerebral arterioles dilation to intraluminal ATP results from

P₂Y₂-stimulation (98). These receptors were confirmed to exist on hamster skeletal muscle arterioles (unpublished observations).

Regardless of the receptor that is activated, ATP-induced vasodilation is the result of the synthesis and activity of endothelium-derived relaxing factors. In skeletal muscle, McCullough (59) and Collins (10) each observed that the conducted vasodilation to intraluminal ATP was eliminated following administration of a nitric oxide synthase inhibitor implicating NO as an important vascular mediator in these vessels. In addition, in large cerebral arterioles endothelial P₂Y₂-specific stimulation was shown to release NO as well as a non-NO, non-cyclooxygenase dependent factor (97,98). Similarly it was reported that in cerebral arterioles, endothelial P₂Y₁-specific stimulation releases both NO and a non-NO, non-cyclooxygenase dependent factor while P₂Y₂-specific stimulation releases only the non-NO, non-cyclooxygenase dependent factor (46) possibly a cytochrome P₄₅₀ monooxygenase product such as epoxyeicosanoic acids (EETs) (16). EETs can activate calcium sensitive potassium channels resulting in hyperpolarization (8,31). In brain artery endothelial cells purines induced strong potassium currents with ATP>ADP>AMP (48). In cerebral arterioles, the dilation to ATP was preceded by hyperpolarization and the dilation to ATP depended on large conductance BK_{Ca} and intermediate conductance IK_{Ca} calcium sensitive potassium channels but not small conductance SK_{Ca} channels (16). Thus, the mediator released in response to activation of endothelial purinergic receptors by ATP will depend on the identity of the specific receptor activated and the signaling pathway present in a particular blood vessel (Figure 4).

Although there is significant evidence to suggest that nitric oxide (NO) is a regulator of vascular perfusion, controversy remains as to its source and mechanism of action. In 1996, Stamler and his colleagues (51) proposed that the erythrocyte was responsible for regulating O₂ delivery through the transport of nitric oxide, produced in the lungs, to the periphery in the form of bioactive compound, S-nitrosothiol, SNO. SNO, reported to be a potent vasodilator, is carried by hemoglobin and released as the hemoglobin O₂ saturation falls in response to local O₂ demand. Although there is support for this hypothesis, (58,87), questions remain as to its role under physiological conditions (35,36,71,74). In 2003, Gladwin's group provided evidence that deoxyhemoglobin acts as a nitrite reductase, converting nitrite to NO, and hence making it possible for the erythrocyte to vasodilate arterioles in response to hypoxia (11). However, it is not clear that this mechanism has sufficient temporal resolution to account for the observed rapid changes in the distribution of perfusion in response to local decreases in O₂ tension. Importantly, unlike erythrocyte-derived ATP, neither SNO or nitrite have been shown to be associated with conducted vasodilation of upstream resistance vessels in an intact vascular bed. Thus, although each of these mechanisms could play a role in the regulation of certain aspects of tissue perfusion, neither would appear to fulfill the role we propose for the release of ATP from erythrocytes in response to local O₂ need in the normal microvasculature.

Control of ATP Release in vivo: Feedback Mechanisms

Although ATP can stimulate the synthesis and release of multiple endothelium-derived vasodilators, ATP-induced increases in NO are important in both the cerebral and skeletal muscle microcirculations. It is important to recognize that NO synthesized in endothelial cells diffuses not only to vascular smooth muscle where it stimulates vasodilation, but it is also released into the vascular lumen. When erythrocytes enter a microcirculation in which large amounts of NO are already present, additional ATP release would be unnecessary. Indeed, NO was shown to attenuate agonist-induced ATP release from erythrocytes in a negative-feedback fashion. In these studies, incubation of erythrocytes with the NO donor, spermine NONOate, results in inhibition of ATP release from rabbit and human erythrocytes exposed to decreased oxygen tension (67). It has also been proposed that ADP, the first degradation product of ATP, can inhibit ATP release from erythrocytes (95). Under this hypothesis, ATP released from the

erythrocytes in response to physiological stimuli is metabolized by ecto-enzymes resulting in the generation of ADP. ADP can then activate P_{2Y13} receptors present on erythrocytes resulting in decreases in intracellular cAMP and reduced ATP release (95). The finding that ATP release from erythrocytes is inhibited by the endothelium-derived vasodilator released from the endothelium in response to ATP (NO) as well as the first degradation product of that nucleotide (ADP) demonstrates the potential for negative feedback regulation of this physiologically important signaling pathway.

Pathological Consequences of a Defect in this Control Mechanism

Recently, it was reported that erythrocytes of humans with type 2 diabetes (DM2), a condition in which cardiovascular disease accounts for nearly half of associated deaths, express decreased amounts of the α_2 subunit of the heterotrimeric G protein, G_i when compared with erythrocytes of healthy humans (86). Interestingly, decreased G_i expression is present in animal models of diabetes as well (40,41,42,63,66,99). In humans, $G_{i\alpha 2}$ expression was decreased selectively, that is, expression $G_i \alpha$ subtypes, $G_{s\alpha}$ and adenylyl cyclase II were unaltered (86). This selective decrease in $G_{i\alpha 2}$ expression in human erythrocytes was associated with impaired cAMP synthesis as well as decreased ATP release in response to an agent that directly activates G_i , mastoparan 7 (86). It is of interest that the degree of impairment of ATP release correlated inversely with glycemic control. That is, the higher the average blood sugar, as measured by glycated hemoglobin level (HbA1C), the greater the defect in ATP release (86). Since vascular complications in humans with DM2 also correlate inversely with HbA1c level, this finding raises the possibility of a connection between the failure of erythrocytes to release ATP and the vascular complications of DM2 (12,61). Moreover, such reports suggest that the erythrocyte could be a novel target for the development of drugs for the treatment of vascular insufficiency.

How the Controlled Release of ATP from Erythrocytes in the Microcirculation Changed the Conceptual Approach to our Understanding of Oxygen Supply

Our understanding of the mechanisms that control matching of O_2 supply with demand has evolved from the concept of a single, idealized capillary supplying oxygen to a simple Krogh cylinder into a vastly more complex system of oxygen delivery and regulation. This evolution resulted from a more complete understanding of the diverse rheological and transport properties of the microvasculature as well as the concept of the erythrocyte as a sensor of O_2 need and regulator of vascular tone via its release of ATP. As a result, a significantly more complex modeling approach than that provided by the pioneering work of Krogh is required to describe this system. It is clear that to make the model more comprehensive, additional variables must be included such as: a) 3D network geometry of capillaries, arterioles and venules, b) realistic hemodynamics of erythrocyte flow and O_2 distribution within these networks, c) 3D oxygen transport between blood and tissue, d) changes in erythrocyte-derived ATP release as a function of oxygen content, e) local and conducted changes in microvessel diameter, and f) other microvascular control mechanisms based on local wall shear rate (shear dependent vasodilation) and blood pressure (myogenic factors). Krogh's original model of oxygen supply assumed that increased oxygen delivery to tissue resulted from an increase in the number of perfused capillaries ("capillary recruitment"). However, *in vivo* experiments in skeletal muscle (20) indicate that most capillaries are perfused under resting conditions. As such, increased flow would result in a more uniform distribution of erythrocytes to already perfused capillaries rather than perfusion of additional capillaries.

Most of the above features have been included in theoretical models of diameter adaptation in microvascular networks (73) and more recently in a theoretical model of blood flow regulation (3). This latter model is consistent with the hypothesis that regulation of microvascular O_2 delivery based on O_2 saturation-dependent release of ATP by erythrocytes leading to conducted

vasodilation can account for experimentally observed increases in perfusion in response to increased oxygen demand. However, this idealized model used a simulated network consisting of only seven representative segments and did not consider diffusive exchange of O₂ between capillaries and arterioles. Therefore, it remains to be shown whether the features described above, including erythrocyte-derived ATP, when included in a more comprehensive model, can fully explain the O₂-dependent microvascular flow regulation that occurs *in vivo*. In addition, other key issues which need to be addressed include: a) whether there is a primary location within the network where the need for changes in O₂ supply are sensed or control is exercised throughout the arteriolar tree to ensure proper distribution of O₂ supply, b) the inherent time scale of the regulatory system (i.e., how fast can the system respond and what are the limiting factors?), c) the vessels in the network that are involved in the response to a given stimulus, and d) the limitations of the system's ability to control local O₂ delivery. Future progress toward understanding the role of erythrocyte-derived ATP in the regulation of O₂ delivery within the microcirculation will depend on a combination of new *in vitro* experiments, detailed computational modeling, and model testing and validation using *in vivo* experiments under a range of physiological and pathophysiological conditions.

Acknowledgments

Original work in the authors' laboratories is supported by the National Institutes of Health, American Diabetes Association, Heart and Stroke Foundation of Ontario and Canadian Institutes of Health Research.

References

1. Abbracchio MP, Burnstock G, Boeynaems JM, Barnard EA, Boyer JL, Kennedy C, Knight GE, Fumagalli M, Gachet C, Jacobson KA, Weisman GA. International Union of Pharmacology LVIII: update on the P2Y G protein-coupled nucleotide receptors: from molecular mechanisms and pathophysiology to therapy. *Pharmacol Rev* 2006;58:281–341. [PubMed: 16968944]
2. Ances BM, Buerk DG, Greenberg JH, Detre JA. Temporal dynamics of the partial pressure of brain tissue oxygen during functional forepaw stimulation in rats. *Neuroscience Letters* June 22;2001 306 (12):106–10. [PubMed: 11403969]
3. Arciero JC, Carlson BE, Secomb TW. Theoretical model of metabolic blood flow regulation: roles of ATP release by red blood cells and conducted responses. *Am J Physiol* 2008;295:H1562–71.
4. Bayewitch ML, Avidor-Reiss T, Levy R, Pfeuffer T, Nevo I, Simonds WF, Vogel Z. Differential modulation of adenylyl cyclase I and II by various G β subunits. *J Biol Chem* 1998;273:2273–2276. [PubMed: 9442071]
5. Bergfeld GR, Forrester T. Release of ATP from human erythrocytes in response to a brief period of hypoxia and hypercapnia. *Cardiovas Res* 1992;26:40–47.
6. Bourguignon LY, Iida N, Sobrin L, Bourguignon GJ. Identification of an IP₃ receptor in endothelial cells. *J of Cell Physiol* 1994;159:29–34. [PubMed: 8138588]
7. Busse R, Trogisch G, Bassenge E. The role of endothelium in the control of vascular tone. *Basic Res Cardiol* 1985;80:475–490. [PubMed: 3000343]
8. 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]
9. Carvalho H, Pittman RN. Longitudinal and radial gradients of PO₂ in the hamster cheek pouch microcirculation. *Microcirc* 2008;15(3):215–224.
10. Collins DM, McCullough WT, Ellsworth ML. Conducted vascular responses: Communication across the capillary bed. *Microvasc Res* 1998;56:43–53. [PubMed: 9683562]
11. Cosby K, Partovi KS, Crawford JH, Patel RP, Reiter CD, Martyr S, Yang BK, Wacławski MA, Zalos G, Xu X, Huang KT, Shields H, Kim-Shapiro DB, Schechter AN, Cannon RO 3rd, Gladwin MT. Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. *Nat Med* 2003;9(12):1498–505. [PubMed: 14595407]

12. Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complication of insulin-dependent diabetes mellitus. *N E J M* 1993;329:977–986.
13. Dietrich HH. Effect of locally applied epinephrine and norepinephrine on blood flow and diameter in capillaries of rat mesentery. *Microvasc Res* 1989;38:125–135. [PubMed: 2796759]
14. Dietrich HH, Kajita Y, Dacey RG Jr. Local and conducted vasomotor responses in isolated rat cerebral arterioles. *Am J Physiol* 1996;271:H1109–H1116. [PubMed: 8853348]
15. Dietrich HH, Ellsworth ML, Sprague RS, Dacey RG Jr. Red blood cell regulation of microvascular tone through adenosine triphosphate. *Am J Physiol* 2000;278:H1294–H1298.
16. 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 Vas Res* 2008;46:253–264.
17. Dietrich HH, Tynl K. Capillary as a communicating medium in the microvasculature. *Microvasc Res* 1992;43:87–99. [PubMed: 1318992]
18. Duling B. Oxygen sensitivity of vascular smooth muscle. II. In vivo studies. *Am J Physiol* 1974;227(1):42–49. [PubMed: 4843354]
19. 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]
20. Ellis CG, Wrigley SM, Groom AC. Heterogeneity of red blood cell perfusion in capillary networks supplied by a single arteriole in resting skeletal muscle. *Circ Res* 1994;75:357–368. [PubMed: 8033345]
21. Ellis CG, Milkovich S, Goldman D. Experimental protocol investigating local regulation of oxygen supply in rat skeletal muscle in vivo. *J Vasc Res* 2006;43:45–55.
22. Ellsworth ML. The red blood cell as an oxygen sensor: what is the evidence? *Acta Physiol Scand* 2000;168:551–559. [PubMed: 10759592]
23. Ellsworth ML, Ellis CG, Popel AS, Pittman RN. Role of microvessels in oxygen supply to tissue. *NIPS* 1994;9:119–123.
24. 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]
25. Ellsworth ML, Pittman RN. Evaluation of photometric methods for quantifying convective mass transport in microvessels. *Am J Physiol* 1986;251:H869–H879. [PubMed: 3766764]
26. 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]
27. Ellsworth ML, Popel AS, Pittman RN. Assessment and impact of heterogeneities of convective oxygen transport parameters in capillaries of striated muscle. *Microvasc Res* 1988;35:341–362. [PubMed: 3393095]
28. Federman AD, Conklin BR, Schrader KA, Reed RR, Bourne HR. Hormonal stimulation of adenylyl cyclase through Gi-protein beta gamma subunits. *Nature* 1992;356:159–161. [PubMed: 1312225]
29. Forrester T, Harper AM, MacKenzie ET. Effects of intracarotid adenosine triphosphate infusions on cerebral blood flow and metabolism in the anaesthetized baboon. *J Physiol* 1975;250:38P–39P.
30. Forrester T, Harper AM, MacKenzie ET, Thomson EM. Effect of adenosine triphosphate and some derivatives on cerebral blood flow and metabolism. *J Physiol (Lond)* 1979;296:343–355. [PubMed: 119042]
31. 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]
32. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980;288:373–376. [PubMed: 6253831]
33. Gao BN, Gilman AG. Cloning and expression of a widely distributed (type IV) adenylyl cyclase. *Proc Natl Acad Sci USA* 199;88:10178–10182. [PubMed: 1946437]
34. Gever JR, Cockayne DA, Dillon MP, Burnstock G, Ford AP. Pharmacology of P2X channels. *Pflugers Arch* 2006;452:513–537. [PubMed: 16649055]

35. Gladwin MT. Role of the red blood cell in nitric oxide homeostasis and hypoxic vasodilation. *Adv Exp Med Biol* 2006;588:189–205. [PubMed: 17089890]
36. Gladwin MT, Crawford JH, Patel RP. The biochemistry of nitric oxide, nitrite, and hemoglobin: role in blood flow regulation. *Free Radical Biology and Medicine* 2004;36(6):707. [PubMed: 14990351]
37. Goldman D, Popel AS. A computational study of the effect of capillary network anastomoses and tortuosity on oxygen transport. *J Theor Biol* 2000 Sep 21;206(2):181–94. [PubMed: 10966756]
38. González-Alonso J, Olsen DB, Saltin B. Erythrocyte and the regulation of human skeletal muscle blood flow and oxygen delivery: role of circulating ATP. *Circ Res* 2002;91:1046–55. [PubMed: 12456491]
39. González-Alonso J, Mortensen SP, Dawson EA, Secher NH, Damsgaard R. Erythrocytes and the regulation of human skeletal muscle blood flow and oxygen delivery: role of erythrocyte count and oxygenation state of haemoglobin. *J Physiol* 2006;572:295–305. [PubMed: 16439430]
40. Grawer D, Milligan G, Spiegel AM, Unson CG, Houslay MD. Abolition of the expression of inhibitory guanine nucleotide regulatory protein Gi activity in diabetes. *Nature* 1987;327:229–232. [PubMed: 3106832]
41. Hashim S, Liu YY, Wand R, Anand-Srivastava MB. Streptozotocin-induced diabetes impairs G-protein linked signal transduction in vascular smooth muscle. *Molecular and Cellular Biochemistry* 2000;240:57–63. [PubMed: 12487372]
42. Hashim S, Li Y, Nagakura TS, Anand-Srivastava MB. Modulation of G-protein expression and adenylyl cyclase signaling by high glucose in vascular smooth muscle. *Cardiovas Res* 2004;63:709–718.
43. Hester RL. Uptake of metabolites by postcapillary venules: mechanism for the control of arteriolar diameter. *Microvasc Res* 1993;46:254–261. [PubMed: 7504161]
44. Hester RL, Hammer L. Venular-arteriolar communication in the regulation of blood flow. *Am J Physiol* 2002;282:R1280–R1285.
45. Hollenberg M. Effect of oxygen on growth of cultured myocardial cells. *Circ Res* 1971;28:148–157. [PubMed: 4927074]
46. Horiuchi T, Dietrich HH, Hongo K, Dacey RG Jr. Comparison of P2 receptor subtypes producing dilation in rat intracerebral arterioles. *Stroke* 2003;34:1473–1478. [PubMed: 12730558]
47. Horiuchi T, Dietrich HH, Tsugane S, Dacey RG Jr. Analysis of purine- and pyrimidine-induced vascular responses in the isolated rat cerebral arteriole. *Am J Physiol* 2001;280:H767–H776.
48. Ikeuchi Y, Nishizaki T. ATP activates the potassium channel and enhances cytosolic Ca²⁺ release via a P2Y purinoceptor linked to pertussis toxin- insensitive G-protein in brain artery endothelial cells. *Biochemical & Biophysical Research Communications* 1995;215:1022–1028. [PubMed: 7488026]
49. Jackson WF. Arteriolar oxygen reactivity : where is the sensor. *Am J Physiol* 1987;253(22):H1120–H1126. [PubMed: 3318502]
50. Jagger JE, Bateman RM, Ellsworth ML, Ellis CG. Role of erythrocyte in regulating local O₂ delivery mediated by hemoglobin oxygenation. *Am J Physiol* 2001;280:H2833–H2839.
51. Jia L, Bonaventura C, Bonaventura J, Stamler JS. S-nitrosohaemoglobin: a dynamic activity of blood involved in vascular control. *Nature* 1996;380(6571):221–6. [PubMed: 8637569]
52. Jiang H, Zhu AG, Wong PY-K, McGiff JC. Stimulation of rat erythrocyte P2X7 receptor induces the release of epoxyeicosatrienoic acids. *FASEB J* 2006;20:A483.
53. Kajita Y, Dietrich HH, Dacey RG Jr. Effects of oxyhemoglobin on local and propagated vasodilatory responses induced by adenosine, adenosine diphosphate, and adenosine triphosphate in rat cerebral arterioles. *J Neurosurg* 1996;85:908–916. [PubMed: 8893731]
54. Koller A, Rodenburg JM, Wolin MS, Messina EJ, Kaley G. Modified arteriolar responses to ATP after impairment of endothelium by light-dye techniques in vivo. *Microvasc Res* 1991;41:63–72. [PubMed: 1904977]
55. Krogh A. The number and distribution of capillaries in muscles with calculations of the oxygen pressure head necessary for supplying the tissue. *J Physiol (Lond)* 1919;52:409–415. [PubMed: 16993405]
56. Kurjiaka DT, Segal SS. Conducted vasodilation elevates flow in arteriole networks of hamster striated muscle. *Am J Physiol* 1995;269:H1723–H1728. [PubMed: 7503270]

57. Lewis CJ, Ennion SJ, Evans RJ. P2 purinoceptor-mediated control of rat cerebral (pial) microvasculature; contribution of p2x and p2y receptors. *J Physiol* Sep 1;2000 527(Pt 2):315–324. [PubMed: 10970432]
58. Liu L, Yan Y, Zeng M, Zhang J, Hanes MA, Ahearn G, McMahon TJ, Dickfeld T, Marshall HE, Que LG, Stamler JS. Essential Roles of S-Nitrosothiols in Vascular Homeostasis and Endotoxic Shock. *Cell* 2004;116(4):617–628. [PubMed: 14980227]
59. McCullough WT, Collins DM, Ellsworth ML. Arteriolar responses to extracellular ATP in striated muscle. *Am J Physiol* 1997;272:H1886–H1891. [PubMed: 9139975]
60. McIntire WE, MacCleery G, Garrison JC. The G protein β subunit is a determinant in the coupling of Gs to the β 1-adrenergic and A2a adenosine receptors. *J Biol Chem* 2001;276:15801–15809. [PubMed: 11278863]
61. McLellan AC, Thornalley PJ, Benn J, Sonksen PH. Glyoxalase system in clinical diabetes mellitus and correlation with diabetic complications. *Clin Sci* 1994;87:21–29. [PubMed: 8062515]
62. Malmfors M, Erlinge D, Hogestatt ED, Zygmunt PM. Endothelial P2Y receptors induce hyperpolarisation of vascular smooth muscle by release of endothelium-derived hyperpolarising factor. *European Journal of Pharmacology* 1999;364:169–173. [PubMed: 9932720]
63. Matsuda N, Hattori Y, Gando S, Watanuki S, Kemmotsu O, Kanno M. Differential gene transcription regulation of Gi isoforms and Gs protein expression in diabetic rat hearts. *Naunyn-Schmiedeberg's Arch Pharmacol* 2000;361:53–60.
64. Messina EJ, Sun D, Koller A, Wolin MS, Kaley G. Increases in oxygen tension evoke arteriolar constriction by inhibiting endothelial prostaglandin synthesis. *Microvasc Res* 1994;48:151–160. [PubMed: 7854203]
65. Miseta A, Bogner P, Berenyi E, Kellermayer M, Galambos C, Wheatley D, Cameron I. Relationship between cellular ATP, potassium, sodium and magnesium concentrations in mammalian and avian erythrocytes. *Biochim Biophys Acta* 1993;1175:133–139. [PubMed: 8418892]
66. Moxham CM, Malbon CC. Insulin action impaired by deficiency of the g-protein subunit Gia2. *Nature* 1996;379:840–844. [PubMed: 8587610]
67. Olearczyk JJ, Ellsworth ML, Stephenson AH, Lonigro AJ, Sprague RS. Nitric oxide inhibits ATP release from erythrocytes. *Journal of Pharmacology & Experimental Therapeutics* 2004;309:1079–1084. [PubMed: 14766946]
68. Olearczyk J, Stephenson AH, Lonigro AJ, Sprague RS. Receptor-mediated activation of the heterotrimeric G-protein Gs results in ATP release from erythrocytes. *Med Sci Monit* 2001;7:669–674. [PubMed: 11433193]
69. 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.
70. 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. *American Journal of Physiology - Heart & Circulatory Physiology* 2004;287:H748–754. [PubMed: 15072952]
71. Patel RP, Hogg N, Spencer NY, Kalyanaraman B, Matalon S, Darley-Usmar VM. Biochemical characterization of human S-nitrosohemoglobin. Effects on oxygen binding and transnitrosation. *J Biol Chem* 1999;274(22):15487–92. [PubMed: 10336440]
72. Pittman RN, Duling BR. Oxygen sensitivity of vascular smooth muscle. *Microvasc Res* 1973;6:202–211. [PubMed: 4748020]
73. Pries AR, Secomb TW. Control of blood vessel structure: insights from theoretical models. *Am J Physiol Heart Circ Physiol* 2005 Mar;288(3):H1010–5. [PubMed: 15706037]
74. Rassaf T, Feelisch M, Kelm M. Circulating NO pool: assessment of nitrite and nitrosp species in blood and tissues. *Free Radic Biol Med* 2004;36:413–422. [PubMed: 14975444]
75. Roach RC, Koskolou MD, Jose ALC, Saltin B. Arterial O₂ content and tension in regulation of cardiac output and leg blood flow during exercise in humans. *Am J Physiol* 1999;276:H438–H445. [PubMed: 9950843]
76. Segal SS, Damon DN, Duling BR. Propagation of vasomotor responses coordinates arteriolar resistances. *Am J Physiol* 1989;256:H832–H837. [PubMed: 2923241]

77. Segal SS, Duling BR. Propagation of vasodilation in resistance vessels of the hamster: development and review of a working hypothesis. *Circ Res* 1987;61(suppl II):II-20–II-25. [PubMed: 3664984]
78. Segal SS, Duling BR. Conduction of vasomotor responses in arterioles: a role for cell-to-cell coupling? *Am J Physiol* 1989;256:H838–H845. [PubMed: 2923242]
79. Silva AC, Lee SP, Iadecola C, Kim SG. Early temporal characteristics of cerebral blood flow and deoxyhemoglobin changes during somatosensory stimulation. *J Cereb Blood Flow Metab* 2000;20:201–206. [PubMed: 10616809]
80. Sprague RS, Bowles EA, Hanson MS, DuFaux EA, Sridharan M, Adderley S, Ellsworth ML, Stephenson AH. Prostacyclin Analogues Stimulate Receptor-mediated cAMP Synthesis and ATP Release from Rabbit and Human Erythrocytes. *Microcirculation* 2008;15:461–471. [PubMed: 18574748]
81. 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]
82. Sprague RS, Ellsworth ML, Stephenson AH, Lonigro AJ. ATP: the red blood cell link to NO and local control of the pulmonary circulation. *Am J Physiol* 1996;271:H2717–H2722. [PubMed: 8997335]
83. Sprague RS, Ellsworth ML, Stephenson AH, Kleinhenz M, Lonigro A. Deformation-induced ATP release from red blood cells requires cystic fibrosis transmembrane conductance regulator activity. *Am J Physiol* 1998;275:H1726–H1732. [PubMed: 9815080]
84. 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 Cell Physiol* 2001;281:C1158–C1164. [PubMed: 11546651]
85. Sprague RS, Olearczyk JJ, Spence DM, Stephenson AH, Sprung RW, Lonigro AJ. Extracellular ATP signaling in the rabbit lung: erythrocytes as determinants of vascular resistance. *Am J Physiol* 2003;285:H693–H700.
86. Sprague RS, Stephenson AH, Bowles EA, Stumpf MS, Lonigro AJ. Reduced expression of Gi in erythrocytes of humans with type 2 diabetes is associated with impairment of both cAMP generation and ATP release. *Diabetes* 2006;55:33588–93.
87. Stamler JS, Jia L, Eu JP, McMahon TJ, Demchenko IT, Bonaventura J, Gernert K, Piantadosi CA. Blood Flow Regulation by S-Nitrosohemoglobin in the Physiological Oxygen Gradient. *Science* 1997;276(5321):2034–2037. [PubMed: 9197264]
88. Stein JC, Ellsworth ML. Capillary oxygen transport during severe hypoxia: role of hemoglobin oxygen affinity. *J Appl Physiol* 1993;75:1601–1607. [PubMed: 8282609]
89. Sunahara RK, Dessauer CW, Gilman AG. Complexity and diversity of mammalian adenylyl cyclases. *Annu Rev Pharmacol Toxicol* 1996;36:461–480. [PubMed: 8725398]
90. Swain DP, Pittman RN. Oxygen exchange in the microcirculation of hamster retractor muscle. *Am J Physiol* 1989;256:H247–55. [PubMed: 2912188]
91. Tang W, Gilman AG. Type-specific regulation of adenylyl cyclase by G protein $\beta\gamma$ subunits. *Science* 1991;254:1500–1503. [PubMed: 1962211]
92. Tsai AG, Johnson PC, Intaglietta M. Oxygen Gradients in the Microcirculation. *Physiol Rev* 2003;83:933–963. [PubMed: 12843412]
93. Tuml K, Ellis CG, Safranyos RG, Fraser S, Groom AC. Temporal and spatial distributions of red cell velocity in capillaries of resting skeletal muscle, including estimates of red cell transit times. *Microvasc Res* 1981;22:14–31. [PubMed: 6974295]
94. Vanhoutte PM, Lüscher TF, Gräser T. Endothelium-dependent contractions. *Blood Vessels* 1991;28:74–83. [PubMed: 1900447]
95. Wang L, Olivecrona G, Gotberg M, Olsson ML, Winzell MS, Erlang D. ADP acting on P2Y₁₃ receptors is a negative feedback pathway for ATP release from human red blood cells. *Circ Res* 2005;96:189–196. [PubMed: 15604418]
96. Walley KR. Heterogeneity of oxygen delivery impairs oxygen extraction by peripheral tissues: theory. *J Appl Physiol* 1996;81:885–894. [PubMed: 8872660]
97. 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–H1477. [PubMed: 9321839]

98. You JP, Johnson TD, Marrelli SP, Bryan RM Jr. Functional heterogeneity of endothelial P2 purinoceptors in the cerebrovascular tree of the rat. *Am J Physiol* 1999;277:H893–H900. [PubMed: 10484408]
99. Zheng X-L, Guo JH, Wang H-Y, Malbon CC. Expression of constitutively activated G_{i2} in vivo ameliorates streptozotocin-induced diabetes. *J Biol Chem* 1998;273:23649–51. [PubMed: 9726968]

Oxygen Delivery to Tissue

A: Original Krogh Concept

B: Current Understanding of Complexity

Figure 1.

A. Oxygen supply to tissue is generally conceptualized based on the simple Krogh tissue cylinder model in which all oxygen exchange occurs across the walls of the capillaries. As such, oxygen content within the capillary falls linearly as oxygen diffuses out of the vessel at a constant rate.

B. Our current understanding of the complex oxygen transport pathways controlling oxygen supply include convective oxygen transport through the microvessels (solid line) coupled with diffusive oxygen exchange (dotted lines) among all orders of microvessels. The appropriate delivery of oxygen to the tissue requires a mechanism to sense oxygen need and alter blood flow to meet that need which can be flexible enough to compensate for alterations in both convective and diffusive transport.

Figure 2.

Impact of diffusional oxygen losses on the distribution of oxygen into downstream vessels. The lower hematocrit (a consequence of the cell free plasma layer) and oxygen saturation (due to diffusional oxygen losses) near the arteriolar wall results in the distribution of red blood cells and oxygen into the side branch which is not proportional to the blood flow (panel B). Vasoconstriction of the side branch (panel A) reduces both hematocrit and O_2 saturation in that vessel while vasodilation (panel C) increases both hematocrit and O_2 saturation. Higher O_2 saturation along vessel centerline designated by bright red erythrocytes; lower saturation near wall by cells with increasing levels of purple

Figure 3.

The entrance of erythrocytes into tissue regions with a high oxygen demand [decreased oxygen tension (PO_2)] results in diffusion of oxygen to the tissue and a decrease in the oxygen saturation (SO_2) of the hemoglobin within erythrocytes in the microcirculation. This decrease in SO_2 stimulates the release of ATP from the erythrocyte via activation of a signaling pathway (see text) with the amount released proportional to the decrease in SO_2 . The erythrocyte-derived ATP can then interact with endothelial purinergic receptors resulting in the production of mediators that initiate vasodilation. This vasodilation is conducted in a retrograde fashion resulting in increased blood flow (oxygen supply) to areas of increased oxygen demand. Abbreviations: Gi = heterotrimeric G protein; ATP = adenosine triphosphate; cAMP = 3'5'-adenosine monophosphate; PKA = protein kinase A; CFTR = cystic fibrosis transmembrane conductance regulator; ? = an as yet unidentified conduit for ATP release; PR = purinergic receptors; + = stimulation, endo = endothelium; SMC = smooth muscle cell.

Figure 4.

Proposed activation of endothelium-dependent and -independent vasodilation which occurs following the release of ATP from erythrocytes. Activation of erythrocyte G_i results in increased adenylyl cyclase (4,28) resulting in increased cAMP followed downstream by increased ATP release. Paracrine activation of endothelial P_{2y} receptors (46,47,97) by erythrocyte-derived ATP release couples with $G_{q/11}$ to stimulate PLC that increases $InsP_3$ synthesis. $InsP_3$ activates its receptor on the ER promoting the release of ER stored Ca^{++} (8). Depletion of Ca^{++} from the ER stimulates Ca^{++} entry through membrane SOC channels. Increased intracellular Ca^{++} activates eNOS to synthesize NO, cPLA₂ to release AA from membrane phospholipids for synthesis of PGI₂ and EDHF (7,32,62). Endothelium-released NO activates sGC in the underlying smooth muscle to increase cGMP synthesis (32) and inhibits erythrocyte G_i to reduce ATP release (67,69). PGI₂ acts on its smooth muscle G_s -coupled IP receptor to stimulate adenylyl cyclase to increase cAMP synthesis. Both increased cGMP and cAMP promote vascular smooth muscle cell relaxation, and dilation. EDHF acts on a $K_{Ca^{++}}$ channel on the vascular smooth muscle plasma membrane to increase K^+ efflux, hyperpolarizing the smooth muscle cell that results in its relaxation. ATP released from the erythrocyte can act in an autocrine manner by activating erythrocyte P_{2x7} receptors (34) that increase Ca^{++} uptake activating PLA₂ activity and EET synthesis and release (52). The EETs act directly on a vascular $K_{Ca^{++}}$ channel to hyperpolarize and relax the smooth muscle cell (8,31). Also in an autocrine manner, ADP, derived from released ATP can activate the

erythrocyte P2y₁₃ receptor that results in a decrease in erythrocyte cAMP and decreases erythrocyte ATP release (95). Endothelial and/or smooth muscle cell hyperpolarization resulting from K⁺ efflux can conduct through gap junctions to adjacent cells resulting in conducted vasodilation. (76-78) AA, arachidonic acid; ACII, adenylyl cyclase II; ADP adenosine diphosphate; ATP, adenosine triphosphate; cAMP, 3',5' - cyclic adenosine monophosphate; cGMP, 3',5' - cyclic guanosine monophosphate; EDHF, endothelium-derived hyperpolarizing factor; EETs, epoxyeicosatrienoic acids; eNOS, endothelial nitric oxide synthase; ER, endoplasmic reticulum; G_i, G_q, and G_s: guanine nucleotide protein I, q and s-coupled receptors, respectively; IP, prostacyclin receptor; K_{Ca++}, calcium-activated potassium channel; K_{IR}, inward rectifying potassium channel; NO, nitric oxide; P2x, ligand gated ion channel purinergic receptor; P2y, G protein-coupled purinergic receptor; PGI₂, prostacyclin; cPLA₂, cytosolic phospholipase A₂; PLC, phospholipase C; sGC, soluble guanylyl cyclase; SOC, store-operated calcium channel, GAP, gap junction.