Erythrocytes: Oxygen Sensors and Modulators of Vasomotor Tone

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 microvesicles 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.

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, 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 over 30 years ago, Duling and Berne (19) reported that oxygen levels in the blood diminished along the arteriolar tree with up to two-thirds lost before 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 O2 that occurs among microvessels.

The Krogh model permitted Krogh to propose that tissue oxygenation was dependent 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\textsubscript{2} will be skipped into this branch (FIGURE 2). As flow into the side branch increases as a result of downstream vasoconstriction, 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\textsubscript{2} saturation to enter that branch. Thus an increase in O\textsubscript{2} demand in one region of tissue would induce a redistribution of erythrocytes (O\textsubscript{2} delivery) not solely described by the simple redistribution of blood flow. In light of these complications, 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\textsubscript{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\textsubscript{2} requirements in a tissue (18, 23, 26, 43, 49, 64, 72). However, the exquisite accuracy in matching O\textsubscript{2} supply with O\textsubscript{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 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 needs is the requirement that the need be detected, quantified, and subsequently coupled to a mechanism that will appropriately alter blood flow (O\textsubscript{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, 44, 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 diffusional 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 components of the oxygen transport pathway that 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 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 whenever and whenever the need might arise.

The establishment that the erythrocyte, the major supplier of oxygen, also functions as a sensor of oxygen requirements for local tissue would have several implications to local vascular control and the maintenance of oxygen supply to the extremities. Erythrocytes contain phospholipid-soluble GTPase-activating protein (GAP) G proteins Gs (86), which is a signaling component of the endothelial cascade for oxygen-sensitive signaling pathways (FIGURE 3). These studies have demonstrated a signaling pathway from oxygen sensors in the RBC to the endothelium that is mediated by transduction of ATP from erythrocyte mitochondria to the RBC membrane-bound G protein Gs (85). An important question is how these cells translate an oxygen tension-sensitive signal to a cellular response to erythrocyte oxygen content reductions that result in local hypoxia and changes in NO release from the erythrocyte. Erythrocytes express nitric oxide synthetase, and NO release from the RBC to the surrounding tissue has been demonstrated in vitro (22, 50, 70). In addition, studies have confirmed in vivo in rats and hamsters that oxygen delivery to tissues is significantly impaired in the absence of Gs (45). The finding that NO release is impaired in conditions of hypoxia suggests that this signaling cascade is present throughout the past several years of studies on oxygen-sensing mechanisms. In summary, a signaling cascade from oxygen sensors in the RBC to the endothelial cell (FIGURE 3) including Gs (86), phosphatidylinositol hydrolysis, and NO release from the RBC may provide the basis for oxygen-sensitive signaling.
triggers the delivery of ATP from erythrocytes in response to both physiological and pharmacological stimuli. 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 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 requirements and affector 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, Bregfeld 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 Torr) alone is sufficient to stimulate ATP release from erythrocytes of hamsters (24), rabbits (85), rats (50), and humans (84). Although 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 changes in the intracellular ATP concentration of the microenvironment (80). This conclusion was further supported by an observed inhibition of ATP release on exposure of the erythrocyte to carbon monoxide, which would prevent the conformational change of hemoglobin in response to a drop in oxygen tension (56). The importance of hemoglobin oxygen saturation in ATP release was later confirmed in human studies by González-Alonso and his collaborators (38, 39, 75). 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 in the erythrocyte connecting the stimulus to the release.

A Proposed Signal Transduction Pathway for ATP Release from Erythrocytes

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 (B). Vasodilation of the side branch (A) reduces both hematocrit and O2 saturation in that vessel, whereas vasoconstriction (C) increases both hematocrit and O2 saturation. Higher O2 saturation along vessel centerline is detected by bright red erythrocytes, lower saturation near wall is indicated by cells with increasing levels of purple.
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 SO₂, 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 (O₂ 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 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).

FIGURE 3. The entrance of erythrocytes into tissue regions with a high oxygen demand

The entrance of erythrocytes into tissue regions with a high oxygen demand (decreased oxygen tension [P O₂]) results in diffusion of oxygen to the tissue and a decrease in the oxygen saturation (S O₂) of the hemoglobin within erythrocytes in the microcirculation. This decrease in S O₂ 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 S O₂. 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. 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.
An increase in oxygen tension to the core of a tissue occurs when the oxygen supply is increased. In the microcirculation, ATP is released from erythrocytes in a manner that allows it to pass through the endothelium and activate downstream targets, such as smooth muscle cells, resulting in a conducted vasodilation. Several studies have demonstrated that ATP released from erythrocytes is known to activate specific purinergic receptors on the vascular endothelium. Although ATP is simpler to consider two distinct classes of these P2 receptors: P2Y and P2X, more recent evidence indicates that the mammalian P2X and P2Y receptors are actually families of receptors consisting of seven P2X receptors (P2X1–7) and eight P2Y receptors (P2Y1, P2Y2, P2Y4, P2Y6, P2Y11–14) (1, 34). Studies in cerebral microvessels have delineated the specific receptors present and the vasoactive response to their stimulation. In isolated nonpressurized pial arterioles, Lewis et al. confirmed that P2Y-receptors are responsible for the observed vessel constriction (57).

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 vasoconstrictor in the baboon cerebral circulation (28) and that topically applied ATP dilated cat pial vessels at a much lower concentration than adenosine (30). These reports led 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 vasoconstrictors (14, 53), only ATP and ADP consistently caused conducted vasoactive 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, whereas 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.

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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, McCulloch (59) and Collins (10) each observed that the conducted vasodilation to intraluminal ATP was eliminated following administration of a NO synthase inhibitor implicating NO as an important vascular mediator in these vessels. In addition, in large cerebral arterioles, endothelial P2Y1-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, whereas P2X7-specific stimulation releases only the...
non-NO, non-cyclooxygenase-dependent factor (46), possibly a cytochrome P450 monoxygenase product such as epoxysaccaric 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 arteries, the dilution of ATP was preceded by hyperpolarization, and the dilution to ATP dependent on large conductance BKCa and intermediate conductance IKCa calcium-sensitive potassium channels but not small conductance IKCa 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 NO is a regulator of vascular perfusion, controversy remains as to its source and mechanism of action. In 1996, Stamler and colleagues (51) proposed that the erythrocyte could aoract on the NO synthase in the underlying smooth muscle to increase cGMP synthesis (32) and inhibit erythrocyte Gi to promote vasodilation, which occurs following the release of ATP from erythrocytes in a negative-feedback fashion. Indeed, NO was shown to attenuate agonist-induced ATP release from erythrocytes in vivo (23).

**Feedback Mechanisms**

**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 vasodilatation, 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.
brain artery 

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 ATP, 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. 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 one-half of associated deaths, express decreased amounts of the subunit of the heterotrimeric G protein Gi compared with erythrocytes of healthy humans (86). Interestingly, decreased Gi expression is present in animal models of diabetes as well (40–42, 63, 86, 99). In humans, Gi2 subunit expression was decreased selectively; that is, expression of other Gi subtypes, G(o), and AC II was unaltered (86). This selective decrease in Gi2 subunit expression in human erythrocytes was associated with impaired cAMP synthesis as well as decreased ATP release in human erythrocytes (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.

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**Microcirculation Changed the Conceptual Approach to our Understanding of Oxygen Supply**

Our understanding of the mechanisms that control matching of O2 supply with demand has evolved from an 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 O2 delivery based on O2 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. 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 that need to be addressed
include: 1) whether there is a primary location within the network where the need for changes in O$_2$ supply are sensed or control is exercised throughout the arteriolar tree to ensure proper distribution of O$_2$ supply; 2) the inherent time scale of the regulatory system (i.e., determining how fast can the system respond and what the limiting factors are); 3) the vessels in the network where the need for changes in O$_2$ supply are sensed; 4) whether there is a primary location within the network where the need for changes in O$_2$ supply are sensed or control is exercised throughout the arteriolar tree to ensure proper distribution of O$_2$ supply; and 4) the limitations of the system’s ability to control local O$_2$ delivery. Future progress toward understanding the role of erythrocyte-derived ATP in the regulation of O$_2$ delivery within the microcirculation will depend on combining new in vitro experiments, detailed computational modeling, and model testing and validation using its in vitro recordings under a range of physiological and pathophysiological conditions.

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19. Duling BR, Berne RM. Longitudinal gradients in arteriolar tree to ensure proper distribution of O$_2$ supply; and 4) the limitations of the system’s ability to control local O$_2$ delivery. Future progress toward understanding the role of erythrocyte-derived ATP in the regulation of O$_2$ delivery within the microcirculation will depend on combining new in vitro experiments, detailed computational modeling, and model testing and validation using its in vitro recordings under a range of physiological and pathophysiological conditions.

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