Erythrocytes: Oxygen Sensors and Modulators of Vascular 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 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.

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. FIGURE 1A, the current view is that the microcirculatory exchange occurs between capillaries with different O2 levels (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 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 convective oxygen flow (blood flow) and diffusive oxygen transfer (23, 37).

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 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 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 convective oxygen flow (blood flow) and diffusive oxygen transfer (23, 37).

Therefore, 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 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 that is not accounted for by the Krogh model. Although it is not fully understood why there is such a large precapillary decrease in O2, Ellsworth and Pittman (26) provided evidence that some of the O2 leaving the arterioles diffuses to erythrocytes flowing through nearby capillaries, resulting in an increase in their O2 saturation. Since O2 is transported by diffusion from arterioles to capillaries, it is also likely that O2 exchange occurs between capillaries with different O2 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 O2 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 O2 requirements and uniform O2 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).

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very low, only plasma and the erythrocytes with lower O2 will be skinned into this branch (FIGURE 2). As flow into the side branch increases as a result of downstream vasodilatation, 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 O2 saturation to enter that branch. Thus an increase in O2 demand in one region of tissue would induce a redistribution of erythrocytes (O2 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 O2 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 O2 requirements in a tissue (18, 23, 26, 27, 43, 49, 64, 72). However, the exquisite accuracy in matching O2 supply with tissue O2 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 needs is the requirement that the need be detected, quantified, and subsequently coupled to a mechanism that will appropriately alter blood flow (O2 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 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 component 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 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 metabolically needed oxygen, 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 to local vessels would add a unique sense cell to local vascular control and physiology. Indeed, the erythrocyte, a living bag of oxygen, is the organ that continuously monitors and responds to local tissue gas exchange to appropriate oxygen supply directly or indirectly with the endothelium to appropriately alter microvascular perfusion. This conclusion is shared by numerous studies that have demonstrated the critical role of the erythrocytes in maintaining local tissue oxygenation in the intact organism. 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.
gene requirements and afferent of changes in oxygen supply would provide an important level of precision to local vascular control. How could such a small, nucleated bag of hemoglobin accomplish this task? Erythrocytes contain millimolar quantities of adenosine 5′-triphosphate (ATP), 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 hemoglobin O₂ saturation, suggesting that the conformational change of hemoglobin, as it desaturates during a fall in oxygen levels, elicits the release of ATP. This conclusion was further supported by an observed inhibition of ATP release on exposure of the erythrocytes to carbon monoxide, which would prevent the conformational change of hemoglobin in response to a drop in oxygen tension (50). 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**

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, Oleareczyk et al. demonstrated that the G protein that is activated when erythrocytes are exposed to deformation or reduced 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). Therefore, the major sensor of oxygen decrease localized to the blood supply. Could such a small, nucleated bag of hemoglobin accomplish this task? Erythrocytes contain millimolar quantities of adenosine 5′-triphosphate (ATP), 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 hemoglobin O₂ saturation, suggesting that the conformational change of hemoglobin, as it desaturates during a fall in oxygen levels, elicits the release of ATP. This conclusion was further supported by an observed inhibition of ATP release on exposure of the erythrocytes to carbon monoxide, which would prevent the conformational change of hemoglobin in response to a drop in oxygen tension (50). 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.

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oxygen tension is not Gi 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 sub
unit 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, 66, 89, 91), have been
shown to activate AC isoforms II, IV, and VII.

Importantly, Giα, β subunits 1 through 4, and AC II
are all components of human erythrocyte membrane
(81). A central role for Gi in the pathway for ATP
release from erythrocytes in response to reduced oxy-
genesis was demonstrated by the finding that
incubation of erythrocytes with pertussis toxin, which
binds to Gi preventing its dissociation into the compo-
nent subunits, prevented ATP release in response to
this physiological stimulus (70). Although Gi
activation is clearly involved in ATP release from ery-
throcytes exposed to reduced oxygen tension, the
mechanism that directly couples the decrease in oxy-
genesis of the hemoglobin molecule to the
activation of Gi remains under investigation. One pos-
sibility is that the conformational change in the hemo-
globin 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 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 perfu-
sion (O2 delivery) (56) (FIGURE 3). Using arterioles in
the intact hamster cheek pouch retractor muscle,
McCullough et al. (59) demonstrated a dose-depend-
ent conducted vasodilator response to the intralumi-
nal application of ATP, occurring at 1
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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 (SO2) of the hemoglobin within erythro-
cytes in the microcirculation. This decrease in SO2 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 SO2. 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'-ado-
sine 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.
Adenosine release

(O2)delivery to the tissue. One possibility is that the hypoxic tissue membrane of the release arterioles in a region of tissue perfused by a conducted arteriole would be sensitive to the decrease in oxygen tension 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 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 to collecting venular beds, observing no conducted vasodilation, the speed of which was influenced by the architecture of the intervening vasculature. It is important to note that the intra-arteriolar application of $10^{-6}$ M ATP, the concentration that produced the maximum conducted vasodilatation, 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 580 ms, 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 stepwise fashion using a computer-controlled gas flow chamber, the increases in flow that occurred within the capillary bed were consistent with the conducted vasodilatation. When ATP is released into the vascular lumen, it can interact with receptors present on the endothelium that 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 vaso dilators. These vasodilators include nitric oxide (NO) 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 [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 vasodilators [14, 53], only ATP and ADP consistently caused conducted vaso motor responses [14, 53]. Since at equimolar concentrations ADP was 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 Ikemoto 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. ATP released from erythrocytes is known to activate specific P2 purinergic receptors on the vascular endothelium. Although it is simpler to consider two distinct classes of these P2 receptors, P2Y and P2X, more recent evidence indicates that the mammalian P2Y2 and P2Y6 receptors are actually families of receptors consisting of seven P2Y receptors (P2Y1-7) and eight P2X receptors (P2X1-7, 11) (1, 34). Studies in cerebral microvessels have delineated the specific receptors present and the vasoactive response to their stimulation. In isolated nonpresurized pial arterioles, Lewis et al. concluded that P2Y1 and P2X1-like receptors are responsible for the observed vessel constriction [57]. In isolated and pressurized rat penetrating arterioles, Hiroiuchi et al. reported that ATP constricts the vessels transientsly via smooth muscle P2X1 receptors and dilates the vessels via endothelial P2Y11 receptors (47). Similarly, You et al. found that, in larger size cerebral arterioles, dilation to intraluminal ATP results from P2Y11 stimulation [98]. These receptors were confirmed to exist on hamster skeletal muscle arterioles (unpublished observations).

Regardless of the receptor that is activated, ATP-induced vasodilatation 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 vasodilatation 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 P2Y11-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 P2X1-specific stimulation releases both NO and a non-NO, non-cyclooxygenase-dependent factor, whereas P2Y1-specific stimulation releases only the
non-NO, non-cyclooxygenase-dependent factor (46), possibly a cytochrome P450 monooxygenase product such as epoxyeicosanoids (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 depended 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 the responders (FIGURE 3).

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 can act in an autocrine manner by activating erythrocyte P2x7 receptors (34) that releases ATP, which can inhibit AT-driven vascular relaxation. This hypothesis was supported by the fact that ATP inhibits blood flow in the rat ear (52). ATP acts on its smooth muscle Gβγ-coupled IP3 receptor to increase IP3 synthesis. IP3 activates its receptor on the sarcoplasmic reticulum (SR) to increase Ca2+ uptake, activating PLA2 activity and EET synthesis and release (52). The EETs act directly on a vascular KCa2+ channel to hyperpolarize and relax the smooth muscle cell that results in its relaxation. ATP released from the erythrocyte can act in an autocrine manner by activating erythrocyte P2x7 receptors (34) that releases ATP, which can inhibit AT-driven vascular relaxation. This hypothesis was supported by the fact that ATP inhibits blood flow in the rat ear (52).

Non-NO, non-cyclooxygenase-dependent factors such as EETs (16) are generated in the erythrocyte and released into the plasma upon cell damage, resulting in hyperpolarization (8, 31). These factors can interact with the endothelium to promote vascular smooth muscle cell relaxation and dilation. ATP, a non-NO, non-cyclooxygenase-derived factor, released by damaged erythrocytes, acts on the endothelium to induce EET synthesis and release. EETs act directly on vascular KCa2+ channels to hyperpolarize and relax smooth muscle cells.

**Control of ATP Release in Vivo: Feedback Mechanisms**

Although ATP can stimulate the synthesis and release of multiple endothelial-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, but also to the periphery in the form of the 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). Recent work from the laboratories of Gladwin and Patel has provided evidence that deoxygenhemoglobin acts as a nitric reductase, converting nitrate to NO, and hence making it possible for the erythrocyte vasodilator to act as an autocrine regulator of NO production (11, 36, 71).

**Pathological Defects in Erythrocyte Function**

Recently, it was discovered that with Type 2 diabetes mellitus, the subunit of the human ADP receptor is decreased (70). This subunit has a significant role in the unique response of the human erythrocyte to ADP, and the cAMP synthesis response to ATP (86). Since vascular microcirculation correlates inversely with the possibility of ATP release from erythrocytes to release ATP, there is an increase in DM2 (12, 61). In these studies, donor sperm containing ATP from normal donors can inhibit ATP release from erythrocytes, which results in the inhibition of the ischemic response to ATP (96). This suggests that the release of ATP could be a potential therapy for the treatment of diabetes mellitus.

**How the Cerebral Microcirculation Can Change**

Our understanding of the concept of the cerebral microcirculation is evolving rapidly. The concept of the cerebral microcirculation is that the cerebral microcirculation is controlled by local metabolic and neurovascular signals. These signals are generated in the brain itself and are transmitted to the cerebral microcirculation through local metabolic and neurovascular signals. The cerebral microcirculation can change in response to changes in these signals, such as increased metabolic demand, increased blood flow, or decreased blood flow. The cerebral microcirculation can also change in response to changes in the local metabolic and neurovascular signals, such as increased metabolic demand, increased blood flow, or decreased blood flow. The cerebral microcirculation can change in response to changes in these signals, such as increased metabolic demand, increased blood flow, or decreased blood flow. The cerebral microcirculation can change in response to changes in these signals, such as increased metabolic demand, increased blood flow, or decreased blood flow. The cerebral microcirculation can change in response to changes in these signals, such as increased metabolic demand, increased blood flow, or decreased blood flow.
In these studies, incubation of erythrocytes with the NO donor nitroso-N-d-nitrosoglutethimide (NOSG) results in inhibition of ATP release from rabbit and human erythrocytes exposed to decreased oxygen tension (87). 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 NO (96) 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 α2 subunit of the heterotrimeric G protein Gα compared with erythrocytes of healthy humans (86). Interestingly, decreased Gα expression is present in animal models of diabetes such as C57bl/6j and db/db mice (88). In humans, decreased Gα expression was decreased selectively; that is, expression of other Gα subtypes, Gαi, and AC II was unaltered (86). This selective decrease in Gαi2 expression in human erythrocytes was associated with impaired cAMP synthesis as well as with decreased ATP release in response to an agent that directly activates Gi, mastoparan 7 (86). It is of interest that the degree of response to an agent that directly activates Gi, cAMP synthesis as well as decreased ATP release in response to an agent that directly activates Gi, mastoparan 7 (86). It is of interest that the degree of response to an agent that directly activates Gi, mastoparan 7 (86).

**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 O2 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 O2 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 1) 3D network geometry of capillaries, arterioles, and venules; 2) realistic hemodynamics of erythrocyte flow and O2 distribution within these networks; 3) 3D oxygen transport between blood and tissue; 4) changes in erythrocyte-derived ATP release as a function of oxygen content; 5) local and conducted changes in microvascular diameter; and 6) 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 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. 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 that need to be addressed...
include 1) whether there is a primary location within the network where the need for changes in O\textsubscript{2} supply are sensed or controlled is exercised throughout the arteri- otree to ensure proper distribution of O\textsubscript{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 net- work that are most likely to respond to a given stimul- us; and 4) the limitations of the system’s ability to control local O\textsubscript{2} delivery. Future progress toward understanding the role of erythrocyte-derived ATP in the regulation of O\textsubscript{2} delivery within the microcircula- tion will depend on a combination of new in vitro experiments, detailed computational modeling, and testing and validation using in vivo ret- ina under a range of physiological and pathophysi- ological conditions.

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