

SYMPOSIUM REVIEW

Regulation of blood flow distribution in skeletal muscle: role of erythrocyte-released ATP

Mary L. Ellsworth and Randy S. Sprague

Department of Pharmacological and Physiological Science, Saint Louis University School of Medicine, 1402 South Grand Boulevard, St Louis, MO 63104, USA

Abstract The maintenance of adequate tissue O₂ levels in skeletal muscle is vital for normal physiology and requires a well regulated and appropriately distributed convective O₂ supply. Inherent in this fundamental physiological process is the requirement for a mechanism which both senses tissue O₂ need and locally adjusts flow to appropriately meet that need. Over the past several years we and others have suggested that, in skeletal muscle, O₂ carrying erythrocytes participate in the regulation of total blood flow and its distribution by releasing ATP. Importantly, the release of this vasoactive molecule must be both rapid and well controlled if it is to serve an important physiological role. Here we provide insights into three distinct regulated signalling pathways within the erythrocyte that are activated by exposure to reduced O₂ tension or in response to binding of agonists to the prostacyclin or β -adrenergic receptors. Although much has been learned about the role of the erythrocyte in perfusion of skeletal muscle, much remains to be understood. However, what is clear is that the long established passive carrier of O₂ also contributes to the regulation of the distribution of microvascular perfusion in skeletal muscle by virtue of its capacity to release ATP.

(Received 26 March 2012; accepted after revision 9 May 2012; first published online 14 May 2012)

Corresponding author M. L. Ellsworth: Department of Pharmacological and Physiological Science, Saint Louis University School of Medicine, 1402 South Grand Boulevard, St Louis, MO 63104, USA. Email: ellsworthm@slu.edu

The concept that blood gains a ‘vital essence’ in the lungs which it then transports throughout the body has been considered doctrine since it was first described by Greek philosophers of the Ionian school in Asia Minor in the 6th century BC (Cournand, 1982). However, it was not until 1628 that a detailed description of the systemic

circulation and the observation that blood is pumped by the heart were provided by the English physician William Harvey (1889). Thirty years later, the Dutch biologists Jan Swammerdam and Anton van Leeuwenhoek each reported that they had visualized erythrocytes, seeing them as flexible discs in a ‘milky’ medium

Mary L. Ellsworth, PhD (Albany Medical College) and **Randy S. Sprague**, MD (St Louis University) have collaborated on studies related to the role of ATP released from erythrocytes in the regulation of the distribution of microvascular perfusion. In association with colleagues and students from the US and Canada, they have described the effect of low oxygen tension on erythrocyte ATP release and the impact of that release on vascular function and oxygen supply, and have characterized the signal transduction pathways which regulate the ATP release in response to both physiological and pharmacological stimuli. Recent studies have focused on the implications of defects in these release pathways on oxygen transport in a number of pathological conditions including diabetes, prediabetes and pulmonary hypertension.



This report was presented at *The Journal of Physiology* Symposium on *Red blood cell mechanisms of tissue blood flow control: physiological insights in health and disease*, which took place at the Experimental Biology 2012 Meeting, San Diego, CA, USA on 22 April 2012. It was commissioned by the Editorial Board and reflects the views of the authors.

(Bessis & Delpuch, 1981). Despite acknowledgement that erythrocytes were present in the circulation, it was not until 1840 that the oxygen (O₂) carrying protein they contain, haemoglobin, was discovered by F. L. Hünefeld (1840). The reversible oxygenation and competitive binding of O₂ to haemoglobin was detailed a few years later by Felix Hoppe-Seyler (1866). For most of the next century, the erythrocyte was considered to be a flexible, haemoglobin containing cell whose sole purpose is to carry oxygen from the lungs to the tissues.

In a 1914 book entitled *The Respiratory Function of the Blood*, Joseph Barcroft stated that 'the cell takes what it needs and leaves the rest'. Barcroft then suggested that it is only logical that the following sequence must occur. 'Firstly the call for oxygen, secondly the mechanism by which the call elicits a response, the immediate response consisting of the carriage of oxygen to the tissue by the blood and its transference from the blood to the cell. Thirdly in the background you have the further mechanism by which the blood acquires its oxygen' (Barcroft, 1914). Barcroft explored possible scenarios to explain how these three components are able to satisfy the tissues' 'call for oxygen' but none could account for the complexity of the response.

As Christian Bohr's assistant, August Krogh became interested in problems connected with gas exchange in the living organism in the early part of the 20th century. His studies demonstrated an increased utilization of the O₂ of the blood during muscular work, which he explained by an increase in the diffusion surface resulting from the opening of previously closed capillaries of skeletal muscle (Krogh, 1959). However he was unable to determine a mechanism by which perfusion distribution was altered to precisely meet the metabolic needs of that tissue. Since the time of Krogh, investigations have revealed that there is not a preponderance of closed capillaries in resting skeletal muscle which would be opened during exercise (Poole *et al.* 2011) and that, in addition, the capillary is not the sole site of oxygen transfer (Ellsworth *et al.* 2009). These findings make the determination of factors responsible for appropriate oxygen supply significantly more complex. In the intervening years, most studies have focused on mechanisms by which the resistance vessels increase and decrease their diameter via alterations in the activity of the autonomic nervous system and/or levels of vasoactive mediators. Although both mechanisms are likely to contribute to overall tissue perfusion, neither appears capable of the exquisite control necessary for the optimal matching of O₂ supply with demand in skeletal muscle.

Release of ATP from erythrocytes in response to reduced O₂ tension

A 1993 study of capillary O₂ transport in hamster skeletal muscle (Stein & Ellsworth, 1993) set the stage for a series

of studies exploring the possibility that the erythrocyte, by virtue of its capacity to release the vasoactive mediator ATP in response to a decrease in O₂ saturation, could serve as the mechanism by which Barcroft's 'call for oxygen' might be answered. In an earlier paper, Bergfeld & Forrester (1992) showed that ATP was released from healthy human erythrocytes upon exposure to reduced O₂ tension in the presence of hypercapnia. It has subsequently been established that isolated human, rat, rabbit and hamster erythrocytes release ATP when exposed to reduced O₂ tension in the presence of normal levels of CO₂ indicating that it is the decrease in O₂ which is the driving force for ATP release (Ellsworth *et al.* 1995; Ellsworth, 2000; Dietrich *et al.* 2000). It has subsequently been suggested that ATP release is evoked by the conformational change in the membrane associated haemoglobin molecules which occurs as they desaturate in response to exposure to reduced O₂ levels (Jagger *et al.* 2001) with the optimal response in the range of the animal's P₅₀ (Sprague & Ellsworth, 2012).

ATP, once released from the erythrocyte, can bind to purinergic receptors located on the vascular endothelium, which initiates a conducted vasodilator response (McCullough *et al.* 1997; Collins *et al.* 1998) augmenting blood flow to the specific local tissue region in need (Sprague *et al.* 2010). Thus, the mobile erythrocyte, by releasing both O₂ and ATP in regions of low O₂ tension contributes to the optimization of O₂ delivery to skeletal muscle enabling it to function appropriately.

The egress of ATP from erythrocytes upon exposure to low O₂ tension occurs by a mechanism that is both rapid and well regulated (Dietrich *et al.* 2000; Wan *et al.* 2008). Critical for this release is the activation of a discrete signalling pathway within the erythrocyte likely initiated by the conformational change induced by the offloading of O₂ from membrane-associated haemoglobin (Jagger *et al.* 2001; Wan *et al.* 2008; Sridharan *et al.* 2010b; Forsyth *et al.* 2011). Key components of this signalling pathway include the heterotrimeric G protein, Gi (Sprague *et al.* 2002; Olearczyk *et al.* 2004a), adenylyl cyclase (Sprague *et al.* 1998, 2001, 2008), protein kinase A (PKA) (Sprague *et al.* 2001), the cystic fibrosis transmembrane conductance regulator (CFTR) (Sprague *et al.* 1998) and pannexin 1, which serves as the final ATP conduit (Sridharan *et al.* 2010a). The release of ATP via this pathway requires an increase in intracellular cAMP. The level of this second messenger is controlled by the relative activities of adenylyl cyclase and phosphodiesterase 3. The details of this pathway are depicted in Fig. 1 and have been the subject of several recent reviews (Ellsworth *et al.* 2009; Sprague *et al.* 2011; Sprague & Ellsworth, 2012). ATP release in response to exposure to low O₂ tension has been suggested to contribute to the appropriate distribution of perfusion in skeletal muscle. However, ATP release from erythrocytes also occurs when these cells are exposed to

the endogenous vasoactive mediators, prostacyclin (PGI₂) (Olearczyk *et al.* 2001; Sprague *et al.* 2008) and adrenaline (Olearczyk *et al.* 2001; Sprague *et al.* 2001), both of which can be released locally into the circulation.

Receptor mediated ATP release from erythrocytes: prostacyclin

In microvessels, increased endothelial shear stress (Ngai & Yao, 2010), as would occur with an increase in the velocity of blood flow, results in the synthesis and/or release of vasodilators, which include nitric oxide (NO) and several products of arachidonic acid metabolism, including PGI₂ (Koller & Kaley, 1990; Osanai *et al.* 2000). Both NO and PGI₂ stimulate vasodilatation via direct action on vascular smooth muscle. However, in addition, exposure of erythrocytes to PGI₂ analogues, including iloprost and UT-15C, stimulates receptor-mediated increases in cAMP synthesis and ATP release from human erythrocytes in a concentration-dependent manner (Sprague *et al.* 2008). Thus, blood vessels exposed to increased shear stress vasodilate as the result of both direct effects of locally released NO and PGI₂ on vascular smooth muscle and the initiation of a conducted vasodilatation secondary to PGI₂-mediated release of ATP from erythrocytes.

The regulated release of ATP in response to PGI₂ requires that erythrocytes possess the prostacyclin receptor (IPR) and that activation of the IPR initiates a signalling pathway. Over the past several years it has been established that the IPR signalling pathway is distinct from the pathway for ATP release activated by exposure of these cells to reduced O₂ tension (Fig. 2). In non-erythrocytes, the IPR is coupled to the heterotrimeric G protein (Gs)

and its activation results in increased production of cAMP via adenylyl cyclase. Erythrocytes of humans and rabbits possess both the IPR and the heterotrimeric G protein, Gs, and exposure of these cells to PGI₂ analogues results in increases in cAMP and ATP release (Olearczyk *et al.* 2001, Sprague *et al.* 2008). That these responses were receptor mediated was confirmed by studies in which increases in cAMP and ATP release were prevented by pre-incubation of erythrocytes with the IPR receptor antagonist CAY10441 (Sprague *et al.* 2008). In subsequent studies, it was shown that increases in cAMP in this pathway are regulated by the activity of phosphodiesterase 3 (PDE3) (Hanson *et al.* 2008). This PDE has two distinct isoforms, one cytosolic (PDE3A) and the other membrane bound (PDE3B), both of which are present in human erythrocytes (Hanson *et al.* 2008, Adderley *et al.* 2010a, 2011b). PDE3A has been associated with IPR activation in platelets and pulmonary vessels and thus is the PDE3 isoform likely to be associated with this pathway in human erythrocytes.

In many intracellular signalling pathways, protein kinase A (PKA) is phosphorylated (activated) by cAMP. Activation of both PKA and PKC occurs in the IPR signalling pathway in rabbit erythrocytes (Adderley *et al.* 2010b). Although PKA and PKC have numerous intracellular targets, their role in the activation of CFTR is crucial for IPR-mediated ATP release from human erythrocytes (Sprague *et al.* 1998; Sridharan *et al.* 2012). Although previously thought to be an ATP conduit, more recent evidence supports the hypothesis that CFTR regulates the movement of anions such as chloride into and out of cells (Gadsby *et al.* 2006). The influx of an anion is essential for the release of the highly negatively charged ATP to maintain the ionic balance of the erythrocyte

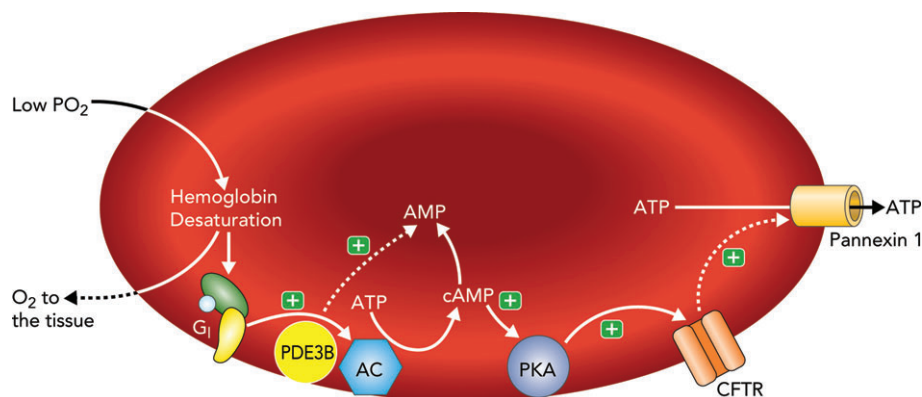


Figure 1. Proposed signalling pathway for low O₂-induced ATP release from erythrocytes

When exposed to low O₂, membrane-associated haemoglobin releases O₂. The associated conformational change in the haemoglobin molecule associated with its desaturation results in local membrane deformation and activation of Gi. This leads to activation of AC and increase in cAMP, the level of which is regulated by PDE3B activity. Increases in cAMP activate PKA and, subsequently, CFTR. The final conduit for ATP release in this pathway is pannexin 1. Abbreviations: Gi, heterotrimeric G protein; PDE3B, phosphodiesterase 3B; cAMP, cyclic adenosine monophosphate; AMP, adenosine monophosphate; AC, adenylyl cyclase; PKA, protein kinase A; CFTR, cystic fibrosis transmembrane conductance regulator; ATP, adenosine triphosphate; (+), activation; (–), inhibition.

(Bergfeld & Forrester, 1992). Recently, the conduit for ATP release from human erythrocytes in response to IPR activation has been identified as the voltage-dependent anion channel (VDAC) (Sridharan *et al.* 2012).

Comparison of the low O₂- and prostacyclin-induced pathways for ATP release from erythrocytes

Although the release of ATP in response to endothelial-derived PGI₂ would amplify a local vasodilatation, such a response would be less effective in directing microvascular perfusion to regions of O₂ need in skeletal muscle. Indeed, computational modelling has confirmed that general vasodilatation, such as that produced by PGI₂, is less effective in providing appropriate amounts of O₂ to skeletal muscle when compared with low O₂-induced ATP release (Sprague *et al.* 2010). The incorporation of a functional regulatory system that directs blood flow only to areas of need provides optimal tissue oxygenation by eliminating the likely oversupply of O₂ to regions which were already adequately supplied, a theoretical consequence of non-directed general vasodilatation. However, the observation that erythrocytes release ATP in response to both low O₂ exposure (directed vasodilatation) and prostacyclin (general vasodilatation) places these cells in a unique position to contribute to the dramatic increase in total skeletal muscle blood flow during exercise and, simultaneously, direct that flow to

specific tissue regions. Importantly, the dual mechanisms provide for a redundant control mechanism should one of the signalling pathways be impaired, as can occur under pathological conditions (Sprague & Ellsworth, 2012).

Although these two mechanisms for ATP release from erythrocytes can work together to augment blood flow to skeletal muscle, the signalling pathways responsible for that release are distinct (Table 1). In spite of the fact that in both pathways ATP release requires increases in cAMP and activation of CFTR (Sprague *et al.* 1998, 2008; Sridharan *et al.* 2010), the G protein involved in initiating the response is different. In the low O₂ pathway G_i activation is required (Sprague *et al.* 2002; Olearczyk *et al.* 2004*b*) while binding of an agonist to IPR activates G_s (Olearczyk *et al.* 2001). In addition, it is now clear that the final conduits for ATP release in response to low O₂ exposure and IPR activation are likewise distinct having been identified as pannexin 1 (Sridharan *et al.* 2010*a*) and VDAC (Sridharan *et al.* 2012), respectively.

The mechanism of receptor-mediated activation of G_s in the IPR signalling pathway has been well defined (Nobles *et al.* 2005). In contrast, the mechanism by which exposure to low O₂ stimulates G_i is less well understood. It is clear that mechanical deformation of erythrocytes results in ATP release by activation of a signalling pathway that appears to be identical to the pathway activated by exposure of erythrocytes to low O₂ (Olearczyk *et al.* 2004*a,b*). This finding led to the hypothesis that upon exposure of erythrocytes to low O₂, the desaturation of the

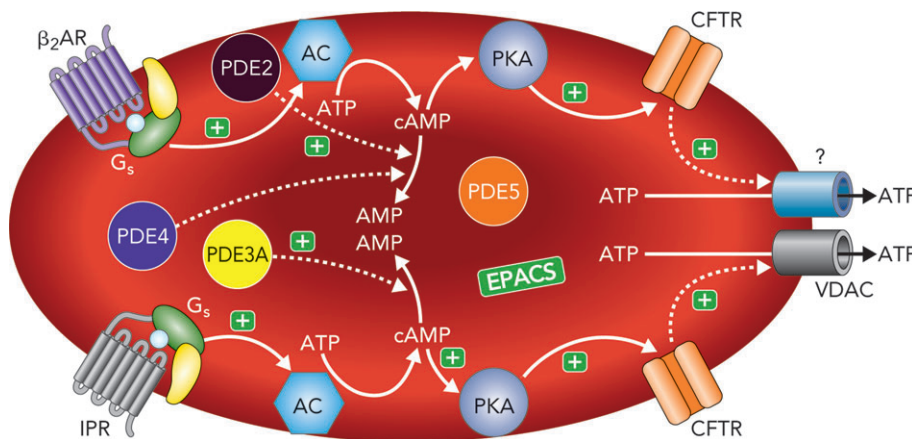


Figure 2. Proposed signalling pathways for ATP release from erythrocytes via activation of the G_s-coupled IPR or β AR

Binding of selective agonists to the IPR or β AR results in activation of G_s and, subsequently, AC leading to increases in cAMP. In the IPR pathway, increases in cAMP are regulated by PDE3 while in the β AR pathway, cAMP is hydrolysed by PDEs 2 and 4. In both pathways increases in cAMP activate PKA and, subsequently, CFTR. The final conduit for ATP release in the IPR signalling pathway is VDAC while the distinct channel responsible for ATP release in the β AR signalling pathway remains to be determined. Abbreviations: β ₂AR, β ₂-adrenergic receptor; IPR, prostacyclin receptor; G_s, heterotrimeric G protein; PDE3A, phosphodiesterase 3A; PDE2, phosphodiesterase 2; PDE4, phosphodiesterase 4; cAMP, cyclic adenosine monophosphate; AMP, adenosine monophosphate; AC, adenylyl cyclase; PKA, protein kinase A; CFTR, cystic fibrosis transmembrane conductance regulator; VDAC, voltage dependent anion channel; ATP, adenosine triphosphate; (+), activation; (–), inhibition. Both phosphodiesterase 5 (PDE5) (Adderley *et al.* 2011*b*) and exchange proteins activated by cAMP (EPACs) (Adderley *et al.* 2011*a*) are present in erythrocytes and their interaction with the IPR and β AR signalling pathways is under investigation.

Table 1. Distinguishing characteristics of discrete signalling pathways for ATP release from erythrocytes

Signalling pathway	Heterotrimeric			Final ATP conduit
	G protein	Phosphodiesterase(s)	Protein kinases	
Low O ₂ Mechanical Stress	Gi	PDE3 (B?)	PKA	Pannexin 1
IPR	Gs	PDE3 (A?)	PKA and PKC	VDAC
β AR	Gs	PDE2 and PDE4	PKA	Unknown

Abbreviations: IPR, prostacyclin receptor; β AR, β -adrenergic receptor; VDAC, voltage dependent anion channel.

haemoglobin molecules associated with the erythrocyte membrane produces a local membrane deformation (Wan *et al.* 2008) which results in the activation of Gi. A similar mechanism for Gi activation has been described in smooth muscle cells (Li & Xu, 2000). Studies in which low O₂-induced ATP release was prevented by either decreased membrane deformability (Sridharan *et al.* 2010b) or by incubation of erythrocytes with carbon monoxide to hold haemoglobin in the 'oxygenated' conformation (Jagger *et al.* 2001) provide strong support for this mechanism.

The IPR and low O₂ signalling pathways are also distinct in their response to exogenous NO. Exposure of erythrocytes to the NO donor spermine NONOate results in inhibition of low O₂-induced ATP release (Olearczyk *et al.* 2004b). Although the exact mechanism responsible for this inhibition has yet to be confirmed, it was suggested that NO might stimulate ADP-ribosylation of the α subunit of Gi (Olearczyk *et al.* 2004b) thereby preventing its activation and thus initiation of the signalling cascade for ATP release. In contrast, NO does not inhibit IPR-mediated ATP release from erythrocytes (Olearczyk *et al.* 2004a).

Receptor mediated ATP release from erythrocytes: β -adrenergic activation

In addition to pathways for ATP release that are activated by exposure to low O₂, mechanical deformation, or binding of an agonist to the IPR, activation of β -adrenergic receptors (β ARs) on erythrocytes also stimulates increases in cAMP and ATP release (Fig. 2). It has been known for some time that erythrocytes possess β ARs and available evidence suggests that the predominant receptor expressed is β_2 AR (Horga *et al.* 2000). The β AR signalling pathway for ATP release from erythrocytes is distinct from the other well characterized signalling pathways in that the PDEs that regulates increases in cAMP levels are PDEs 2 and 4 (Adderley *et al.* 2009). It is important to note that amounts of ATP released in response to incubation of erythrocytes with the β AR agonists isoproterenol or adrenaline are not as great as that associated with activation of the IPR signalling pathway (Olearczyk *et al.* 2001). Moreover, it is not clear what physiological role β AR-mediated ATP release from erythrocytes plays. It has been suggested that

β AR signalling in mature erythrocytes represents residual activity that remains from signalling pathways that were important for erythrocyte maturation prior to their release into the circulation (Kaiser *et al.* 1974; Rodan *et al.* 1975).

Perspective

The distribution of a 'vital essence' first suggested by Ionian philosophers in the 6th century BC has become universally accepted as a basic physiological concept (Courmand, 1982). Barcroft, in the early 20th century, suggested that the tissue must send out a 'call for oxygen' which the circulation provides (1914). Recent research suggests that the erythrocyte, first described in the 1600s (Bessis & Delpech, 1981), serves an important role in helping to answer that call. This mobile cell, by virtue of its ability to respond to both physiological and pharmacological stimuli by releasing ATP into the vasculature, enhances flow to specific tissue regions. The release of ATP in tissue regions with low O₂ tension directs flow to that specific region via the initiation of a conducted vasodilator response. An increase in wall shear stress induces the release of PGI₂ from the endothelial cells, which via its direct action on smooth muscle cells, induces a local dilatation. Importantly, this released PGI₂ also augments the release of ATP from the erythrocyte further contributing to ATP-induced increases in blood flow. This redundancy, a hallmark of important physiological processes, suggests that the erythrocyte, although not the sole controller of total perfusion and its distribution within skeletal muscle, does serve a critical role possibly as a mechanism to fine tune the distribution of flow to meet local tissue need.

References

- Adderley SP, Dufaux EA, Sridharan M, Bowles EA, Hanson MS, Stephenson AH, Ellsworth ML & Sprague RS (2009). Iloprost- and isoproterenol-induced increases in cAMP are regulated by different phosphodiesterases in erythrocytes of both rabbits and humans. *Am J Physiol Heart Circ Physiol* **296**, H1617–H1624.
- Adderley SP, Sprague RS, Stephenson AH & Hanson MS (2010a). Regulation of cAMP by phosphodiesterases in erythrocytes. *Pharmacol Rep* **62**, 475–482.

- Adderley SP, Sridharan M, Bowles EA, Stephenson AH, Sprague RS & Ellsworth ML (2011a). Inhibition of ATP release from erythrocytes: a role for EPACs and PKC. *Microcirculation* **18**, 128–135.
- Adderley SP, Sridharan M, Bowles EA, Stephenson AH, Ellsworth ML & Sprague RS (2010b). Protein kinases A and C regulate receptor-mediated increases in cAMP in rabbit erythrocytes. *Am J Physiol Cell Physiol* **298**, C587–C593.
- Adderley SP, Thuet KM, Sridharan M, Bowles EA, Stephenson AH, Ellsworth ML & Sprague RS (2011b). Identification of cytosolic phosphodiesterases in the erythrocyte: a possible role for PDE5. *Med Sci Monit* **17**, CR241–CR247.
- Barcroft J (1914). *The Respiratory Function of the Blood*. Cambridge University Press, Cambridge.
- Bergfeld GR & Forrester T (1992). Release of ATP from human erythrocytes in response to a brief period of hypoxia and hypercapnia. *Cardiovasc Res* **26**, 40–47.
- Bessis M & Delpech (1981). Discovery of the red blood cell with notes on priorities and credits of discoveries, past, present and future. *Blood Cells* **7**, 447–480.
- Collins DM, McCullough WT & Ellsworth ML (1998). Conducted vascular responses: communication across the capillary bed. *Microvasc Res* **56**, 43–53.
- Cournand A (1982). Air and blood. In *Circulation of the Blood: Men and Ideas*, ed. Fishman AP & Richards DW, p. 3. American Physiological Society, Bethesda, MD.
- Dietrich HH, Ellsworth ML, Sprague RS & Dacey RG Jr (2000). Red blood cell regulation of microvascular tone through adenosine triphosphate. *Am J Physiol Heart Circ Physiol* **278**, H1294–H1298.
- Ellsworth ML (2000). The red blood cell as an oxygen sensor: what is the evidence? *Acta Physiol Scand* **168**, 551–559.
- Ellsworth ML, Forrester T, Ellis CG & Dietrich HH (1995). The erythrocyte as a regulator of vascular tone. *Am J Physiol Heart Circ Physiol* **269**, H2155–H2161.
- Ellsworth ML, Ellis CG, Goldman D, Stephenson AH, Dietrich HH & Sprague RS (2009). Erythrocytes: oxygen sensors and modulators of vascular tone. *Physiology (Bethesda)* **24**, 107–116.
- Forsyth AM, Wan J, Owrutsky PD, Abkarian M & Stone HA (2011). Multiscale approach to link red blood cell dynamics, shear viscosity, and ATP release. *Proc Natl Acad Sci U S A* **108**, 10986–10991.
- Gadsby DC, Vergani P & Csanády L (2006). The ABC protein turned chloride channel whose failure causes cystic fibrosis. *Nature* **440**, 477–483.
- Hanson MS, Stephenson AH, Bowles EA, Sridharan M, Adderley S & Sprague RS (2008). Phosphodiesterase 3 is present in rabbit and human erythrocytes and its inhibition potentiates iloprost-induced increases in cAMP. *Am J Physiol Heart Circ Physiol* **295**, H786–H793.
- Harvey W (1889). *Motion of the Heart and Blood in Animals*. Translated by Bowie A. George Bell and Sons, London.
- Hoppe-Seyler F (1866). Über die oxydation in lebendem blute. *Medizinisch-chemische Untersuchungen* **1**, 133–140.
- Horga JF, Gisbert J, Agustin D, Hernandez M & Zapater P (2000). A beta-2 adrenergic receptor activates adenylate cyclase in human erythrocyte membranes at physiological calcium plasma concentrations. *Blood Cells Mol Dis*. **26**, 223–228.
- Hünefeld FL (1840). *Die Chemismus in der thierischen Organization*. F.A. Brockhaus, Leipzig.
- Jagger JE, Bateman RM, Ellsworth ML & Ellis CG (2001). Role of erythrocyte in regulating local O₂ delivery mediated by hemoglobin oxygenation. *Am J Physiol Heart Circ Physiol* **280**, H2833–H2839.
- Kaiser G, Quiring K, Gauger D, Palm D, Becker H & Schoeppe W (1974). Occurrence of adenyl cyclase activity in human erythrocytes. *Blut* **29**, 115–122.
- Koller A & Kaley G (1990). Endothelium regulates skeletal muscle microcirculation by a blood flow velocity-sensing mechanism. *Am J Physiol Heart Circ Physiol* **258**, H916–H920.
- Krogh A (1959). Lecture II (1929): The distribution and number of capillaries in selected organs. In *The Anatomy and Physiology of Capillaries*, pp. 22–46, Hafner Publishing Company, New York.
- Li C & Xu Q (2000). Mechanical stress-initiated signal transductions in vascular smooth muscle. *Cell Signal* **12**, 435–445.
- McCullough WT, Collins DM & Ellsworth ML (1997). Arteriolar responses to extracellular ATP in striated muscle. *Am J Physiol Heart Circ Physiol* **272**, H1886–H1891.
- Nobles M, Benians A & Tinker A (2005). Heterotrimeric G proteins precouple with G protein-coupled receptors in living cells. *Proc Natl Acad Sci U S A* **102**, 18706–18711.
- Ngai CY & Yao X (2010). Vascular responses to shear stress: the involvement of mechanosensors in endothelial cells. *Open Circ Vasc J* **3**, 85–94.
- Olearczyk JJ, Stephenson AH, Lonigro AJ & Sprague RS (2001). Receptor-mediated activation of the heterotrimeric G-protein G_s results in ATP release from erythrocytes. *Med Sci Monit* **7**, 669–674.
- Olearczyk JJ, Stephenson AH, Lonigro AJ & Sprague RS (2004a). Heterotrimeric G protein G_i is involved in a signal transduction pathway for ATP release from erythrocytes. *Am J Physiol Heart Circ Physiol* **286**, H940–H945.
- Olearczyk JJ, Stephenson AH, Lonigro AJ & Sprague RS (2004b). NO inhibits signal transduction pathway for ATP release from erythrocytes via its action on heterotrimeric G protein G_i. *Am J Physiol Heart Circ Physiol* **287**, H748–H754.
- Osanaï T, Fujita N, Fujiwara N, Nakano T, Takahashi K, Guan W & Okumura K (2000). Cross talk of shear-induced production of prostacyclin and nitric oxide in endothelial cells. *Am J Physiol Heart Circ Physiol* **278**, H233–H238.
- Poole DC, Copp SW, Hirai DM & Musch TI (2011). Dynamics of muscle microcirculatory and blood-myocyte O₂ flux during contractions. *Acta Physiol (Oxf)* **202**, 293–310.
- Rodan SB, Rodan GA & Sha'afi RI (1975). Demonstration of adenylate cyclase activity in human red blood cell ghosts. *Biochim Biophys Acta* **428**, 509–515.
- Sprague RS, Bowles EA, Achilleus D & Ellsworth ML (2011). Erythrocytes as controllers of perfusion distribution in the microvasculature of skeletal muscle. *Acta Physiol (Oxf)* **202**, 285–292.
- Sprague RS, Bowles EA, Hanson MS, DuFaux E, Sridharan M, Adderley S, Ellsworth ML & Stephenson AH (2008). Prostacyclin analogues stimulate receptor-mediated cAMP synthesis and ATP release from rabbit and human erythrocytes. *Microcirculation* **15**, 461–471.

- Sprague RS, Bowles EA, Olearczyk JJ, Stephenson AH & Lonigro AJ (2002). The role of G protein β subunits in the release of ATP from human erythrocytes. *J Physiol Pharmacol* **53**, 667–674.
- Sprague RS & Ellsworth ML (2012). Erythrocyte-derived ATP and perfusion distribution: Role of intracellular and intercellular communication. *Microcirculation* DOI: 10.1111/j.1549-8719.2012.00158.x
- Sprague RS, Ellsworth ML, Stephenson AH, Kleinhenz ME & Lonigro AJ (1998). Deformation-induced ATP release from red blood cells requires cystic fibrosis transmembrane conductance regulator activity. *Am J Physiol Heart Circ Physiol* **275**, H1726–H1732.
- Sprague RS, Ellsworth ML, Stephenson AH & Lonigro AJ (2001). Participation of cAMP in a signal-transduction pathway relating erythrocyte deformation to ATP Release. *Am J Physiol Cell Physiol* **281**, C1158–C1164.
- Sprague RS, Goldman D, Bowles EA, Achilleus D, Stephenson AH, Ellis CG & Ellsworth ML (2010). Divergent effects of low O₂ tension and iloprost on ATP release from erythrocytes of humans with type 2 diabetes: Implications for O₂ supply to skeletal muscle. *Am J Physiol Heart Circ Physiol* **299**, H566–H573.
- Sridharan M, Adderley SP, Bowles EA, Egan TM, Stephenson AH, Ellsworth ML & Sprague RS (2010a). Pannexin 1 is the conduit for low oxygen tension-induced ATP release from human erythrocytes. *Am J Physiol Heart Circ Physiol* **299**, H1146–H1152.
- Sridharan M, Bowles EA, Richards JP, Krantic M, Davis KL, Dietrich KA, Stephenson AH, Ellsworth ML & Sprague RS (2012). Prostacyclin receptor-mediated ATP release from erythrocytes requires the voltage-dependent anion channel (VDAC). *Am J Physiol Heart Circ Physiol* **302**, H553–H559.
- Sridharan M, Sprague RS, Adderley SP, Bowles EA, Ellsworth ML & Stephenson AH (2010b). Diamide decreases deformability of rabbit erythrocytes and attenuates low oxygen tension-induced ATP release. *Exp Biol Med (Maywood)* **235**, 1142–1148.
- Stein JC & Ellsworth ML (1993). Capillary oxygen transport during severe hypoxia: role of hemoglobin oxygen affinity. *J Appl Physiol* **75**, 1601–1607.
- Wan J, Ristenpart WD & Stone HA (2008). Dynamics of shear-induced ATP release from red blood cells. *Proc Natl Acad Sci U S A* **105**, 16432–16437.

Acknowledgements

The authors thank Kelly Paralis of Penumbra Design, Inc. for creating the figures and Dr Hans H. Dietrich for assistance in translating the German papers. Original work from the authors' laboratories was supported by the National Institutes of Health, the American Diabetes Association and United Therapeutics. The authors thank the many students, research assistants and colleagues who contributed to the author's research presented here. In addition, we thank J. L. Sprague for inspiration.