Red blood cell regulation of microvascular tone through adenosine triphosphate

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Dietrich, Hans H., Mary L. Ellsworth, Randy S. Sprague, and Ralph G. Dacey, J. R. Red blood cell regulation of microvascular tone through adenosine triphosphate. Am J Physiol Heart Circ Physiol 278: H1294–1298, 2000.—The matching of blood flow with metabolic need requires a mechanism for sensing the needs of the tissue and communicating that need to the arterioles, the ultimate controllers of tissue perfusion. Despite significant strides in our understanding of blood flow regulation, the identity of the O2 sensor has remained elusive. Recently, the red blood cell, the Hb-containing O2 carrier, has been implicated as a potential O2 sensor and contributor to this vascular control by virtue of its concomitant carriage of millimolar amounts of ATP, which it is able to release when exposed to a low-O2 environment. To evaluate this possibility, we exposed perfused cerebral arterioles to low extraluminal O2 in the absence and presence of red blood cells or 6% dextran and determined both vessel diameter and ATP in the vessel effluent. Only when the vessels were perfused with red blood cells did the vessels dilate in response to low extraluminal O2. In addition, this response was accompanied by a significant increase in vessel effluent ATP. These findings support the hypothesis that the red blood cell itself serves a role in determining O2 supply to tissue.

rat; cerebral arterioles; microvascular regulatory mechanism; oxygen tension

RED BLOOD CELLS (RBCs) are the efficient, Hb-containing carriers of O2. Recently, RBCs have been hypothesized to serve an additional and equally important role in the circulation as a regulator of both vascular resistance and the distribution of microvascular perfusion (10). Previous reports have suggested that, in addition to the respiratory gases, the Hb within RBCs also binds and transports the endothelium-derived relaxing factor nitric oxide (NO), which it releases in the peripheral tissues to increase perfusion (25). Although there is substantial chemical evidence to support the ability of Hb to avidly bind NO, recent kinetics studies suggest that its release in the peripheral circulation is unlikely to play an important role in tissue perfusion, with this mechanism of vascular control possibly operative only in the smallest microvessels (28).

Thus, if the RBC is to be an important contributor to the regulation of tissue perfusion, some other mechanism needs to be considered.

It has been known for many years that RBCs contain millimolar amounts of ATP (18), and, in addition, that they possess the membrane-bound glycolytic enzymes necessary for its production. Bergfeld and Forrester (2) reported in 1992 that human RBCs release ATP when exposed to severe hypoxia in the presence of hypercapnia (2). Later, Ellsworth et al. (10) reported that hamster RBCs similarly release ATP when exposed to a less severe hypoxia in the absence of hypercapnia and that acidic pH also could serve as a stimulator of release. This result has been extended to other species and other stresses, including mechanical deformation (23, 24).

Numerous studies have implicated ATP in the regulation of vascular perfusion. Burnstock and Kennedy (4, 5) showed that ATP causes endothelium-dependent vasorelaxation in a wide variety of species and tissues. In recent studies McCullough et al. (16) demonstrated that ATP, when applied to the lumen of striated muscle arterioles and venules in intact hamsters, induces a conducted, NO-dependent vasodilator response in the feed arterioles, resulting in a localized increase in perfusion. Similarly, ATP applied locally caused conducted vasodilatation in isolated rat cerebral arterioles (8).

On the basis of the data presented above, one could hypothesize that the RBC, by virtue of its ability to release ATP under conditions associated with a limitation of perfusion, may serve as an O2-need sensor and as an ultimate controller of perfusion to meet that need. To ascertain whether there exists a physiological basis for such speculation, we utilized the isolated, perfused rat cerebral arteriole as a model and tested the ability of RBCs to release ATP on exposure to a low extraluminal PO2 environment and thereby decrease vascular resistance.

MATERIALS AND METHODS

Vessel preparation and protocol. All animal procedures were carried out in accordance with regulations of the Washington University Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (343 ± 41 g, n = 12) were anesthetized with pentobarbital sodium (65 mg/kg ip), autologous blood was collected via cardiac puncture, and the rats were decapitated. The procedure for preparing and cannulat-
HYPoxic ATP Release From RBCs and Vessel Dilation

The technique developed by Strehler and McElroy (26) in 1957 that utilizes the ATP concentration-dependent emission of light induced by the reaction of ATP with firefly tail extract. In our system, the sensitivity of the assay was augmented by the addition of synthetic 0-luciferin to the crude firefly tail extract. The vessel effluent of RBC-perfused vessels was diluted with the perfusion buffer, and a 200-µl sample of the RBC suspension fluid was injected into a cuvette containing 100 µl of crude firefly tail extract (FTE 50, Sigma) and 100 µl of a solution of synthetic 0-luciferin (50 mg/100 ml distilled water; Sigma). The signal was measured using a luminometer (TD2020, Turner Designs) that provides a digital output of both the peak signal and the area under the curve. A cell count was determined for the diluted sample, and the ATP measured was corrected to the cell count in the perfusate to determine the effective ATP content within the vessel. In the absence of red cells, the measured ATP concentration was corrected to account for the sample dilution. A standard curve was obtained on the day of the experiment just before each sample analysis.

Statistics. Data are presented as means ± SE. An ANOVA followed by either a Mann Whitney or Wilcoxon signed rank test was used to evaluate statistical significance of unpaired and paired data, respectively. All data analysis was done using a statistical software package (InStat, GraphPad Software, San Diego, CA). Significance was assigned at P < 0.05.

RESULTS

Vessels perfused with RBCs. Vessels used for RBC perfusion had a length of 1,868 ± 162 µm (n = 9) and a passive maximum diameter of 76.4 ± 4.5 µm. At 60 mmHg without perfusion, they developed spontaneous tone, constricted by 24.4% to a diameter of 57.8 ± 3.3 µm (control), and responded to pH 6.8 with a dilatation to 70.9 ± 3.9 µm. An alkaline pH of 7.65 constricted the vessels to a diameter of 42.2 ± 2.9 µm. Perfusion of the vessels with buffer at 3 µl/min lowered the perfusion pressure to 4.8 ± 1.0 mmHg and increased the diameter to 59.4 ± 3.2 µm. In buffer-perfused arteries, decreasing the O2 content of the fluid surrounding the vessel had no effect on either vessel diameter (59.6 ± 3.2 vs. 59.4 ± 3.2 µm at control) and the ATP content in the vessel effluent (Fig. 1). Indeed, the ATP efflux dropped during hypoxia from 0.9 ± 0.2 to 0.5 ± 0.1 µM, although this decrease was not significant (P = 0.06; Fig. 2). After normoxic recovery, the vessel diameter (61.7 ± 2.9 µm, P = not significant (NS)) and the ATP content (0.4 ± 0.2 µM; Fig. 2) were unchanged compared with normoxic control values. Switching from buffer to RBC perfusion increased perfusion pressure from 4.8 ± 1.0 to 15.7 ± 3.1 mmHg, which was accompanied by an average vessel constriction of 5.9 ± 2.1 µm. This constriction was due to the increased intraluminal pressure (Bayliss effect). The ATP level increased over that seen with buffer perfusion alone (Fig. 2), a likely consequence of mechanical deformation-induced ATP release from the RBCs (24). In contrast to vessels perfused with buffer alone, in the presence of RBCs a reduction in P02 in the surrounding fluid induced both a significant increase in vessel diameter (56.7 ± 3.6 to 60.6 ± 4.2 µm, P < 0.05; Fig. 1) and an increase in ATP in the vessel effluent (6.1 µM to
12.5 µM, P < 0.05; Fig. 2). Vessel diameter and ATP level decreased to values not different from baseline after a return to normoxic conditions.

Vessels perfused with dextran. In a separate series of experiments, we replaced the RBC perfusate with 6% dextran to ascertain the role that increased viscosity associated with RBC perfusion might have on both vessel diameter and effluent ATP. Vessels in this group were 1,506 ± 110 µm long and had a passive maximum diameter of 89.3 ± 2.9 µm (n = 3). They constricted by 29.4% to a spontaneous tone diameter of 63.0 ± 5.5 µm, and constricted to alkaline pH by 21.9% to a diameter of 51.7 ± 3.0 µm. We found that switching from buffer to dextran resulted in an increase in perfusion pressure from 8.0 ± 5.0 to 24.7 ± 6.6 mmHg, similar to that seen when perfusion was switched from buffer to RBCs. This was accompanied by a small but significant increase in vessel diameter from control to 67.7 ± 3.2 µm. However, the vessel did not dilate in response to low extraluminal P O2 (Fig. 3), and there was no increase in ATP in the vessel effluent (Fig. 4).

Figure 5, A and B, depicts the distal end of a single 1,300-µm-long cerebral arteriole perfused with RBCs during exposure to a normal and low-PO2 environment. On exposure of this arteriole to low P O2, the vessel dilated from 50.0 to 56.7 µm, and ATP in the effluent doubled from 3.55 × 10⁻⁶ to 7.04 × 10⁻⁶ M. Previously, when the same vessel was perfused with buffer in the absence of RBCs, similar exposure to low PO2 resulted in a small, <1-µm increase in diameter with a decrease in effluent ATP.

**DISCUSSION**

The data presented demonstrate that only in the presence of RBCs does exposure of the perfused vessel to a low-PO2 environment result in both vessel dilation...
and an increase in effluent ATP. Thus the data provide strong support for the idea that the RBC is an O2-need sensor and a controller of tissue perfusion.

In our studies we observed an average 8% increase in vessel diameter that is consistent with the results obtained in hamster striated muscle arterioles after intraluminal application of ATP (16). Although an 8% increase is small, it is important to remember that, on the basis of Poiseuille’s law, vascular resistance and flow are determined by the fourth power of the radius. Therefore, even a small change in vessel diameter will have a dramatic effect on both parameters. In our constant flow system, an 8% increase in diameter would be correlated with a 25% decrease in vascular resistance of this arteriole. Our results also establish that the time course for the response to low PO2 exposure is well within that required by a physiologically relevant control mechanism. In our system, at a blood flow of 3 µl/min and average vessel length of 1,800 µm, the transit time of the RBCs through the vessel is 500 ms. During this time, the RBCs sense the hypoxic environment and release ATP. This ATP then diffuses to the vascular endothelium and binds to its receptors, resulting in activation of a vasodilator mechanism operative in these vessels, possibly NO (12). Thus our observation that a response occurred that could be correlated with an enhanced ATP level supports a cause-and-effect relationship in this system.

In vitro, RBCs release ATP in response to low PO2 (2, 10). However, it is also known that endothelial cells release ATP when exposed to elevated shear stress (3). Thus it is possible that the increase in ATP that we observed in these studies was derived from the endothelial cells rather than the RBCs. To address this point, we replaced the RBCs with 6% dextran, a solution that, on the basis of literature values, has a viscosity of 3.16 cP (13), ~1.5 times higher than that of a 20% RBC suspension (14). We found that in the presence of dextran, perfusion pressure was similar to that observed during RBC perfusion, which would suggest a similar viscosity response in the vessels. However, in contrast to perfusion with RBCs, we observed no increase in either vessel diameter or effluent ATP on exposure to low PO2, with the results not differing from those obtained during buffer perfusion. These data suggest that it is the RBC rather than the endothelial cell that is responsible for the increase in ATP and the alteration in vessel diameter. This result is supported by data reported by Sprague et al. (24) in the pulmonary circulation.

Under normoxic conditions, switching to RBC perfusion induced vessel constriction coincident with an increase in perfusate ATP concentration. Although this may seem contradictory to our hypothesis, it is important to note that switching to RBC perfusion also resulted in a significant viscosity-induced increase in perfusate pressure. This pressure increase would evoke a myogenic constriction (Bayliss effect), the magnitude of which may have been attenuated by the presence of the ATP.

Several studies have looked at the effect of low PO2 on vessel diameter in the absence of perfusion with varying levels of PO2 tested. In general, these experiments show that isolated, pressurized, but not perfused, skeletal muscle, cardiac, and cerebral arterioles or cerebral arteries dilate to the hypoxia (7, 17, 19, 21, 27), with lung and kidney vessels observed to respond either by constriction or dilation (15, 22).

Studies in isolated and perfused vessels are limited. Fredricks et al. (11) in their study of cannulated and perfused middle cerebral arteries observed changes in vessel diameter in response to combinations of hypoxic extra- and/or intraluminal PO2 in the absence of RBCs. In these studies dilation was only observed with hypoxic perfusate. This is in contrast to the studies presented here, in which only the extraluminal environment was hypoxic. Interestingly, Fredericks et al. (11) found that the vessels did not dilate but, rather, constricted to extraluminal hypoxia when the intraluminal perfusate was normoxic, a situation similar to that in our experiment. Thus it appears that isolated and pressurized buffer-containing vessels will only dilate to hypoxia when the intraluminal buffer is made hypoxic either by allowing the intraluminal buffer to equilibrate, as in nonperfused vessels, or by perfusing the vessels with hypoxic buffer. In our studies, only
with RBC perfusion did our vessels dilate to hypoxia, which supports a contribution of the RBC in vascular regulation to hypoxia.

In the work presented here, we provide evidence in support of the hypothesis that RBCs have the capacity to modulate the increase in blood flow in response to metabolic demand and suggest that this mechanism may subserve the role of the long-sought regulator of flow distribution within a tissue to meet the local O₂ or flow demands.

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