Impaired Release of ATP from Red Blood Cells of Humans with Primary Pulmonary Hypertension

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Previously, we reported that in the isolated perfused rabbit lung, red blood cells (RBCs) obtained from either rabbits or healthy humans were a required component of the perfusate to unmask evidence of nitric oxide (NO) participation in regulation of the pulmonary circulation. In addition, we found that mechanical deformation of rabbit and healthy human RBCs released ATP, a known agonist for enhanced NO synthesis. In contrast, RBCs obtained from patients with cystic fibrosis (CF) did not release ATP in response to mechanical deformation. The coexistence of airway disease and alveolar hypoxia in patients with CF precluded the drawing of conclusions relating a defect in RBC ATP release with the pulmonary hypertension associated with CF. Airway disease and alveolar hypoxia are not, however, features of primary pulmonary hypertension (PPH), a human condition of unknown etiology. We postulated that a defect in NO generation might contribute to the increased pulmonary vascular resistance in PPH, and as a first step, we hypothesized that RBCs obtained from patients with PPH would not release ATP. In contrast to RBCs of healthy humans, when RBCs of PPH patients were passed through filters (average pore size 12, 8, or 5 μm), ATP was not released and the RBCs exhibited reduced deformability. Moreover, when incubated with the active cAMP analogue, Sp-cAMP (100 μM), an activator of the CF transmembrane conductance regulator, ATP was not released. These results demonstrate that RBCs obtained from patients with PPH fail to release ATP whether the stimulus is mechanical or pharmacological. Thus, failure of RBCs to release ATP in patients with PPH might be a major pathogenetic factor that accounts for the heretofore unknown etiology of their pulmonary hypertension. [Exp Biol Med Vol. 226(5):434–439, 2001]

Key words: erythrocyte; lungs; cystic fibrosis; prostacyclin; epoprostenol

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Under physiological conditions, resistance to blood flow in the pulmonary circulation is maintained at a value 10-fold less than that of the systemic circulation. It is well recognized that the endothelium of the pulmonary circulation synthesizes and releases factors that relax the underlying vascular smooth muscle, leading, thereby, to increased vascular caliber and reduced vascular resistance (1, 2). Previously, we reported that the red blood cell (RBC) is a determinant of endogenous nitric oxide (NO) synthesis in the pulmonary circulation; i.e., in the isolated perfused rabbit lung, RBCs obtained from either rabbits or healthy humans were a required component of the perfusate in order to demonstrate flow-induced endogenous NO synthesis (3). In the absence of these RBCs, increments in perfusate flow rate did not stimulate NO synthesis in the pulmonary circulation (3). RBCs contain concentrations of ATP adequate to activate the endothelial P_{2y} purinergic receptors, resulting, thereby, in synthesis of NO (4, 5). In addition, RBCs are deformed as they traverse microvascular beds such as in the pulmonary circulation (6). Recently, we reported that RBCs of rabbits and healthy humans release ATP in response to mechanical deformation (7). It is important to note that as the degree of deformation was increased, ATP release increased, i.e., ATP was released in a stimulus-dependent fashion.

The finding that RBCs are required for flow-induced NO synthesis in the isolated perfused rabbit lung, coupled with the finding that mechanical deformation of RBCs results in ATP release, which, in turn, can stimulate NO synthesis in the lung, suggests a novel mechanism for the control of pulmonary vascular caliber. In this construct, as the RBC is increasingly deformed by increments in the velocity of blood flow through a vessel and/or by reductions in vascular caliber, it releases ATP, which stimulates endothelial synthesis of NO, resulting in relaxation of vascular smooth muscle and thereby, an increase in vascular caliber. Failure of this mechanism for deformation-induced ATP release from RBCs could be expected to lead, ultimately, to the development of pulmonary hypertension. Indeed, we re-

ported that RBCs of patients with cystic fibrosis (CF) in which the activity of the cystic fibrosis transmembrane conductance regulator (CFTR) is either diminished or lost, do not release ATP in response to mechanical deformation (8), suggesting that CFTR is a component of a signal-transduction pathway that links mechanical deformation of the RBC to the release of ATP. CF patients develop pulmonary hypertension. However, the co-existence of severe airways disease, alveolar hypoxia, and pulmonary hypertension in these individuals makes investigation of the contribution of defective ATP release from RBCs to the development of pulmonary hypertension difficult (9, 10). Severe airways disease and alveolar hypoxia are not, however, features of primary pulmonary hypertension (PPH), a human condition of unknown etiology (11, 12, 21–23). Indeed, it was suggested that a defect in NO generation might contribute to the increased pulmonary vascular resistance in PPH.

In the present study, we investigated the hypothesis that RBCs of patients with PPH fail to release ATP in response to increasing mechanical deformation. In addition, we hypothesized that since cAMP is a stimulus for the activity of CFTR (13–15), the active cAMP analogue, Sp-cAMP, would stimulate ATP release from RBCs of healthy humans, but not from those of patients with PPH.

Materials and Methods

Preparation of RBCs. Sixty milliliters of blood was collected into a syringe containing heparin (50 units) via a central venous catheter or by venipuncture without the use of a tourniquet. Immediately after blood collection, RBCs were separated from other formed elements and plasma by centrifugation at 500*g* at 4°C for 10 min. The supernatant and buffy coat were removed by aspiration. Packed RBCs were re-suspended and washed three times in a physiological salt solution (PSS; in millimoles: 4.7 KCl, 2.0 $CaCl_2$, 1.2 $MgSO_4$, 140.5 NaCl, 15.7 *N*-2-Hydroxyethylpiprazine-*N'*-2-ethanesulfonic acid [HEPES], 11.1 dextrose, and 0.5% bovine serum albumin, pH adjusted to 7.4). RBCs were prepared on the day of use. The protocol for removal of blood from humans was approved by the Institutional Review Board of Saint Louis University.

Deformation of RBCs. RBCs were subjected to mechanical deformation using the St. George's Blood Filtrometer (Carri-Med Ltd., Dorking, UK) (16). This device develops a calibrated pressure gradient across a vertically mounted filter. A 13-mm diameter polycarbonate filter (Nucleopore) with a 9.53-mm exposed surface diameter and average pore size of 12, 8, or 5 μm was placed in the filter chamber and the outflow channel was filled with PSS. Flow was prevented by an outflow channel tap. For calibration, proximal to the filter, an open-ended capillary tube was filled with PSS. The time taken for the PSS to pass four fiberoptic detectors was recorded with a computer. This process was repeated until coefficients of variance between runs were 1% or less.

Deformation of RBCs was achieved by passing cells suspended in PSS at a hematocrit of 10% through the filters. The filtration rate of the RBC suspension relative to PSS alone, the red cell transit time (RCTT), was calculated as described previously (7, 8, 16). The RCTT is dependent on the deformability of the RBCs, the hematocrit, and the size of the filter pores relative to the size of the RBC studied. Thus, the St. George's Blood Filtrometer can be used to determine the deformability of RBCs (16), as well as ATP released in response to increasing deformation (decreasing pore size) (7, 8). The basal release of ATP from RBCs was determined by measurement of ATP present in a 10% suspension of RBCs not passed through a filter. All solutions and RBC suspensions were warmed to 37°C for a minimum of 30 min before use. The concentration of ATP present in the effluent from the various filters or under basal conditions was normalized to that released from 2×10^5 RBCs/mm^3.

Incubation of RBCs with Sp cAMP or Its Vehicle. The active cAMP analogue, adenosine 3′ 5′-cyclic monophosphorothioate, Sp-isomer (Sp-cAMP, Biomol Research Labs, Plymouth Meeting, PA)(17) was dissolved in distilled water. RBCs (hematocrit 20%) were incubated for 30 min at 37°C with either Sp-cAMP (100 μM) or its vehicle. This dose of Sp-cAMP was chosen based both on reports in the literature (17) and on preliminary results obtained in our laboratory. Importantly, this amount of Sp-cAMP did not cause RBC lysis and did not interfere with the ATP assay. The concentration of ATP released in response to Sp-cAMP or its vehicle was normalized to that released from 5×10^5 RBCs/mm^3.

Measurement of ATP and Hemoglobin. ATP was measured by the luciferin-luciferase technique (7, 8, 18) in which the amount of light generated by the reaction of ATP with firefly tail extract is dependent on the ATP concentration. Sensitivity was augmented by addition of synthetic D-luciferin to the crude firefly tail extract. A 200-μl sample of RBC-containing solution was injected into a cuvette containing 100 μl of crude firefly tail extract (5 mg/5 ml distilled water, FLE 50, Sigma, St. Louis, MO) and 100 μl of a solution of synthetic D-luciferin (50 mg/100 ml distilled water, Sigma). The peak light efflux from cuvettes to which either known ATP standards or samples are added was determined using a luminometer (model 20/20; Turner Designs, Sunnyvale, CA). An ATP standard curve was obtained on the day of each experiment.

To exclude the presence of significant hemolysis, after ATP determinations, RBC suspension were centrifuged at 500*g* at 4°C for 10 min and the presence of hemoglobin in the supernatant was determined by light absorption at wavelengths of 385, 405, 560, 577, and 630 nm (8, 19, 20). This technique for hemoglobin determination is sufficiently sensitive to detect hemolysis of 0.5% of RBCs added to the filtrometer. The amount of ATP released in association with this level of hemolysis was not detectable in the luciferin-luciferase assay. Finally, in all experiments, ATP content of

RBCs was determined by measurement of ATP in solution following lysis of a known number of RBCs in distilled water.

Statistical Methods. Statistical significance between experimental periods and groups was determined with analysis of variance. In the event the F ratio indicated that changes had occurred, a Tukey's protected T test was used to identify individual differences. A P value of 0.05 or less was considered statistically significant. Results are reported as the means ± SEM.

Results

Human Subjects Studied. RBCs from five patients (three male, two female) with the diagnosis of PPH as defined by recognized criteria (11, 12, 21–23) were studied. No patient had a family history of PPH. Patient 4 had a history of appetite suppressant usage (a derivative of fenfluramine). The average age of the patients was 36 ± 4 years. The data for the individual patients with PPH is summarized in Table I. In addition, RBCs of five healthy human volunteers (three male, two female, average age 39 ± 6 years) on no medications and with no history of pulmonary disease were studied.

Deformability of RBCs of Healthy Humans and Humans with PPH. As the average pore size through which RBCs were passed was decreased, the time taken for the cells to traverse the filters (RCTT) increased (Fig. 1). The RCTT for RBCs of patients with PPH passed through filters with a pore average diameter of 5 μm was greater than that for RBCs of healthy humans (Fig. 1). In these studies both filter pore size and driving pressure were held constant, thus the finding of an increase in RCTT for RBCs of patients with PPH is consistent with previous studies (28) that reported decreased RBC deformability relative to cells of healthy control subjects.

Effect of Deformation of RBCs on ATP Release. Mechanical deformation of RBCs of healthy humans (n = 5) by passage of the cells through filters with decreasing average pore size (12, 8, or 5 μm) produced increments in ATP release (Fig. 2). Basal release of ATP from RBCs of healthy humans was 0.06 ± 0.03 μM ATP per 2×10^5 RBCs/mm^3 and was significantly less than amounts of ATP released in response to passage of these cell through filters with average pore diameters of 8 or 5 μm ($P < 0.01$).

In contrast to RBCs of healthy humans, RBCs of patients with PPH (n = 5) failed to release ATP in response to mechanical deformation, i.e., these was no increase in ATP released in response to passage of RBCs through filters with decreasing average pose size (Fig. 2). The inability of RBCs of patients with PPH to release ATP was not the

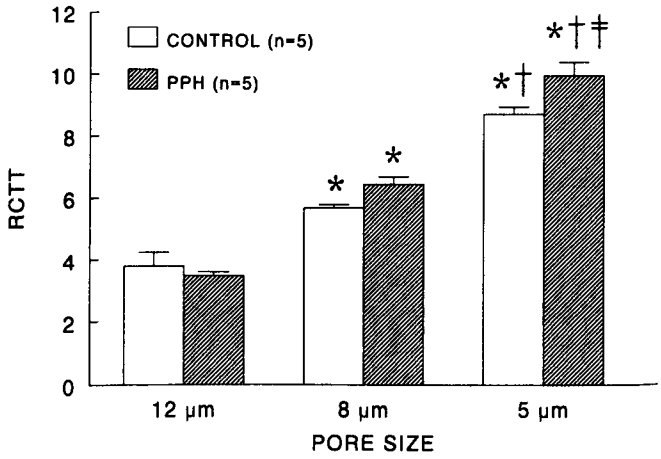

Figure 1. RCTT in response to passage of RBCs through filters with pore sizes of 12, 8, or 5 μm. Open bars, RBCs of healthy humans (n = 5); cross hatched bars, RBCs of patients with PPH (n = 5). Asterisk, different from respective 12 μm value ($P < 0.001$); †, different from respective 8 μm value ($P < 0.001$); ‡, different from value for RBCs of healthy humans (control; $P < 0.05$). Values are means ± SEM.

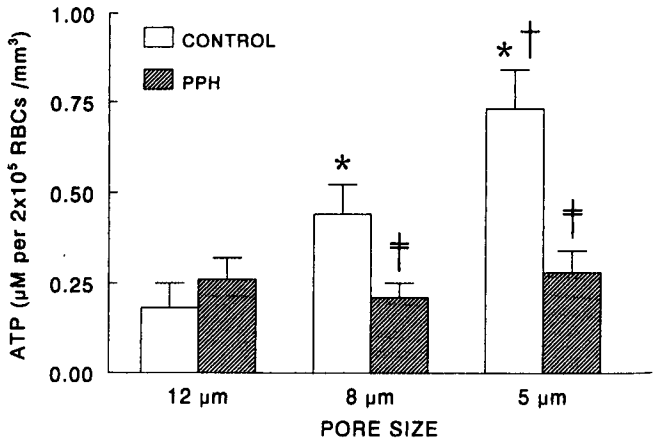

Figure 2. ATP release (ATP concentration in filter effluent per 2 × 10^5 RBCs/mm^3) in response to passage of RBCs through filters with pore sizes of 12, 8, or 5 μm. Open bars, RBCs of healthy humans (n = 5); cross hatched bars, RBCs of patients with PPH (n = 5). Asterisk, different from respective 12 μm value ($P < 0.01$); †, different from respective 8-μm value ($P < 0.01$); ‡, different from value for RBCs of healthy humans (control; $P < 0.01$). Vales are means ± SEM.

Table I. Characteristics of Patients with PPH at the Time of Study

Patient	Ppa (mmHg)	Cardiac output (l/min)	PVR (dyn/sec/cm^5)	Medication	Age (years)	Sex
1	35	5.58	330	Epoprostenol; diltiazem	46	M
2	60	6.54	588	Epoprostenol	25	M
3	53	4.33	831	None	42	F
4	20	7.57	106	Epoprostenol; enalapril; diltiazem	27	F
5	45	6.46	348	None	38	M

Note. Ppa, mean pulmonary arterial pressure; PVR, pulmonary vascular resistance.

result of a decrease in total ATP content of the cells compared with RBCs of healthy humans (Table II). Basal ATP release from RBCs of patients with PPH was 0.12 ± 0.03 μM ATP per 2×10^5 RBCs/mm^3 and was not different from that released from RBCs of healthy humans under these conditions. In addition, in contrast to RBCs of healthy humans, amounts of ATP released from RBCs of PPH patients did not increase significantly above this basal value in response to mechanical deformation (Fig. 2).

Effect of Incubation of RBCs with an Active cAMP Analogue (Sp-cAMP). Incubation of RBCs of healthy humans with the active cell-permeable cAMP analogue, Sp-cAMP (100 μM, $n = 5$) resulted in a 3.0- ± 0.7-fold increase in ATP release ($P < 0.01$, Fig. 3). In contrast, incubation of RBCs of three patients with PPH with Sp-cAMP did not resulted ATP release (Fig. 3).

Discussion

PPH is a condition in which pulmonary vascular resistance is increased in humans in the absence of known etiology (11, 12, 21–24). The disease is progressive with a mean survival time after diagnosis of 2 to 3 years (22). PPH occurs in individuals of all ages and both sexes, but is most commonly diagnosed in young to middle-aged females (20). Despite the findings that as many as 10% of cases are familial in nature (11, 12) and that the development of PPH may follow the use of some appetite suppressants (25), those pathophysiological mechanisms responsible for the development and progression of PPH have not been defined.

The results presented here demonstrate that in contrast to RBCs of healthy humans, RBCs of patients with PPH fail to release ATP in response to mechanical deformation such as occurs in the intact pulmonary circulation (6). One interpretation of these findings is that the failure of RBCs of patients with PPH to release ATP, a stimulus for the endogenous synthesis of endothelium-derived relaxing factors such as NO and prostacyclin (PGI_2) (4, 5, 26), deprives the pulmonary circulation of a mechanism for the maintenance of normal pulmonary vascular resistance. The finding of a decrease in NO in the lungs of patients with PPH suggests that there may indeed be a relationship between impaired synthesis of NO and the disease process (27). This interpretation is strengthened by the finding that in patients with PPH, amounts of biological reaction products of NO present in bronchoalveolar lavage fluid were correlated inversely with both the pulmonary arterial pressure and the duration of disease (27).

Table II. Concentration of ATP in Red Blood Cells (RBCs) of Healthy Humans (control, $n = 5$) and Humans with PPH ($n = 5$)

	Total ATP
Control	2.3 ± 0.6
PPH	2.9 ± 1.1

Note. Values are represented as millimoles of ATP per RBC. Values are means ± SEM.

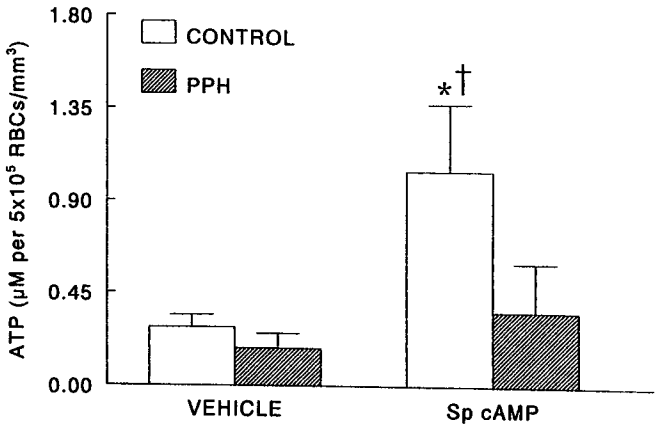

Figure 3. ATP release (ATP concentration in filter effluent per 5×10^5 RBCs/mm^3) from RBCs of healthy humans (open bars, $n = 5$) and patients with PPH (cross hatched bars, $n = 3$) in response to incubation of RBCs with the active cAMP analogue, Sp-cAMP (100 μM). Asterisk, different from respective vehicle value ($P < 0.01$); †, different from patients with PPH before and after incubation with Sp-cAMP ($P < 0.01$). Vales are means ± SEM.

Previously, it was reported that the RBCs of patients with PPH are distinct from those of healthy humans, i.e., the deformability of RBCs of patients with PPH was found to be decreased when compared with those of healthy humans (28). Blood viscosity was increased in patients with PPH, while plasma viscosity was normal, suggesting that decreased RBC deformability resulted in rheological changes of physiological significance (28). In support of this hypothesis it was reported that perfusion of isolated rabbit lungs with human RBCs with decreased deformability resulted in an impaired oxygen diffusing capacity and an increase in perfusion pressure when compared to lungs perfused with human RBCs in which deformability was unaltered (29). Taken together, these results are consistent with the hypothesis that in PPH, decreased RBC deformability could contribute to increased vascular resistance. In the present work we confirm the observation that RBC deformability is decreased in patients with PPH (Fig. 1).

The results of the present study demonstrate that RBCs of patients with PPH have a defect in addition to decreased deformability, i.e., these cells do not release ATP in response to mechanical deformation (Fig. 2) or incubation with an active cAMP analogue (Fig. 3). The hypothesis that the release of ATP from RBCs is an important mechanism for the maintenance of normal pulmonary vascular resistance is supported by the results of several studies (3, 7, 8, 30, 31). It was reported that in isolated perfused lungs, flow-induced increases in NO synthesis required the presence of RBCs (3, 30, 31). In addition, endogenous NO synthesis was reported to be increased in isolated rabbit lungs in response to increments in perfusate flow rate solely in the presence of RBCs that release ATP in response to mechanical deformation, i.e., RBCs of rabbits and healthy humans (7). Finally, it was reported that the passage of rabbit RBCs through the circulation of isolated rabbit lungs resulted in the release of ATP from these cells (32). Taken

together, the results of these studies suggest that ATP released from RBCs of healthy humans could serve as an important stimulus for the endogenous release of NO, a potent pulmonary vasodilator. These results are consistent with the hypothesis that in humans with PPH, failure of RBCs to release ATP in response to deformation could contribute to the increase in pulmonary vascular resistance that characterizes this disease process.

Examination of the pulmonary vasculature of patients with PPH reveals one of three histological patterns described as either veno-occlusive disease, thrombosis *in situ*, or plexogenic arteriopathy (23, 33). The latter condition is the most common pathological lesion reported in patients with PPH and is proliferative in nature (31, 33). Indeed, it has been suggested that PPH may represent an inflammatory process resulting in vascular remodeling of pulmonary resistance vessels (34). In addition to its effects on smooth muscle tone, NO has been reported to inhibit vascular smooth muscle proliferation (35). Thus, it is possible that the failure of deformation-induced ATP release from RBCs and the consequent decrease in this stimulus for endogenous NO synthesis could, in addition to depriving the vascular smooth muscle of a physiological stimulus for vasodilation, contribute to the vascular lesions that are present in patients with PPH. Resolution of this issue is beyond the scope of the present study.

Like patients with PPH, humans with CF develop pulmonary hypertension (9, 10). Interestingly, both PPH patients (27) and patients with CF (36) were reported to demonstrate decreased NO synthesis in the lung when compared with those of healthy humans. Although several different genetic defects result in the clinical manifestations of CF, the feature common to all CF patients is reduced or absent activity of the CFTR (37). Previously, we reported that RBCs of humans with CF demonstrate normal RBC deformability, but fail to release ATP in response to mechanical deformation (8). Moreover, incubation of rabbit RBCs with either of two chemically dissimilar inhibitors of CFTR resulted in inhibition of deformation-induced ATP release without any effect on RBC deformability (8). One interpretation of these results is that CFTR is a component of a signal-transduction pathway for deformation-induced ATP release from RBCs (8). A major stimulus for the activation of CFTR is an increase in intracellular cAMP (14). Here we report that incubation of RBCs of healthy humans with Sp-cAMP, an active cAMP analogue (17), results in ATP release (Fig. 3). In contrast, RBCs of patients with PPH did not release ATP in response to incubation with Sp-cAMP (Fig. 3). Although Sp-cAMP could have effects in addition to activation of CFTR, these findings demonstrate that in contrast to RBCs of healthy humans, RBCs of patients with PPH fail to release ATP in response to a pharmacological stimulus. Importantly, this finding demonstrates that the failure of mechanical deformation to induce ATP release from RBCs of patients with PPH cannot be attributed to an effect on RBC deformability *per se*.

In summary, we have presented evidence in support of the hypothesis that RBCs of humans with PPH have decreased deformability and do not release ATP in response to mechanical deformation. In contrast to results obtained with RBCs of healthy humans, RBCs of PPH patients do not release ATP in response to Sp-cAMP, suggesting that a cAMP-mediated increase in CFTR activity may be a necessary component in a signal-transduction pathway that couples mechanical deformation to ATP release in RBCs of healthy humans. These findings suggest a new and heretofore unexplored mechanism for the development of increased vascular resistance in humans with PPH.

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1. Ignaro LJ, Harbison RG, Wood KS, Kadowitz PJ. Activation of purified guanylate cyclase by endothelium-derived relaxing factor from intrapulmonary artery and vein: Stimulation by acetylcholine, bradykinin and arachidonic acid. J Pharmacol Exp Ther **237:**893–900, 1986.
2. Ignaro LJ, Buga G, Chaydhuri G. EDRF generation and release from perfused bovine pulmonary artery and vein. Eur J Pharmacol **149:**79–88, 1988.
3. Sprague RS, Stephenson AH, Dimmitt RA, Weintraub NL, McMurdo L, Lonigro AJ. Effect of L-NAME on pressure-flow relationships in isolated rabbit lungs. Am J Physiol **269:**H1941–H1948, 1995.
4. Bogle RG, Coade SB, Moncada S, Pearson JD, Mann GE. Bradykinin and ATP stimulate L-arginine uptake and nitric oxide release in vascular endothelial cells. Biochem Biophys Res Commun **80:**926–932, 1991.
5. Busse R, Ogilvie A, Pohl U. Vasomotor activity of diadenosine triphosphate and diadenosine tetraphosphate in isolated arteries. Am J Physiol **254:**H828–H832, 1988.
6. Robard S. Vascular caliber. Cardiology **269:**4–49, 1975.
7. Sprague RS, Ellsworth ML, Stephenson AH, Lonigro AJ. ATP: The red blood cell link to NO and local control of the pulmonary circulation. Am J Physiol **271:**H2717–H2722, 1996.
8. Sprague RS, Ellsworth ML, Stephenson AH, Kleinhenz ME, Lonigro AJ. Deformation-induced ATP release from red blood cells requires cystic fibrosis transmembrane conductance regulator activity. Am J Physiol **275:**H1726–H1732, 1998.
9. Goldring RM, Fishman AP, Turino GM, Cohen HI, Denning CR, Andersen DH. Pulmonary hypertension and cor pulmonale in cystic fibrosis of the pancreas. Pediatrics **65:**501–524, 1964.
10. Symchych PS. Pulmonary hypertension in cystic fibrosis. Arch Pathol **92:**409–414, 1971.
11. Gaine SP, Rubin LJ. Primary pulmonary hypertension. Lancet **352:**719–725, 1998.
12. Loyd J, Newman J. Familial primary pulmonary hypertension: clinical patterns. Am Rev Resp Dis **129:**194–197, 1984.
13. Kopelman H, Ferretti E, Gauthier C, Goodyer PR. Rabbit pancreatic acini express CFTR as a cAMP-activated chloride efflux pathway. Am J Physiol **38:**C626–C631, 1994.
14. Sutts MJ, Canessa CM, Olsen JC, Hamrick M, Cohn JA, Rossier BC, Boucher RC. CFTR as a cAMP-dependent regulator of sodium channels. Science **269:**847–850, 1995.
15. Illek B, Yankaskas JR, Machen TE. cAMP and genistein stimulate HCO_3^- conductance through CFTR in human airway epithelium. Am J Physiol **272:**L752–L761, 1997.

16. Dormandy J, Flute P, Matrai A, Boger L, Mikita J. The new St. George's blood filtrometer. Clin Hemorheol **5**:975–983, 1987.
17. Holen B, Gordon PB, Stromhaug PE, Seglen PO. Role of cAMP in the regulation of hepatotoxic autophagy. Eur J Biochem **236**:169–170, 1996.
18. Strehler BL, McElroy WC. Assay of adenosine triphosphate. In: Colowick SP, Kaplan NO, Eds. Methods in Enzymology. New York: Academic Press, pp871–873, 1957.
19. van Kampen EJ, Zijlstra WG. Spectrophotometry of hemoglobin and hemoglobin derivatives. Adv Clin Chem **23**:199–257, 1983.
20. Brunelle JA, Degtiarov AM, Moran RF, Race LA. Simultaneous measurement of total hemoglobin and its derivatives in blood using CO-oximeters: Analytical principals: Their application in selecting analytical wavelengths and reference methods; a comparison of the results of the choices made. Scand J Clin Lab Invest **224**(suppl):47–69, 1996.
21. Dresdale DT, Schultz M, Michtom RJ. Primary Pulmonary Hypertension. Am J Med **11**:686–694, 1951.
22. Stuart R, Rich S, Dantzker DR, Ayres SM, Bergofsky EH, Brundage BH, Detre KM, Fishman AP, Goldring RM, Groves BM, Koerner SK. Primary pulmonary hypertension: A national prospective study. Ann Int Med **107**:216–223, 1987.
23. Rubin LJ. Pathology and pathophysiology of primary pulmonary hypertension. Am J Cardiol **75**:51A–54A, 1995.
24. Lee S-D, Shroyer KR, Markham NE, Cool CD, Voelkel NF, Tuder RM. Monoclonal endothelial cell proliferation is present in primary but not secondary pulmonary hypertension. J Clin Invest **101**:927–934, 1998.
25. Abenhaim L, Moride Y, Brenot F, Rich S, Benichou J, Kurz X, Higenbottam T, Oakley C, Wouters E, Aubier M, Simonneau G, Begaud B. Appetite-suppressant drugs and the risk of primary pulmonary hypertension. N Engl J Med **335**:609–616, 1996.
26. Forsberg E, Feuerstein G, Shohami E, Pollard H. Adenosine triphosphate stimulates inositol phospholipid metabolism and prostacyclin formation in adrenal medullary endothelial cells by means of P2-purinergic receptors. Proc Natl Acad Sci U S A **87**:5630–5634, 1987.
27. Kaneko FT, Arroliga AC, Dweikl RA, Comhair SA, Laskowski D, Oppedisano R, Thomassen MJ, Erzurum SC. Biochemical reaction products of nitric oxide as quantitative markers of primary pulmonary hypertension. Am J Respir Crit Care Med **158**:917–923, 1998.
28. Persson SU, Gustavasson CG, Larsson H, Persson S. Studies on blood rheology in patients with primary pulmonary hypertension. Angiology **42**:836–842, 1991.
29. Betticher DC, Reinhart WH, Geiser J. Effect of RBC shape and deformability on pulmonary O_2 diffusing capacity and resistance to flow in rabbit lungs. J Appl Physiol **78**:778–783, 1995.
30. Wilson PS, Khimenko P, Moore TM, Taylor AE. Perfusate viscosity and hematocrit determine pulmonary vascular responsiveness to NO synthase inhibitors. Am J Physiol **270**:H1757–H1765, 1996.
31. Uncles DR, Daugherty MO, Frank DU, Roos CM, Rich GF. Nitric oxide modulation of pulmonary vascular resistance is red blood cell dependent in isolated rat lungs. Anesthiol Analg **83**:1212–1217, 1996.
32. Sprague RS, Ellsworth ML, Stephenson AH, Lonigro AJ. Increases in flow rate stimulate adenosine triphosphate release from red blood cells in isolated rabbit lungs. Exp Clin Cardiol **3**:73–77, 1998.
33. Palevsky HI, Schloo BL, Pietra GG, Weber KT, Janici JS, Rubin E, Fishman AP. Primary pulmonary hypertension: Vascular structure, morphology, and responsiveness to vasodilator agents. Circulation **80**:1207–1202, 1989.
34. Tuder RM, Voelkel NF. Pulmonary hypertension and inflammation. J Lab Clin Med **132**:16–24, 1998.
35. Garg UC, Hassid A. Nitric oxide-generating vasodilators and 8-bromocyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. J Clin Invest **83**:1774–1777, 1989.
36. Lundberg JON, Nordvall SL, Weitzberg E, Kollberg K, Alving K. Exhaled nitric oxide in pediatric asthma and cystic fibrosis. Arch Dis Child **75**:323–326, 1998.
37. Cheng SH, Gregory RJ, Marshall J, Paul S, Souza DW, White GR, O'Riordan CR, Smith AE. Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. Cell **63**:827–834, 1990.