Isoproterenol attenuates high vascular pressure-induced permeability increases in isolated rat lungs

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Parker, James C., and Claire L. Ivey. Isoproterenol attenuates high vascular pressure-induced permeability increases in isolated rat lungs. J. Appl. Physiol. 83(6): 1962–1967, 1997.—To separate the contributions of cellular and basement membrane components of the alveolar capillary barrier to the increased microvascular permeability induced by high pulmonary venous pressures (Ppv), we subjected isolated rat lungs to increases in Ppv, which increased capillary filtration coefficient (Kfc) without significant hemorrhage (31 cmH2O) and with obvious extravasation of red blood cells (43 cmH2O). Isoproterenol (20 μM) was infused in one group (Iso) to identify a reversible cellular component of injury, and residual blood volumes were measured to assess extravasation of red blood cells through ruptured basement membranes. In untreated lungs (High Ppv group), Kfc increased 6.2 ± 1.3 and 38.3 ± 15.2 times baseline during the 31 and 43 cmH2O Ppv states. In Iso lungs, Kfc was 36.2% (P < 0.05) and 64.3% of that in the High Ppv group at these Ppv states. Residual blood volumes calculated from tissue hemoglobin contents were significantly increased by 53–66% in the high Ppv groups, compared with low vascular pressure controls, but there was no significant difference between High Ppv and Iso groups. Thus isoproterenol significantly attenuated vascular pressure-induced Kfc increases at moderate Ppv, possibly because of an endothelial effect, but it did not affect red cell extravasation at higher vascular pressures.

pulmonary hypertension; pulmonary edema; mechanical stress failure; capillary filtration coefficient

INCREASED MICROVASCULAR PERMEABILITY in the lung, induced by high pulmonary vascular pressures, may have a role in the pathogenesis of neurogenic pulmonary edema (18), high-altitude pulmonary edema (32, 41), and bleeding into the lung during intense exercise (40). The “stretched-pore phenomenon” was originally described in systemic vessels after acute exposure to venous hypertension in relation to an increased transcapillary clearance of fluid and protein (30). Acute increases in pulmonary venous pressure (Ppv) not only increase pulmonary lymph fluid and protein clearances (22) and pulmonary capillary filtration coefficient (Kfc) (18, 19, 28) but also cause extravasation of red blood cells (18).

West et al. (41) proposed that “stress failure” of pulmonary capillaries at high vascular pressure results from rupture of the alveolar capillary barrier, with the threshold pressure for failure determined by the high tensile strength of the basement membrane. Electron-microscopic studies revealed tears predominantly through cell layers of the alveolar capillary membrane, with fewer tears extending through the basement membranes after exposure to high vascular pressures (35). However, the relative contributions of the endothelial, epithelial, and basement membrane layers to microvascular fluid conductance in lungs at high vascular pressure have not been evaluated at different vascular pressures (4). The cellular layers significantly restrict passage of fluid and protein and generally prohibit the free passage of cells, whereas the basement membrane restricts passage of cells with much less impediment to fluid movement (32).

The purpose of the present study was to separate the contributions of endothelial openings from capillary ruptures extending through the basement membrane to transvascular fluid conductance at high pulmonary vascular pressures. The threshold pulmonary vascular pressures required to increase microvascular permeability in rat lungs were assessed using the capillary filtration coefficient (Kfc), a measure of capillary hydraulic conductivity. Because isoproterenol has been shown to reverse increases in Kfc produced by ischemia-reperfusion, the efficacy of isoproterenol for reversing the increases in permeability induced by mechanical injury was studied to determine whether endothelial permeability increases could be attenuated in a similar manner (1, 17). Extravasated blood volumes were used to indicate rupture of basement membranes, because we assumed that red blood cells would not pass across intact basement membranes. Isoproterenol attenuated the increased permeability at moderately high vascular pressures but had no significant effect on the amount of hemorrhage.

METHODS

Isolated Rat Lung Preparation

The isolated rat lung preparation has been previously described (12, 43). Briefly, male Charles River CD rats weighing between 199 and 320 g (247 ± 6 g) were anesthetized with an intraperitoneal injection of pentobarbital sodium (65 mg/kg). The trachea was cannulated, and the rats were ventilated with 20% O2-5% CO2-75% N2 by using a Harvard rodent ventilator (model 683, South Natick, MA) with a tidal volume of 2.5 ml and a positive end-expiratory pressure of 3 cmH2O at 40 breaths/min. The chest was opened, and 300 U heparin sodium were injected into the right ventricle. The pulmonary artery and left atrium were then cannulated, and the heart and lungs were excised en bloc and suspended from a force transducer. Lungs were perfused with 5% bovine albumin in Krebs-bicarbonate buffer (37°C) at 6 ml/min per gram of predicted (initial) lung weight by using a Minipuls-2 roller pump (Gilson, Middleton, WI). Homologous blood (~10 ml) was obtained from a donor rat and added to the perfusate to obtain a hematocrit of ~20%, which was measured with the use of a microcentrifuge. Arterial, venous, and airway pressures were measured with Cobe pressure transducers (Lakewood, CO), and the lung weight was continuously recorded by using a Grass model 7 polygraph (Grass, Quincy, MA). At the end of the experiments, the lungs were weighed and divided into pieces as described (12, 43). Briefly, male Charles River CD rats weighing between 199 and 320 g (247 ± 6 g) were anesthetized with an intraperitoneal injection of pentobarbital sodium (65 mg/kg). The trachea was cannulated, and the rats were ventilated with 20% O2-5% CO2-75% N2 by using a Harvard rodent ventilator (model 683, South Natick, MA) with a tidal volume of 2.5 ml and a positive end-expiratory pressure of 3 cmH2O at 40 breaths/min. The chest was opened, and 300 U heparin sodium were injected into the right ventricle. The pulmonary artery and left atrium were then cannulated, and the heart and lungs were excised en bloc and suspended from a force transducer. Lungs were perfused with 5% bovine albumin in Krebs-bicarbonate buffer (37°C) at 6 ml/min per gram of predicted (initial) lung weight by using a Minipuls-2 roller pump (Gilson, Middleton, WI). Homologous blood (~10 ml) was obtained from a donor rat and added to the perfusate to obtain a hematocrit of ~20%, which was measured with the use of a microcentrifuge. Arterial, venous, and airway pressures were measured with Cobe pressure transducers (Lakewood, CO), and the lung weight was continuously recorded by using a Grass model 7 polygraph (Grass, Quincy, MA). At the end of the experiments, the lungs were weighed and divided into pieces as described (12, 43). Briefly, male Charles River CD rats weighing between 199 and 320 g (247 ± 6 g) were anesthetized with an intraperitoneal injection of pentobarbital sodium (65 mg/kg). The trachea was cannulated, and the rats were ventilated with 20% O2-5% CO2-75% N2 by using a Harvard rodent ventilator (model 683, South Natick, MA) with a tidal volume of 2.5 ml and a positive end-expiratory pressure of 3 cmH2O at 40 breaths/min. The chest was opened, and 300 U heparin sodium were injected into the right ventricle. The pulmonary artery and left atrium were then cannulated, and the heart and lungs were excised en bloc and suspended from a force transducer. Lungs were perfused with 5% bovine albumin in Krebs-bicarbonate buffer (37°C) at 6 ml/min per gram of predicted (initial) lung weight by using a Minipuls-2 roller pump (Gilson, Middleton, WI). Homologous blood (~10 ml) was obtained from a donor rat and added to the perfusate to obtain a hematocrit of ~20%, which was measured with the use of a microcentrifuge. Arterial, venous, and airway pressures were measured with Cobe pressure transducers (Lakewood, CO), and the lung weight was continuously recorded by using a Grass model 7 polygraph (Grass, Quincy, MA). At the end of the experiments, the lungs were weighed and divided into pieces
Kfc and total vascular resistance

The procedure for measuring Kfc in isolated rat lungs has been previously described (12, 43). After an isogravimetric state was obtained, the venous reservoir was raised to obtain the desired venous pressure. For baseline Kfc measurements, Ppv was increased to 15 cmH2O and maintained for 20 min. Capillary pressure (Ppc) was calculated from pulmonary arterial pressure (Ppa) and Ppv at baseline and at increased Ppv by using

\[ Ppc = (Ppa - Ppv)/2 \]  

and the increase in capillary filtration pressure (ΔPpc) was the change in Ppc between vascular pressure states. The rate of weight gain over the last 2 min (ΔWt / Δt, where t is time) at increased Ppv were used to calculate Kfc by using

\[ Kfc = \Delta Wt / \Delta Ppc \]  

All Kfc values were normalized to 100 g predicted lung weight (PLW), which was based on body weight (BW) according to

\[ \text{PLW} = 0.0053 \text{BW} - 0.48 \]  

and expressed as ml · min⁻¹ · cmH2O⁻¹ · 100 g⁻¹. Perfusion flow (Q) for each experiment was calculated from the PLW and set at 6 ml · min⁻¹ · g PLW⁻¹. Total vascular resistance (Rt) was calculated by using

\[ Rt = (Ppa - Ppv)/Q \]  

Experimental Protocols

Low vascular pressure perfusion group (Low Ppv; n = 5). The lungs of these rats were isolated and perfused at baseline vascular and airway pressures. High vascular pressure control group (High Ppv; n = 7). These rat lungs were isolated and prepared as described above. The general time course of Ppv increases are shown in Fig. 1. After a baseline period of 20 min, a baseline Kfc was performed with Ppv increased to 15 cmH2O for a period of 18–20 min. After 15-min recovery, the venous outflow reservoir was raised as described above to 30 cm for 10 min, followed by 15-min recovery and a third Ppv increase to ~45 cmH2O for 5 min. After perfusion was stopped, the lung was weighed, divided into 0.3- to 1.0-g pieces and, together with 1-ml perfusate, was frozen in liquid nitrogen.

High vascular pressure isoproterenol group (Iso; n = 7). These rat lungs were isolated and prepared as described above. After a baseline period of 20 min, a baseline Kfc was performed, with Ppv increased to 15 cmH2O for a period of 18–20 min. Isoproterenol (10 µM) was infused into the venous reservoir, and the protocol shown in Fig. 1 was performed. After 15-min recovery, the venous outflow reservoir was raised as described above to 30 cm for 10 min, followed by 15-min recovery and a third Ppv increase to ~45 cmH2O for 5 min. After perfusion was stopped, the lung was weighed, divided into 0.3- to 1.0-g pieces and, together with 1-ml perfusate, was frozen in liquid nitrogen.

Hemoglobin Assay and Tissue Volumes

Lung samples were homogenized in distilled water (3.33 ml/g tissue; water added, see Eq. 6) and centrifuged for 1 h at 15,000 revolutions/min, and the supernatant was retained. Total hemoglobin (Hb) was measured in perfusate (hb) and tissue supernatant (hs) by using the cyanomethemoglobin method, and absorbance was read at 540 nm (6). Samples of perfusate and tissue homogenate (hs) were also weighed and dried to a stable weight at 60°C to obtain wet (WW) and dry weights (DW) and water fraction [FW = (WW − DW)/WW]. Lung tissue perfusate volume (Qb) was calculated by using

\[ Qb = Qh · (Hb/hs) · (FWh/FWs) \]  

and tissue water volume (Qwl) was calculated by using

\[ Qwl = Qh · FWh - WA \]  

where WA is water added. The total lung water and perfusate volumes were calculated from the lung weight-to-sample-weight ratio, and the volumes were normalized to 100 g PLW (Eq. 3).

Statistics

All values are expressed as means ± SE unless otherwise stated. The Kfc values were compared among groups by using an analysis of variance with repeated measures and a Newman-Keuls posttest with CRUNCH4 statistical software and a Gateway 2000 digital computer. A logarithmic transformation was used to minimize the within-group variance effects, and a significant difference was determined at P < 0.05.

RESULTS

Hemodynamics

Mean vascular and airway pressures and hemodynamic variables for the Low Ppv, High Ppv, and Iso groups are summarized in Table 1. All lungs were perfused at 6 ml · min⁻¹ · g PLW⁻¹. Vascular pressures in the Low Ppv group were maintained constant throughout the experiments. Vascular pressures shown in Table 1 for other groups are those present during the Kfc measurement, whereas baseline vascular pressures in these groups were not significantly different from those in the Low Ppv group. The peak inflation pressures (PIPs) are those recorded at the end of the Kfc measurement. An analysis of variance comparing the High Ppv and Iso groups indicated no differences between groups in any vascular pressures or resistance at the baseline state before the Ppv was increased or...
between groups at the 31 cmH2O Ppv state. Relative to Low Ppv, Iso, and High Ppv groups increased by 7.0

Lung Water and Blood Volumes

and 25.6 ± 6 during increased venous pressure Vascular and peak airway pressure Table 1.

Kfc values

The Kfc values at the three Ppv states during the Kfc measurements in the High Ppv and Iso groups are shown in Fig. 2. Kfc in both groups increased significantly at Ppv of 30 and 43 cmH2O compared with baseline, but Kfc values were only significantly different between groups at the 31 cmH2O Ppv state. Relative to baseline values, Kfc increased 6.2 ± 1.3-fold and 38.3 ± 15.2-fold in the High Ppv group during the respective Ppv increases to 31 and 43 cmH2O, and 2.6 ± 0.6-fold and 25.6 ± 6.5-fold in the Iso group, respectively.

Lung Water and Blood Volumes

As a percent of the PLW, the final lung weights of the Low Ppv, Iso, and High Ppv groups increased by 7.0 ± 2.9, 144 ± 22, and 191 ± 29%, respectively. Figure 3 indicates the total tissue water and blood volumes in lungs of the Low Ppv, High Ppv, and Iso groups at the end of the experiments. Relative to the Low Ppv group, the respective blood volumes and total lung water were increased significantly in the Iso (51 and 103%) and High Ppv (64 and 131%) groups. These differences in blood volumes represent extravasated blood and edema fluid. Blood volume and total lung water values were not significantly different between the Iso and High Ppv groups. The estimated contributions of each Kfc measurement to total lung water were 4, 31, and 65% for the 15, 31, and 43 cmH2O Ppv states, respectively, which could account for the absence of a significant difference in lung water volumes, even though Kfc values differed during the 32 cmH2O Ppv measurement. Lungs in both the High Ppv and Iso groups had grossly obvious hemorrhages, but the blood volume-total lung water ratios were 0.16, 0.12, and 0.12 for the Low Ppv, Iso, and High Ppv groups, respectively.

DISCUSSION

High pulmonary vascular pressures induce pulmonary microvascular injury and increased leakage of fluid, protein, and blood cells into the interstitial and alveolar spaces. Increased vascular permeability has been demonstrated in lungs of sheep (22), dogs (9, 18, 28, 34), and rabbits (4, 20, 26, 36) and may contribute to the high-permeability pulmonary edemas observed in such diverse conditions as neurogenic pulmonary edema, high-altitude pulmonary edema, or exercise-induced pulmonary hemorrhage (32, 40, 41). In isolated dog lung studies, Rippe et al. (28) demonstrated variable increases in Kfc after brief (5-min) Ppv increases. Mean Kfc increased significantly at Ppv values greater than −42 cmH2O, but the Kfc response was variable. Kfc generally recovered to baseline values after 15–20 min in most experiments. Also, in isolated dog lungs, Maron
(18) reported a decreased reflection coefficient and increased residual tissue blood after transient Ppv increases above 70 mmHg, and Townsley et al. (34) and Ehrhart and Hofman (9) reported an increased Kfc following Ppv increases of ~50 Torr. Interestingly, the osmotic reflection coefficient, based on the relative concentration changes of perfusate protein and red blood cells, did not decrease significantly in these latter two studies. Edema formed by hemorrhage of whole blood into the tissue would not affect these reflection coefficient measurements.

The major new findings of the present study were that 1) Kfc increased at relatively low Ppv values in rat lungs, indicating a susceptibility to mechanical injury similar to rabbit lungs (20); 2) the Kfc in isoproterenol-treated lungs at ~31 cmH2O was only 36% that of untreated controls; and 3) there were no differences in residual blood volumes between High Ppv and Iso groups. Isoproterenol was assumed to have little effect on the yield pressure of basement membranes but was expected to decrease the size of openings through endothelial cells by relaxing the cytoskeleton and spreading the endothelial cell layer (15). The dose of isoproterenol used exceeded that which completely reversed the Kfc increases induced by periods of lung ischemia (15). To our knowledge, this appears to be the first example of use of a pharmacological intervention to significantly attenuate mechanically induced increases in microvascular permeability. At the highest Ppv of 43 cmH2O, Kfc in the Iso group was only 64% that in the untreated group, but this trend did not reach significance. The similar hemoglobin contents in the Iso and High Ppv groups indicate that isoproterenol had no effect on the number of breaks through the basement membrane and the number of extravasated red blood cells. Most of the hemorrhage appeared to occur during the 43 cmH2O Ppv state, as few surface hemorrhages were observed during the 31 cmH2O Ppv increase. Compared with the Low Ppv lungs, the increases in total lung water and blood volumes indicate that only ~8.5% of the total fluid volume increase in both high Ppv groups occurred from bleeding and that 91.5% of the increase occurred through pathways that largely excluded red blood cells. The total lung water content of the Iso group was 88% that of the untreated lungs, despite the lower Kfc values, because most filtration occurred at the highest Ppv values.

Isoproterenol, a β-adrenergic agonist, attenuates microvascular permeability by increasing intracellular adenosine 3,5'-cyclic monophosphate through a pathway involving Gs protein and adenyl cyclase. In turn, adenosine 3,5'-cyclic monophosphate inhibits the active ATP and Ca2+-dependent contraction of cytoskeletal myofibrils in endothelial and epithelial cells mediated by myosin light chain kinase, causing spreading of endothelial cells with reduction of intercellular gaps in venules and cultured monolayers (17, 42). Histamine, H2O2, and α-thrombin applied to endothelial cell layers cause reorganization of F-actin fibers from peripheral bands to cell-spanning stress fibers (5, 31), and contraction of these fibrils is thought to retract the cell margins to allow increased paracellular transport (17). Endothelial permeability increases caused by ischemia-reperfusion in isolated perfused rat lungs can be reversed by isoproterenol, as well as by inhibition of calcium calmodulin, protein kinase A, or myosin light chain kinase and appear to be mediated by these cytoskeletal mechanisms (1, 2, 15, 17, 23). However, electron microscopic studies indicate that vascular pressure-induced permeability increases occur through parajunctional openings through the cell bodies of epithelial and endothelial cells rather than at the junctions (7). The isoproterenol response in the present study indicates that isoproterenol may also induce closure of these mechanical transcellular “breaks” as well as receptor-mediated junctional openings.

An alternative mechanism for the decrease in Kfc after isoproterenol could be an enhanced absorption of alveolar fluid by the alveolar epithelium (13, 29). Alveolar epithelium normally transports fluid out of the alveolar spaces coupled to Na+ transport, and this transport is increased by β-agonists (21). Although the final lung weight gains in both the High Ppv and Iso groups exceeded the 35–50% increase generally associated with initiation of alveolar flooding (32), lung weights had only increased by 35% at the end of the statistically different 31-cmH2O Kfc measurements based on recorded weight transients. Thus fluid reabsorption is unlikely to account for the significantly lower Kfc in the Iso group at a Ppv of 32 cmH2O but could have contributed to the lower Kfc at 43 cmH2O. However, in an isolated rat lung ischemia-reperfusion model, Khimenko et al. (14) showed that isoproterenol returned Kfc toward baseline and prevented further edema formation in the presence or absence of amiloride, a Na+-channel blocker. If a Na+-driven fluid reabsorption had accounted for the isoproterenol effect, then amiloride should have prevented the protective effect of isoproterenol and caused persistence of edema formation. In isolated dog lungs, fluid reabsorption occurred when Kfc was normal (16) but ceased when Kfc was elevated (M. I. Townsley, personal communication). Even if fluid reabsorption had occurred in the present study, the absorption rates could not account for the observed differences in Kfc caused by isoproterenol. Jayr et al. (13) observed fluid absorption rates of 0.90 ml·min−1·100 g lung weight−1 in isolated perfused rat lungs, which increased to 1.32 ml·min−1·100 g lung weight−1 after terbutaline, an increase of 0.42 ml·min−1·100 g lung weight−1. These alveolar fluid clearance rates are approximately two times those reported by other investigators for isolated rat lungs (3, 8). However, the respective filtration rates in the High Ppv and Iso groups at a Ppv of ~31 cmH2O were 7.02 and 2.42 ml·min−1·100 g lung weight−1, calculated as (Ppc − isogravimetric Ppc)×Kfc, or a difference of 4.60 ml·min−1·100 g lung weight−1 (28). Thus, fluid reabsorption would be an order of magnitude less than the observed effect of isoproterenol on Kfc in the present study.

The ability of isoproterenol to attenuate these vascular pressure-induced increases in fluid conductance warrants a revision of both of the current hypotheses of
capillary leak after mechanical injury, which generally consider injury as a passive process of structural failure. The first hypothesis of Shirley et al. (30) envisioned a stretched-pore phenomenon caused by stress-induced separation of endothelial junctions to account for increased lymph flow and reduced size selectivity for protein sieving. Neal and Michel (24, 25) made detailed microscopic studies of hydrostatic pressure-induced endothelial gap formation and measured hydraulic conductivities of frog mesentery capillaries at high vascular pressures. Over 80% of these gaps occurred through, rather than between, endothelial cells, with large increases in single capillary filtration occurring at pressures ~10 mmHg below these required for capillary rupture. Infrequent red blood cells were observed passing through the basement membrane below the rupture pressure. These authors postulated that gap formation resulted from insufficient plasmalemma membrane to cover the thinning areas of endothelium. Elegant electron-microscopic studies by West and colleagues (7, 10, 11, 35, 41) indicate that leakage of fluid at high pulmonary vascular pressures also occurs via parajunctional "breaks" through the endothelial and epithelial layers in dog and rabbit lungs. About one-half of all breaks extended through the basement membrane at the highest transmural pressures (35). The number of breaks was as a function of vessel transmural pressure and decreased when vascular pressure was reduced (35). The isoproterenol effects observed here suggest that an active retractile tension of endothelial cytoskeleton may be a major determinant of the fluid conductance response to vascular pressure-induced injury.

A second hypothesis advanced by West et al. (39, 41) attributes capillary leak and hemorrhage to a stress failure of the capillary wall, with the limiting tensile strength determined by that of the basement membrane. West and Mathieu-Costello (39) have discussed the engineering problem confronting design of the lung where the walls of alveolar capillaries must be thin to minimize diffusion distances but have sufficient tensile strength to withstand the microvascular hydrostatic pressure (39–41). The thin side of the pulmonary capillaries is only ~0.3 µm thick, with 0.15 µm of basement membrane contributing most of the tensile strength to the alveolar-capillary barrier (37, 39). Welling et al. (37) concluded that the basement membrane was the major tensile component of intact renal tubules, which exhibited the same pressure-volume curve when the epithelium was removed. Rupture occurred at 20 × 10⁵ N/m² in renal tubules, a value approximately an order of magnitude higher than that required for stress failure of pulmonary capillaries. Thickening of basement membrane and cellular layers could account for the higher threshold for edema formation in patients with chronic pulmonary venous hypertension (27) and the increased threshold vascular pressure for microvascular injury, as assessed by Kfc, in lungs of dogs with chronic congestive heart failure (33). The equal residual blood volumes in the High Ppv and Iso groups suggest that the number of capillaries ruptured at the highest Ppv (43 cmH₂O) was not affected by isoproterenol. However, the extravasated portion of this residual blood volume, calculated by subtracting that in the Low Ppv group, accounted for only ~8.5% of the total fluid gain by the High Ppv and Iso groups. This suggests that most of the increased fluid filtration occurred through openings that restricted red blood cells. While tensile strength of the basement membrane undoubtedly determines the amount of hemorrhage at very high pulmonary vascular pressures in racehorses and elite athletes (38, 40), the barrier function of the endothelium is the major determinant of fluid filtration over a lower range of pulmonary microvascular pressures. Furthermore, there appears to be an active "stretch recoil" that augments pressure-induced openings in this barrier and significantly contributes to filtration rates at high vascular pressures.

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