β-Adrenergic agonist therapy accelerates the resolution of hydrostatic pulmonary edema in sheep and rats

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Frank, James A., Yibing Wang, Oscar Osorio, and Michael A. Matthay. β-Adrenergic agonist therapy accelerates the resolution of hydrostatic pulmonary edema in sheep and rats. J Appl Physiol 89: 1255–1265, 2000.—To determine whether β-adrenergic agonist therapy increases alveolar liquid clearance during the resolution phase of hydrostatic pulmonary edema, we studied alveolar and lung liquid clearance in two animal models of hydrostatic pulmonary edema. Hydrostatic pulmonary edema was induced in sheep by acutely elevating left atrial pressure to 25 cmH2O and instilling 6 ml/kg body wt isotonic 5% albumin (prepared from bovine albumin) in normal saline into the distal air spaces of each lung. After 1 h, sheep were treated with a nebulized β-agonist (salmeterol) or nebulized saline (controls), and left atrial pressure was then returned to normal. β-Agonist therapy resulted in a 60% increase in alveolar liquid clearance over 3 h (P < 0.001). Because the rate of alveolar fluid clearance in rats is closer to human rates, we studied β-agonist therapy in rats, with hydrostatic pulmonary edema induced by volume overload (40% body wt infusion of Ringer lactate). β-Agonist therapy resulted in a significant decrease in excess lung water (P < 0.01) and significant improvement in arterial blood gases by 2 h (P < 0.03). These preclinical experimental studies support the need for controlled clinical trials to determine whether β-adrenergic agonist therapy would be of value in accelerating the resolution of hydrostatic pulmonary edema in patients.

hydrostatic edema; congestive heart failure; respiratory failure

THE MOST COMMON CAUSE OF CLINICAL hydrostatic pulmonary edema is elevated pulmonary microvascular pressure resulting from left ventricular dysfunction or valvular disease. Less often, hydrostatic pulmonary edema results from hemodynamic and osmotic changes that accompany volume overload. In either condition, the changes in Starling forces result in increased transudation of fluid from the pulmonary capillaries, producing interstitial pulmonary edema (45). Fluid is cleared from the interstitium in several ways. Because a pressure gradient exists between the alveolar and extra-alveolar compartments of the interstitial space, even in the presence of edema (6), transudated fluid first moves to the extra-alveolar interstitium. A significant portion of the edema fluid is then directly absorbed into the circulation or is returned to the circulation via lymphatics. Edema fluid is also cleared from the interstitium directly into the pleural space across the low-resistance visceral pleural mesothelium (9). If the underlying pathophysiology that produces interstitial edema is not corrected, and the capacity of the above clearance mechanisms is exceeded, flooding of the alveolar air compartment ensues (45, 55). Clearance of fluid from the alveolar air compartment depends on the active transport of sodium across the alveolar epithelium by alveolar type II cells (3, 12, 15, 21, 54). Water follows passively through aquaporins on alveolar type I cells (14) and other pathways. The active transport of sodium across type II cells can be stimulated by β-adrenergic agonists as well as by catecholamine-independent pathways (2, 4, 5, 8, 11, 13, 16, 18, 20, 22, 25, 27, 28, 33, 38, 40, 41, 47, 48, 50, 53).

Data from previous clinical studies by our laboratory have demonstrated that an intact, functional alveolar epithelium is required for clearance of alveolar edema (32). Furthermore, intact alveolar liquid clearance is associated with more rapid improvement in oxygenation in patients with hydrostatic pulmonary edema (52). The potential therapeutic role of β-agonists for hastening the resolution of alveolar pulmonary edema has been suggested by animal and ex vivo human lung studies (4, 30, 38, 39, 50). Interestingly, in a recent clinical study, patients with intact alveolar liquid clearance were more likely to have received nebulized β-adrenergic agonist than patients with impaired alveolar liquid clearance (85% positive predictive value for intact alveolar liquid clearance with β-agonist therapy) (52). Although these data suggest a potential therapeutic benefit of exogenous β-adrenergic agonists in these patients, the study did not have sufficient power to detect the observed differences with statistical significance.

Therefore, the primary objective of the present study was to determine whether β-adrenergic agonist therapy would increase alveolar liquid clearance during the resolution phase of hydrostatic pulmonary edema in sheep. The second objective was to determine whether
the increase in alveolar liquid clearance from β-agonist therapy was associated with an increase in lung lymph flow and clinically important changes in gas exchange. The third objective was to measure the systemic and pulmonary hemodynamic effects of β-agonist therapy for hydrostatic pulmonary edema. The advantages of a sheep model of hydrostatic pulmonary edema include the ability to directly elevate left atrial pressure with a Foley balloon, to monitor cardiac output and pulmonary artery pressures, and to quantify lung lymph flow as a measure of alveolar liquid clearance and pulmonary vascular filtration. Because the sheep experiments in the present study demonstrated that salmeterol treatment resulted in an increase in alveolar liquid clearance but did not significantly decrease excess lung water or improve blood gases, the fourth objective was to carry out a similar series of experiments in a rat model of hydrostatic pulmonary edema (3, 4, 9, 11, 19, 24, 36, 38–40). Both baseline and maximal alveolar liquid clearance rates in sheep are significantly slower than in humans and rats (Table 1). Therefore, the main advantage of a rat model is that alveolar liquid clearance rates are similar to the rate of alveolar fluid clearance in humans (38). The experimental conditions in both studies were designed to approximate the clinical condition of patients with hydrostatic pulmonary edema who have received pharmacological therapy to lower left atrial pressure.

**METHODS**

*Sheep Preparation and General Experimental Protocol*

Sheep weighing 28 ± 4 kg were anesthetized with intravenous thiopental sodium (15 mg/kg body wt). A tracheotomy was performed via a midline incision. A cuffed tracheotomy tube was inserted, and the sheep were ventilated with constant-volume ventilation (Harvard Apparatus, Dover, MA). Tidal volume was set to 13–15 ml/kg. Positive end-expiratory pressure was set to 3 cmH2O, and the fraction of inspired oxygen was 1.0. Anesthesia was maintained with 1% halothane. A catheter was placed in the carotid artery for the measurement of arterial blood gases, left atrial pressure, and pulmonary vascular pressure, and to obtain blood samples. The respiratory rate was adjusted to maintain an arterial partial pressure of CO2 (Paco2) between 30 and 40 Torr. Pancuronium bromide (0.3 mg·kg⁻¹·h⁻¹; Pavulon, Organon, West Orange, NJ) was administered for neuromuscular blockade. Sheep were placed prone, with the head and thorax elevated 5°. The protocol was approved by the Committee on Animal Research at the University of California, San Francisco.

The sheep were surgically prepared for collection of lung lymph, as previously described (4, 29). The surgical preparation usually required 1.5–2 h. A Foley catheter (20-Fr, Bard, Covington, GA) was inserted into the left atrium and secured as described previously (26).

After a 1 h-baseline period of stable heart rate, systemic blood pressure, pulmonary vascular pressure, and arterial blood gases, left atrial pressure was raised to 25 cmH2O by inflation of the Foley catheter balloon. Left atrial pressure was continuously monitored with a separate left atrial catheter. One hour after left atrial pressure was increased, a fiber-optic bronchoscope was used to instill 12 ml/kg body wt of test solution directly into the lower lobes of the lungs, as described elsewhere (10). The test solution was isotonic 5% albumin (prepared from bovine albumin; Sigma Chemical, St. Louis, MO) in 0.9% saline with 10 μCi 125I-labeled albumin (Frosst Laboratories, Montreal, Canada) and 1 mg/l Evans blue dye (Aldrich, Milwaukee, WI). One-half of the test solution (6 ml/kg body wt or ~170 ml) was instilled into the lower lobe of each lung. Immediately after instillation, sheep were randomized to receive either 5 mg salmeterol (Glaxo, Greenford, Middlesex, UK) in 5 ml saline or an equal volume of saline, by aerosolization, using a whisper jet nebulizer system (model 123014, Marquest). Left atrial pressure remained at 25 cmH2O for 1 h after intratracheal instillation of the test solution. The balloon was then deflated, and left atrial pressure was allowed to return to normal (Fig. 1).

Lung lymph was collected continuously, and aliquots were separated every 15 min. Arterial blood samples were collected each hour for measurement of arterial blood gases, plasma protein, and plasma epinephrine. At the end of the experiment, animals were exsanguinated. Alveolar fluid samples were obtained from the distal air spaces of the lower lobes by gently guiding a premeasured 8-Fr feeding catheter (Sims Smith Industries Medical Systems, Kneene, NH) into a wedged position and aspirating fluid (4, 29). A median sternotomy was performed, and the lungs were removed. The distribution of the instilled fluid into both lungs was confirmed by examining the distribution of Evans blue dye. Gravimetric lung water was then determined by standard methods (43).

*Specific Experimental Protocols for Sheep Studies*

**Group I: Left atrial hypertension (25 cmH2O) with aerosolized salmeterol.** After the surgical preparation, elevation of left atrial pressure to 25 cmH2O for 1 h, and intratracheal instillation of 12 ml/kg 5% albumin in 0.9% saline solution, 5 mg salmeterol in 5 ml 0.9% saline were nebulized over 1 h (n = 5). Left atrial pressure was maintained at 25 cmH2O for

| Table 1. Alveolar liquid clearance in sheep, rats and humans under basal and stimulated conditions and with and without hydrostatic pulmonary edema |
|-----------------------------------|-----------------------------------|---------------------------------|---------------------------------|---------------------------------|
|                                  | Alveolar Liquid Clearance Without | Alveolar Liquid Clearance With   |                                  |
|                                  | Hydrostatic Pulmonary Edema, %/h  | Hydrostatic Pulmonary Edema, %/h |
|                                  | Basal | Stimulated | % Increase | Basal | Stimulated | % Increase |
| Sheep                            | 8     | 13         | 62         | 5     | 8          | 60         |
| Rat                              | 20    | 35         | 75         | 16*   | 22*        | 38         |
| Human                            | 54‡   |            |            | 25†   |            |            |

*Lung liquid clearance (%/h). †Rate was the mean value for patients in the highest clearance group (see Ref. 52). ‡Based on data obtained from lavage fluid of patients with pulmonary alveolar proteinosis (M. Chesnutt, J. Golden, T. Nuckton, and M. Matthay, unpublished observations).
a total of 2 h. The Foley catheter balloon was then deflated and left atrial pressure was allowed to return to normal (Fig. 1). Measurements were continued for a total of 3 or 4 h (n = 3 and n = 2, respectively) after administration of β-agonist therapy. Our initial experiments were 3 h; however, when no difference in excess lung water after 3 h was observed, we extended the experiments to 4 h.

**Group II: Left atrial hypertension (25 cmH₂O) with aerosolized saline control.** After surgical preparation, elevation of left atrial pressure to 25 cmH₂O for 1 h, and intratracheal instillation of 12 ml/kg body wt 5% albumin in 0.9% saline solution, 5 ml 0.9% saline were nebulized over 1 h (n = 6). The protocol was otherwise identical to group I. Measurements were continued for a total of 3 or 4 h (n = 4 and n = 2, respectively) after administration of nebulized saline.

**Measurements for Sheep Studies**

**Hemodynamics, airway pressures, arterial blood gases and protein concentration.** Systemic blood pressure, pulmonary artery pressure, left atrial pressure, and peak airway pressure were monitored continuously using calibrated pressure transducers (Pd23 ID, Gould, Oxnard, CA) and recorded on a polygraph (Grass Instruments, Quincy, MA). Arterial blood gases were measured every hour. Samples of blood, instillate, and alveolar fluid aspirated from the distal air spaces 3 h after the administration of nebulized salmeterol or saline were collected to measure total protein concentration and ¹²⁵I-albumin activity.

**Measurement of alveolar liquid clearance.** Alveolar liquid clearance (ALC) was calculated as

\[ ALC = \frac{\left( V_i - V_f \right)}{V_i} \times 100 \] (1)

where \( V_i \) is the volume of instilled fluid (ml) and \( V_f \) is the final alveolar fluid volume (ml). \( V_f \) was estimated as

\[ V_f = V_i \times \frac{cpg_i}{cpg_f} \] (2)

where \( cpg \) refers to the radioactivity (counts per min per gram of sample) of ¹²⁵I-albumin in the instilled fluid and \( cpg_f \) refers to the radioactivity of ¹²⁵I-albumin in the alveolar fluid aspirated at 3 h.

**Measurement of excess lung water.** We measured extravascular lung water in sheep using standard methods (4, 10, 29). Excess lung water (ELW) in the lungs was calculated as

\[ ELW = \left( W_e - P \right) - \left( W_d - P \right) \] (3)

where \( W_e \) is the weight of the instilled lungs, \( D_d \) is the dry weight of the instilled lungs and \( P \) is the weight of protein that was instilled. \( W_e \) and \( D_d \) are reference data from control lungs (n = 6) from previous studies.

**Determination of plasma epinephrine concentration.** Plasma epinephrine was measured in 1-ml heparinized blood samples by high-performance liquid chromatography (HPLC) (4, 10). HPLC data were collected by a laboratory technician blinded to the conditions of the experiments. In each sheep experiment, four blood samples were obtained: at baseline, at the time of intratracheal instillation, after deflation of the Foley catheter balloon, and at the end of the experiment.
Rat Preparation and General Experimental Protocol

In an effort to extend the results of the sheep studies to an animal model with a maximal alveolar liquid clearance rate closer to that of humans (38), we developed a rat model of hydrostatic pulmonary edema. Male Sprague-Dawley rats weighing 275–300 g were intraperitoneally anesthetized with 50 mg/kg body weight pentobarbital sodium. A tracheostomy was performed, and the trachea was cannulated with PE-205 tubing (Becton Dickinson, Parsippany, NJ). Rats were ventilated with a constant-volume ventilator (Harvard Apparatus, South Natick, MA). Tidal volume was 8 ml/kg, the fraction of inspired oxygen was 1.0, and positive end-expiratory pressure was 3 cmH2O. Respiratory rate was adjusted to maintain $P_{a\text{CO}_2}$ at 30–40 Torr. Neuromuscular blockade was maintained with pancuronium bromide (2 mg/kg iv, every hour). A catheter was inserted into the right carotid artery to monitor systemic blood pressure, arterial blood gases, and to obtain blood samples. Two additional vascular catheters (PE-50 tubing) were then placed into each jugular vein. One of the venous catheters was used to monitor central venous pressure, and the other was used for intravenous infusion of fluid. After surgical preparation (30–60 min), rats were placed in the supine position and covered with a warming blanket set at 38°. After a 1-h baseline period of stable hemodynamics and arterial blood gases, an intravenous infusion of Ringer lactate (38°C) was given over 2 h (volume = 40% of body wt, with 40% of the total volume given over the first 20 min). The infusion rate was controlled using an infusion pump (model 22, Harvard Apparatus). Preliminary experiments, based on previously published protocols (7, 9, 44, 55), confirmed that this protocol resulted in the development of primarily interstitial pulmonary edema (i.e., lung water increased but oxygenation changed minimally). At the end of 2 h, rats were randomized to receive intratracheal instillation of 6 ml/kg of isotonic 5% albumin solution in 0.9% saline with or without salmeterol ($10^{-5}$ M). This concentration was selected because measured alveolar fluid levels of salmeterol, administered via a nebulizer, were $10^{-6}$ M–$10^{-5}$ M in our laboratory’s previous sheep study (10). That study also demonstrated the equal efficacy of nebulized or instilled salmeterol (10). The solution was prepared using bovine albumin (Sigma Chemical), as described previously (24, 36, 37). Evans blue dye (1 mg/l) was added to the instillate to confirm adequate distribution in all lung lobes. After intratracheal instillation, mechanical ventilation was immediately resumed, and measurements were continued for 4 h (Fig. 2). The protocol was approved by the Committee on Animal Research at the University of California, San Francisco.

Specific Experimental Protocols for Rat Studies

**Group I: Volume overload-induced pulmonary edema and intratracheal instillation of 5% albumin solution with salmeterol.** After the 2-h intravenous infusion of Ringer lactate, 6 ml/kg of 5% albumin in 0.9% saline with Evans blue dye and salmeterol ($10^{-5}$ M; $n=4$) was instilled into the trachea. Measurements were continued for 4 h after instillation.

**Group II: Volume overload-induced pulmonary edema and intratracheal instillation of 5% albumin solution.** The protocol was the same as for group I except salmeterol was not added to the instillate and $n=5$.

Measurements for Rat Studies

**Hemodynamics, airway pressures, and arterial blood gases.** Systemic blood pressure, central venous pressure, and peak airway pressure were monitored continuously using calibrated pressure transducers and recorded on a polygraph, as described in Measurements for Sheep Studies. Arterial blood gases were measured every hour before instillation and every 30 min after. Hematocrit was measured at baseline, after the intravenous infusion, and at the end of the experiment to determine the amount of blood dilution.

**Measurement of excess lung water and lung liquid clearance.** We determined extravascular lung water in rats using standard techniques (24, 36, 37). The excess lung water was calculated as described in Eq. 3. The mean measured lung water of the reference control group ($n=6$) was used as

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**Fig. 2.** Effects of intravascular volume overload and intratracheal instillation of isotonic 5% albumin solution on hemodynamics and airway pressure in rats. Central venous pressure (CVP) and mean systemic arterial blood pressure (MAP) increased acutely during the initial phase of the intravenous infusion of Ringer lactate. When the infusion rate was decreased after 20 min, CVP decreased but remained above baseline. MAP remained elevated throughout the infusion. When the intravenous infusion was stopped and the isotonic 5% albumin solution was instilled into the trachea, MAP decreased to baseline. There were no significant differences in CVP or MAP between salmeterol-treated (solid lines) and control (dashed lines) rats.
described in Measurements for Sheep Studies. Lung liquid clearance (LLC) was calculated from the measured ELWf and the EMLW for each rat

\[
\text{LLC} = \left( \frac{(\text{EMLW} - \text{ELW}_f) / \text{EMLW}}{100} \right)
\]

where EMLW is the volume of fluid instilled into the trachea in each experiment plus the mean excess lung water in a group of rats \((n = 6)\) immediately after a 40% body wt infusion of Ringer lactate, and ELWf is the measured excess lung water at the end of the experiment. LLC is expressed as the percent of the EMLW volume cleared from the lungs.

**Statistics**

The data are summarized as means ± SD. Experimental interventions were compared with controls using an unpaired \(t\)-test. One-way ANOVA and Newman-Keuls tests were used to compare data from hemodynamic measurements, airway pressure, and epinephrine levels between the groups in each experiment. Additionally, measurements of epinephrine within groups I and II in the sheep experiments were compared by paired \(t\)-tests. Statistical significance was defined by a \(P\) value ≤ 0.05.

**RESULTS**

Hemodynamic Measurements in Sheep During and After Elevation of Left Atrial Pressure

Baseline pulmonary artery pressure, left atrial pressure and cardiac output were similar in all sheep (Fig. 1). As expected, the pulmonary artery pressure increased when left atrial pressure was increased to 25 cmH\(_2\)O. Neither the increase in left atrial pressure nor treatment with salmeterol significantly reduced cardiac output; however, there was a trend toward an increase in heart rate in both groups after left atrial hypertension. The maximal heart rate was not different in salmeterol-treated sheep (166 ± 25 beats/min) compared with control sheep (187 ± 37 beats/min). There were no significant differences in cardiac output, left atrial pressure, or pulmonary artery pressure between the groups at any time points (Fig. 1).

Alveolar Liquid Clearance and Excess Lung Water After Left Atrial Hypertension in Sheep Treated With Salmeterol

During the resolution phase of hydrostatic pulmonary edema, alveolar liquid clearance increased significantly in salmeterol-treated animals compared with controls (34 ± 4 vs. 21 ± 6%, \(P < 0.001\)) (Fig. 3A). However, despite this 60% increase in clearance, there was no difference in the measured excess lung water between salmeterol-treated animals and controls 3 h after treatment was initiated (Fig. 3B). The absolute rate of alveolar liquid clearance in the salmeterol-treated sheep was only 8% per hour. Because no difference in lung water was observed at 3 h after initiation of therapy, we extended the length of the experiment by 1 h. In these longer experiments, there was still no difference in excess lung water between salmeterol-treated and control animals. The 3-h and 4-h time point data are combined in Fig. 3B. Plasma epinephrine levels were not different between the two groups (Fig. 4). The median plasma epinephrine level in salmeterol-treated sheep at the end of the experiment was 950 pg/ml, with a range of 600–1,940 pg/ml.
The median plasma epinephrine level in the control animals was 860 pg/ml, with a range of 760–1,200 pg/ml \((n = 5)\). Plasma epinephrine levels increased slightly in both groups after elevation of left atrial pressure; however, this increase was not statistically significant.

**Lung Lymph Flow in the Resolution Phase of Hydrostatic Pulmonary Edema in Sheep**

As expected, lung lymph flow increased in response to left atrial hypertension (Fig. 5). After the intratracheal instillation of isotonic 5% albumin solution, there was a further increase in lymph flow in both groups. However, the administration of salmeterol was associated with a significantly greater increase in lymph flow compared with controls \((\bullet)\). This effect was sustained for the duration of the experiment. Total lung lymph flow was 139 ml for salmeterol-treated sheep and 90 ml for saline-treated (control) sheep. \(*P < 0.01.\)

\((n = 4)\). The median plasma epinephrine level in the control animals was 860 pg/ml, with a range of 760–1,200 pg/ml \((n = 3)\). Plasma epinephrine levels increased slightly in both groups after elevation of left atrial pressure; however, this increase in was not statistically significant.

**Measurements of Oxygenation and Airway Pressure in Sheep After Left Atrial Hypertension**

The alveolar-arterial oxygen difference increased after intratracheal instillation of isotonic 5% albumin solution. In spite of the more rapid alveolar liquid clearance in salmeterol-treated sheep, the improvement in oxygenation was similar between salmeterol- and saline-treated sheep (Fig. 6). There were no differences in \(P_{\text{aCO}_2}\) or pH between the groups at any time. There was a modest trend toward lower peak airway pressures in sheep treated with salmeterol compared with control animals 2 h after administration of the drug. Two hours after administration of aerosolized salmeterol or saline, peak airway pressure in salmeterol-treated sheep was \(26 \pm 5\ \text{cmH}_2\text{O} (n = 5)\) compared with \(29 \pm 6\ \text{cmH}_2\text{O} in controls (n = 6; P = 0.4).\)

**Hemodynamic Measurements for Rats With Hydrostatic Pulmonary Edema**

There were no differences in baseline mean arterial blood pressure or central venous pressure between rats in the control and salmeterol groups (Fig. 2). Mean arterial blood pressure and central venous pressure increased similarly in the two groups with volume infusion. After intratracheal instillation of isotonic 5% albumin solution, central venous pressure changed minimally, and mean systemic arterial blood pressure decreased slightly in both groups. No significant differences were observed in these hemodynamic variables after the administration of salmeterol (Fig. 2). There was no difference in heart rate between the two groups at any time point (mean maximum heart rate in salmeterol-treated rats was \(282 \pm 25 vs. 285 \pm 18\) beats/...
min in control rats). Blood hematocrit was monitored as a measure of hemodilution. There were no differences in hematocrit between the groups at any time point.

**Excess Lung Water, Lung Liquid Clearance, and Catecholamine Levels in Rats After Volume Overload**

Figure 7 compares measured extravascular lung water at baseline (n = 6), after 40% body wt infusion of Ringer lactate (n = 6), and at the end of the experiments (n = 4 in the salmeterol-treated group and n = 5 in the control group). After the intravascular infusion of Ringer lactate, lung water increased by 0.6 ± 0.2 ml (P < 0.01). This volume is termed excess lung water. The maximum excess lung water immediately after intratracheal instillation of 6 ml/kg body wt albumin solution was calculated by adding the instilled fluid volume to the excess lung water measured after the intravascular infusion. Therefore, the mean maximum excess lung water was 3.2 ± 0.5 ml (n = 6). Four hours after intratracheal instillation, the measured excess lung water in rats that received salmeterol was 0.3 ± 0.1 ml (n = 4) compared with 0.8 ± 0.2 ml in control rats (n = 5; P < 0.01; see Fig. 7). Accordingly, lung liquid clearance, as determined by Eq. 4, after volume overload and intratracheal instillation of albumin solution in rats treated with salmeterol, was significantly higher than in controls over 4 h (87 ± 2 vs. 69 ± 9%, P < 0.01; see Table 1). Plasma norepinephrine was measured in two salmeterol-treated rats and one control rat at 6 h and was found to be similar and low in all three animals.

**Measurements of Oxygenation, Airway Pressure, and Alveolar Edema in Rats After Volume Overload**

The alveolar-arterial oxygen difference after volume overload and instillation increased to 407 ± 163 Torr in control rats and 421 ± 122 Torr in rats that received salmeterol (Fig. 8). There was a significantly greater (P = 0.03) improvement in the alveolar-arterial oxygen difference over the first 2 h in the rats treated with salmeterol compared with controls. However, by 4 h, the alveolar-arterial oxygen difference in the two groups was nearly identical (160 ± 29 Torr in controls vs. 166 ± 28 Torr in salmeterol-treated rats, P = 0.8; Fig. 8). There were no differences in PaCO2 or pH. There was a trend toward lower peak airway pressures in rats that received salmeterol compared with control rats. Four hours after administration of salmeterol or saline, peak airway pressure in salmeterol-treated rats was 16 ± 3 cmH2O compared with 18 ± 2 cmH2O in controls (P = 0.22).

Fig. 7. Excess lung water in rats. Baseline lung water was determined by subtracting the dry weight from the blood-free wet weight in untreated control rats (n = 3). This baseline value was used as the reference value for excess lung water in the other experiments. The excess lung water measured at the end of the intravascular infusion (end infusion, time 0) was 0.6 ± 0.2 ml (n = 6). The excess lung water immediately after intratracheal instillation (alveolar instillation) was calculated by adding the volume of instillate to the lung water measured after the intravascular infusion in each of the 6 rats (end infusion). The excess lung water was measured in the salmeterol-treated (n = 4) and control rats (n = 5) 4 h after intratracheal instillation. Excess lung water was significantly higher in control rats compared with salmeterol-treated rats (P < 0.01). Four hours after treatment, excess lung water in the salmeterol-instilled rats was significantly lower than immediately after the intravascular infusion but remained significantly higher than baseline. *P < 0.03; †P < 0.03.

Fig. 8. Alveolar-arterial oxygen difference in rats during the resolution of hydrostatic pulmonary edema. There were no differences in arterial blood gases and alveolar-arterial oxygen difference between the groups during the intravascular (IV) infusion or after intratracheal instillation of fluid. Two hours after intratracheal instillation of isotonic 5% albumin solution, rats treated with salmeterol (●) showed significantly greater improvement in the alveolar-arterial oxygen difference compared with control rats (○). By 4 h, there were no differences between the groups. *P < 0.03.
DISCUSSION

Recently, studies from our laboratory demonstrated that alveolar liquid clearance in sheep persists at a normal rate in the presence of hydrostatic pulmonary edema produced by left atrial hypertension (10). However, the administration of a long-acting, lipophilic, β₂-adrenergic agonist (salmeterol), did not increase alveolar liquid clearance if moderate left atrial hypertension persisted (10, 36). In the absence of left atrial hypertension, aerosolized salmeterol increased alveolar liquid clearance by 60% above the basal rate (10). The mechanism for the lack of stimulation during left atrial hypertension was unclear but may have been due to an inhibitory effect of atrial natriuretic factor on sodium transport (10, 34). Alternatively, severe interstitial edema may have been associated with ongoing alveolar flooding, thus obscuring an increase in alveolar liquid clearance from β-agonist therapy. The sheep experiments in the present study were initially designed to determine whether the stimulatory effect of salmeterol on alveolar liquid clearance was restored during the resolution phase of hydrostatic pulmonary edema, when left atrial pressure was returned to normal. The major finding of the sheep studies was that salmeterol increased alveolar liquid clearance by 60% during the resolution of hydrostatic pulmonary edema.

The measured difference in alveolar liquid clearance was consistent with the difference in lung lymph flow. The salmeterol-treated sheep had significantly greater lymph flow than the control sheep (139 vs. 90 ml; Fig. 5). Lung lymph reflects both vascular filtration and alveolar fluid transported to the interstitium (4). Therefore, this increase in lymph flow is attributable, in part, to the increase in alveolar liquid clearance but also to an increase in lung vascular filtration. Our laboratory previously found that ~50% of the increase in lymph flow that follows the administration of β-agonist is attributable to alveolar liquid clearance and ~50% is attributable to increased lung vascular filtration (4).

There was not, however, a measurable difference in excess lung water at the end of the 4-h experiments. How can this result be explained? The average volume of fluid instilled into the lungs was 336 ml (12 ml/kg). In sheep treated with salmeterol, an average of 114 ml was removed from the air spaces (34%) compared with 71 ml in control sheep (21%), a difference of 43 ml. This represents only 12% of the volume instilled into the lungs. Because the determination of extravascular lung water is associated with a coefficient of variation of 10–15%, we would not expect to detect a difference of this magnitude. Furthermore, the increase in alveolar liquid clearance after β-agonist therapy seems to be of limited significance in the sheep because there was no difference in arterial blood gases between the groups (Fig. 6).

Because alveolar liquid clearance may depend in part on endogenous catecholamines (10, 36, 37, 52), we measured epinephrine levels during and after left atrial hypertension (Fig. 4). Although there was no statistically significant difference in plasma epinephrine between the salmeterol-treated sheep and controls, there was a trend toward increased plasma epinephrine in both groups in response to left atrial hypertension. This finding is consistent with our laboratory’s previous study (10).

Basal and maximal alveolar liquid clearance in sheep is significantly lower than in humans and rats (Table 1) (29, 31, 38, 52). Therefore, we designed a second set of experiments in a rat model of hydrostatic pulmonary edema to determine whether the higher clearance rates after β-agonist therapy resulted in significant improvements in oxygenation and lung water in this species. The rate of basal and maximal alveolar liquid clearance in rats is closer to estimates of alveolar liquid clearance in the ex vivo human lung and in patients during the resolution of hydrostatic pulmonary edema (Table 1) (38, 52). The major findings of the rat experiments were that salmeterol administration resulted in a 62% greater reduction in excess lung water (Fig. 7) as well as a significant improvement in arterial blood gases (Fig. 8). The difference in oxygenation was evident over the first 2 h after instillation. By 4 h, there were no difference in arterial blood gases between the groups. The most plausible explanation for this finding is that salmeterol markedly increased alveolar liquid clearance, resulting in more rapid resolution of alveolar edema; however, because alveolar liquid clearance in rats is fast, most alveolar edema was cleared by 4 h, even in the control animals. The difference observed in excess lung water at 4 h likely represents, primarily, a difference in the magnitude of interstitial pulmonary edema (7, 44, 55). Compared with the excess lung water in rats immediately after the intravascular infusion, the amount of edema in the salmeterol-treated rats at 4 h was lower. Therefore, it seems likely that the majority of alveolar edema was cleared well before 4 h in the salmeterol-treated rats, thus allowing for greater clearance of interstitial edema by the end of 4 h.

Importantly, in both the sheep and rat studies, there were no measured untoward hemodynamic or other adverse effects of salmeterol therapy. Although the sample size in these experiments was small, leaving the possibility of a type II error, these data suggest that β-agonist therapy for hydrostatic pulmonary edema is safe in sheep and rats and may not be associated with adverse effects in human patients. Inhaled salmeterol has been shown to be safe in asthmatic individuals (48, 49) and is probably safe in patients with preexisting cardiac arrhythmia (50). The hemodynamic data also indicate that the observed differences in alveolar liquid clearance were not due to hemodynamic changes.

There are some potential limitations to these studies. Also, the conditions in these experiments may not precisely approximate the clinical condition of hydrostatic pulmonary edema. The immediate return of left atrial pressure to normal in the sheep experiments may be an overestimate of the effectiveness of clinical therapies for left ventricular failure. In addition, neu-
roendocrine changes in patients with left atrial hypertension could also affect alveolar liquid clearance. Patients with left ventricular failure often have chronic, modest elevations in circulating catecholamine levels (35), although catecholamine levels in these patients, and even in patients with septic shock (52), are below the theoretical maximum dose response of the alveolar epithelium to β-agonist stimulation (16). Chronic stimulation, however, could result in downregulation of β-receptors and could adversely affect the response of the alveolar epithelium to exogenous catecholamines. The long-term effect of increased endogenous catecholamine levels on alveolar type II cell β-receptor expression in humans is largely unknown; however, administration of inhaled β2-agonist has been found to result in decreased expression of β2-receptors and downregulation of intracellular cyclic AMP in human airway epithelium and alveolar macrophages in vivo (51). In addition to the increase in plasma catecholamines, atrial natriuretic factor is elevated in patients with left atrial hypertension. Atrial natriuretic factor decreases sodium transport in vitro and in the ex vivo rat lung (34, 49) and may decrease alveolar liquid clearance by inhibiting sodium transport. Our laboratory’s previous sheep studies demonstrated a modest elevation in atrial natriuretic factor with persistent left atrial hypertension; however, plasma concentrations of atrial natriuretic factor were lower in the sheep than was required for sodium transport inhibition in vitro (10, 34).

There are at least three mechanisms by which these models could underestimate the effect of exogenous β-agonists on alveolar fluid clearance in patients with hydrostatic pulmonary edema. First, in the rat experiments, two Starling forces, plasma protein osmotic pressure and intravascular hydrostatic pressure, were changed. Although the volume of fluid infused produced primarily lung interstitial edema, the decrease in plasma protein osmotic pressure could conceivably slow clearance of the instilled fluid from the lung (45, 46). Second, the alveolar epithelium in patients with chronic left ventricular failure may not be identical to normal individuals. For example, histology studies have found alveolar type II cell proliferation in patients with chronically elevated left atrial pressure (1), and type II cell proliferation increases the rate of alveolar fluid clearance (17, 53). Third, patients with chronic congestive heart failure have enlarged lymphatics in the extraalveolar interstitium (23), a finding that could result in increased fluid clearance via the lymphatics compared with normals.

In summary, these experimental studies demonstrate that a lipid-soluble, long-acting, β2-adrenergic agonist (salmeterol) accelerated the rate of alveolar liquid clearance during the resolution of hydrostatic pulmonary edema in both sheep and rats. Salmeterol significantly increased alveolar liquid clearance during the resolution phase of hydrostatic pulmonary edema in sheep and in rats without altering systemic or pulmonary hemodynamics. Our previous sheep studies found that 3 h after the administration of nebulized salmeterol (5 mg), therapeutic levels could be measured in edema fluid (10). There were not, however, significant plasma levels of salmeterol (10). In the present sheep study, the increase in alveolar liquid clearance was not associated with an improvement in arterial blood gases. In the rat experiments, there was a significant improvement in arterial blood gases 2 h after salmeterol administration. This rapid improvement in arterial blood gases could have clinical importance. For example, administration of β-agonist could potentially obviate the need for intubation and mechanical ventilation in patients by resolving hydrostatic pulmonary edema due to more rapid improvement in oxygenation and possibly a decrease in the work of breathing. The latter possibility is suggested by a modest trend toward lower airway pressures in the salmeterol-treated sheep and rats. Furthermore, nebulized β-agonist therapy could potentially decrease the duration of mechanical ventilation and hospitalization. A recent clinical study found that administration of exogenous β-agonist was associated with intact alveolar liquid clearance in patients with hydrostatic pulmonary edema (52), and intact alveolar liquid clearance was associated with a significant improvement in arterial blood gases at 24 h and a trend toward a decrease in the duration of mechanical ventilation. Interestingly, salmeterol has recently been shown to prevent high-altitude pulmonary edema in at-risk patients brought to altitude (42). The data from these new experiments and the recent clinical study by our laboratory (52) suggest that inhaled β-agonist therapy for hydrostatic pulmonary edema is probably safe and may be associated with a more rapid improvement in oxygenation. This therapy may be especially useful in patients receiving systemic β-blockers after acute myocardial infarction and congestive heart failure, because systemic β-blockers could slow alveolar fluid clearance and the improvement in oxygenation. In this setting, use of a nebulized β-agonist could improve alveolar fluid clearance without adversely impacting systemic hemodynamics. These data support the need for controlled clinical trials of the safety and efficacy of this therapy in patients with hydrostatic pulmonary edema.

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