Beta₁-Adrenergic Agonist Is a Potent Stimulator of Alveolar Fluid Clearance in Hyperoxic Rat Lungs

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ABSTRACT—Because it was still uncertain whether a stimulation of β₁-adrenoceptors accelerated alveolar fluid clearance in hyperoxic lung injury, the effect of denopamine, a selective β₁-adrenergic agonist, on alveolar fluid clearance was determined in rats exposed to 93% oxygen for 48 and 56 h. Alveolar fluid clearance was measured by the progressive increase in the concentration of Evans blue labeled albumin instilled into the alveolar spaces over 1 h at 37°C in isolated rat lungs. The principle results were as follows: 1) Although lung water volume increased in rats exposed to hyperoxia for 48 and 56 h, basal alveolar fluid clearance did not change for up to 56 h; 2) Denopamine increased alveolar fluid clearance in rats exposed to hyperoxia as well as in rats without exposure to hyperoxia; 3) Denopamine primarily increased amiloride-insensitive alveolar fluid clearance in rats exposed to hyperoxia; 4) The potency of denopamine was similar to that of terbutaline, a selective β₂-adrenergic agonist. In summary, denopamine is a potent stimulator of alveolar fluid clearance in rats exposed to hyperoxia.

Keywords: Pulmonary edema, Lung injury, Sodium transport, Alveolar epithelium

The stimulation of β₂-adrenoceptors accelerates trans-epithelial sodium transport out of the alveolar spaces through apical sodium channels and basolateral Na⁺-K⁺ ATPase on type II alveolar epithelial cells (1). Alveolar fluid clearance occurs in accordance with sodium transport and results in the resolution of pulmonary edema (2, 3). Terbutaline, epinephrine and salmeterol are potent stimulators of β₂-adrenoceptors on type II alveolar epithelial cells in rat lungs (4 – 9). Amiloride, a sodium channel inhibitor, inhibits the increase in alveolar fluid clearance in the presence of β₂-adrenergic agonists (10, 11). However, inconsistent with rat lungs, β₂-adrenergic agonists did not increase alveolar fluid clearance in rabbit and guinea pig lungs (12, 13). Interestingly, isoproterenol increased alveolar fluid clearance via β₁-adrenoceptors in guinea pig lungs (13), whereas isoproterenol increased alveolar fluid clearance via β₂-adrenoceptors in rat lungs (14). Recently, we reported that denopamine, a selective β₁-adrenergic agonist, increased alveolar fluid clearance in normal rat and guinea pig lungs (15).

The exposure to hyperoxia (>95%) induces lung injury and results in high mortality in rats (16, 17). Recently, it was reported that isoproterenol increased alveolar fluid clearance by stimulating β₂-adrenoceptors in severely injured rat lungs exposed to hyperoxia for 64 h (18). The stimulating effect by terbutaline on alveolar fluid clearance was also reported in moderately injured rat lungs exposed to 100% oxygen for 40 h (6). However, it was uncertain whether a selective β₁-adrenergic agonist increased alveolar fluid clearance in rats exposed to hyperoxia.

Therefore, the first objective was to determine whether alveolar fluid clearance changed in rats with hyperoxic lung injury. Alveolar fluid clearance was measured in rats exposed to 93% oxygen for 48 or 56 h. The second objective was to determine whether denopamine, a selective β₁-adrenergic agonist, stimulated alveolar fluid clearance in rats exposed to hyperoxia. Inasmuch as denopamine stimulated alveolar fluid clearance in rats exposed to hyperoxia, the third objective was to determine whether the increase was mediated by amiloride-sensitive sodium channels. The final objective was to compare the potency of denopamine with that of terbutaline, a commonly used β₂-adrenergic agonist, in rats exposed to hyperoxia.

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MATERIALS AND METHODS

Materials

Materials were obtained from the following commercial sources: denopamine (Tanabe Pharmaceutical Co., Ltd., Tokyo); amiloride and terbutaline (Sigma, St. Louis, MO, USA); and Evans blue (Tokyo Kasei, Tokyo).

General protocol

Exposure to hyperoxia: Male Sprague-Dawley rats (200 – 250 g) were exposed to 93% oxygen for up to 72 h in a chamber (70 × 60 × 40 cm) flooded with 97% oxygen at 91/min. Rats had access to food and water ad libitum throughout the exposure period. Although the oxygen concentration in the chamber was 97% before the rats were exposed to hyperoxia, the concentration decreased to 93% during exposure. All of the rats survived for 48 h. Although the mortality was low (8%) in rats exposed to hyperoxia for 56 h, the mortality increased to 75% and 100% in rats exposed to hyperoxia for 64 and 72 h, respectively. Therefore, we measured alveolar fluid clearance in rats exposed to hyperoxia for 48 or 56 h.

Measurement of alveolar fluid clearance: As previously reported (9, 15, 19), we isolated rat lungs and measured alveolar fluid clearance in the absence of either pulmonary perfusion or ventilation. Briefly, rats were anesthetized by an intraperitoneal administration of pentobarbital sodium (50 mg/kg body wt). An endotracheal tube was inserted through a tracheostomy. The animals were exsanguinated through the abdominal aorta. Through a median sternotomy, the left hilum was ligated and the left lung was excised through the abdominal aorta. Through a tracheostomy. The animals were exsanguinated through the abdominal aorta. Through a median sternotomy, the left hilum was ligated and the left lung was excised for the measurement of lung water-to-dry lung weight ratio.

Then, the trachea, the right lung, and the heart were excised en bloc for the measurement of alveolar fluid clearance. The right lungs were wrapped by saran wrap to prevent dehydration and were placed in a humid incubator at 37°C. A warmed aqueous solution with 0.9% NaCl (4 ml/kg body wt) containing 5% albumin and Evans blue dye (0.15 mg/ml) was instilled into the right lungs followed by 2 ml of oxygen to deliver all of the instilled solution into the alveolar spaces. The lungs were inflated with 100% oxygen at an airway pressure of 7 cmH2O. Alveolar fluid was aspirated 1 h after instillation. To estimate alveolar fluid clearance for 1 h, the concentrations of Evans blue labeled albumin in the instilled and aspirated solutions were measured by a spectrophotometer at a wavelength of 621 nm (BioSpec-1600; Shimadzu, Kyoto).

Alveolar fluid clearance was estimated by measuring the progressive increase in the concentrations of alveolar Evans blue labeled albumin (9, 15, 19). Alveolar fluid clearance (AFC) was calculated as follows:

\[ \text{AFC} = \frac{(V_i - V_f)}{V_i} \times 100 \]

where \( V \) is the volume of instilled albumin solution (i) and final alveolar fluid (f).

\[ V_f = (V_i \times EBi) / EBi, \]

where EB is the concentration of Evans blue in the instilled albumin solution (i) and final alveolar fluid (f).

Measurement of extravascular lung water: The water content of the left lung was measured by drying the lungs to a constant weight at 70°C for 48 h. Lung water-to-dry lung weight ratio (LW/DL) was calculated as LW/DL = (wet lung weight – dry lung weight) / (dry lung weight).

Specific protocols

Group 1. Effects of hyperoxia on alveolar fluid clearance and lung water volume (n = 20): Inasmuch as mortality was low in rats exposed to hyperoxia for up to 56 h, we measured alveolar fluid clearance and lung water-to-dry lung weight ratio in rats exposed to hyperoxia for 48 h (n = 6) and 56 h (n = 6). As controls, we measured alveolar fluid clearance in rats in the absence of exposure to hyperoxia (n = 8).

Group 2. Effects of denopamine on alveolar fluid clearance in rats exposed to hyperoxia (n = 19): To determine the effect of denopamine on alveolar fluid clearance in hyperoxic rat lungs, an isosmolar albumin solution in the presence of 10^-5 M denopamine was instilled into the alveolar spaces in rats exposed to hyperoxia for 48 h (n = 7) and 56 h (n = 6). As controls, an isosmolar albumin solution in the presence of 10^-5 M denopamine was instilled into the alveolar spaces in rats in the absence of exposure to hyperoxia (n = 6).

Group 3. Effects of denopamine on amiloride-sensitive alveolar fluid clearance in rats exposed to hyperoxia (n = 8): To determine whether hyperoxia altered the effects of denopamine on amiloride-sensitive sodium channels, an isosmolar albumin solution in the presence of 10^-5 M denopamine plus 3 × 10^-4 M amiloride was instilled into the alveolar spaces in rats exposed to hyperoxia for 48 h (n = 4). As controls, an isosmolar albumin solution in the presence of amiloride (3 × 10^-4 M) without denopamine was instilled into the alveolar spaces in rats exposed to hyperoxia for 48 h (n = 4).

Group 4. Comparison with terbutaline in rats exposed to hyperoxia (n = 6): To compare with the effect of terbutaline on alveolar fluid clearance in rats exposed to hyperoxia, an isosmolar albumin solution in the presence of 10^-5 M terbutaline was instilled into the alveolar spaces in rats exposed to hyperoxia for 56 h (n = 6).

Statistics

The data are summarized as the mean and standard deviation. The data were analyzed by one-way analysis of variance (ANOVA) with the Student-Newman-Keuls post hoc test when multiple comparisons were needed. When comparisons were made between two experimental groups, an
unpaired Student’s t-test was used. We regarded as significant those differences with a P value of <0.05.

RESULTS

Group 1. Effects of hyperoxia on alveolar fluid clearance and lung water volume

The lung water-to-dry lung weight ratio increased to 4.88 ± 0.49 g H$_2$O/g dry lung weight and 5.17 ± 0.44 g H$_2$O/g dry lung weight in rats exposed to hyperoxia for 48 and 56 h, respectively, from 4.04 ± 0.09 g H$_2$O/g dry lung weight in control rats (Fig. 1). However, alveolar fluid clearance did not change in rats exposed to hyperoxia up to 56 h (Fig. 1).

Group 2. Effects of denopamine on alveolar fluid clearance in rats exposed to hyperoxia

Although hyperoxia did not alter basal alveolar fluid clearance, 10$^{-5}$ M denopamine significantly increased alveolar fluid clearance by 88% and 90% in rats exposed to hyperoxia 48 and 56 h, respectively (Fig. 2). There was no significant difference among the effects of denopamine in the alveolar fluid clearance in rats exposed to hyperoxia for 48 h, in rats exposed to hyperoxia for 56 h, and in rats without exposure to hyperoxia.

Group 3. Effects of denopamine on amiloride-sensitive alveolar fluid clearance in rats exposed to hyperoxia

Amiloride decreased basal alveolar fluid clearance by 46% and inhibited the increase in alveolar fluid clearance by denopamine in rats exposed to hyperoxia for 48 h (Fig. 3). Denopamine significantly increased amiloride-sensitive alveolar fluid clearance in rats exposed to hyperoxia for 48 h. *P<0.05 vs normoxia.

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**Fig. 1.** Effects of hyperoxia on alveolar fluid clearance and lung water volume in rats exposed to hyperoxia. Although lung water-to-dry lung weight ratio increased in rats exposed to hyperoxia for 48 and 56 h, alveolar fluid clearance did not change for up to 56 h. *P<0.05 vs normoxia.

**Fig. 2.** Effects of denopamine on alveolar fluid clearance in rats exposed to hyperoxia. Denopamine increased alveolar fluid clearance in rats exposed to hyperoxia for 48 and 56 h as well as in rats without exposure to hyperoxia. *P<0.05 vs normoxia.

**Fig. 3.** Effects of hyperoxia on amiloride-sensitive alveolar fluid clearance. Denopamine increased amiloride-insensitive alveolar fluid clearance in rats exposed to hyperoxia for 48 h. *P<0.05 vs hyperoxia (48 h). †P<0.05 vs hyperoxia (48 h) + denopamine (10$^{-5}$ M).
Effects of terbutaline on alveolar fluid clearance in rats exposed to hyperoxia. Terbutaline increased alveolar fluid clearance as well as denopamine in rats exposed to hyperoxia for 56 h. *P<0.05 vs hyperoxia (56 h).

**DISCUSSION**

In the present study, rats were exposed to 93% oxygen for up to 72 h and alveolar fluid clearance was measured in rats exposed to hyperoxia for 48 and 56 h because an exposure for longer than 64 h was lethal. Although the oxygen concentration was lower than the 95% that had been used to test the effects of hyperoxia on alveolar fluid clearance (6, 7, 17, 20), the oxygen concentration was sufficient to cause lung injury because mortality and the wet-to-dry lung weight ratio increased to levels similar to those in rats exposed to >95% oxygen (6, 17).

The first finding was that basal alveolar fluid clearance did not change for 56 h. Similar results were observed in the studies where basal alveolar fluid clearance was sustained (6, 21); and terbutaline, a selective β2-adrenergic agonist, increased alveolar fluid clearance in rats exposed to >95% oxygen for 40 and 60 h (6, 7). In contrast to this study, rats exposed to 100% oxygen for 60 or 64 h had a decreased basal rate in alveolar fluid clearance, active sodium transport and Na+ -K+ ATPase activity (20, 22). These different observations are probably due to the difference of alveolar epithelial injury. Recently, we reported that lung collapse with ischemia decreased alveolar fluid clearance in rabbit and rat lungs (19). Injury of the alveolar epithelial barrier was observed by electron microscopy in collapsed rat lungs followed by reperfusion (23). Therefore, it is probable that decreased alveolar fluid clearance might be measured in lung injury with higher intensity under a longer period of exposure to hyperoxia (20, 22). Another explanation was that the difference may be related to rat age: younger rats are more resistant to hyperoxic injury than the adult rats (20). Rats used in this study were younger than those in the previous studies (7, 18, 20).

Although β1- and β2-adrenoceptor binding sites coexist in different proportions within the rat lung tissue and the percentage of β1 binding sites, 25%, was lower than that of β2 binding sites, 75% (24), there was no difference between the magnitude of denopamine-stimulated alveolar fluid clearance and that of terbutaline-stimulated alveolar fluid clearance in rats exposed to hyperoxia. Several explanations may be possible. First, the magnitude of alveolar fluid clearance stimulated by denopamine or terbutaline was probably at the plateau of the dose-response curve. This explanation is supported by the observation that the addition of 10⁻⁵ M terbutaline to 10⁻⁵ M denopamine did not produce further increase in alveolar fluid clearance (15). Second, although 10⁻⁵ M terbutaline increased cAMP levels in cultured type II alveolar epithelial cells as well as alveolar fluid clearance in rat lungs, 10⁻⁵ M denopamine increased alveolar fluid clearance, but not cAMP levels in type II cells (15). Therefore, the distribution of binding sites of β1- and β2-adrenergic agonists may be different between type II cells and rat lung tissue. Third, it was recently reported that isoproterenol increased Na⁺-K⁺ ATPase activity by membrane insertion of α-subunits in lung alveolar cells (25). There might be a limitation in recycling of Na⁺-K⁺ ATPase, cAMP or β-adrenoceptors in this study because the alveolar fluid clearance was measured in the absence of pulmonary perfusion.

Alveolar fluid clearance consists of amiloride-sensitive and -insensitive alveolar fluid clearance (1, 2). Denopamine primarily stimulated amiloride-insensitive alveolar fluid clearance in this study. The fractions of amiloride-sensitive and -insensitive alveolar fluid clearance were different in different animal species (26). The amiloride-sensitive fraction was lower in human and rat lungs (approximately 40 – 50%; 11, 27) than in mouse and rabbit lungs (>80%; 12, 26). Although knock-out of the amiloride-sensitive α1-ENaC (epithelial sodium channel) is critical for removal of alveolar fluid at birth in mice (28), amiloride-insensitive alveolar fluid clearance is also important because the fraction is high in the human lungs and the clearance capacity is also
critical in patients with pulmonary edema (3, 29, 30). Therefore, irrespective of the animal species, the preservation of amiloride-sensitive and insensitive alveolar fluid capacity and of the response to \( \beta \)-adrenergic agonists may be important in the resolution of pulmonary edema.

What are the clinical implications of this study? Since denopamine has been administered to patients with congestive heart failure (31), if denopamine can accelerate the resolution of clinical alveolar edema, this vasoactive agent may be beneficial for hastening the resolution of pulmonary edema as well as improving cardiac function in patients with hyperoxic lung injury.

In summary, basal alveolar fluid clearance was preserved in rats exposed to hyperoxia (93% oxygen) for up to 56 h. Denopamine primarily increased amiloride-insensitive alveolar fluid clearance in rats exposed to hyperoxia. The potency of denopamine was similar to that of terbutaline in rats exposed to hyperoxia (93% oxygen) for up to 56 h. Denopamine primarily increased amiloride-insensitive alveolar fluid clearance in rats exposed to hyperoxia. In summary, denopamine is a potent stimulator of alveolar fluid clearance in rats exposed to hyperoxia.

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