

Catecholamine Clearance from Alveolar Spaces of Rat and Human Lungs

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Key Words

Norepinephrine · Epinephrine · Amiloride · Benzamil · Alveolar epithelium

Abstract

Background: Although aerosolized β -adrenergic agonists have been used as a therapy for the resolution of pulmonary edema, the mechanisms of catecholamine clearance from the alveolar spaces of the lung are not well known. **Objective:** To determine whether catecholamine clearance from the alveolar spaces is correlated with the fluid transport capacity of the lung. **Methods:** Albumin solution containing epinephrine (10^{-7} M) or norepinephrine (10^{-7} M) was instilled into the alveolar spaces of isolated rat and human lungs. Alveolar fluid clearance rate was estimated by the progressive increase in the albumin concentration over 1 h. Catecholamine clearance rate was estimated by the changes in catecholamine concentration and alveolar fluid volume over 1 h. **Results:** The norepinephrine clearance rate was faster than the epinephrine clearance rate in the rat and human lungs. In the rat lungs, amiloride (a sodium channel blocker) caused a greater decrease in alveolar fluid clearance and epinephrine clearance rate than propranolol (a nonselective β -adrenergic antagonist). Although

propranolol and phentolamine (an α -adrenergic antagonist), and 5-(N-ethyl-N-isopropyl)amiloride (a Na^+/H^+ antiporter blocker) changed neither the alveolar fluid clearance nor the norepinephrine clearance rate, amiloride and benzamil (a sodium channel blocker) decreased both clearance rates. As in the rat lungs, amiloride decreased alveolar fluid and norepinephrine clearance rates in the human lungs. **Conclusion:** These results indicate that the catecholamine clearance rate from the alveolar spaces is correlated with alveolar fluid clearance in rat and human lungs.

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Introduction

The mechanism responsible for ion and fluid transport across the alveolar epithelia has been studied over two decades. The initial step in alveolar fluid clearance is the absorption of alveolar sodium ions through apical sodium channels into the alveolar epithelial cells, and then the exchange of sodium and potassium ions through basolateral Na^+/K^+ ATPase [1–3]. Amiloride is a potent blocker of sodium channels on the apical membrane of alveolar epithelial cells and has been shown to impair the rate of alveolar fluid clearance [2, 4]. In contrast, β -adrenergic

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agonists stimulate alveolar fluid clearance under normal and pathological conditions in mice [5], rats [6–9], dogs [10, 11] and sheep [12, 13]. We have reported that terbutaline and salmeterol, two selective β_2 -adrenergic agonists, increase alveolar fluid clearance in isolated human lungs [14–17]. In addition, exogenous catecholamine (epinephrine or norepinephrine) increase alveolar fluid clearance in sheep [12], guinea pig [18], and rat [19]. Therefore, β -adrenergic agonists have been considered as therapeutic agents for accelerating the resolution of pulmonary edema [3, 20].

Another important function of the alveolar epithelial barrier is that it provides a large surface area for the transfer of instilled materials to the circulation [21–24]. Therefore, catecholamines have been administered into the airspaces with the goal of delivering them into the pulmonary circulation and heart in patients requiring cardiopulmonary resuscitation [25]. Since the effect of a β -adrenergic agonist therapy on alveolar fluid clearance depends on its concentration in the alveolar fluid [9, 16], rapid clearance from the alveolar space could diminish its efficacy. Indeed, amiloride concentrations of 10^{-4} – 10^{-3} M are necessary in vivo to inhibit alveolar fluid clearance because of its rapid clearance rate from the alveolar spaces [26]. In rabbit lung preparations in which saline solution containing 10^{-3} M amiloride was instilled, approximately 23% of the initial amiloride was cleared from the alveolar spaces over 1 h [27]. However, little is known regarding the mechanism responsible for the clearance of β -adrenergic agonists from the distal airspaces.

Therefore, our first objective was to determine the clearance rates of catecholamines (epinephrine and norepinephrine) in isolated rat lungs. Our second objective was to determine whether α - and β -adrenergic antagonists changed the catecholamine clearance rate. Our third objective was to determine whether sodium channel blockers changed the catecholamine clearance rate. Our final objective was to determine the catecholamine clearance rate in the isolated human lungs.

Methods

Materials

Amiloride, benzamil, epinephrine, 5-(N-ethyl-N-isopropyl)amiloride, norepinephrine, phentolamine, and propranolol were obtained from Sigma (St. Louis, Mo., USA).

Experimental Protocol

All rats received humane care and this study was approved by the Committee for Animal Experiments at Kanazawa Medical University.

Alveolar fluid clearance was measured in the isolated rat lungs in the absence of pulmonary perfusion or ventilation [9, 16, 28, 29]. Briefly, male Sprague-Dawley rats (200–250 g, Japan SLC, Inc., Hamamatsu, Japan) were anesthetized with intraperitoneal pentobarbital sodium (50 mg/kg). An endotracheal tube was inserted through a tracheostomy. The rats were exsanguinated via the abdominal aorta and the trachea, bilateral lungs, and heart were excised en bloc through a median sternotomy. Warmed physiological saline solution (7 ml/kg, 37 °C) containing 5% bovine albumin was instilled into both lungs, followed by 4 ml oxygen to deliver all the instilled fluid into the alveolar spaces. The lungs were placed in a humid incubator at 37 °C and inflated with 100% oxygen at an airway pressure of 7 cm H₂O. Alveolar fluid was aspirated 1 h after instillation.

Catecholamine Clearance Rate in Isolated Rat Lungs

To determine the catecholamine clearance rate under basal conditions, albumin solution containing epinephrine (10^{-7} M, n = 8) or norepinephrine (10^{-7} M, n = 8) was instilled into the alveolar spaces of isolated rat lungs. As a control, albumin solution in the absence of any catecholamine was instilled into the alveolar spaces of isolated rat lungs (n = 8).

Effects of Propranolol and Amiloride on the Epinephrine Clearance Rate in Isolated Rat Lungs

To determine if epinephrine clearance was mediated via β -adrenergic receptors, propranolol (10^{-5} M), a β -adrenergic antagonist, was added to an albumin solution containing epinephrine (10^{-7} M) and instilled into the alveolar spaces of isolated rat lungs (n = 4). In addition, to determine whether epinephrine clearance was mediated via amiloride-sensitive sodium channels, amiloride (10^{-3} M) was added to albumin solution containing epinephrine (10^{-7} M) and instilled into the alveolar spaces of isolated rat lungs (n = 4).

Effects of Adrenergic Antagonists and Sodium Channel Blockers on the Norepinephrine Clearance Rate in Isolated Rat Lungs

Since the norepinephrine clearance rate was faster than the epinephrine clearance rate, we determined the effect of adrenergic antagonists and sodium channel blockers on the faster norepinephrine clearance rate in rat lungs. Phentolamine (10^{-5} M, n = 4), an α -adrenergic antagonist, or propranolol (10^{-5} M, n = 4) was added to albumin solution containing norepinephrine (10^{-7} M) and instilled into the alveolar spaces of rat lungs. In addition, amiloride (10^{-3} M, n = 6) or benzamil (10^{-3} M, n = 6), a sodium channel blocker, or 5-(N-ethyl-N-isopropyl)amiloride (EIPA, 10^{-3} M, n = 4), an Na⁺/H⁺ antiport blocker, was added to albumin solution containing norepinephrine (10^{-7} M) and instilled into the alveolar spaces of isolated rat lungs.

Effects of Initial Norepinephrine Concentration on the Norepinephrine Clearance Rate in Isolated Rat Lungs

To determine whether the norepinephrine clearance rate was dependent on the instilled concentration of norepinephrine, an albumin solution containing a higher concentration of norepinephrine (10^{-5} M) was instilled into the alveolar spaces of isolated rat lungs (n = 5).

Catecholamine Clearance Rate in Isolated Human Lungs

This study was approved by the Human Research Committee in Kanazawa Medical University. Human lungs were obtained from patients who underwent pulmonary resections for bronchogenic car-

cinoma. Informed consent was given by each patient before the operation. There were no fibrous or emphysematous lesions as assessed by preoperative chest radiographs and computed tomograms. Nor were any macroscopic emphysematous changes found when the lungs were removed from the thorax. The pulmonary function tests before the operation were normal (vital capacity: $93 \pm 8\%$ of the predicted value, forced expiratory volume in 1 s: $82 \pm 11\%$ of forced vital capacity). The surgical procedure was described previously [14–16]. The segmental bronchus was occluded by a 10-french balloon catheter immediately after removal of the lung. We chose the occluded segment that was located furthest away from the tumor. A warmed physiological saline solution (45 ml, 37°C) containing 5% bovine albumin and catecholamine (10^{-7} M epinephrine; $n = 4$, or 10^{-7} M norepinephrine; $n = 4$) was instilled into the distal airspaces through the catheter. To determine the effect of amiloride on the norepinephrine clearance rate, a warmed physiological saline solution containing 5% bovine albumin and norepinephrine (10^{-7} M) and amiloride (10^{-3} M) was instilled into the distal airspaces ($n = 4$). After instillation, the lungs were inflated with 100% oxygen at an airway pressure of 7 cm H_2O . Alveolar fluid was aspirated 1 h after instillation. Aspirated alveolar fluid (1–2 ml) was centrifuged at 3,000 rpm for 10 min, and supernatant was obtained for the measurement of the protein and catecholamine concentrations.

Metabolism of Catecholamine in the Alveolar Spaces

To determine whether exogenous norepinephrine and epinephrine were metabolized in the alveolar spaces, we measured vanillyl-mandelic acid (VMA), a catecholamine metabolite, in plasma, final alveolar fluid, and flushing fluid of pulmonary vasculature from rats with instillation of 5% albumin solution containing epinephrine (10^{-7} M, $n = 4$), instillation of 5% albumin solution containing norepinephrine (10^{-7} M, $n = 4$), or instillation of 5% albumin solution alone ($n = 4$). After anesthesia and tracheostomy, heparin sodium (3,000 units/kg) was intravenously injected and arterial blood was obtained 60 s after heparinization. Then, the rats were exsanguinated and the lungs were used for the measurement of alveolar fluid clearance for 1 h. After the measurement of alveolar fluid clearance, the pulmonary vasculature was flushed with a warmed 0.9% saline solution (10 ml) through a catheter placed in the trunk of the pulmonary artery. Samples of flushing fluid were collected from the left atrium. VMA levels were measured by high-performance liquid chromatography (SRL, Inc., Tokyo, Japan).

Measurements

Alveolar Fluid Clearance. The protein concentrations in instilled and aspirated solutions were measured by a spectrophotometer at a wavelength of 280 nm (BioSpec-1600, Shimadzu, Kyoto, Japan). Alveolar fluid clearance was estimated by measuring the progressive increase in the concentration of albumin [9, 16, 28, 29]. Alveolar fluid clearance (AFC) was calculated as follows:

$$\text{AFC} = [(V_i - V_f)/V_i] \times 100 \quad (1)$$

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

$$V_f = (V_i \times P_i)/P_f \quad (2)$$

where P is the concentration of protein in the instilled albumin solution (i) and the final alveolar fluid (f).

The term ‘alveolar’ does not imply that all reabsorption occurs across the alveolar epithelium because the distal bronchial epithelia can also transport sodium and fluid [28].

Catecholamine Concentration. The samples of fluid were stored at -80°C until analysis. Catecholamine concentrations in the instilled and aspirated albumin solutions were determined by high-performance liquid chromatography with a trihydroxyindole reaction [19, 29]. The catecholamine clearance rate from the alveolar spaces was calculated as the quantity of absorbed catecholamine divided by the quantity of instilled catecholamine:

$$\text{Clearance rate} = (C_i \times V_i - C_f \times V_f)/(C_i \times V_i) \quad (3)$$

where C is the concentration of catecholamine in the instilled albumin solution (i) and the final alveolar fluid (f).

Statistics

Data are summarized as the mean and standard deviation. The data were analyzed by a one-way analysis of variance (ANOVA) with the Student-Newman-Keuls post hoc test when multiple comparisons were needed (Prism 4, GraphPad Software, Inc., San Diego, Calif., USA). When comparisons were made between two experimental groups, an unpaired Student's t test was used. Differences with $p < 0.05$ were regarded as significant.

Results

Epinephrine (10^{-7} M) increased alveolar fluid clearance by 38% in the rat lungs. However, norepinephrine (10^{-7} M) did not change alveolar fluid clearance (fig. 1). The norepinephrine clearance rate ($89 \pm 5\%/h$) was greater than the epinephrine clearance rate ($51 \pm 5\%/h$). In the control rat lungs, neither epinephrine nor norepinephrine was identified in the samples of alveolar fluid 1 h after instillation.

Propranolol abolished the increase in alveolar fluid clearance induced by epinephrine and decreased the epinephrine clearance rate in the rat lungs (fig. 2). Propranolol plus amiloride further decreased both alveolar fluid clearance and the epinephrine clearance rate.

Neither propranolol nor phentolamine changed alveolar fluid clearance and the norepinephrine clearance rate in the rat lungs (fig. 3). Amiloride and benzamil decreased both alveolar fluid clearance and the norepinephrine clearance rate. However, EIPA neither changed the alveolar fluid clearance nor the norepinephrine clearance rate.

A higher concentration (10^{-5} M) of norepinephrine increased alveolar fluid clearance (fig. 4). However, the higher concentration did not change the norepinephrine clearance rate. The quantity of norepinephrine that was removed from the alveolar spaces depended on the initial concentration of norepinephrine in the alveolar spaces.

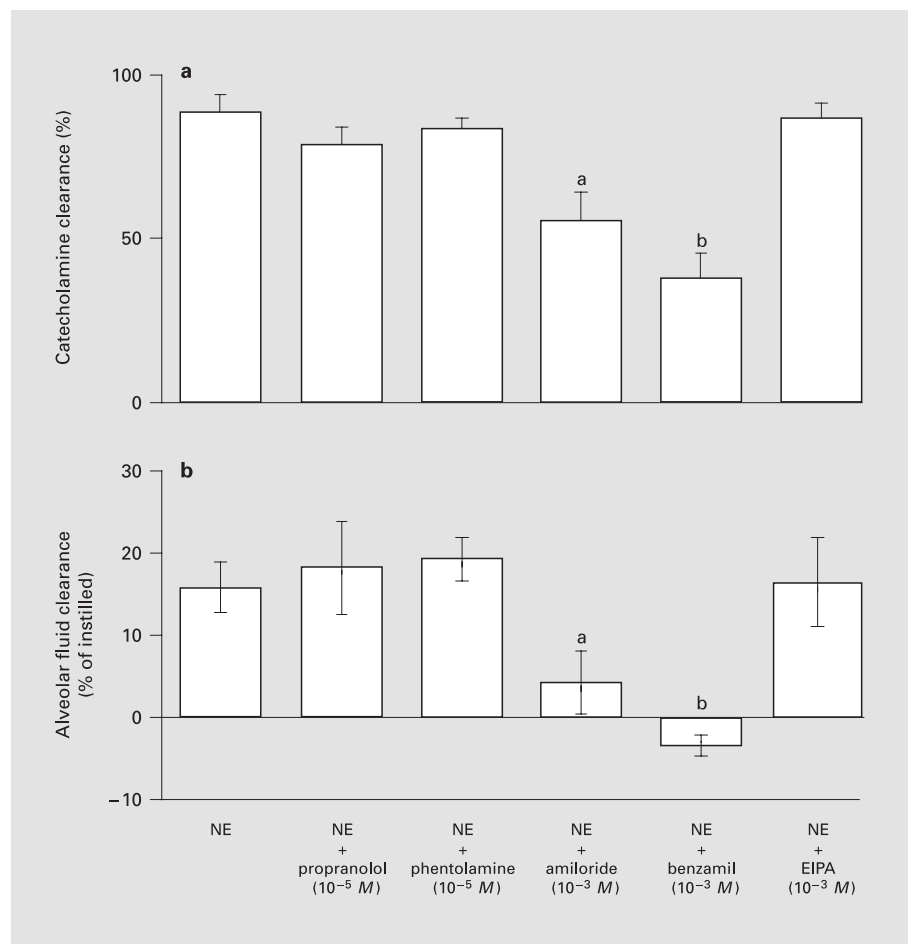
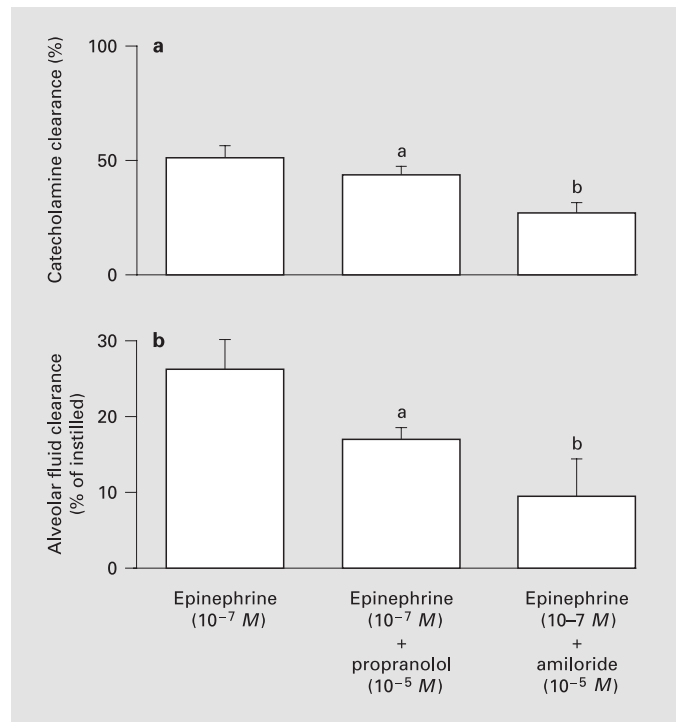
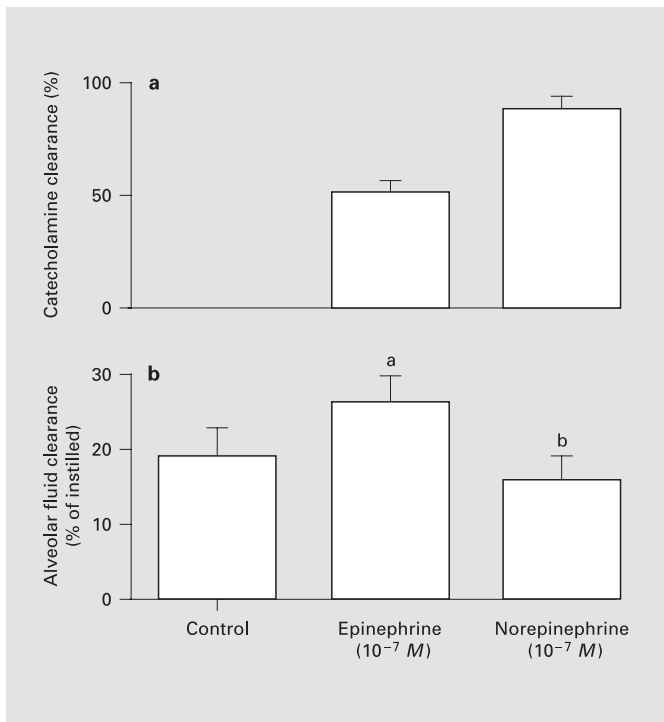
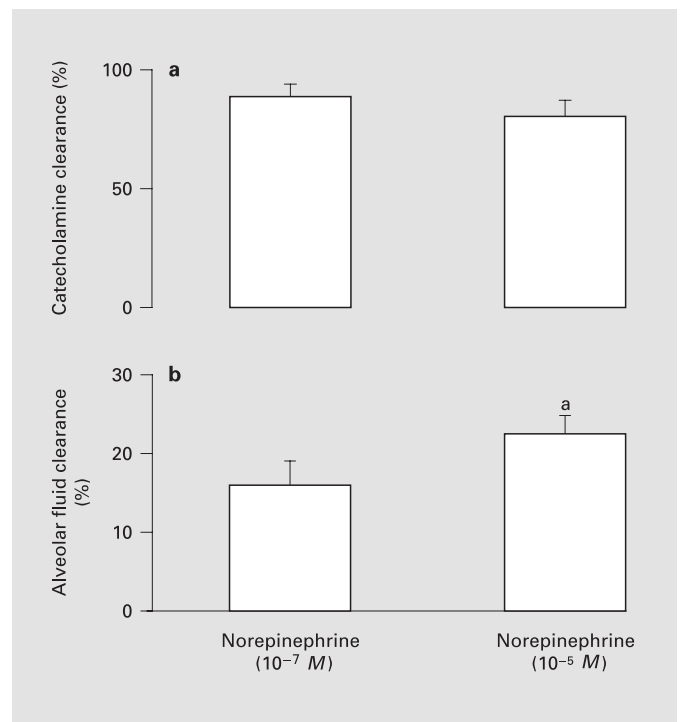


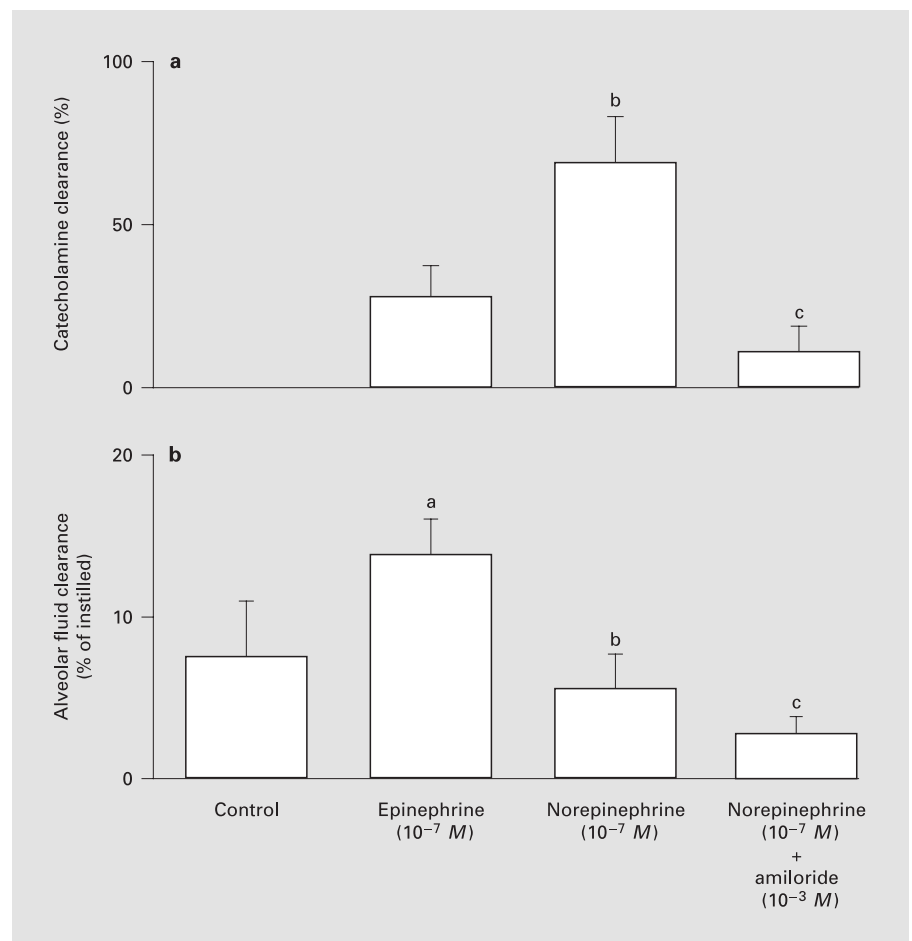
Fig. 1. Comparison between clearance rates of epinephrine and norepinephrine in isolated rat lungs. ^a $p < 0.05$ vs. control value; ^b $p < 0.05$ vs. corresponding values in the rat lungs treated with epinephrine. **a** Catecholamine clearance. **b** Alveolar fluid clearance.
Fig. 2. Effects of propranolol and amiloride on the epinephrine clearance rate in isolated rat lungs. ^a $p < 0.05$ vs. corresponding values in epinephrine-treated rat lungs. ^b $p < 0.05$ vs. corresponding values in rat lungs treated with epinephrine plus propranolol. **a** Catecholamine clearance. **b** Alveolar fluid clearance.
Fig. 3. Effects of adrenergic antagonists and sodium channel blockers on the norepinephrine (NE) clearance rate in isolated rat lungs. ^a $p < 0.05$ vs. corresponding values in norepinephrine-treated rat lungs. ^b $p < 0.05$ vs. corresponding values in rat lungs treated with norepinephrine plus amiloride. **a** Catecholamine clearance. **b** Alveolar fluid clearance.

In the human lungs, epinephrine increased alveolar fluid clearance, whereas norepinephrine did not (fig. 5). As in rat lungs, the norepinephrine clearance rate ($69 \pm 14\%/h$) was faster than the epinephrine clearance rate ($28 \pm 9\%/h$). Amiloride decreased alveolar fluid clearance and the norepinephrine clearance rate.

The volume of flushing fluid collected from the left atrium was 9.4 ± 0.4 ml and there was no difference among the volumes from control rat lungs, epinephrine-instilled and norepinephrine-instilled rat lungs. The VMA levels in plasma were not different among the groups (10.3 ± 1.4 ng/ml in the control lungs, 10.2 ± 1.8 ng/ml in the epinephrine-instilled, and 11.0 ± 2.0 ng/ml in the norepinephrine-instilled lungs). The VMA levels in final alveolar fluid and flushing fluid were below the detection levels (<1.0 ng/ml).



4



5

Fig. 4. Effects of the initial norepinephrine concentration on the norepinephrine clearance rate in isolated rat lungs. ^a $p < 0.05$ vs. corresponding value in the rat lungs treated with norepinephrine 10^{-7} M. **a** Catecholamine clearance. **b** Alveolar fluid clearance. **Fig. 5.** Catecholamine clearance rates isolated human lungs. ^a $p < 0.05$ vs. corresponding control value. ^b $p < 0.05$ vs. corresponding values in human lungs treated with epinephrine 10^{-7} M. ^c $p < 0.05$ vs. corresponding values in the control lungs and in the lungs treated with norepinephrine 10^{-7} M. **a** Catecholamine clearance. **b** Alveolar fluid clearance.

Discussion

Several experimental preparations have been used to measure ion and fluid transport from the alveolar spaces [30]. We have developed an isolated lung preparation in which fluid clearance across the alveolar epithelial barrier was measured in the absence of perfusion and ventilation [9, 14–16, 28, 29]. Therefore, the catecholamine clearance rate from the alveolar spaces was examined in the isolated rat lungs in the absence of pulmonary perfusion and ventilation.

Clearance of catecholamines from the alveolar spaces may proceed via two major pathways: a transcellular and a paracellular pathway. Several findings in this study indicate that catecholamine clearance occurred primarily by diffusion through a paracellular pathway. First, the rate of catecholamine clearance was much higher than the rate of alveolar fluid clearance that is driven by an active sodium transport mechanism [30]. The fast catecholamine clearance in the isolated lungs was consistent with the epinephrine clearance rate in anesthetized dogs [31]. Second, α - and β -adrenergic antagonists did not impair the norepinephrine clearance rate. If catecholamine clearance was mediated through adrenergic receptors, the adrenergic antagonists should have impaired the catecholamine clearance rate as the antagonists inhibited the stimulating effect of terbutaline on alveolar fluid clearance [14]. Third, the quantity of norepinephrine that was removed from the alveolar spaces depended on the initial norepinephrine concentration in the alveolar spaces. The results are consistent with the report that the paracellular pathway has the characteristics of a dose-dependent movement [32]. These observations suggest that norepinephrine clearance is driven by diffusion through a paracellular pathway.

This study was carried out in the isolated rat lungs in the absence of perfusion and ventilation. This preparation eliminates the confounding effect of increased transvascular fluid and protein flux that may occur in injured lungs and permits independent assessment of the permeability and transport properties of the alveolar epithelium [30]. However, it has been known that stretching of alveolar epithelial cells can have a profound effect of on surfactant secretion [33], releasing growth factors [34], and the activity of extracellular signal-regulated kinase [35]. These effects may alter the ion transport capacity of alveolar epithelial cells [36]. In our previous study, alveolar fluid clearance was smaller in nonperfused and nonventilated rat lungs than in rat lungs *in vivo* [16]. Therefore, it is probable that catecholamine clearance might be smaller

in this study than in the *in vivo* study. Further studies are necessary to determine the effect of ventilation or pulmonary perfusion on catecholamine clearance from the alveolar spaces.

Amiloride has been used as a potent blocker of sodium transport and net alveolar fluid clearance [2]. The effect of amiloride on alveolar fluid clearance in the rat and human lungs in this study was consistent with our prior results [14, 15]. In addition, benzamil abolished alveolar fluid clearance. These results were consistent with the results that amiloride and benzamil are potent blockers of sodium transport in cultured type II alveolar epithelial cells [37, 38].

To determine if catecholamine clearance was correlated with alveolar fluid clearance, we tested the effect of amiloride, benzamil, and EIPA on the norepinephrine clearance rate. Although amiloride and benzamil inhibited both alveolar fluid clearance and the norepinephrine clearance rate, EIPA did not inhibit either of them. Since amiloride-sensitive sodium channels play an important role in active ion and fluid transport across the alveolar epithelial cells [3, 4], it is likely that amiloride-sensitive sodium channels also play an important role in catecholamine clearance from the alveolar spaces of rat lungs. There are reports indicating that amiloride inhibits the clearance of substances through a paracellular pathway. In the perfused fluid-filled rat lungs, amiloride reduced the permeability rate of mannitol, a trace of paracellular transport from the alveolar spaces [1, 39]. In addition, amiloride inhibited both active sodium transport and mannitol permeability in the presence of these stimulatory agents [32]. Similar effects of amiloride with and without isoproterenol were reported in the study performed on cultured Clara cell epithelium from rabbit [40]. Therefore, catecholamine clearance is correlated with alveolar fluid clearance in isolated rat and human lungs.

To answer the question whether catecholamine moved into the alveolar space and affected the final catecholamine concentration in the alveolar fluid, we measured the catecholamine concentration in the final alveolar fluid of control rat lungs. Since no catecholamine was identified in the final alveolar fluid, it is unlikely that endogenous catecholamines moved into the alveolar spaces and affected the clearance rate of catecholamine.

We also investigated whether exogenous norepinephrine and epinephrine were metabolized in the alveolar spaces. VMA, a catecholamine metabolite, was measured in final alveolar fluid and flushing fluid of the pulmonary vasculature from rat lungs instilled with epinephrine and norepinephrine and found to be below the detection level

in both fluids. Therefore, it is unlikely that exogenous norepinephrine and epinephrine were metabolized in the alveolar spaces during the 1-hour experiment.

It is unclear why epinephrine and norepinephrine are cleared at different rates from the distal airspaces. Several characteristics, e.g., molecular size, radius, electric charges, may affect the absorption rates of substances [22]. The molecular sizes of epinephrine and norepinephrine are similar, but epinephrine has a methyl group and a hydroxyl group. Further studies are needed to determine the mechanisms responsible for the difference between the clearance rates of epinephrine and norepinephrine.

Summary and Conclusions

The rate of norepinephrine clearance from the alveolar spaces was faster than that of epinephrine clearance in rat and human lungs. Amiloride and benzamil decreased

both alveolar fluid clearance and the norepinephrine clearance rate, whereas EIPA did not. These results indicate that the catecholamine clearance rate is correlated with alveolar fluid clearance in rat and human lungs. Since sodium channel blockers impair catecholamine clearance from the alveolar spaces, sodium channel may play a role in the mechanism responsible for catecholamine clearance from the alveolar spaces.

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