Effects of hypoxia on alveolar fluid transport capacity in rat lungs

TSUTOMU SAKUMA,1 MIEKO HIDA,2 YOSHIHIRO NAMBU,2 KAZUHIRO OSANAI,2 HIROHISA TOGA,2 KEIJI TAKAHASHI,2 NOBUO OHYA,2 MASAO INOUE,3 AND YOH WATANABE1

1Thoracic Surgery, 2Pulmonary Medicine, and 3Basic Medical Science, Kanazawa Medical University, Uchinada, Ishikawa 920–0293, Japan

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Sakuma, Tsutomu, Mieko Hida, Yoshihiro Nambu, Kazuhiro Osanai, Hirohisa Toga, Keiji Takahashi, Nobuo Ohya, Masao Inoue, and Yoh Watanabe. Effects of hypoxia on alveolar fluid transport capacity in rat lungs. J Appl Physiol 91: 1766–1774, 2001.—There is little information regarding the effect of hypoxia on alveolar fluid clearance capacity. We measured alveolar fluid clearance, lung water volume, plasma catecholamine concentrations, and serum osmolality in rats exposed to 10% oxygen for up to 120 h and explored the mechanisms responsible for the increase in alveolar fluid clearance. The principal results were 1) alveolar fluid clearance did not change for 48 h and then increased between 72 and 120 h of exposure to hypoxia; 2) although nutritional impairment during hypoxia decreased basal alveolar fluid clearance, endogenous norepinephrine increased net alveolar fluid clearance; 3) the changes of lung water volume and serum osmolality were not associated with those of alveolar fluid clearance; 4) an administration of β-adrenergic agonists further increased alveolar fluid clearance; and 5) alveolar fluid clearance returned to normal within 24 h of reoxygenation after hypoxia. In conclusion, alveolar epithelial fluid transport capacity increases in rats exposed to hypoxia. It is likely that a combination of endogenous norepinephrine and nutritional impairment regulates alveolar fluid clearance under hypoxic conditions.

catecholamine; fluid balance; alveolar epithelium; denopamine

ALVEOLAR FLUID CLEARANCE CAPACITY is inversely associated with mortality in patients with pulmonary edema (19). Recently, it was reported that the stimulation of alveolar fluid clearance accelerated the resolution of pulmonary edema and facilitated gas exchange across the alveolar epithelium (9). The initial step in alveolar fluid clearance is to transport alveolar sodium through apical sodium channels into the alveolar epithelial cells, then to exchange sodium and potassium through basolateral Na+-K+-ATPase (3, 6, 17, 18, 29, 31). Osmotic gradients created by those transported ions drive alveolar fluid across the alveolar epithelium (18). Amiloride inhibits apical sodium channels (16), and β-adrenergic agonists accelerate sodium and fluid transport (1, 17, 18, 31). Endogenous catecholamines increase alveolar fluid clearance in rats with septic (21) and hemorrhagic shock (20) and in dogs with neurogenic pulmonary edema (14).

The effect of hypoxia on alveolar epithelial fluid transport is an important issue because alveolar spaces are exposed to hypoxia in patients with pulmonary edema or during ascent to high altitudes (5). Hypoxia induced a downregulation of the expression and activity of sodium channels and Na+-K+-ATPase in cultured type II alveolar epithelial cells from rat lungs and in A549 cells (15, 22, 23). However, the oxygen concentrations used in those studies were too low (2% oxygen) to replicate in an in vivo study. Recently, Suzuki et al. (32) reported that alveolar fluid clearance decreased in rats exposed to 10% oxygen for 72 h. However, their results are inconsistent with the report that ≥5% oxygen did not change the expression and activity of sodium channels and Na+-K+-ATPase in cultured type II cells (22).

The objectives in this study were to determine 1) whether hypoxia affected alveolar fluid transport capacity and lung water volume in rat lungs, 2) the mechanisms responsible for such an increase in alveolar fluid clearance, 3) whether β-adrenergic agonists could further increase alveolar fluid clearance in rats exposed to hypoxia, and 4) whether alveolar fluid clearance returned to normal after reoxygenation following hypoxia.

METHODS

Materials

Materials were obtained as follows: denopamine from Tanabe Pharmaceutical (Tokyo, Japan); salmonel from Glaxo Wellcome (Middlesex, UK); Evans blue from Tokyo Kasei (Tokyo, Japan); and amiloride, norepinephrine, phentolamine, and propranolol from Sigma Chemical (St. Louis, MO).

General Protocol

Hypoxic exposure. This study was approved by the Animal Care Committee in Kanazawa Medical University. Specific
pathogen-free Sprague-Dawley rats (250–300 g, Japan SLC, Hamamatsu, Japan) were exposed to normobaric hypoxia (10% oxygen) for up to 120 h in a sealed chamber that was continuously flooded with hypoxic gas at 6 l/min (low oxygen generator, Teijin, Tokyo, Japan). Oxygen concentration in the chamber was analyzed twice a day with an oxygen analyzer (TED 60T, Teledyne Brown Engineering, City of Industry, CA). The rats were permitted access to food and water ad libitum.

Measurement of alveolar fluid clearance. We isolated the rat lungs and measured alveolar fluid clearance in the absence of either pulmonary perfusion or ventilation as previously reported (24, 27, 28). Briefly, rats were anesthetized by intraperitoneal administration of pentobarbital sodium (50 mg/kg). An endotracheal tube was inserted through a tracheostomy. Blood samples for the measurements of plasma catecholamines and serum osmolality were obtained from the abdominal aorta, and rats were exsanguinated. Through a median sternotomy, the left hilum was ligated with a silk suture and the left lung was isolated for the subsequent measurement of lung water-to-dry lung weight ratio (LW/DL). The trachea, right lung, and heart were excised en bloc. The trachea, right lung, and heart were excised en bloc.

A warmed physiological saline solution (0.7 ml/kg, 37°C) containing 5% albumin and Evans blue dye (0.15 mg/ml) was instilled into the alveolar spaces through the endotracheal tube. After instillation, the lungs were inflated with 100% nitrogen for 1 h. As controls, the lungs were inflated with 100% oxygen for 1 h at 37°C after instillation of the albumin solution (n = 7). Alveolar fluid clearance and oxygen partial pressure in the instilled albumin solution were measured 5 min and 1 h after instillation. Because Evans blue dye could not be used in a blood gas analyzer, an albumin solution without Evans blue dye was used in this group. Alveolar fluid clearance was estimated by the progressive increase in the albumin concentration measured by a spectrophotometer at a wavelength of 280 nm.

Group 2: time course of alveolar fluid clearance and lung water volume in rats exposed to hypoxia (n = 41). Alveolar fluid clearance, LW/DL, plasma catecholamine and cortisol concentrations, and serum osmolality were measured in rats exposed to hypoxia (10% oxygen) for 3 h (n = 10), 48 h (n = 7), 72 h (n = 7), 96 h (n = 6), and 120 h (n = 7) and in rats not exposed to hypoxia (n = 10, as controls).

Group 3: effects of a sodium channel inhibitor on alveolar fluid clearance in rats exposed to hypoxia (n = 12). Inasmuch as alveolar fluid clearance increased in rats exposed to hypoxia (10% oxygen) for 120 h, we determined whether increased alveolar fluid clearance depended on amiloride-sensitive sodium channels. An albumin solution containing amiloride (5 × 10⁻⁴ M), a sodium channel inhibitor, was instilled in rats exposed to hypoxia for 120 h (n = 6) and in rats not exposed to hypoxia (n = 6).

Group 4: effects of nutritional deprivation on alveolar fluid clearance and lung water volume (n = 12). Intake of food and water was impaired during hypoxia (10% oxygen). Weight gain over 120 h was 30 ± 10 (SD) g less in rats exposed to hypoxia than in rats not exposed to hypoxia. Therefore, we tested the hypothesis that nutritional impairment increased serum osmolality and resulted in the increase in alveolar fluid clearance in rats exposed to hypoxia due to osmotic gradients between the alveolar spaces and the pulmonary vasculature drove the alveolar fluid across the alveolar epithelial barrier (18). Alveolar fluid clearance, LW/DL, plasma catecholamine concentrations, and serum osmolality were measured in rats exposed to nutritional deprivation under normoxic conditions for 120 h (n = 7) and in rats exposed to nutritional deprivation under hypoxic conditions for 120 h (n = 5). Rats were allowed access to water but not to food. In the measurement of alveolar fluid clearance, the lungs were inflated with 100% nitrogen for 1 h after instillation.

Group 5: effects of α- or β-adrenergic antagonists on alveolar fluid clearance in rats exposed to hypoxia (n = 22). We determined whether the increase in alveolar fluid clearance was mediated by the stimulation of α-adrenoceptors or β-adrenoceptors. An albumin solution containing 10⁻⁴ M phenylephrine (n = 5), an α-adrenergic antagonist, or 10⁻⁵ M propranolol (n = 5), a β-adrenergic antagonist, was instilled into the alveolar spaces in rats exposed to hypoxia (10% oxygen) for 120 h. In addition, to determine whether physiological levels of norepinephrine could further increase alveolar fluid clearance in rats exposed to hypoxia, an albumin

Specific Protocol

Group 1: effects of deoxygenation on alveolar oxygen tension and alveolar fluid clearance (n = 18). We determined whether the alveolar spaces were hypoxic during the measurement and whether deoxygenation of the albumin solutions would alter alveolar fluid clearance in rats not exposed to 10% oxygen. We instilled 5% albumin solution exposed to room air for 2 h at 37°C before instillation (n = 7) or 5% albumin solution in which oxygen was removed with 100% nitrogen for 2 h at 37°C before instillation (n = 7). After instillation, the lungs were inflated with 100% nitrogen for 1 h. As controls, the lungs were inflated with 100% oxygen for 1 h at 37°C after instillation of the albumin solution (n = 7). Alveolar fluid clearance and oxygen partial pressure in the instilled albumin solution were measured 5 min and 1 h after instillation. Because Evans blue dye could not be used in a blood gas analyzer, an albumin solution without Evans blue dye was used in this group. Alveolar fluid clearance was estimated by the progressive increase in the albumin concentration measured by a spectrophotometer at a wavelength of 280 nm.

**Measurement of alveolar fluid clearance.** We isolated the rat lungs and measured alveolar fluid clearance in the absence of pulmonary perfusion or ventilation as previously reported (24, 27, 28). Briefly, rats were anesthetized by intraperitoneal administration of pentobarbital sodium (50 mg/kg). An endotracheal tube was inserted through a tracheostomy. Blood samples for the measurements of plasma catecholamines and serum osmolality were obtained from the abdominal aorta, and rats were exsanguinated. Through a median sternotomy, the left hilum was ligated with a silk suture and the left lung was isolated for the subsequent measurement of lung water-to-dry lung weight ratio (LW/DL). The trachea, right lung, and heart were excised en bloc. The trachea, right lung, and heart were excised en bloc.

A warmed physiological saline solution (0.7 ml/kg, 37°C) containing 5% albumin and Evans blue dye (0.15 mg/ml) was instilled into the alveolar spaces through the endotracheal tube. After instillation, the lungs were inflated with 100% nitrogen at an airway pressure of 7 cmH₂O. Alveolar fluid was aspirated 1 h after instillation. 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, Japan). By using trichloroacetic acid, >99.5% of Evans blue dye was bound to albumin in the instilled and aspirated solutions.

Alveolar fluid clearance was estimated by the progressive increase in the concentration of alveolar Evans blue-labeled albumin (24, 27, 28). Alveolar fluid clearance (AFC) was calculated as follows

$$AFC = \left( \frac{V_i - V_f}{V_i} \right) \times 100$$

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

$V_f = \left( \frac{V_i \times EB_i}{EB_f} \right)$

where EB is the concentration of Evans blue in i and f.

The term alveolar does not imply that all reabsorption occurs across the alveolar epithelium because the distal bronchial epithelium can also transport sodium and fluid (18).

Measurement of lung water volume. Water volume in the left lung was measured by drying the isolated lung to a constant weight at 70°C for 48 h. LW/DL was calculated as LW/DL = (wet lung weight – dry lung weight)/(dry lung weight).

Plasma catecholamine concentrations. Blood samples were obtained from the abdominal aorta and transferred immediately to chilled tubes containing 0.02 ml heparin sodium. The blood samples were then centrifuged (1,200 g, 10 min, 4°C), and the plasma samples were separated and stored at −80°C. Plasma catecholamine concentrations were determined by high-performance liquid chromatography with a trihydroxyindole reaction.

Serum osmolality. Serum osmolality was measured by a freezing-point depression method using an osmometer (Fiske one-ten osmometer, Fiske Associates, Norwood, MA).
solution containing $10^{-7}$ M norepinephrine was instilled in rat lungs exposed to hypoxia for 120 h ($n = 4$). To determine the effect of phenolamine alone or propranolol alone on alveolar fluid clearance in normal rats, an albumin solution containing $10^{-4}$ M phenolamine ($n = 4$) or $10^{-6}$ M propranolol ($n = 4$) was instilled into the alveolar spaces in rats not exposed to hypoxia.

**Group 6:** effects of exogenous norepinephrine on alveolar fluid clearance in rats exposed to nutritional deprivation ($n = 15$). To determine whether the elevated concentrations of plasma norepinephrine were sufficient to increase alveolar fluid clearance, the effects of exogenous norepinephrine at concentrations similar to plasma norepinephrine concentrations on alveolar fluid clearance were determined in rats exposed to nutritional deprivation. An albumin solution containing $10^{-8}$ M ($n = 5$) or $10^{-7}$ M ($n = 5$) norepinephrine was instilled in rats exposed to nutritional deprivation for 120 h under normoxic conditions. In addition, to determine whether the effect of norepinephrine was mediated by β-adrenoceptors, $10^{-4}$ M propranolol ($n = 5$) was added to an albumin solution containing $10^{-7}$ M norepinephrine and instilled into the alveolar spaces. After instillation, the lungs were inflated with 100% nitrogen as described in **General Protocol**. In five rats with instillation of $10^{-7}$ M norepinephrine, the concentrations of norepinephrine in the instilled albumin solution were measured before instillation and 1 h after instillation.

**Group 7:** potency of exogenous norepinephrine on alveolar fluid clearance in normal rats ($n = 15$). The alveolar fluid clearance rates were slower in rats with nutritional impairment in the presence of exogenous $10^{-7}$ M norepinephrine and in rats exposed to hypoxia for 120 h than the rates in rats with an administration of β-adrenergic agonists in the previous studies (24, 27, 28). Therefore, we determined whether the potency of norepinephrine was lower than that of β-adrenergic agonists. An albumin solution containing $10^{-5}$ M norepinephrine was instilled into the normal rat lungs ($n = 5$) and the potency of norepinephrine was compared with that of the identical concentrations of β-adrenergic agonists (24, 27, 28). In addition, to determine whether the effect of norepinephrine was mediated by α-adrenoceptors or β-adrenoceptors, $10^{-4}$ M phenolamine ($n = 5$) or $10^{-4}$ M propranolol ($n = 5$) was added to an albumin solution containing $10^{-5}$ M norepinephrine and instilled into the alveolar spaces. After instillation, the lungs were inflated with 100% nitrogen as described in **General Protocol**.

**Group 8:** effects of β-adrenergic agonists on alveolar fluid clearance in rats exposed to hypoxia ($n = 19$). Inasmuch as the rate of alveolar fluid clearance in rats exposed to hypoxia for 120 h was less than that produced by β-adrenergic agonists in rats not exposed to hypoxia (24, 27, 28), we determined whether exogenous β-adrenergic agonists would further increase alveolar fluid clearance in rats exposed to hypoxia. An albumin solution containing $10^{-5}$ M denopamine, a selective β1-adrenergic agonist (28), was instilled in the rat lungs exposed to hypoxia (10% oxygen) for 120 h ($n = 5$) and in the rat lungs not exposed to hypoxia ($n = 5$). In addition, an albumin solution containing $10^{-6}$ M salmeterol, a lipophilic β2-adrenergic agonist (24), was instilled in the rat lungs exposed to hypoxia for 120 h ($n = 5$) and in the rat lungs not exposed to hypoxia ($n = 4$).

**Group 9:** recovery of alveolar fluid clearance capacity in rats after reoxygenation ($n = 5$). To determine whether reoxygenation after hypoxia could restore the normal clearance rates, alveolar fluid clearance, LW/DL, plasma catecholamine concentrations, and serum osmolality were measured in rats exposed to normoxia for 24 h after hypoxia (10% oxygen) for 96 h ($n = 5$).

**Statistics**

The data are summarized as means and SD. The data were analyzed by one-way ANOVA with 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 differences with a $P$ value of $<0.05$ as significant.

**RESULTS**

**Group 1:** Effects of Deoxygenation on Alveolar Oxygen Tension and Alveolar Fluid Clearance

In the rat lungs inflated with 100% nitrogen after instillation of the normoxic albumin solutions, the oxygen partial pressure in the alveolar albumin solutions decreased to 75 and 39 Torr at 5 min and 1 h after instillation, respectively. When oxygen in the albumin solution was exchanged with 100% nitrogen for 2 h before instillation, the oxygen partial pressure in the albumin solution before instillation decreased to 50 Torr. Then, the oxygen partial pressure decreased to 36 Torr at 5 min after instillation and was maintained at 36 Torr up to 1 h after instillation. Deoxygenation of the instilled albumin solutions had no effect on alveolar fluid clearance (Fig. 1).

**Group 2:** Time Course of Alveolar Fluid Clearance and Lung Water Volume in Rats Exposed to Hypoxia

Alveolar fluid clearance and lung water volume did not change in rats exposed to hypoxia (10% oxygen) for 3 and 48 h (Fig. 2A). However, alveolar fluid clearance significantly increased in rats exposed to hypoxia for 72, 96, and 120 h. In contrast, LW/DL decreased in rats exposed to hypoxia for 72, 96, and 120 h (Fig. 2B). Plasma norepinephrine concentrations increased significantly in rats exposed to hypoxia for 72, 96, and 120 h ($0.9 \times 10^{-8}$, $1.1 \times 10^{-8}$, and $1.6 \times 10^{-8}$ M at 72, 96, and 120 h, respectively; Table 1). There were no
major changes in plasma epinephrine, dopamine, and cortisol concentrations in rats exposed to hypoxia for 72, 96, and 120 h. Serum osmolality did not change in rats exposed to hypoxia for 120 h (Table 2).

**Group 3: Effects of a Sodium Channel Inhibitor on Alveolar Fluid Clearance in Rats Exposed to Hypoxia**

Amiloride decreased alveolar fluid clearance in control rats and in rats exposed to hypoxia (10% oxygen) for 120 h (Fig. 3). The fractions of amiloride-insensitive alveolar fluid clearance were similar in control rats and in rats exposed to hypoxia for 120 h.

**Group 4: Effects of Nutritional Deprivation on Alveolar Fluid Clearance and Lung Water Volume**

Alveolar fluid clearance decreased in rats exposed to nutritional deprivation for 120 h under normoxic conditions (Fig. 4). However, hypoxia (10% oxygen) increased alveolar fluid clearance in rats exposed to nutritional deprivation. The lung water volume decreased in rats exposed to nutritional deprivation as well as in rats exposed to hypoxia for 120 h. An additional exposure to hypoxia did not change the lung water volume in rats exposed to nutritional deprivation. Although serum osmolality did not change in rats exposed to hypoxia for 120 h, the level increased in rats exposed to nutritional deprivation under normoxic and hypoxic conditions for 120 h (Table 2). Body weight loss was larger in rats exposed to nutritional deprivation than in rats exposed to hypoxia for 120 h because rats in the latter group took some diet. Plasma norepinephrine concentrations did not change in rats exposed to nutritional deprivation under normoxic conditions. However, hypoxia increased the plasma norepinephrine concentrations in rats exposed to nutritional deprivation.

**Group 5: Effects of α- or β-Adrenergic Antagonists on Alveolar Fluid Clearance in Rats Exposed to Hypoxia**

Propranolol, but not phentolamine, inhibited the increase in alveolar fluid clearance in rats exposed to hypoxia (10% oxygen) for 120 h (Fig. 5). There was no additional increase in the presence of $10^{-7}$ M norepinephrine in rats exposed to hypoxia for 120 h. Propranolol alone or phentolamine alone had no effect on alveolar fluid clearance in rats not exposed to hypoxia.

**Group 6: Effects of Exogenous Norepinephrine on Alveolar Fluid Clearance in Rats Exposed to Nutritional Deprivation**

In rats exposed to nutritional deprivation for 120 h under normoxic conditions, $10^{-7}$ M norepinephrine, but not $10^{-8}$ M norepinephrine, significantly increased alveolar fluid clearance (Fig. 6). Propranolol inhibited alveolar fluid clearance increased by $10^{-7}$ M norepinephrine in rats exposed to nutritional deprivation. Norepinephrine concentrations in the alveolar albumin solution decreased from $15.4 \pm 0.4$ to $2.3 \pm 0.6$ ng/ml over 1 h (from $9.1 \times 10^{-8}$ to $1.4 \times 10^{-8}$ M).

**Group 7: Potency of Exogenous Norepinephrine on Alveolar Fluid Clearance in Normal Rats**

In normal rats, $10^{-5}$ M norepinephrine increased alveolar fluid clearance (Fig. 7). Although the increase

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### Table 1. Plasma catecholamines in rats exposed to hypoxia

<table>
<thead>
<tr>
<th>Catecholamines</th>
<th>Control</th>
<th>48 h</th>
<th>72 h</th>
<th>96 h</th>
<th>120 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine, ng/ml</td>
<td>$0.16 \pm 0.06$</td>
<td>$0.47 \pm 0.13$</td>
<td>$0.32 \pm 0.08$</td>
<td>$0.25 \pm 0.076$</td>
<td>$0.38 \pm 0.24$</td>
</tr>
<tr>
<td>Norepinephrine, ng/ml</td>
<td>$0.36 \pm 0.11$</td>
<td>$0.33 \pm 0.14$</td>
<td>$1.47 \pm 0.52^*$</td>
<td>$1.82 \pm 0.41^*$</td>
<td>$2.75 \pm 0.39^*$</td>
</tr>
<tr>
<td>Dopamine, ng/ml</td>
<td>$0.11 \pm 0.10$</td>
<td>$0.11 \pm 0.10$</td>
<td>$0.24 \pm 0.12$</td>
<td>$0.12 \pm 0.09$</td>
<td>$0.22 \pm 0.02$</td>
</tr>
</tbody>
</table>

Values are means ± SE. $^*P < 0.05$ vs. control levels. $^\dagger P < 0.05$ vs. control and 72-h levels.

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### Table 2. Serum osmolarity in rats exposed to hypoxia and/or nutritional deprivation

<table>
<thead>
<tr>
<th>Experiments</th>
<th>n</th>
<th>Osmolarity, mosmol/kgH2O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>$313 \pm 3$</td>
</tr>
<tr>
<td>Hypoxia (120 h)</td>
<td>7</td>
<td>$311 \pm 2$</td>
</tr>
<tr>
<td>Nutritional deprivation (120 h)</td>
<td>7</td>
<td>$321 \pm 6^*$</td>
</tr>
<tr>
<td>+Hypoxia (120 h)</td>
<td>5</td>
<td>$331 \pm 8^*$</td>
</tr>
</tbody>
</table>

Values are means ± SE. $^*P < 0.05$ vs. control levels.

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Fig. 2. Time course of alveolar fluid clearance (A) and lung water volume (B) in rats exposed to hypoxia. Alveolar fluid clearance increased, but the lung water-to-dry lung weight ratio (LW/DL) decreased in rats exposed to hypoxia for 72, 96, and 120 h. $^*$Significantly different vs. corresponding controls ($P < 0.05$). $^\dagger$Significantly different vs. LW/DL at 72 h ($P < 0.05$).
was comparable with that observed in rats exposed to hypoxia for 120 h, the increase was lower than that in the presence of identical concentrations of terbutaline, denopamine, or salmeterol (24, 27, 28). Propranolol, but not phentolamine, inhibited the rates of alveolar fluid clearance increased by $10^{-5}$ M norepinephrine in normal rats (Fig. 7).

**Group 8: Effects of β-Adrenergic Agonists on Alveolar Fluid Clearance in Rats Exposed to Hypoxia**

Denopamine increased alveolar fluid clearance in control rats and in rats exposed to hypoxia (10% oxygen) for 120 h (Fig. 8). Although basal alveolar fluid clearance was faster in rats exposed to hypoxia for 120 h than in control rats, there was no difference between the rates of alveolar fluid clearance increased by denopamine in control rats and in rats exposed to hypoxia for 120 h. Salmeterol increased alveolar fluid clearance as well as denopamine in normal rats and in rats exposed to hypoxia for 120 h (Fig. 8).

**Group 9: Recovery of Alveolar Fluid Clearance in Rats After Reoxygenation**

Alveolar fluid clearance and plasma norepinephrine concentrations returned to normal in rats exposed to normoxia for 24 h after hypoxia (10% oxygen) for 96 h (Fig. 9). However, the lung water volume remained decreased after reoxygenation for 24 h.

**DISCUSSION**

The major findings in this study are that alveolar fluid transport capacity did not change for 48 h and then increased after 72, 96, and 120 h of exposure to hypoxia with 10% oxygen. These findings are inconsistent with the finding that alveolar fluid clearance decreased in rats exposed to 10% oxygen for 72 h (32) but are consistent with the finding that moderate hypoxia (5% oxygen) had no effect on the sodium channel activ-

Inflation of the lungs with 100% nitrogen reduced oxygen concentration in the alveolar albumin solutions ~5–10% oxygen for 1 h. Deoxygenation of the albumin solution before instillation reduced the oxygen concentration ~5% oxygen. However, deoxygenation before instillation had no effect on alveolar fluid clearance. These results are consistent with the finding that a short term of hypoxia did not affect the alveolar fluid clearance capacity in previous studies (27, 28). One of the limitations in these preparations is that it is impossible to expose the alveolar spaces in the isolated rat lung to more severe hypoxia (<5% oxygen) that can decrease amiloride-sensitive $^{22}$Na influx in cultured type II cells (22).

The upregulation of amiloride-sensitive alveolar fluid clearance has been reported in the rat lungs’...
responses to several stimuli: endotoxin (11), keratinocyte growth factor (33), transforming growth factor-α (8), and β-adrenergic agonists (1, 24, 25, 29). We tested three hypotheses accounting for the increase in amiloride-sensitive alveolar fluid clearance in rats exposed to hypoxia. The first hypothesis was the effect of osmotic gradients (18). Recently, Fukuda et al. (10) evaluated the effect of osmotic difference between the alveolar spaces and the pulmonary vasculature on alveolar fluid clearance in the mouse lung. The difference of 65 mosmol/kg H2O resulted in a 20% slower clearance. However, serum osmolality did not increase in rats exposed to hypoxia for 120 h in this study. Therefore, it is unlikely that serum osmolality played a role in the increase in alveolar fluid clearance.

The second hypothesis was that the decrease in the lung water volume played a role in the increase in alveolar fluid clearance in rats exposed to hypoxia. Fukuda et al. (10) also reported that lung interstitial fluid volume representing a part of lung water volume played an important role in the regulation of alveolar fluid clearance in the in situ mouse lung model. How-

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Fig. 5. Effects of α- or β-adrenoceptor blocker on alveolar fluid clearance in rats exposed to hypoxia for 120 h. Propranolol, a β-adrenergic antagonist, but not phentolamine, an α-adrenergic antagonist, inhibited the increase in alveolar fluid clearance. Exogenous norepinephrine did not further increase alveolar fluid clearance in rats exposed to hypoxia for 120 h. *Significantly different vs. control (P < 0.05). †Significantly different vs. 120-h hypoxia (P < 0.05).

Fig. 6. Effects of 10−7 M norepinephrine on alveolar fluid clearance in rats exposed to nutritional deprivation under normoxic conditions. An administration of 10−7 M norepinephrine increased alveolar fluid clearance. The increase was inhibited by propranolol, a β-adrenergic antagonist. *Significantly different vs. nutritional deprivation (P < 0.05). †Significantly different vs. nutritional deprivation + norepinephrine (10−7 M; P < 0.05).

Fig. 7. Effects of 10−6 M norepinephrine on alveolar fluid clearance in normal rat lungs. The administration of 10−6 M norepinephrine increased alveolar fluid clearance to the comparable rates observed in rats exposed to hypoxia for 72–120 h. The increase was mediated by β-adrenoceptors, not by α-adrenoceptors. *Significantly different vs. control (P < 0.05). †Significantly different vs. norepinephrine (10−6 M; P < 0.05).

Fig. 8. Effects of β-adrenergic agonists on alveolar fluid clearance in rats exposed to hypoxia. Denopamine increased alveolar fluid clearance in rats exposed to hypoxia for 120 h as well as in normal rats. Salmeterol increased alveolar fluid clearance as well as denopamine. *Significantly different vs. control (P < 0.05). †Significantly different vs. hypoxia 120 h (P < 0.05).
ever, the changes of alveolar fluid clearance were not consistent with those of lung water volume in this study. For example, the lung water volume remained decreased, but alveolar fluid clearance returned to normal after reoxygenation for 24 h following hypoxia for 96 h. Therefore, it is also unlikely that the decrease in the lung water volume played a primary role in the increase in alveolar fluid clearance in rats exposed to hypoxia.

Did the increase in alveolar fluid clearance result in a reduction of the lung water volume? Alveolar fluid clearance measured by the progressive increase in the alveolar albumin concentration changed in parallel with lung fluid clearance measured by lung water volume (1, 2, 12, 14, 21). The stimulation of alveolar fluid clearance by salmeterol, a β2-adrenergic agonist, resulted in a 62% reduction of excess lung water volume as well as a significant improvement in arterial blood gases (9). In the present study, the lung water volume was inversely related to the alveolar fluid clearance rates (Fig. 2). These results support the hypothesis that the increase in alveolar fluid clearance can decrease the lung water volume. However, as observed in rats exposed to nutritional deprivation, an additional exposure to hypoxia increased both plasma norepinephrine concentrations and alveolar fluid clearance but did not change the lung water volume. Therefore, it is impossible to conclude that the increase in alveolar fluid clearance results in a reduction of the lung water volume.

The third hypothesis was that endogenous catecholamines contributed to the increase in alveolar fluid clearance. Although plasma epinephrine and denopamine levels did not significantly change, norepinephrine concentrations significantly increased, consistent with alveolar fluid clearance (Table 1). The increase in plasma norepinephrine concentration was coincident with the results obtained from a human study in which healthy men were transported to a high altitude (4,559 m) for 120 h (13).

There are three lines of evidence that support a role of endogenous norepinephrine in increased alveolar fluid clearance in rats exposed to hypoxia. First, only the plasma norepinephrine concentration changed in association with alveolar fluid clearance. Second, propranolol, but not phentolamine, inhibited the increase in alveolar fluid clearance. The results indicated that increased clearance was mediated by β-adrenoceptors, not by α-adrenoceptors. In addition, exogenous norepinephrine increased alveolar fluid clearance via β-adrenoceptors. These data are consistent with β-adrenoceptor-mediated alveolar fluid clearance in the in vivo lung (18) and sodium transport in cultured type II cells (17). Third, the magnitude of alveolar fluid clearance in the presence of 10⁻⁵ M norepinephrine in normal rats was comparable to that in rats exposed to hypoxia for 120 h. However, the magnitude was less than that in the presence of identical concentrations of β-adrenergic agonists (24, 27, 28).

We determined whether plasma norepinephrine concentrations were sufficient to increase alveolar fluid clearance. We found that 10⁻⁷ M norepinephrine increased alveolar fluid clearance by stimulating β-adrenoceptors in rats exposed to nutritional deprivation. Although the initial concentration (10⁻⁷ M) was higher than the plasma norepinephrine concentration (1–2 × 10⁻⁸ M), the final concentration (1.6 × 10⁻⁸ M) was comparable to plasma norepinephrine levels in rats exposed to hypoxia. The norepinephrine concentration decrease was comparable to the amiloride and salmeterol concentration decreases (2, 12). The higher norepinephrine concentrations are consistent with the results that denopamine and salmeterol higher than 10⁻⁷ M were necessary to increase alveolar fluid clearance (24, 28). Therefore, it is likely that endogenous norepinephrine concentration was sufficient to increase some alveolar fluid clearance in rats exposed to hypoxia.

The results in this study are inconsistent with the finding that endogenous or exogenous norepinephrine...
did not stimulate alveolar fluid clearance in dog lungs (14, 16). Because alveolar fluid clearance was slower in dogs and faster in rats (18), it is likely that the effect of norepinephrine was masked in the dog lungs. In the present study, it is uncertain whether hypoxia or nutritional deprivation increased sensitivity of β-adrenoceptors.

Although alveolar fluid clearance was slower in rats exposed to hypoxia for 120 h than in normal rats with the treatment of denopamine and salmeterol (24, 28), these agonists increased alveolar fluid clearance to normal levels in rats exposed to hypoxia. These results suggest that the response to β-adrenergic agonists persisted at normal levels in rats exposed to hypoxia for 120 h. The preservation of the response to β-adrenergic agonist is consistent with that in human lungs exposed to severe hypothermia (26) or in rat lungs exposed to hyperoxia (30). Therefore, it is likely that β1- and β2-adrenoceptors are resistant to moderate hypoxia (10% oxygen) for 120 h. The resistance may be beneficial in the resolution of pulmonary edema when alveolar epithelial cells are exposed to hypoxia for several days.

Because nutritional impairment was observed in rats exposed to hypoxia, we determined whether nutritional deprivation affected alveolar fluid clearance in rats under normoxic conditions. Nutritional deprivation for 120 h decreased both alveolar fluid clearance and lung water volume but increased serum osmolality and did not change plasma catecholamine concentrations. Therefore, it is likely that nutritional impairment decreased the alveolar fluid transport capacity under normoxic conditions. Then, we proceeded with the study to determine whether hypoxia affected alveolar fluid clearance in rats exposed to nutritional deprivation. Similar to the results in rats in the absence of hypoxia, both alveolar fluid clearance and plasma norepinephrine concentrations increased in the presence of hypoxia and nutritional deprivation. These results suggested that nutritional impairment during the hypoxic exposure decreased alveolar fluid clearance, but endogenous norepinephrine increased net alveolar fluid clearance in rats exposed to hypoxia. Recently, it was reported that continuous nutrition attenuated pulmonary edema in rats exposed to 100% oxygen (7). Taken together, nutritional improvement may be important in the resolution of pulmonary edema as well as in the attenuation of the development of pulmonary edema.

Last, we determined whether alveolar fluid clearance returned to normal within 24 h of reoxygenation after 96 h of hypoxia. We found that plasma norepinephrine concentrations and alveolar fluid clearance returned to normal 24 h after reoxygenation. However, the lung water volume remained decreased. Therefore, the results indicate that the change of alveolar fluid clearance was related to the change of plasma norepinephrine concentrations but not to the lung water volume. The reversibility of alveolar fluid clearance in this study is consistent with the effect of epinephrine on alveolar fluid clearance in anesthetized rats (4). In summary, alveolar fluid clearance capacity was sustained for 48 h and then increased between 72 and 120 h of exposure to hypoxia (10% oxygen) in rats. Although nutritional impairment during hypoxia decreases basal alveolar fluid clearance, the increase in plasma norepinephrine concentration increased net alveolar fluid clearance by stimulating β-adrenoceptors.

The response to exogenous β1- and β2-adrenergic agonists was preserved in rats exposed to hypoxia for 120 h. Reoxygenation for 24 h after hypoxia could restore normal alveolar fluid clearance. It is likely that alveolar epithelial cells are resistant to moderate hypoxia. β-Adrenergic agonist therapy may be effective in the resolution of pulmonary edema in patients exposed to hypoxia.

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