Malnutrition impairs alveolar fluid clearance in rat lungs

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Sakuma, Tsutomu, Yabin Zhao, Makoto Sugita, Motoyasu Sagawa, Hirohisa Toga, Takaharu Ishibashi, Matomo Nishio, and Michael A. Matthy. Malnutrition impairs alveolar fluid clearance in rat lungs. Am J Physiol Lung Cell Mol Physiol 286: L1268–L1274, 2004.—Inadequate nutrition complicates the clinical course of critically ill patients, and many of these patients develop pulmonary edema. However, little is known about the effect of malnutrition on the mechanisms that resolve alveolar edema. Therefore, we studied the mechanisms responsible for the decrease in alveolar fluid clearance in rats exposed to malnutrition. Rats were allowed access to water, but not to food, for 120 h. Then, the left and right lungs were isolated for the measurement of lung water volume and alveolar fluid clearance, respectively. The rate of alveolar fluid clearance was measured by the progressive increase in the concentration of Evans blue dye that was instilled into the distal air spaces with an isosmolar 5% albumin solution over 1 h. Malnutrition decreased alveolar fluid clearance by 38% compared with controls. Amiloride (10−3 M) abolished alveolar fluid clearance in malnourished rats. Either refeeding for 120 h following nutritional deprivation for 120 h or an oral supply of sodium glutamate during nutritional deprivation for 120 h restored alveolar fluid clearance to 91 and 86% of normal, respectively. Dibutyryl-cGMP, a cyclic nucleotide-gated cation channel agonist, increased alveolar fluid clearance in malnourished rats supplied with sodium glutamate. Terbutaline, a β2-adrenergic agonist, increased alveolar fluid clearance in rats under all conditions (control, malnutrition, refeeding, and glutamate-treated). These results indicate that malnutrition impairs primarily amiloride-insensitive and dibutyryl-cGMP-sensitive alveolar fluid clearance, but this effect is partially reversible by refeeding, treatment with sodium glutamate, or β-adrenergic agonist therapy.

Pulmonary edema; alveolar epithelium; β-adrenergic agonist; diet; sodium glutamate

The mechanisms responsible for alveolar epithelial fluid clearance have been studied over the past two decades (16, 18). Alveolar epithelial type II cells primarily transport sodium ions from the alveolar spaces through apical sodium channels, and the sodium is then pumped out through basolateral Na+-K+-ATPase (1, 4, 6, 15). Osmotic gradients created by vectorial ion transport move water from the alveolar spaces, resulting in the resolution of alveolar edema (17, 32).

Amiloride inhibits sodium uptake through apical sodium channels and reduces alveolar fluid transport (15). However, since amiloride is unable to completely abolish alveolar fluid clearance in several species, including the rat and human lung (16), it is clear that amiloride-insensitive alveolar fluid clearance exists (12, 21).

Malnutrition develops in many experimental and clinical conditions. Adult rats exposed to hyperoxia develop anorexia, weight loss, and lung injury characterized by increased lung water volume (5). We recently reported that malnutrition as well as a hypoxic insult contribute to the decrease in alveolar fluid clearance in rats exposed to hypoxia for 120 h (25). Critically ill patients with acute lung injury or the acute respiratory distress syndrome frequently suffer from nutritional impairment because of an increase in catabolism associated with their medical illnesses and suboptimal nutritional therapy. However, the mechanisms responsible for a possible deleterious effect of malnutrition on alveolar fluid clearance have not been elucidated.

Therefore, the first objective of this study was to determine whether nutritional impairment reduced the rate of alveolar fluid transport in rats. Alveolar fluid clearance was measured in rats exposed to nutritional deprivation for 120 h, the same time period that has been studied previously in rats exposed to hypoxia (25). The second objective was to determine the mechanisms responsible for the decrease in alveolar fluid clearance in acutely malnourished rats. Since the decreased fraction of alveolar fluid clearance in malnourished rats was amiloride insensitive, we hypothesized that a cyclic nucleotide-gated channel might play a role in the decrease in fluid transport. Therefore, we tested the effects of dibutyryl-cGMP (DB-cGMP) on alveolar fluid clearance in malnourished rats. The third objective was to determine whether restoration of normal nutrition or a supply of sodium glutamate during nutritional deprivation restored alveolar fluid clearance. The final objective was to determine whether the decrease in alveolar fluid clearance in malnourished rats was reversible with β2-adrenergic agonist therapy.

Materials and Methods

Materials

Materials were obtained as follows: amiloride, terbutaline sulfate, and DB-cGMP were from Sigma (St. Louis, MO); Evans blue was from Tokyo Kasei (Tokyo, Japan); and sodium glutamate was from Ajinomoto (Tokyo, Japan).

General Protocol

All rats received humane care, and this study was approved by the Committee on Animal Experiments at Kanazawa Medical University. Male Sprague-Dawley rats (250–330 g, Japan SLC, Hamamatsu, Japan) were maintained under conditions of nutritional deprivation for 120 h. The rats were allowed access to water but not to food. Alveolar fluid clearance was measured by the progressive increase in the concentration of Evans blue dye that was instilled into the distal air spaces with an isosmolar 5% albumin solution over 1 h. Malnutrition decreased alveolar fluid clearance by 38% compared with controls. Amiloride (10−3 M) abolished alveolar fluid clearance in malnourished rats. Either refeeding for 120 h following nutritional deprivation for 120 h or an oral supply of sodium glutamate during nutritional deprivation for 120 h restored alveolar fluid clearance to 91 and 86% of normal, respectively. Dibutyryl-cGMP, a cyclic nucleotide-gated cation channel agonist, increased alveolar fluid clearance in malnourished rats supplied with sodium glutamate. Terbutaline, a β2-adrenergic agonist, increased alveolar fluid clearance in rats under all conditions (control, malnutrition, refeeding, and glutamate-treated). These results indicate that malnutrition impairs primarily amiloride-insensitive and dibutyryl-cGMP-sensitive alveolar fluid clearance, but this effect is partially reversible by refeeding, treatment with sodium glutamate, or β-adrenergic agonist therapy.

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fluid clearance, the lung water-to-dry lung weight ratio (LW/DL), and the serum osmolality were measured after nutritional deprivation for 120 h.

Alveolar fluid clearance was measured in the isolated rat lungs in the absence of either pulmonary perfusion or ventilation (24–26, 28). Briefly, rats were anesthetized by an intraperitoneal administration of pentobarbital sodium (50 mg/kg). An endotracheal tube was inserted through a tracheostomy. The rats were exsanguinated through the abdominal aorta. Through a median sternotomy, the left hilum was ligated with a silk suture and the left lung was isolated to measure lung water volume. The trachea, right lung, and heart were isolated en bloc to measure the rate of alveolar fluid clearance capacity.

Alveolar fluid clearance was estimated by measuring the progressive increase in the concentrations of alveolar Evans blue dye, as in our prior studies (25, 26, 28). Briefly, a warmed saline solution (1.5 ml) containing 5% albumin and 0.15 mg/ml Evans blue dye was instilled into the right lung followed by 1 ml of oxygen to deliver all of the instilled fluid into the alveolar spaces. The lung was placed in a humid incubator at 37°C and was inflated with 100% oxygen at an airway pressure of 8 cmH₂O. Alveolar fluid was aspirated 1 h after instillation. Alveolar fluid clearance (AFC) was calculated as follows

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

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

\[ V_f = \frac{(V_i \times EBi)}{EBf} \]  

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

This does not imply that all reabsorption may occur across the alveolar epithelial cells, because the distal airway epithelia can also transport sodium.

**Measurements**

*Evans blue dye concentration.* 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 bound to albumin in the instilled and aspirated solutions.

*Extravascular lung water volume.* Water volume in the left lung was measured by drying the 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.

*Serum osmolality.* Serum osmolality was measured by a freezing point depression method using an osmometer (Fiske One-Ten Osmometer; Fiske Associates, Norwood, MA).

*Concentration of nitric oxide metabolites.* The concentration of nitric oxide (NO) metabolites (NO₂⁻ and NO₃⁻) stable end products of NO in bronchoalveolar lavage (BAL) fluid was measured by a high-performance liquid chromatography-Griess system (Eicom, Kyoto, Japan) consisting of a separation column, a reduction column (to reduce NO₂⁻ to NO₃⁻), a flow reactor (with Griess reagent), and a detector at 540 nm as previously described (11). The sensitivity of the setup was 0.1 μM for both NO₂⁻ and NO₃⁻ with a loading volume of 10 μl. Under these specifications, the sensitivity limit was 0.01 μM for NO₂⁻ and 0.1 μM for NO₃⁻. To minimize NO₃⁻ contamination, all laboratory ware was washed five times with pure water (resistance > 18.3 MΩ and almost NO metabolite-free through MILLI-Q SP; Millipore, Bedford, MA).

*Concentration of ATP in the lung.* Following perfusion with PBS, the right lungs were isolated and homogenized in 5 ml of 0.5 N perchloric acid on ice and then centrifuged at 3,000 rpm for 10 min at 4°C. The supernatant was neutralized with 0.5 M triethanolamine plus 2.0 M K₂CO₃ and centrifuged at 3,000 rpm for 10 min at 4°C. The concentration of ATP in the supernatant fluid was measured by an ultraviolet method with phosphoglycerate kinase (ATP assay kit 366-A; Sigma).

**Specific Protocol**

*Group 1: effects of malnutrition on alveolar fluid clearance in the rat lungs.* To determine whether alveolar fluid clearance changes in rats under conditions of malnutrition, rats were allowed access to water, but not to any food, for 120 h (n = 10). As control, rats were allowed access to both rat chow and water for 120 h (n = 8). In addition, to determine whether the alveolar fluid clearance capacity was restored to the normal rates after refeeding, rats were allowed access to both rat chow and water for 120 h after nutritional deprivation for 120 h (n = 8). Alveolar fluid clearance, lung water volume, and serum osmolality were measured in this group.

*Group 2: effects of a sodium channel inhibitor on alveolar fluid clearance in the rat lungs.* Since the alveolar fluid clearance capacity was decreased in malnourished rats, we asked whether the fraction of alveolar fluid clearance that remained in malnourished rats was sensitive to amiloride, a sodium channel inhibitor. An albumin solution containing 10⁻⁴ M amiloride was instilled into the lungs of rats maintained under conditions of nutritional deprivation for 120 h (n = 4). This concentration of amiloride was chosen because the same concentration has been previously shown to differentiate amiloride-sensitive alveolar fluid clearance from amiloride-insensitive alveolar fluid clearance in adult rats (21). To compare the fractions of amiloride-sensitive and -insensitive alveolar fluid clearance in malnourished rats with those in control rats and in rats under conditions of refeeding after nutritional deprivation, an albumin solution containing 10⁻³ M amiloride was instilled into the lungs of control rats (n = 4) and the lungs of rats under conditions of refeeding for 120 h after nutritional deprivation for 120 h (n = 4).

*Group 3: effects of sodium glutamate on alveolar fluid clearance in malnourished rats.* Glutamate is commonly used in culture medium for isolated type II alveolar epithelial cells (38). When patch-clamped in the whole cell mode using symmetrical solutions (150 mM sodium glutamate), the type II alveolar epithelial cells exhibited outwardly rectified sodium ion currents that were diminished by amiloride (10–100 μM) instilled into the bath solution (14). Therefore, we hypothesized that a supply of sodium glutamate might be necessary to restore the normal alveolar fluid clearance capacity in nutritionally deprived rats. To test this hypothesis, rats were allowed access to water containing 1% sodium glutamate for 120 h (n = 6) but were not allowed access to food. To compare the effect of sodium glutamate on alveolar fluid clearance in malnourished rats with that in control rats and that in rats under conditions of refeeding after nutritional deprivation, rats were allowed access to both rat chow and water containing 1% sodium glutamate for 120 h (n = 4) and were allowed access to both rat chow and water containing 1% sodium glutamate for 120 h after nutritional deprivation for 120 h (n = 4). Alveolar fluid clearance, lung water volume, and serum osmolality were measured in this group.

*Group 4: effects of DB-cGMP on alveolar fluid clearance in malnourished rats.* Since malnutrition impaired primarily amiloride-insensitive alveolar fluid clearance, we studied the effect of DB-cGMP in malnourished rats. Our hypotheses were that DB-cGMP-sensitive cyclic nucleotide-gated cation channels play a role in amiloride-insensitive alveolar fluid clearance and that sodium glutamate is necessary to maintain DB-cGMP-sensitive alveolar fluid clearance. To determine the effect of DB-cGMP alone, an albumin solution containing 10⁻⁴ M DB-cGMP was instilled into the lungs of rats maintained under conditions of malnutrition for 120 h (n = 4). To determine whether DB-cGMP could stimulate alveolar fluid clearance in rats supplied with sodium glutamate, an albumin solution containing 10⁻⁴ M DB-cGMP was instilled into the lungs of rats supplied with sodium glutamate for 120 h (n = 4). To compare the effect of DB-cGMP on alveolar fluid clearance in malnourished rats with those
in control rats and in rats under conditions of refeeding after nutritional deprivation, an albumin solution containing $10^{-4}$ M DB-cGMP was instilled into the lungs of control rats ($n = 4$) and the lungs of rats under conditions of refeeding for 120 h following nutritional deprivation for 120 h ($n = 4$).

Group 5: effects of malnutrition on NO concentration in BAL fluid. Because release of NO in the alveolar spaces can contribute to inhibition of alveolar fluid clearance (7), we determined whether malnutrition increases the concentrations of NO metabolites (NO$_2$ and NO$_3$) in the alveolar spaces in malnourished rats. Immediately after the isolation of both lungs and the heart en bloc, BAL was performed three times with 5 ml of PBS and ~80% of instilled PBS was collected. There was no difference among quantities of collected BAL fluid. The concentrations of NO metabolites in BAL fluid were measured in control rats ($n = 8$), in rats exposed to nutritional deprivation for 120 h ($n = 8$), and in rats supplied with sodium glutamate under conditions of nutritional deprivation for 120 h ($n = 8$).

Group 6: effects of ATP levels on alveolar fluid clearance in malnourished rats. Since malnutrition decreases energy levels in several tissues and glutamine can be a major energy source in HeLa cells (23), we determined whether lung ATP content played a role in impaired alveolar fluid clearance in malnourished rats and in restoration of alveolar fluid clearance in rats supplied with sodium glutamate. The concentration of ATP in the right lung was measured in control rats ($n = 3$), rats exposed to nutritional deprivation for 120 h ($n = 3$), and rats supplied with sodium glutamate during nutritional deprivation for 120 h ($n = 3$).

Group 7: effects of terbutaline on alveolar fluid clearance in malnourished rats. To determine whether the lungs of malnourished rats were responsive to a $\beta_2$-adrenergic agonist, the effect of terbutaline on alveolar fluid clearance was determined in malnourished rats. An albumin solution containing $10^{-5}$ M terbutaline was instilled into the lungs of rats exposed to nutritional deprivation for 120 h ($n = 6$). To determine whether the effect of terbutaline was present in rats supplied with sodium glutamate or complete refeeding after nutritional deprivation, an albumin solution containing $10^{-5}$ M terbutaline was instilled into the lungs of rats supplied with sodium glutamate ($n = 6$) and rats under conditions of refeeding for 120 h following nutritional deprivation for 120 h ($n = 6$). In the control group, an albumin solution containing $10^{-5}$ M terbutaline was instilled into the lungs of rats without exposure to malnutrition ($n = 6$). To determine whether the effect of terbutaline was mediated via $\beta_2$-adrenoceptors, $10^{-4}$ M propranolol was added to an albumin solution containing $10^{-5}$ M terbutaline and instilled into the lungs of rats maintained under conditions of control ($n = 4$), malnutrition ($n = 4$), supply of sodium glutamate during malnutrition ($n = 4$), and refeeding after malnutrition ($n = 4$).

Statistics

The data were summarized as means and standard deviations. 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. A $P$ value of <0.05 was considered a significant difference.

RESULTS

Effects of Malnutrition on Body Weight, Lung Water Volume, and Serum Osmolarity

Nutritional deprivation for 120 h resulted in body weight loss by 53 ± 7 g (baseline weight 285 ± 21 g), a decrease in LW/DL by 8%, and an increase in serum osmolality by 30 mosmol/kgH$_2$O (Table 1). Refeeding for 120 h restored body weight and LW/DL to the levels present at the beginning of the period of malnutrition. Refeeding restored serum osmolality to a lesser degree. A supply of sodium glutamate normalized serum osmolality levels in part but did not change the body weight or LW/DL.

Effects of Malnutrition on Alveolar Fluid Clearance in the Rat Lungs

Alveolar fluid clearance decreased by 38% of control rate in malnourished rats (Fig. 1). Refeeding following nutritional deprivation restored the rate of alveolar fluid clearance to the clearance rate of control rats.

Effects of Amiloride on Alveolar Fluid Clearance in Malnourished Rats

To determine whether the fraction of decreased alveolar fluid clearance in malnutrition was amiloride sensitive, we tested the effect of amiloride in rats maintained under conditions of nutritional deprivation for 120 h. Amiloride abolished alveolar fluid clearance in these rats (Fig. 2), thus indicating that malnutrition had primarily reduced the amiloride-insensitive fraction of alveolar fluid clearance. Refeeding after malnutrition restored the amiloride-insensitive fraction of alveolar fluid clearance.

Effects of Sodium Glutamate on Alveolar Fluid Clearance in Malnourished Rats

Because a patch-clamped study has shown that treatment with sodium glutamate maintained amiloride-sensitive sodium transport in type II alveolar epithelial cells (14), we determined whether sodium glutamate would restore alveolar fluid clearance in malnourished rats by supplying sodium glutamate to rats under conditions of nutritional deprivation for 120 h. The intake of sodium glutamate per day was 0.8 ± 0.3 g/kg body wt. A supply of sodium glutamate in malnourished rats restored alveolar fluid clearance to 86% of the control level (Fig. 3). However, a combined supply of sodium glutamate and rat chow did not change alveolar fluid clearance in normal rats and in rats exposed to refeeding after malnutrition.

Effects of DB-cGMP on Alveolar Fluid Clearance in Malnourished Rats

In rats supplied with sodium glutamate during nutritional deprivation, DB-cGMP ($10^{-4}$ M) alone did not change alveolar fluid clearance (Fig. 4). However, in rats supplied with

Table 1. Lung water-to-dry lung weight ratio and serum osmolality

<table>
<thead>
<tr>
<th>Experiments</th>
<th>No. of Rats</th>
<th>Lung Water-to-Dry Lung Weight Ratio, g/g</th>
<th>Serum Osmolality, mosmol/kgH$_2$O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>4.07 ± 0.09</td>
<td>294 ± 3</td>
</tr>
<tr>
<td>Malnutrition, 120 h</td>
<td>12</td>
<td>3.74 ± 0.05*</td>
<td>324 ± 5†</td>
</tr>
<tr>
<td>Malnutrition + refeeding, 120 h each</td>
<td>12</td>
<td>3.94 ± 0.17</td>
<td>302 ± 9*</td>
</tr>
<tr>
<td>Malnutrition + sodium glutamate, 120 h each</td>
<td>8</td>
<td>3.78 ± 0.09*</td>
<td>303 ± 6*</td>
</tr>
</tbody>
</table>

*P < 0.05 vs. corresponding control values. †P < 0.05 vs. serum osmolality levels in all other groups.
sodium glutamate during nutritional deprivation, DB-cGMP significantly increased the rate of alveolar fluid clearance by 30% over the rate in malnourished rats. In rats under normal conditions and rats under conditions of refeeding after malnutrition, DB-cGMP \(10^{-4}\) M did not significantly change alveolar fluid clearance.

Concentrations of NO Metabolites in the Alveolar Spaces

There was no significant difference among the concentrations of NO metabolites (NO\(_2\) and NO\(_3\)) in control rats \((0.26 \pm 0.13 \mu\text{M})\), in malnourished rats \((0.19 \pm 0.19 \mu\text{M})\), and in rats supplied with sodium glutamate \((0.22 \pm 0.12 \mu\text{M})\).

Concentration of ATP in the Right Lung

The concentration of ATP in the right lung was 1.49 \(\pm 0.05 \mu\text{mol/g lung}\) in control rats and significantly decreased to 1.08 \(\pm 0.14 \mu\text{mol/g lung}\) in malnourished rats \((P < 0.05)\) and to 1.19 \(\pm 0.13 \mu\text{mol/g lung}\) in rats supplied with sodium glutamate \((P < 0.05)\). There was no difference between the concentration of ATP in malnourished rats and that in rats supplied with sodium glutamate.

Effect of Terbutaline on Alveolar Fluid Clearance in Malnourished Rats

To determine whether lungs exposed to malnutrition were responsive to a \(\beta_2\)-adrenergic agonist, the effect of terbutaline on alveolar fluid clearance was measured in rats maintained under conditions of malnutrition for 120 h. Although basal alveolar fluid clearance was decreased, the capacity of terbutaline to stimulate alveolar fluid clearance was intact in malnourished rats (Fig. 5). In addition, the ability of terbutaline to stimulate alveolar fluid clearance was also intact in rats supplied with sodium glutamate during nutritional deprivation and in rats under conditions of refeeding after nutritional deprivation. Propranolol abolished the effect of terbutaline to stimulate alveolar fluid clearance in all groups.

DISCUSSION

The major findings in this study are that malnutrition reduces primarily amiloride-insensitive alveolar fluid clearance and that a supply of sodium glutamate during malnutrition or...
refeeding after malnutrition restores alveolar fluid clearance in rats. Also, β-adrenergic agonist therapy restores normal cAMP-stimulated clearance in malnourished rats.

Amiloride is a potent inhibitor of sodium uptake by the apical membrane of alveolar epithelial and distal airway epithelium (15). Amiloride inhibits 40–60% of basal fluid clearance in several species, including rat and human, indicating that there is a substantial fraction of fluid clearance that cannot be inhibited by amiloride (16). Therefore, it has been concluded that alveolar fluid clearance consists of amiloride-sensitive and amiloride-insensitive fractions (16). Previously, we reported that amiloride inhibited alveolar fluid clearance by 64% in control rats (26). In the present study, alveolar fluid clearance was decreased by ~38% in rats maintained under conditions of malnutrition, and the addition of amiloride abolished alveolar fluid clearance in these rats. These results indicate that malnutrition primarily impairs amiloride-insensitive alveolar fluid clearance.

Recent studies have suggested that amiloride-insensitive alveolar fluid clearance may be partially mediated through cyclic nucleotide-gated cation channels. In an in vivo adult rat study, DB-cGMP increased alveolar fluid clearance through (+)-cis-diltiazem-sensitive pathways, which are cyclic nucleotide-gated cation channels (21). In cultured alveolar epithelial type II cells from the adult rat lungs, DB-cGMP increased whole cation conductance in the presence of amiloride (12), and amiloride-insensitive sodium conductance increased in the presence of pulmonary edema (22). Therefore, in this study we tested whether the reduction in alveolar fluid clearance in malnourished rats was due to the impaired function of cyclic nucleotide-gated cation channels. An albumin solution containing DB-cGMP was instilled into the alveolar spaces in rats maintained under conditions of strict malnutrition and in rats supplied with sodium glutamate. Although DB-cGMP alone did not stimulate alveolar fluid clearance in strictly malnourished rats, DB-cGMP alone increased alveolar fluid clearance in rats if sodium glutamate was supplied during nutritional deprivation. These results indicate that malnutrition impaired primarily amiloride-insensitive alveolar fluid clearance and that the function of cyclic nucleotide-gated cation channels was probably impaired by nutritional deprivation. The effect of DB-cGMP on alveolar fluid clearance in the present study is similar to the results of a recent in vivo rat study (21).

The amiloride-sensitive fraction of alveolar fluid clearance in rats after refeeding changed from that in malnourished rats. Therefore, it is probable that amiloride-sensitive alveolar fluid clearance was also affected by malnutrition.

Reactive nitrogen species can decrease alveolar fluid clearance in pathological rats (7,10). Increased levels of NO₂⁻ and NO₃⁻ in edema fluid samples were associated with slower rates of alveolar fluid clearance in patients with acute lung injury (39). These reports led us to measure NO₃⁻ (total NO₂⁻ and NO₃⁻) concentrations in the BAL fluid of malnourished rats by a high-performance liquid chromatography-Griess system (11). It is unlikely that reactive nitrogen species played a role in the impairment of alveolar fluid clearance in malnourished rats, because there was no difference among the concentrations of BAL fluid NO₃⁻ in control rats, malnourished rats, and rats supplied with sodium glutamate.

Because osmotic gradients generated across the alveolar epithelium drive alveolar fluid out of the alveolar spaces (16,17), we hypothesized that an increase in serum osmolality would provide an increase in driving force that could result in an increase in alveolar fluid clearance. However, alveolar fluid clearance was decreased in malnourished rats, although serum osmolality was increased under these conditions. If the increase in osmolality of the serum was taken into account, it is likely that the real rate of alveolar fluid clearance was lower than the observed rate in malnourished rats.

Although glutamine is a principal energy source for intestinal enterocytes and is considered essential for gut metabolism, structure, and function, glutamine did not restore the intracellular ATP level in the jejunal mucosal cells of malnourished rats (37). Similar to that report, malnutrition decreased ATP content in the right lung and an oral supply of sodium glutamate did not restore ATP content in the lung. Therefore, it is unlikely that lung ATP levels played a role in the restoration of alveolar fluid clearance in rats supplied with sodium glutamate under conditions of nutritional deprivation.

A supply of sodium glutamate in malnourished rats restored alveolar fluid clearance to normal levels. Although the mechanism responsible for the restoration of alveolar fluid clearance was not established in this study, previous reports indicate important roles of glutamate or glutamine. For example, a diet containing sodium glutamate induces a significant increase of plasma glutamine levels in rats (2,19). The glutamate transporter EAAC1 mRNA is expressed in the rat lungs (20,30). This transporter modulates intracellular glutamate/glutamine metabolism, paracellular permeability, and intracellular H⁺ concentration (33). Extracellular glutamate flux regulates intracellular glutamate content and glutaminase activity in proximal tubular-like LLC-PK1-F⁺ cells (34). Glutamine can be a major energy source in HeLa cells (23) and serves as a precursor for the synthesis of purine and pyrimidine nucleotides in HeLa cells (35,36). When patch-clamped in the whole cell mode using a solution of 150 mM sodium glutamate, alveolar type II epithelial cells from rats exhibited outwardly rectified sodium currents (14). Further studies are needed to determine the exact mechanism responsible for the role of glutamate in the restoration of alveolar fluid clearance.

Fig. 5. Effect of terbutaline on alveolar fluid clearance. Rates of alveolar fluid clearance in rat lungs without a treatment of terbutaline (open bar), with a treatment of terbutaline (closed bar), and with a combined treatment of terbutaline and propranolol (gray bar) are shown. *P < 0.05 vs. control rat lungs without a treatment of terbutaline. **P < 0.05 vs. corresponding values in rat lungs without a treatment of terbutaline. †P < 0.05 vs. corresponding values in rat lungs with a treatment of terbutaline.
sodium glutamate in maintaining normal alveolar fluid clearance.

β2-Adrenergic agonists are being considered as potential therapeutic agents for accelerating the resolution of pulmonary edema. Indeed, inhalation of a lipid-soluble β2-adrenergic agonist, salmeterol, reduced the incidence of high altitude pulmonary edema in susceptible subjects (29). The stimulatory effect of β-adrenergic agonists on ion and fluid transport has been studied in cultured alveolar type II cells, in situ lungs, and in vivo lungs after various pathological insults (16). For example, β2-adrenergic agonists can accelerate the resolution of alveolar edema in experimental hydrostatic pulmonary edema (3, 8). These agents also increased alveolar fluid clearance in rats in the presence of hyperoxia-induced lung injury (9, 13), in ventilator-associated lung injury (7), and in rats exposed to hypoxia (25, 31). We previously reported (27) that the effect of terbutaline on alveolar fluid clearance was completely preserved in isolated human lungs exposed to severe hypothermia followed by rewarming. Thus the results of the present study are consistent with prior evidence.

Interestingly, previous studies have shown that terbutaline can stimulate both amiloride-sensitive and insensitive alveolar fluid clearance, but DB-cGMP stimulates only the amiloride-insensitive fraction of alveolar fluid clearance (21). These results are consistent with our result that terbutaline, but not DB-cGMP, increased alveolar fluid clearance in rats exposed to nutritional impairment. The upregulation of amiloride-sensitive alveolar fluid clearance has been reported in rat lungs in response to several stimuli: endotoxin, keratinocyte growth factor, transforming growth factor-α, and β-adrenergic agonists (16). It is likely that the amiloride-sensitive sodium channel was resistant to the insult of malnutrition and therefore the effect of terbutaline on alveolar fluid clearance was preserved in malnourished rats.

The results of this study may have clinical relevance. Nutrition is probably important to maintain the normal alveolar fluid clearance capacity for the resolution of alveolar edema, especially in surgical patients following resection of large organs or in medical diseases with a high metabolic requirement such as sepsis.

This study indicates that malnutrition impairs primarily amiloride-insensitive alveolar fluid clearance and that this effect is in part reversible by refeeding, by an oral supply of sodium glutamate, or by administration of a β2-adrenergic agonist.

GRANTS

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