Dexamethasone and thyroid hormone pretreatment upregulate alveolar epithelial fluid clearance in adult rats

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Folkesson, Hans G., Andreas Norlin, Yibing Wang, Parisa Abedinpour, and Michael A. Matthay. Dexamethasone and thyroid hormone pretreatment upregulate alveolar epithelial fluid clearance in adult rats. J. Appl. Physiol. 88: 416–424, 2000.—The in vivo effect of 48-h glucocorticoid and thyroid hormone 3,3′,5-triiodine-L-thyronine (T3) pretreatment on alveolar epithelial fluid transport was studied in adult rats. An isosmolar 5% albumin solution was instilled, and alveolar fluid clearance was studied for 1 h. Compared with controls, dexamethasone pretreatment increased alveolar fluid clearance by 80%. T3 pretreatment stimulated alveolar fluid clearance by 65%, and dexamethasone and T3 had additive effects (132%). Propranolol did not inhibit alveolar fluid clearance in either group, indicating that stimulation was not secondary to endogenous b-adrenergic stimulation. With the use of bromodeoxyuridine in vivo labeling, there was no evidence of cell proliferation. Alveolar fluid clearance was partially inhibited by amiloride in all groups. Fractional amiloride inhibition was greater in dexamethasone- and dexamethasone-plus-T3-pretreated rats than in control animals, but less in T3-pretreated rats. In summary, pretreatment with dexamethasone, T3, or both in combination upregulated in vivo alveolar fluid clearance similarly to short-term b-adrenergic stimulation. The effects are mediated partly by increased amiloride-sensitive Na+ transport, because the stimulated alveolar fluid clearance was more amiloride sensitive than in control rats. These observations may have clinical relevance because glucocorticoid therapy is commonly used with acute lung injury.

acute lung injury; alveolar epithelium; amiloride; b-adrenergic agonists; glucocorticoids; pulmonary edema; sodium transport

FLUID CLEARANCE FROM THE DISTAL air spaces of the lung is driven by active sodium transport across the alveolar epithelial barrier (4, 7, 16, 26, 30, 31, 34, 36, 44). Several studies have demonstrated that exogenous administration of b-adrenergic agonists can stimulate alveolar fluid clearance (6, 7, 13, 22, 26, 34, 42). Also, exogenous addition of cAMP can stimulate alveolar fluid clearance (5, 34). In addition, endogenous release of catecholamines in pathological conditions increases alveolar fluid clearance by b-adrenergic receptor stimulation (39, 40) and under normal conditions at birth (18). However, catecholamine-independent pathways may also increase alveolar fluid clearance. For example, epidermal growth factor and transforming growth factor-a stimulate alveolar fluid transport (8, 22).

Hormones released from the adrenal glands, such as cortisol and aldosterone, and the thyroid gland, such as 3,3′,5-triiodine-L-thyronine (T3) and L-thyroxine (T4), have been implicated as important regulators of Na+ channels (2, 37). Aldosterone primarily affects Na+ channels in the kidney (37), whereas cortisol can affect both kidney and lung Na+ channels (10, 37). Glucocorticoid regulation of alveolar epithelial fluid absorption has been suggested from several studies in developing and adult lungs (12, 25, 45, 47). Molecular investigations have suggested that the epithelial Na+ channel (ENaC) possesses a glucocorticoid regulatory element (10, 41). Glucocorticoids also promote alveolar epithelial type II cell maturation and differentiation in the preterm fetus (24). In the adult lung, glucocorticoids have been recommended by some investigators for the treatment of pulmonary edema and inflammation in established clinical lung injury (32). In addition, many patients with cardiogenic or noncardiogenic pulmonary edema are treated with glucocorticoids for a variety of nonpulmonary disorders. Thus the in vivo effects of glucocorticoids on alveolar epithelial fluid transport are important.

Therefore, we first tested the hypothesis that alveolar epithelial fluid transport could be upregulated by glucocorticoids. Adult rats were injected with intramuscular dexamethasone over 48 h before measurement of in vivo alveolar fluid transport. The dose and administration schedule of dexamethasone were chosen from a study on glucocorticoid-induced expression of aquaporin-1 water channels in the developing rat lung (27). The second objective was to determine whether coadministration of dexamethasone and the thyroid hormone T3 would exert an additive effect on stimulating alveolar fluid clearance. A synergistic effect has been reported in the fetal near-term sheep lung (2), but no studies have been done in the adult lung. The third objective was to determine whether the increase in alveolar fluid clearance after dexamethasone, T3, and dexamethasone-plus-T3 pretreatment was secondary to endogenous catecholamine release or to a proliferative effect on alveolar epithelial type II cells. We also investigated whether the sensitivity to b-adrenergic
stimulation was altered after dexamethasone pretreatment as well as after the combined pretreatment with dexamethasone and $T_3$. The fourth objective was to determine whether the fractional inhibition by amiloride changed after dexamethasone, $T_3$, and dexamethasone plus $T_3$ pretreatment.

**METHODS**

**Animals**

Surgical preparation and ventilation. Male Sprague-Dawley rats ($n = 89$) weighing 300–350 g (B&K Universal, Sollentuna, Sweden) were anesthetized with 50 mg/kg body wt of pentobarbital (Nembutal, Apoteksbolaget, Umeå, Sweden) intraperitoneally (ip). A 2-mm-inner-diameter (PE-240; Clay Adams, Becton Dickinson, Parsippany, NJ) endotracheal tube was inserted through a tracheostomy. A PE-50 catheter (Clay Adams, Becton Dickinson) was sutured into the right carotid artery to monitor systemic blood pressure and to obtain blood samples.

Pancuronium bromide (0.3 mg/kg body wt, Pavulon, Organon Teknika, Boxtel, The Netherlands) was given intravenously hourly for neuromuscular blockade. The rats were maintained in the left lateral decubitus position during the experiments. The rats were ventilated with a constant-volume piston pump (Harvard Apparatus, Dover, MA) with an inspired oxygen fraction of 1.0 and with peak airway pressures of 7–9 cmH$_2$O during the baseline period, supplemented with a positive end-expiratory pressure of 4 cmH$_2$O.

The Ethics Review Committee on Animal Experiments at Lund University approved the experiments.

Preparation of the Instillates

A 5% albumin instillate solution was prepared by dissolving 50 mg/ml of bovine serum albumin (Sigma Chemical, St. Louis, MO) in an aqueous solution of 0.9% NaCl. For studies of the potential effects of endogenous epinephrine release on alveolar fluid clearance after dexamethasone, $T_3$, and dexamethasone plus $T_3$ pretreatment, $10^{-4}$ M of the general $\beta$-adrenergic antagonist propranolol (Sigma Chemical) was added to the 5% albumin instillate solution. To investigate whether $\beta$-adrenergic agonists could stimulate alveolar fluid clearance after dexamethasone and dexamethasone plus $T_3$ treatment, terbutaline ($10^{-4}$ M; Sigma Chemical) was instilled with the 5% albumin solution. For the determination of the fractional inhibition by amiloride on alveolar fluid clearance, amiloride ($10^{-3}$ M; Sigma Chemical) was added with the 5% albumin solution. We used amiloride at the concentration of $10^{-2}$ M because $50\%$ of the amiloride is protein bound and another significant fraction escapes from the air spaces, resulting in functional in vivo concentrations closer to $10^{-4}$ M ($35, 49$).

Glucocorticoid and Thyroid Hormone Pretreatment

The rats were injected intramuscularly (im) with 0.35 mg/kg body wt of dexamethasone (water soluble; Sigma Chemical) dissolved in a 0.9% NaCl solution. The rats were pretreated on 2 consecutive days 24 h apart, and also on the morning of the third day, the same day on which the in vivo alveolar fluid clearance experiment was done. This dose and administration protocol were adapted from a study by King and colleagues (27) on glucocorticoid-induced expression of aquaporin-1 in developing rat lungs. Control rats were treated with 0.9% NaCl alone. In some rats, the acute effects from instillation of dexamethasone simultaneously with the 5% albumin solution were examined.

In a second set of rats, synergistic effects from pretreatment with dexamethasone and the thyroid hormone $T_3$ (dissolved in concentrated NH$_4$ then diluted 100× and pH corrected; Sigma Chemical) were investigated. The rats were injected with 0.35 mg dexamethasone/kg body wt im and 30 $\mu$g $T_3$/rat (im) on 2 consecutive days, 24 h apart, and also on the morning of the third day, the day on which the alveolar fluid clearance experiment was done. This dose and administration protocol of $T_3$ were adapted from a study by Barker and colleagues (2) on synergistic effects of glucocorticoids and thyroid hormone on newborn sheep lungs. Rats were also pretreated with 30 $\mu$g $T_3$ im alone/rat on 2 consecutive days, 24 h apart, and on the morning of the third day, the day on which the alveolar fluid clearance experiment was done.

**General Experimental Protocol**

In all rats, after surgery, a 30-min baseline of stable heart rate and blood pressure was required before fluid instillation. An instillation tubing (PE-50 catheter; Clay Adams, Becton Dickinson) was gently passed through the tracheal tube and into the left lung at the end of the 30-min baseline. Then, 1 ml of fluid (i.e., 3 ml/kg body wt with or without stimulatory or inhibitory substances) was instilled over 25 min into the left lung for the 1-h studies. The 1-h studies began at the start of instillation of the fluid. The fluid was instilled by injecting 0.04 ml/min by using a 1-ml syringe. After the instillation, the tubing was withdrawn. The location of the instilled fluid was clearly visible in the lungs by postmortem examination. At the end of the experiments, a blood sample was obtained, the abdomen was opened, and the rats were exsanguinated by transecting the abdominal aorta. The lungs were removed through a median sternotomy. An alveolar fluid sample (0.1–0.2 ml) was obtained by gently passing the sampling catheter (PE-50 catheter; Clay Adams, Becton Dickinson) into a wedged position in the instilled area of the left lung. We previously reported that fluid aspirated with a catheter wedged into the distal air spaces is a good reflection of alveolar fluid protein concentration (6). Protein concentrations in alveolar fluid samples and in plasma samples were measured by the Lowry method (29) modified for use on microtiter plates.

**Specific Protocols**

**Group 1:** Control (1-h) studies ($n = 9$).

After the 30-min baseline period, 3 ml/kg body wt of the 5% albumin solution were instilled into the left lower lobe. The rats were then studied for 1 h. After this time, the rats were exsanguinated and processed as described in General Experimental Protocol.

**Group 2:** Dexamethasone studies ($n = 13$).

The rats ($n = 9$) were pretreated for 2 consecutive days with dexamethasone as described in Glucocorticoid and Thyroid Hormone Pretreatment. Then, on the third day, the animals were anesthetized and prepared for the alveolar fluid clearance measurements. After the 30-min baseline period,
3 ml/kg body wt of the 5% albumin solution were instilled into the left lower lobe. The rats were studied for 1 h. After this time, the rats were exsanguinated and processed as described in the General Experimental Protocol.

Group 4: Dexamethasone-plus-T3 studies (n = 6). The rats were pretreated for 2 consecutive days with dexamethasone and T3 as described in Glucocorticoid and Thyroid Hormone Pretreatment. Then, on the third day, the animals were anesthetized and prepared for the alveolar fluid clearance measurements. After the 30-min baseline period, 3 ml/kg body wt of the 5% albumin solution were instilled into the left lower lobe. The rats were studied for 1 h. After this time, the rats were exsanguinated and processed as described in General Experimental Protocol.

Group 5: Effect of propranolol (10^-4 M) in dexamethasone (n = 4), dexamethasone-plus-T3-pretreated (n = 4), and control (n = 4) rats. The rats were pretreated for 2 consecutive days with saline, dexamethasone, T3, or dexamethasone plus T3 as described in Glucocorticoid and Thyroid Hormone Pretreatment. Then, on the third day, the animals were anesthetized and prepared for the alveolar fluid clearance measurements. After the 30-min baseline period, 3 ml/kg body wt of the propranolol-containing 5% albumin solution were instilled into the left lower lobe. The rats were studied for 1 h. After this time, the rats were exsanguinated and processed as described in General Experimental Protocol.

Group 6: Effect of terbutaline in dexamethasone (n = 7), dexamethasone-plus-T3-pretreated (n = 6), and control rats (n = 5). The rats were pretreated for 2 consecutive days with dexamethasone or dexamethasone plus T3 as described in Glucocorticoid and Thyroid Hormone Pretreatment. Then, on the third day, the animals were anesthetized and prepared for the fluid clearance measurements. The β2-adrenergic agonist propranolol was added to the instillate and instilled into the saline-, dexamethasone-, T3-, or dexamethasone-plus-T3-pretreated rats to determine whether the effects were mediated by an increased release of endogenous epinephrine. After the 30-min baseline period, 3 ml/kg body wt of the propranolol-containing 5% albumin solution were instilled into the left lower lobe. The rats were studied for 1 h. After this time, the rats were exsanguinated and processed as described in General Experimental Protocol.

Group 7: Effect of amiloride (10^-3 M) in dexamethasone (n = 4), dexamethasone-plus-T3-pretreated (n = 6), and control rats (n = 4). The rats were pretreated for 2 consecutive days with dexamethasone, T3, or dexamethasone plus T3 as described in Glucocorticoid and Thyroid Hormone Pretreatment. Then, on the third day, the animals were anesthetized and prepared for the fluid clearance measurements. Amiloride, a Na+ channel inhibitor, was added to the 5% albumin instillate to determine the fraction of the fluid clearance from the distal air spaces that was mediated by amiloride-sensitive Na+ channels. After the 30-min baseline period, 3 ml/kg body wt of the amiloride-containing 5% albumin solution was instilled into the left lower lobe. The rats were studied for 1 h. After this time, the rats were exsanguinated and processed as described in General Experimental Protocol.

Hemodynamics and Airway Pressure

Peak airway pressure, systemic blood pressure, and heart rate were measured by using calibrated pressure transducers (UFI model 1050BP, BioPac Systems, Goleta, CA) connected to analog-to-digital converters and amplifiers (MP100 and DA100, respectively, BioPac Systems) and continuously recorded on an IBM computer with Acknowledge 3.0 software (BioPac Systems).

Alveolar Fluid Clearance

The increase in alveolar protein concentration over 1 h was used to measure clearance of fluid from the distal air spaces, as we have done before (7, 34, 40, 44). Data on alveolar fluid clearance are shown in two ways. First, alveolar fluid clearance is presented as a ratio between the final aspirated alveolar fluid protein concentration and the instilled fluid protein concentration. The ratio of final to instilled protein concentration provides direct evidence for alveolar fluid clearance, because fluid must be removed for the final alveolar protein concentration to rise. Because there were no changes in the epithelial permeability to protein and very little protein was transported out of the air spaces in any of the groups (see RESULTS), this method is accurate for measuring fluid clearance from the distal air spaces of the lungs. The second method is based on calculating alveolar fluid clearance (AFC) from the following equation

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

where AFC is alveolar fluid clearance, Vf is instilled fluid volume, and Vt is final alveolar fluid volume calculated from the increase in the concentration of protein in the final alveolar aspirate from the instilled protein amount.

The term "alveolar," however, does not imply that all reabsorption of fluid occurs at the alveolar level; some fluid reabsorption may occur across distal bronchial epithelium (1).

Alveolar Type II Cell Proliferation

To investigate whether proliferation of alveolar epithelial type II cells occurred in response to dexamethasone injections with or without T3, four additional rats were used. One rat was used as control and was injected with 0.9% NaCl im three times during 48 h. The second rat was injected with dexamethasone (0.35 mg/kg body wt) at the same times during 48 h, and the third rat was injected with T3 (30 µg/rat). The fourth rat was injected with both dexamethasone (0.35 mg/kg body wt) and T3 (30 µg/rat) together at the same times during 48 h.

Tissue preparation. Forty hours after the first injection (38), the rats were injected with 100 mg/kg body wt of bromodeoxyuridine ip (BrDu; Boehringer Mannheim, Mannheim, Germany). Then, 48 h after the first injection, the rats were killed by an ip overdose of pentobarbital sodium mixed with heparin for anticoagulation. The abdomen was opened, and the abdominal aorta was transected to exsanguinate the animals. The chest was opened through a midline sternotomy. The lungs were perfused blood free via the pulmonary artery. When the lungs were free of blood, the perfusate solution was changed to 4% paraformaldehyde for fixation. The lungs were then left in the paraformaldehyde for 4 h and then cut into 1-cm² cubes and transferred into a 30% sucrose solution. The sucrose-immersed lung tissue was embedded in OCT (Miles, Elkhart, IN), and the tissue was snap-frozen in liquid nitrogen. Then, 4- to 5-µm sections were cut and mounted on 3-aminopropyl-triethoxysilane-coated slides (Fisher Scientific, Pittsburgh, PA). The slides were stored at −70°C until stained for BrDu.

Immunohistochemistry. Cell proliferation was determined by a BrDu assay (38) with a specific antibody. The sections were incubated in 1% H2O2 (Sigma Chemical) in phosphate-
buffered NaCl (PBS) with 0.1% Triton X-100 for 5 min and then washed with PBS. The slides were then incubated with 100 µg/ml Pronase E (Sigma Chemical) in 20 mM Tris·HCl and 20 mM CaCl₂, pH 7.6, for 10–30 min and then rinsed in PBS. To remove the DNA-binding proteins, the sections were incubated in ice-cold 0.1 N HCl for 10 min. The DNA was then denatured by incubating the sections in 2 N HCl for 30 min at 37°C, and then the acid was neutralized with 0.1 M borax (Sigma Chemical), pH 8.5, for 5–10 min. After washing in PBS, the sections were incubated in 3% horse serum in PBS (Sigma Chemical) diluted 1:50 in 3% horse serum for 1 h at room temperature. After washing of the slides in PBS, the sections were incubated with a biotinylated goat anti-mouse IgG Fc (Sigma Chemical) diluted 1:400 in 3% horse serum in PBS for 1 h at room temperature and washed. PBS, the sections were incubated in 3% horse serum in PBS for 1 h at room temperature and washed.

## RESULTS

### Effect of dexamethasone.

There were no changes in systemic blood pressure and airway pressure after fluid instillation in the dexamethasone-pretreated rats or in those that were acutely treated with dexamethasone compared with control rats or with baseline conditions in the 1-h studies.

Dexamethasone pretreatment resulted in a significant increase in the ratio of final to instilled protein concentration over 1 h compared with control rats (Fig. 1). There was a similar increase in the calculated alveolar fluid clearance (Table 1). Dexamethasone alone increased alveolar fluid clearance by 80% compared with in control rats. There was no effect on the ratio of final to instilled protein concentration or alveolar fluid clearance from the acute dexamethasone exposure compared with control rats (Table 1). Saline injections into the control rats did not affect alveolar fluid clearance compared with normal noninjected control rats (data not shown).

### Effect of T₃.

There were no changes in systemic blood pressure and airway pressure after fluid instillation in the T₃-pretreated rats compared with control rats or baseline conditions in the 1-h studies.

Dexamethasone pretreatment resulted in a significant increase in the ratio of final to instilled protein concentration over 1 h compared with control rats, but was not different from that in dexamethasone-pretreated rats (Fig. 2). There were similar increases in the calculated alveolar fluid clearance (Table 1). T₃ alone increased alveolar fluid clearance by 65% compared with in control rats.

### Table 1. Effect of dexamethasone and thyroid hormone pretreatment on alveolar liquid clearance over 1 h in anesthetized rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Alveolar Albumin Concentration, μg/dl</th>
<th>Alveolar Fluid Clearance, % of instilled volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Instilled</td>
<td>Final</td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>4.63 ± 0.48</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>9</td>
<td>4.87 ± 0.33</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pretreatment</td>
<td>9</td>
<td>4.96 ± 0.44</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>6</td>
<td>5.78 ± 0.25</td>
</tr>
<tr>
<td>T₃</td>
<td>4</td>
<td>5.62 ± 0.05</td>
</tr>
<tr>
<td>Terbutaline</td>
<td>5</td>
<td>4.99 ± 0.20</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>7</td>
<td>5.03 ± 0.89</td>
</tr>
<tr>
<td>T₃-terbutaline</td>
<td>4</td>
<td>5.96 ± 0.05</td>
</tr>
<tr>
<td>Propranolol</td>
<td>4</td>
<td>4.98 ± 0.76</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>4</td>
<td>6.66 ± 0.32</td>
</tr>
<tr>
<td>propranolol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>4</td>
<td>6.45 ± 0.29</td>
</tr>
<tr>
<td>T₃- propranolol</td>
<td>4</td>
<td>4.70 ± 0.35</td>
</tr>
<tr>
<td>Amiloride</td>
<td>6</td>
<td>4.71 ± 0.15</td>
</tr>
<tr>
<td>Dexamethasone</td>
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<td>4.80 ± 0.02</td>
</tr>
<tr>
<td>T₃-amiloride</td>
<td>6</td>
<td>5.84 ± 0.50</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>4</td>
<td>5.96 ± 0.05</td>
</tr>
</tbody>
</table>

Values are means ± SD, n. No. of rats; T₃, 3,3',5-triiodo-L-thyronine. *P < 0.05 vs. control; †P < 0.05 vs. dexamethasone; ‡P < 0.05 vs. dexamethasone-terbutaline (ANOVA with Tukey's post hoc test).
Dexamethasone enhances fluid clearance from the lung

Effect of dexamethasone in combination with T₃.
There were no changes in systemic blood pressure and airway pressure after fluid instillation in the dexamethasone-plus-T₃-pretreated rats compared with control rats or with baseline conditions in the 1-h studies. Dexamethasone-plus-T₃ pretreatment resulted in a significant increase in the ratio of final to instilled protein concentration over 1 h compared with control rats and a further increase in the above-mentioned ratio compared with dexmethylasone-pretreated animals (Fig. 2). There were similar increases in the calculated alveolar fluid clearance (Table 1). The combination of dexamethasone and T₃ stimulated alveolar fluid clearance by 132% compared with the 80% stimulation by dexamethasone alone and 65% stimulation by T₃ alone.

Effect of terbutaline and propranolol treatment.
The addition of the β-adrenergic agonist terbutaline to the instillate of control rats increased the ratio of final to instilled protein concentration to 1.44 ± 0.05 vs. 1.21 ± 0.03 for control rats and alveolar fluid clearance was increased by ~80%, similar to that after dexamethasone pretreatment (Fig. 3). However, addition of terbutaline to dexamethasone-pretreated rats did not result in a further increase in the ratio of final to instilled protein concentration (Fig. 3) nor in the alveolar fluid clearance. The combination of terbutaline and propranolol to dexamethasone- and dexamethasone-T₃-pretreated rats (ANOVA with Tukey's post hoc test).

DISCUSSION
Glucocorticoids have been implicated in stimulating alveolar fluid clearance, especially in the near-term lung (25, 45, 47), but no studies have investigated whether glucocorticoids can stimulate alveolar fluid clearance by 10.220.32.246 on July 11, 2017 http://jap.physiology.org/ Downloaded from the distal air spaces of the rats. We also determined whether fractional amiloride inhibition was different among control, dexamethasone-pretreated, and dexamethasone-plus-T₃-pretreated rats. Amiloride inhibited the increase in alveolar fluid clearance from the distal air spaces in dexamethasone-pretreated rats (Fig. 4, Table 1). Also, the fractional inhibition by amiloride was significantly greater in dexamethasone-pretreated rats than in control rats (Fig. 5).

Cell proliferation. Neither of the treatments induced an alveolar epithelial cell proliferation as assessed by the BrdU assay (data not shown).

**Fig. 2.** Alveolar fluid clearance expressed as ratio of final to instilled protein concentration in control rats (n = 9), dexamethasone (n = 9), 3,3′,5-triiodothyronine (T₃; n = 4), and dexamethasone-plus-T₃-stimulated rats (n = 6). Values are means ± SD. Dexamethasone pretreatment continued for 2 days with one intramuscular (im) injection of 0.35 mg dexamethasone/kg body wt with or without 30 µg T₃ daily. Alveolar fluid clearance was done on the 3rd day. Pretreatment with T₃ and dexamethasone resulted in a further significant stimulation of alveolar fluid clearance to 132% of control levels. T₃ pretreatment alone also stimulated alveolar fluid clearance, but to a lesser extent than dexamethasone pretreatment. *P < 0.05 compared with control rats. †P < 0.05 compared with dexamethasone- and dexamethasone-T₃-pretreated rats (ANOVA with Tukey's post hoc test).
Amiloride inhibited most (62%) of the dexamethasone- and dexamethasone-plus-T3-stimulated increase in alveolar fluid clearance. Thus most of the stimulated increase in alveolar fluid clearance probably occurred by increasing Na\(^+\) uptake through amiloride-sensitive channels in the alveolar epithelial cells. In normal rats, amiloride reduced alveolar fluid clearance by \( \sim 48\% \) similar to the fractional inhibition that has been reported previously by us (23, 26) and other investigators (4, 16, 30). After dexamethasone pretreatment, this fraction increased to 62%, suggesting a modest upregulation of the amiloride-sensitive Na\(^+\) transport pathways. The amiloride-sensitive fraction was similar in the rats pretreated with dexamethasone and T3, suggesting that T3 had limited effects on the amiloride-sensitive Na\(^+\) channels in this model. Also, T3 alone had a significantly lower amiloride-sensitive fraction (38%) than dexamethasone-treated (62%) and control animals (48%) (Fig. 5), suggesting that T3 may primarily upregulate the amiloride-insensitive fraction of alveolar fluid clearance. Also, the time required for the stimulatory effect by dexamethasone suggests that the effect occurred by transcriptional and/or translational mechanisms. Other investigators have reported that dexamethasone pretreatment increased the mRNA levels for the three subunits of the ENaC (10, 41, 47) as well as that of the translated protein in the cell membrane (41). There are also data that dexamethasone pretreatment increases the activity and expression of the basolaterally located Na\(^+\)-K\(^+\)-ATPase (3, 25). Thus it is likely that an upregulated capacity to transport Na\(^+\) across the alveolar epithelium is the mechanism responsible for the increased fluid absorption after dexamethasone pretreatment in the adult rat. Our data do not precisely determine whether it was mediated by increases in ENaC expression and function, Na\(^+\)-K\(^+\)-ATPase activity and expression, or both. The data suggest, however, that an increase in ENaC function may be a significant component because the fractional inhibition was greater after dexamethasone and dexamethasone-plus-T3 pretreatment.

One objective of these studies was to test the hypothesis that pretreatment with dexamethasone resulted in stimulation of clearance of fluid from the distal airspaces of the lung and to investigate whether the responsiveness to \( \beta \)-adrenergic stimulation was altered by the dexamethasone and the dexamethasone-plus-T3 pretreatment. We and other investigators have previously reported that \( \beta \)-stimulatory agents, growth factors, and endogenous catecholamines were associated with an increase in liquid removal from the alveolar compartment of the lung (7, 22, 26, 34, 40, 42). It has also been suggested that ENaC possesses a glucocorticoid regulatory element, potentially making it sensitive to glucocorticoid regulation (10, 41). We investigated whether the effects were secondary to endogenous epinephrine release and compared the effects of dexamethasone pretreatment on alveolar fluid clearance with the stimulatory effect of \( \beta \)-adrenergic agonists. We

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**Fig. 4.** Alveolar fluid clearance expressed as final-to-instilled protein concentration ratio in dexamethasone (n = 4), dexamethasone-plus-T3 (n = 6), T3 (n = 4), and saline-pretreated rats instilled with amiloride (10\(^{-3}\) M)-containing instillate (n = 4). Values are means ± SD. Dexamethasone pretreatment continued for 2 days with 1 im injection of 0.35 mg dexamethasone/kg body wt with or without 30 µg T3 daily. Alveolar fluid clearance experiment was done on the 3rd day. Amiloride-containing albumin solution in dexamethasone- and dexamethasone-plus-T3-pretreated rats inhibited alveolar fluid clearance to a higher degree than in control rats. Inhibition by amiloride in T3-pretreated rats did not reach statistical significance (\( P = 0.20 \)). *\( P < 0.05 \) compared with control rats; †\( P < 0.05 \) compared with rats instilled with 5% albumin solution without amiloride (ANOVA with Tukey's post hoc test).

**Fig. 5.** Percent inhibition of alveolar fluid clearance by amiloride (10\(^{-3}\) M) in dexamethasone (n = 4), dexamethasone-plus-T3 (n = 6), T3 (n = 4), and saline-pretreated rats (n = 4). Values are means ± SD. Dexamethasone pretreatment continued for 2 days with 1 im injection of 0.35 mg dexamethasone/kg body wt with or without 30 µg T3 daily. The alveolar fluid clearance experiment was done on the 3rd day. The amiloride-containing albumin solution in the dexamethasone-pretreated rats inhibited alveolar fluid clearance more than in control rats, although amiloride had less effect in T3-pretreated rats. A higher amiloride sensitivity was also observed after dexamethasone-plus-T3 pretreatment. *\( P < 0.05 \) compared with control; †\( P < 0.05 \) compared with T3-pretreated rats (ANOVA with Tukey's post hoc test).
also investigated whether addition of β-adrenergic agonists to dexamethasone- and dexamethasone-plus-T₃-pretreated rats resulted in an additional stimulation of the fluid clearance. Propranolol did not affect the dexamethasone-, T₃-, or the dexamethasone-plus-T₃-stimulated alveolar fluid clearance, so it is not likely that dexamethasone, T₃, or dexamethasone plus T₃ acted via endogenous epinephrine release. Terbutaline, the β-adrenergic agonist, and dexamethasone pretreatment were equally potent in stimulating alveolar fluid clearance. Also, terbutaline had no additional stimulatory effect on alveolar fluid clearance in dexamethasone- or in dexamethasone-plus-T₃-pretreated rats.

Could an increased expression of aquaporins in lung epithelium or endothelium increase alveolar fluid clearance? Aquaporins are present in the lung as observed in both expression and functional studies (9, 15, 19, 20, 33, 46), and steroid-pretreatment over 48 h increases aquaporin-1 expression in developing rat lungs (27). It is not known whether aquaporin-5 expression in alveolar epithelial type I cells (15) is sensitive to glucocorticoid stimulation. Even if that were the case, it is unlikely that the observed increase in alveolar fluid clearance in this study could be explained by increased aquaporin expression. Transepithelial water movement requires an osmotic driving force for water to cross the barrier created by transepithelial Na⁺ transport via amiloride-sensitive and -insensitive pathways and by basolaterally located Na⁺-K⁺-ATPase (17).

In several species, a significant fraction of the stimulatory effect from β-adrenergic agonists on alveolar fluid clearance can also be inhibited by amiloride (7, 26, 34, 40). However, because both basal and stimulated alveolar fluid clearance by β-adrenergic agonists is inhibited similarly by amiloride, the data indicate that β-adrenergic agonists increase alveolar fluid clearance by stimulation of both amiloride-sensitive and -insensitive pathways. The amiloride-insensitive fraction may be related, in part, to cation channels that are not inhibited by amiloride. Recent data (14, 43) suggest the existence of a rodtype, cyclic, nucleotide-gated cation channel in the alveolar epithelium that could be involved in fluid movement in the lung. Other pathways are possible, of course, because no definite proof exists presently concerning the makeup of the amiloride-insensitive fraction. However, the results from this study suggest that dexamethasone pretreatment affects the amiloride-sensitive fraction of alveolar fluid clearance more than it affects the amiloride-insensitive fraction. In contrast, T₃ might stimulate alveolar fluid clearance by stimulating the amiloride-insensitive fraction of Na⁺ channels more than the amiloride-sensitive fraction. These results, together with the time needed after dexamethasone exposure to reach an effect, indicate also that the amiloride-sensitive Na⁺ channel is upregulated after dexamethasone pretreatment. Indeed, amiloride-sensitive ENaC channels possess glucocorticoid regulatory element in their gene sequences that could allow these channels to be upregulated after steroid exposure (10, 41). Also, the basolaterally situated Na⁺-K⁺-ATPase can be upregulated after dexamethasone pretreatment in rats (3, 25). It is also possible that Na⁺ flux through the amiloride-sensitive Na⁺ channels is increased after dexamethasone and dexamethasone-plus-T₃ pretreatment.

The effect from dexamethasone pretreatment was not secondary to endogenous catecholamine release, because propranolol did not block the dexamethasone stimulation of alveolar fluid clearance. We investigated this possibility because several studies have reported that endogenous release of epinephrine can regulate the rate of alveolar fluid clearance (18, 28, 39, 40). However, the longer-term upregulation by dexamethasone, T₃, or dexamethasone-plus-T₃ pretreatment of alveolar fluid clearance in this study was not mediated by endogenous release of catecholamines. The small molecular size of propranolol made it likely that adequate concentrations were reached quickly at the β-adrenergic receptor sites on the basolateral side of the alveolar epithelial cells. Thus complete blockage would be expected within the 1-h experimental time. Another potential concern would be if the per se injections of dexamethasone, T₃, or dexamethasone plus T₃ would cause epinephrine release. To address this concern, we injected normal rats with 0.9% NaCl at the same times as they would have received the stimulants. We found no effects from the injections per se, indicating that no significant epinephrine release occurred. Moreover, a relatively long time period passed between the injections and the start of the fluid clearance experiments. Also, recent data show that the stimulatory effect of epinephrine is short lived in vivo (11).

Could the effect be related to increased cell proliferation after dexamethasone or dexamethasone-plus-T₃ pretreatment? Recent experiments have demonstrated that proliferation of alveolar epithelial type II cells may be correlated with an enhanced capacity to absorb alveolar fluid (21, 48). However, we did not observe an increase in cell proliferation after either of the treatments in this study.

What is the physiological role of glucocorticoids in the lung? Glucocorticoids have been implicated as regulators of fetal and neonatal lung maturation (12). For example, glucocorticoids may be involved together with β-adrenergic agonists in the regulation of reabsorption of fetal lung fluid from the air spaces at delivery and onset of breathing, as has been suggested from several molecular studies (25, 47). Glucocorticoids also upregulate both alveolar ENaC (45, 47) and Na⁺-K⁺-ATPase (3, 25) in near-term lungs, suggesting that they might affect the vectorial Na⁺ transport that is responsible for maintaining dry alveoli. In adult lung, glucocorticoids have been used in the late phase of acute respiratory distress syndrome to hasten recovery from clinical acute lung injury (32).

In summary, dexamethasone pretreatment of rats increased the rate of alveolar fluid clearance. The stimulation was further potentiated by pretreatment with dexamethasone and T₃, suggesting that an additive effect by these two hormones can occur in the adult lung, in contrast to the synergistic effect in the fetal
Pulmonary edema. Pharmacological treatment with glucocorticoids and/or thyroid hormones (T₃) may increase the basal transport capacity of the alveolar epithelium in patients with pulmonary edema.

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