Dopamine increases lung liquid clearance during mechanical ventilation

F. J. Saldías, A. P. Comellas, L. Pesce, E. Lecuona, and J. I. Sznajder. Dopamine increases lung liquid clearance during mechanical ventilation. Am J Physiol Lung Cell Mol Physiol 283: L136–L143, 2002. First published February 22, 2002; 10.1152/ajplung.00089.2000.—Short-term mechanical ventilation with high tidal volume (HVT) causes mild to moderate lung injury and impairs active Na⁺ transport and lung liquid clearance in rats. Dopamine (DA) enhances active Na⁺ transport in normal rat lungs by increasing Na⁺-K⁺-ATPase activity in the alveolar epithelium. We examined whether DA would increase alveolar fluid reabsorption in rats ventilated with HVT for 40 min compared with those ventilated with low tidal volume (LVT) and with nonventilated rats. Similar to previous reports, HVT ventilation decreased alveolar fluid reabsorption by ~50% (P < 0.001). DA increased alveolar fluid reabsorption in nonventilated control rats (by ~60%), LVT ventilated rats (by ~55%), and HVT ventilated rats (by ~200%). In parallel studies, DA increased Na⁺-K⁺-ATPase activity in cultured rat alveolar epithelial type II cells (ATII). Depolymerization of cellular microtubules by colchicine inhibited the effect of DA on HVT ventilated rats as well as on Na⁺-K⁺-ATPase activity in ATII cells. Neither DA nor colchicine affected the short-term Na⁺-K⁺-ATPase activity in alveolar epithelial cells. Thus we reason that DA improved alveolar fluid reabsorption in rats ventilated with HVT by upregulating the Na⁺-K⁺-ATPase function in alveolar epithelial cells.

Na⁺-K⁺-ATPase; alveolar fluid clearance

PULMONARY EDEMA FORMATION results from changes in hydrostatic or colloid-osmotic pressure gradients in the pulmonary circulation and/or increased alveolo-capillary barrier permeability (28). Patients with respiratory failure and pulmonary edema frequently need to be placed on mechanical ventilation. However, mechanical ventilation with high tidal volumes (HVT) may adversely affect lung function and cause or worsen lung injury (7, 9–11, 29–31). It has been reported that mechanical ventilation may cause physiological and morphological alterations similar to the diffuse alveolar damage observed in the acute respiratory distress syndrome (1, 10, 15, 19, 29, 32). Pulmonary edema resolution depends on active Na⁺ transport across the alveolar epithelium (14, 18, 26).

We previously reported that mechanical ventilation with HVT in rats decreased active Na⁺ transport and lung liquid clearance (17). Recently it was reported that dopamine (DA) placed in rat lung air spaces and/or pulmonary circulation increased active Na⁺ transport within 1 h in healthy rat lungs and Na⁺-K⁺-ATPase activity in alveolar epithelial cells within 15 min (2, 3, 16, 21). The purpose of this study was to determine the effects of DA on lung liquid clearance in mechanically ventilated rats.

MATERIALS AND METHODS

A total of 132 rat lungs were studied. Pathogen-free, male Sprague-Dawley rats weighing 280–320 g were purchased from Harlan Sprague Dawley (Indianapolis, IN). All animals were provided food and water ad libitum and maintained on a 12:12-h light-dark cycle. DA, amlodipine, ouabain, colchicine, and β-lumicolchicine were purchased from Sigma (St. Louis, MO).

Mechanical Ventilation

Adult rats were anesthetized with 50 mg of pentobarbital/kg body wt (BW) intraperitoneally, tracheotomized, and mechanically ventilated with a rodent ventilator (model 683; Harvard Apparatus, South Natick, MA). The animals were ventilated for 40 min with HVT of ~40 ml/kg, peak airway pressure of 35 cmH₂O, and respiratory rate (RR) of 40 breath/min without positive end-expiratory pressure and compared with rats ventilated with low tidal volume (LVT) for 40 min (~10 ml/kg; peak airway pressure, 8 cmH₂O; RR, 40 breath/min) and control nonventilated rats. It has been previously reported that HVT ventilation to 35 cmH₂O for 40 min causes mild to moderate lung injury and decreases alveolar epithelial fluid reabsorption in rats (17, 29).

Specific Protocols

Group A. Lung liquid clearance was measured in a control nonventilated group of rats instilled with 5 ml of buffered salt albumin (BSA) solution into the air spaces (n = 10).

Group B. Lung liquid clearance was measured in rats ventilated with LVT for 40 min and instilled with 5 ml of BSA solution into the air spaces (n = 6).

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Group C. Lung liquid clearance was measured in rats ventilated with HVT for 40 min and instilled with 5 ml of BSA solution into the air spaces \((n = 6)\).

Group D. To examine lung liquid clearance modulation by DA in rats ventilated with HVT, we instilled \(10^{-4}\) M DA into the air space of three groups of animals: HVT \((n = 6)\) and LVT \((n = 6)\) ventilated for 40 min and control nonventilated rats \((n = 8)\).

Group E. To study the role of epithelial Na\(^+\) transport on lung liquid clearance modulation by DA in control rats, we studied the effect of 1) the Na\(^+\) channel blocker amiloride \((10^{-4}\) M\) instilled into control rat air spaces with \(10^{-4}\) M DA \((n = 5)\) and without DA \((n = 6)\), and 2) the Na\(^+-K\(^{+}\)-ATPase inhibitor ouabain \((5 \times 10^{-4}\) M\) perfused through the pulmonary circulation in rats treated with \(10^{-4}\) M DA \((n = 5)\) or without DA \((n = 6)\).

Group F. To study the role of epithelial Na\(^+\) transport on lung liquid clearance modulation by DA in rats ventilated with HVT, we studied in four additional groups the effect of 1) the Na\(^+\) channel blocker amiloride \((10^{-4}\) M\) instilled into HVT air spaces with \(10^{-4}\) M DA \((n = 6)\) and without DA \((n = 6)\), and 2) the Na\(^+-K\(^{+}\)-ATPase inhibitor ouabain \((5 \times 10^{-4}\) M\) perfused through the pulmonary circulation in HVT-ventilated rats \((n = 6)\) and HVT rats treated with \(10^{-4}\) M DA \((n = 6)\).

Group G. To study the role of the cellular microtubular system on lung liquid clearance modulation by DA in HVT-ventilated rats, we measured active Na\(^+\) transport in rats treated with colchicine \((0.25\) mg/100 g BW injected intraperitoneally \(\approx 15\) h before experiments) alone or with \(10^{-4}\) M DA instilled into the air spaces \((n = 6\) in each group). We also studied the effects of DA instilled into the air space in rats treated with \(\beta\)-lumicolchicine \((0.25\) mg/100 g BW injected intraperitoneally \(\approx 15\) h before experiments; \(n = 6\) in each group). \(\beta\)-Lumicolchicine is an isomer of colchicine that does not affect the microtubular system, but it shares many properties of colchicine, such as inhibition of protein synthesis \((35)\). Accordingly, it has been used to demonstrate the physiological effects of colchicine on microtubular disruption. The effects of colchicine (as compared with lumicolchicine) on the cellular microtubules disruption have been previously reported in bile secretion studies and lung liquid clearance modulation by \(\beta\)-adrenergic agonists \((12, 25)\).

Isolated Lungs

The isolated perfused rat lung model was used as previously described \((2, 3, 17, 22–25)\). Briefly, rats were anesthetized with 50 mg/kg BW of pentobarbital, tracheotomized, and mechanically ventilated with a tidal volume of 2.5 ml, peak airway pressure of 8–10 cmH\(_2\)O, and 100% oxygen for 5 min. The chest was opened via a median sternotomy, after which 400 units of heparin sodium were injected into the right ventricle. After exsanguination, the heart and lungs were removed en bloc from the thoracic cavity. We catheterized the pulmonary artery and left atrium and flushed the pulmonary circulation of remaining blood by perfusing with BSA solution containing 135.5 mM Na\(^+\), 119.1 mM Cl\(^{-}\), 25 mM HCO\(_3\)\(^{-}\), 4.1 mM K\(^{+}\), 2.8 mM Mg\(^{2+}\), 2.5 mM Ca\(^{2+}\), 0.8 mM SO\(_4\)\(^{2-}\), 8.3 mM glucose, and 3% bovine albumin, osmolality 300 mosmol/kgH\(_2\)O. We maintained the solution at pH 7.40 by bubbling a mixture of 5% CO\(_2\) and 95% O\(_2\) as needed. Two sequential bronchoalveolar lavages (BAL) were performed with 3 ml of BSA solution containing 0.1 mg/ml Evans blue dye (EBD; Sigma), 0.02 µCi/ml \(^{22}\)Na\(^{+}\) (DuPont, NEN, Boston, MA), and 0.12 µCi/ml \(^{3}H\)mannitol (DuPont, NEN). The epithelial lining fluid volume (ELF) was estimated by the dilution of EBD in the first BAL. The lungs were then instilled with the volume necessary to leave 5 ml of BSA in the alveolar space. The lungs were not ventilated during the experimental protocol, and the airway pressures were maintained at \(\approx 0\) cmH\(_2\)O. Finally, the lungs were immersed in a “pleural bath” reservoir containing 100 ml of BSA solution maintained at 37°C. This allowed us to follow markers that had moved across the pleural membrane or were drained by the lung lymphatics.

Perfusion of the lungs was performed with 90 ml of the same BSA solution containing 0.16 mg/ml of fluorescein-tagged albumin (FITC-albumin, Sigma). The perfusate was pumped from a lower reservoir to an upper reservoir by a peristaltic pump and from there flowed through the pulmonary artery and exited via the left atrium. Pulmonary artery and left atrial pressures were maintained at 12 and 0 cmH\(_2\)O, respectively, and recorded via a pressure transducer with a zero reference point at the level of the left atrium. Pulmonary artery and left atrium pressures were recorded continuously in a multichannel recorder (Gould 3000 Oscillograph Recorder; Gould, Cleveland, OH). Pulmonary circulation pressures and flow rates were monitored continuously during the experimental protocol.

Samples were drawn from the three reservoirs: air space instillate, pleural bath, and perfusate 10 and 70 min after the experimental protocol was started. To ensure homogeneous sampling from the air spaces, we aspirated and reintroduced 2 ml of instillate into the air spaces three times before removing each sample. This has been shown to provide a reproducibly mixed sample in our laboratory \((2, 3, 17, 22–25)\). All samples were centrifuged at 1,000 g for 15 min. Colorimetric analysis of the supernatant for EBD (absorbance at 620 nm) was performed in a Hitachi model U2000 Spectrophotometer (Hitachi Instruments, San Jose, CA). Analysis of FITC-albumin (excitation 487 nm and emission 520 nm) was performed in a Perkin-Elmer fluorescence spectrometer (model LS-3B; Perkin-Elmer, Oakbrook, IL). \(^{22}\)Na\(^{+}\) and \(^{3}H\)mannitol were measured in a beta-counter (Packard Tricarb, Downers Grove, IL).

Calculations

We calculated the alveolar lining fluid volume \((V_{ELF})\) by instilling into the air spaces 3 ml of fluid \((V_0)\) containing a known concentration of albumin \((EBD)\), tagged with EBD. After a brief mixing, a sample was removed and the EBD concentration at time \(t\) \((EBD)_t\) was estimated. The amount of EBD is the same in the instillate \((V_0EBD)_0\) and in the lung after initial mixing \((V_0 + V_{ELF})EBD)\). Equating the two yields

\[
V_0EBD)_0 = (EBD)_t(V_0 + V_{ELF}) \tag{1}
\]

or

\[
V_{ELF} = V_0EBD)\_0/(EBD)\_t - V_0 \tag{2}
\]

Similarly, the alveolar fluid volume at time \(t\) is estimated by

\[
V_t = V_0EBD)\_t/(EBD)\_t \tag{3}
\]

The movement of sodium from the alveolar space during a defined period of time is assumed to be accompanied by isotonic water flux and is given by: \(J_{Na,net} = J_{Na, out} - J_{Na, in}\), where \(J_{Na, net}\) is the net or active Na\(^+\) transport, \(J_{Na, out}\) is the total or unidirectional Na\(^+\) outflux from the rat air space, and \(J_{Na, in}\) is the passive bidirectional flux of Na\(^+\) between the air space and the other compartments. The volume flux \(J = J_{Na, net}[Na^+]\) is the average rate of change in the volume and is given by

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As described by Rutschman et al. (22), the passive movement of \( {^{22}}\text{Na}^+ \), \( J_{\text{Na,in}} \), is given by

\[
J_{\text{Na,in}} = \left[ \text{Na}^- \right] \frac{J \left( \ln C(t) - \ln C(0) / (\ln V_i - \ln V_o) \right)}{t}
\]

where \( C(t) \) is the \( {^{22}}\text{Na}^+ \) concentration at time \( t \) and \( \left[ \text{Na}^- \right] \) is the constant Na\(^+\) concentration in the BSA solution.

Similarly, the mannitol flux (typically expressed as \( PA \), the permeability-surface area product) is given by

\[
PA = J \left( \ln M_o - \ln M_i / (\ln V_i - \ln V_o) \right)
\]

where \( M(t) \) is the [\(^3\)H]mannitol mass at time \( t \).

Albumin flux from the pulmonary circulation into the alveolar space was determined from the fraction of FITC-albumin that appears in the air space during the experimental protocol. These calculations were carried out for each sampling period.

### Alveolar Epithelial Type II Cells Isolation and Na\(^+-\)K\(^+\)-ATPase Activity

Alveolar epithelial type II (ATII) cells were isolated from adult rat lungs as previously described (3, 5, 23–25). The lungs were perfused via the pulmonary artery, lavaged, and digested with 30 U/ml of elastase ( Worthington Biochemical, Lakewood, NJ) for 20 min at 37°C. The tissue was minced and filtered through sterile gauze and 70-μm nylon mesh. The crude cell suspension was purified by differential adherence to immunoglobulin G-pretreated dishes, and cell viability was assessed by trypan blue exclusion (>95%). The \( \text{Na}^+ \) pump function was examined in ATII cells cultured for 48 h, pretreated with 10\(^{-5}\) M colchicine for 45 min and then treated in the presence or absence of 0.1 mM DA for 15 min at 37°C. \( \text{Na}^+\)-K\(^+\)-ATPase activity was determined as the rate of \[^{32}\text{P}]\text{ATP} \) hydrolysis of cells in a buffer containing (in mM): 5 KCl, 10 MgCl\(_2\), 1 EGTA, 50 Tris-\( \text{HCl} \), and 3 Na\(_2\)ATP (grade II; Sigma), as well as \[^{32}\text{P}]\text{ATP} \) (Amersham, Arlington Heights, IL) in trace amounts. We added NaCl to a final concentration of 100 mM to examine activity at the concentration that the \( \text{Na}^+ \) pump operates at maximal transport rate (>70 mM \( \text{Na}^+ \)). Cells were transiently permeated by thermal shock to make them permeable to \[^{32}\text{P}]\text{ATP} \) (5). The reaction was carried out for 15 min at 37°C and terminated by placement on ice and addition of TCA-charcoal to absorb nonhydrolyzed \[^{32}\text{P}]\text{ATP} \). The ouabain-insensitive ATPase activity was determined in buffer lacking \( \text{Na}^+ \) and K\(^+\) but including 1 mM ouabain. Nonspecific ATP hydrolysis was determined in samples in the absence of cells. The \(^{32}\text{P} \) liberated was quantified by liquid scintillation counting (Packard Tricarb) and expressed as nanomoles P\(_i\) per milligram of protein per minute.

### Total RNA Isolation and RT-PCR Analysis

Total cellular RNA from ATII cells incubated with 10\(^{-4}\) M DA for 15 min in the presence or absence of 10\(^{-5}\) M colchicine was isolated by use of an RNeasy total RNA kit (Qiagen, Santa Clarita, CA), as described by the manufacturer, based on the method described by Chomczynski and Sacchi (6). RNA was quantified by measurement of absorbance at 260 nm. The reverse transcriptase (RT) reaction was performed using the Superscript Preamplification System (GIBCO-BRL, Gaithersburg, MD) following the manufacturer’s instructions. Briefly, 1 μg of total RNA was converted into cDNA, after denaturing at 70°C for 15 min, by incubation with a buffer containing oligo-dT primers, the RT enzyme, and 2-deoxynucleotide 5’-triphosphate mix for 50 min at 42°C. The RT enzyme was then inactivated by incubation at 70°C for 15 min, and the RNA was removed by digestion with RNase H for 20 min at 37°C. The resultant cDNAs were amplified by polymerase chain reaction (PCR) using specific primers and analyzed by agarose gel electrophoresis. The amplified bands were quantified by densitometric scan (Eagle Eye II; Stratagene, La Jolla, CA) and normalized against the internal control gene glyceraldehyde-3-phosphate dehydrogenase (G3PDH).

The following set of oligonucleotides was used for the amplification of the Na\(^+-\)K\(^+\)-ATPase \( \beta_1 \)-subunit: 5’-AAT CAT GAA CGA GGA GAG CAG-3’ and 5’-AGG TGA GGT TGG TGA ACT GC-3’, which correspond to the positions 418–437 and 786–805, respectively, of the cDNA from the rat gene (consider number 1 the “A” of the first ATG of the gene). For the amplification of the Na\(^+-\)K\(^+\)-ATPase \( \alpha_1 \)-subunit, the following set of oligonucleotides was used: 5’-GCA GGT GTA TCA GAA CAT-3’ and 5’-CTC CGA TGG TGT GGG GTT-3’, which correspond to positions 37–54 and 1,483–1,500, respectively, of the cDNA from the rat gene (consider number 1 the “A” of the first ATG of the gene). For \( \alpha_1 \) and \( \beta_1 \)-isoforms, amplification was performed as follows: 25 cycles of 94°C \times 1 min, 53°C \times 1 min 30 s, and 72°C \times 2 min. For the control gene G3PDH, we performed the amplification using the rat G3PDH Control Amplimer Set from Clontech (Palo Alto, CA). The RT-PCR data were obtained during the exponential phase of the PCR reaction. The control gene was amplified at the same time as the Na\(^+-\)K\(^+\)-ATPase \( \alpha_1 \)- or \( \beta_1 \)-subunit gene.

### Cell Lysate and Western Blot Analysis

ATII cells cultured for 48 h were incubated with 10\(^{-4}\) M DA for 15 min in the presence or absence of 10\(^{-5}\) M colchicine at 37°C, placed on ice, and washed twice with ice-cold PBS. Cells were scraped in 1 mL of lysis buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na\(_3\)VO\(_4\), 1 μg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride; New England Biolabs) and centrifuged at 14,000 g to eliminate all the insoluble material. Protein was quantified by Bradford assay (Bio-Rad, Hercules, CA) and resolved in a 10% polyacrylamide gel. Thereafter, proteins were transferred onto nitrocellulose membranes (Optitran; Schleicher & Schuell, Keene, NH) using a semidry transfer apparatus (Bio-Rad). Incubation with specific antibodies [anti-Na\(^+-\)K\(^+\)-ATPase \( \alpha_1 \)-subunit was purchased from Upstate Biotechnology (Lake Placid, NY), anti-Na\(^+-\)K\(^+\)-ATPase \( \beta_1 \)-subunit was a generous gift of Dr. P. Martin-Vasallo (University of La Laguna, La Laguna, Spain)] was performed overnight at 4°C. Blots were developed with an enhanced chemiluminescence detection kit (ECL+; Amersham, Amersham, UK) used as recommended by the manufacturer. The bands were quantified by densitometric scan (Eagle Eye II; Stratagene).

### Data Analysis

Data are presented as mean values ± SE; \( n \) represents the number of animals in each experimental group. When comparisons were made between two experimental groups, an unpaired Student’s \( t \)-test was used. When multiple comparisons were made, a one-way analysis of variance was used, followed by a multiple comparison test (Tukey) when the \( F \) statistic indicated significance. Results were considered significant when \( p < 0.05 \).
RESULTS

Lung Permeability in Rats Ventilated with HVT

The lung permeability to small solutes ($^{22}$Na$^+$ and $[^3]$Hmannitol) increased in rats ventilated with high tidal volume (HVT) compared with low tidal volume (LVT) ventilated and control nonventilated rats, without significantly changing the movement of FITC-albumin from the pulmonary circulation into rat air space (Table 1). Concordant with previous reports, EBD-bound albumin instilled in the air space was not detected in the perfusate or bath compartments in any of the experimental groups (2, 3, 17, 22–25), confirming that HVT ventilation for 40 min did not significantly increase alveolar epithelial permeability to albumin. Epithelial lining fluid volume was similar in all experimental groups (data not shown).

Lung Liquid Clearance During HVT Ventilation

As depicted in Fig. 1, DA instilled into the air space increased lung liquid clearance ~60% above the basal level in control rat lungs. Lung liquid clearance decreased by ~50% in rats exposed to HVT ventilation for

![Graph A](image)

Table 1. Lung permeability to small solutes

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Passive Na$^+$ Flux, %</th>
<th>Mannitol Flux, %</th>
<th>Albumin Flux, ml/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ct</td>
<td>10</td>
<td>100 ± 5</td>
<td>100 ± 7</td>
<td>0.019 ± 0.009</td>
</tr>
<tr>
<td>LVT</td>
<td>6</td>
<td>91 ± 11</td>
<td>129 ± 21</td>
<td>0.012 ± 0.006</td>
</tr>
<tr>
<td>HVT</td>
<td>6</td>
<td>224 ± 6*</td>
<td>281 ± 19*</td>
<td>0.031 ± 0.007</td>
</tr>
<tr>
<td>DA</td>
<td>8</td>
<td>103 ± 10</td>
<td>120 ± 13</td>
<td>0.015 ± 0.003</td>
</tr>
<tr>
<td>LVT + DA</td>
<td>6</td>
<td>101 ± 9</td>
<td>128 ± 20</td>
<td>0.014 ± 0.007</td>
</tr>
<tr>
<td>HVT + DA</td>
<td>6</td>
<td>278 ± 16*</td>
<td>312 ± 23*</td>
<td>0.017 ± 0.004</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE. Lung permeability to $^{22}$Na$^+$ and $[^3]$Hmannitol increased in rats ventilated with high tidal volume (HVT) for 40 min. Lung permeability to albumin did not change in HVT ventilated rats. Ct, control group; LVT, low tidal volume ventilated rats; HVT, high tidal volume ventilated rats; DA, $10^{-4}$ M dopamine instilled into air space. *P < 0.01 compared with LVT and control nonventilated rats.

![Graph B](image)

Fig. 2. Amiloride (Amil, $10^{-4}$ M) instilled into the air spaces and ouabain (Oua, $5 	imes 10^{-4}$ M) perfused through the pulmonary circulation inhibited lung liquid clearance stimulated by DA ($10^{-4}$ M) in Ct rats (A) and in rats ventilated HVT for 40 min (B). Bars represent means ± SE. ***P < 0.001.

40 min but not in LVT ventilated rats. DA also increased lung liquid clearance in rats ventilated with LVT and HVT to levels similar to those in control nonventilated rats. The Na$^+$ channel blocker amiloride ($10^{-4}$ M), instilled into rat air space, and the specific Na$^+$-K$^+$-ATPase inhibitor ouabain ($5 	imes 10^{-4}$ M), perfused through the pulmonary circulation, inhibited the DA effects in control and HVT ventilated rats (Fig. 2). Pulmonary circulation pressures were maintained at 0 and 12 cmH$_2$O. Pulmonary circulation flow rates and active Na$^+$ transport are depicted in Table 2.

We examined the role of the cellular microtubular system on DA-mediated lung liquid clearance in HVT
ventilated rats. Colchicine (by depolymerizing microtubuli) inhibited the DA-mediated increase in lung liquid clearance in rats ventilated with HVT, whereas the isomer β-lumicolchicine did not affect the DA effects on lung clearance (Fig. 3). Colchicine and β-lumicolchicine did not change lung permeability to small and large solutes in any experimental group (data not shown).

Effect of DA on Na\(^{+}\)-K\(^{+}\)-ATPase in ATII Cells

As shown in Fig. 4, Na\(^{+}\)-K\(^{+}\)-ATPase activity increased ~100% over basal levels in ATII cells incubated with 10\(^{-4}\) M DA for 15 min. Pretreatment with colchicine blocked the stimulatory effect of DA on Na\(^{+}\)-K\(^{+}\)-ATPase activity. To examine whether DA treatment affected Na\(^{+}\)-K\(^{+}\)-ATPase α\(_1\) and β\(_1\) mRNA steady-state levels, we performed an RT-PCR. As shown in Fig. 5, there were no changes in Na\(^{+}\)-K\(^{+}\)-ATPase α\(_1\) and β\(_1\) mRNA steady-state levels in ATII cells incubated with 10\(^{-4}\) M DA or with 10\(^{-5}\) M colchicine for 15 min. Total Na\(^{+}\)-K\(^{+}\)-ATPase α\(_1\) and β\(_1\) protein abundance did not change under the same conditions (Fig. 6).

DISCUSSION

The effects of mechanical ventilation in the treatment of patients with acute hypoxemic respiratory failure have been the focus of several studies. Most of these studies have reported that ventilating patients and animals with HVT causes lung function to deteriorate (19, 29). Several investigators have demonstrated in a rat model that HVT ventilation caused lung injury and high permeability pulmonary edema via capillary stress failure, depletion and inactivation of surfactant components, and release of proteolytic enzymes, such as metalloproteinases and cytokines (7, 9–11, 15, 20, 27–32).

In contrast to the modest effect of static lung inflation on alveolar epithelial permeability, cyclic lung inflation during HVT ventilation caused significant lung injury (9, 10, 13, 29, 31). After short periods of HVT ventilation of rats, there were no lasting changes in lung permeability to solutes; however, higher tidal volumes, previous lung injury, or prolonged HVT ventilation caused severe damage to the alveolo-capillary barrier (7, 9–11, 17). Recently it was reported that HVT ventilation of adult rats (tidal volume of ~40 ml/kg and peak airway pressure of 35 cmH\(_2\)O) increased alveolar epithelial permeability to small solutes and impaired lung edema clearance. These changes of lung liquid clearance were similar to the changes in clearance observed during hyperoxic lung injury (17, 23, 24).
It has been previously reported that DA increased active Na\(^+\) transport in normal and hyperoxia-injured rat lungs (2, 3, 24). In the present study we examined whether DA instilled into the air spaces of HVT ventilated rat lungs would improve alveolar fluid reabsorption. In agreement with previous reports, the lung permeability to albumin did not increase significantly in rats ventilated with HVT for up to 40 min (17, 23). Thus the isolated perfused rat lung model allowed us to accurately estimate the active Na\(^+\)/H\(^+\) transport in rats ventilated with HVT (2, 3, 17, 22–25). HVT ventilation for 40 min decreased lung liquid clearance by 50% and increased alveolar epithelial permeability to small solutes compared with LVT and control nonventilated rats (see Fig. 1 and Table 1). DA increased the lung liquid clearance in rats exposed to HVT ventilation, and the stimulatory effect of DA was proportionally higher in HVT rat lungs compared with LVT and control nonventilated rat lungs (increasing by ~200, ~55, and ~60% above the basal level, respectively). Our data suggest that the alveolar epithelial damage associated with HVT ventilation for 40 min was relatively mild and similar to the hyperoxia lung injury model and did not irreversibly affect the alveolar epithelial function, thus allowing the alveolar epithelium to respond to the dopaminergic stimulation (24). We reason that HVT ventilation probably inhibited Na\(^+\)/K\(^+\)-ATPase activity by promoting its endocytosis into intracellular compartments and not permanently damaging the Na\(^+\) pump proteins (8). We also reason that DA promoted the exocytosis of the Na\(^+\) pumps back into the plasma membrane, thus restoring the lung ability to clear edema.

Additional experiments with the Na\(^+\) channel blocker amiloride, placed into the air spaces, and the Na\(^+\)/K\(^+\)-ATPase inhibitor ouabain, perfused through the pulmonary circulation, confirmed that the DA effects in HVT ventilated rat lungs were mediated by active Na\(^+\) transport modulation. Both amiloride and ouabain inhibited the stimulatory effects of DA on lung liquid clearance in control rats and rats ventilated with HVT, suggesting that DA upregulated the Na\(^+\)/K\(^+\)-ATPase activity.
Na\(^{+}\)-ATPase and Na\(^{+}\) channel function in rat alveolar epithelium (see Fig. 2). Amiloride and ouabain decreased lung liquid clearance by \(-50\%\) in control rats (see Fig. 2A) but not more than HVT alone did in HVT ventilated rats (see Fig. 2B). We propose potential explanations for these discrepancies. One possibility is that HVT ventilation inhibited the same mechanisms that amiloride and ouabain inhibit, namely the Na\(^{+}\) channels and Na\(^{+}\)-K\(^{+}\)-ATPase. In that case, amiloride and ouabain would not inhibit clearance more than the effects of HVT alone. Another explanation is that our physiological assessment of clearance is not very precise when the changes in liquid clearance are very low, in the \(\sim 2\% - 5\%\) range of the total fluid instilled in the lungs.

Alveolar epithelial Na\(^{+}\)-K\(^{+}\)-ATPase may be regulated at different levels, including transcription, translation, translocation from intracellular pools to the plasma membrane, and conformational changes of Na\(^{+}\) pump at the plasma membrane (4, 5). It has been proposed that DA effects could be mediated by the recruitment of Na\(^{+}\) pumps from intracellular pools to the basolateral membranes of ATII cells (16, 21, 24). Thus we also examined whether the DA effects could be explained by translocation of ion-transporting proteins from intracellular pools into the plasma membrane in rats ventilated with HVT. Disruption of the cellular microtubular system by colchicine inhibited the stimulatory effects of DA in HVT ventilated rat lungs, whereas the isomer \(\alpha\)-colchicine but does not depolymerize microtubules, did not inhibit the DA effects (see Fig. 3). We then studied the effect of DA on Na\(^{+}\)-K\(^{+}\)-ATPase function in ATII cells. Short-term exposure of ATII cells to DA (15 min) increased Na\(^{+}\) pump activity by \(-100\%\) above the control group (see Fig. 4). The disruption of the microtubular system by colchicine inhibited the stimulatory effects of DA on Na\(^{+}\)-K\(^{+}\)-ATPase activity in ATII cells without changing \(\alpha_1\) and \(\beta_1\) mRNA and total protein levels (see Figs. 4–6).

In summary, our data suggest that DA improves the lung's ability to reabsorb fluid in rats ventilated with HVT. During mechanical ventilation, lung liquid clearance and active Na\(^{+}\) transport modulation by DA were probably mediated by the recruitment and translocation of ion-transporting proteins from intracellular pools to the cell plasma membrane in rat alveolar epithelium and not by the de novo synthesis of Na\(^{+}\) pumps.

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REFERENCES


