Catecholamines increase lung edema clearance in rats with increased left atrial pressure

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Received 3 May 2000; accepted in final form 12 September 2000

Azzam, Zaher S., Fernando J. Saldivas, Alejandro Comellas, Karen M. Ridge, David H. Rutschman, and Jacob I. Sznapjder. Catecholamines increase lung edema clearance in rats with increased left atrial pressure. J Appl Physiol 90: 1088–1094, 2001.—During hydrostatic pulmonary edema, active Na+ transport and alveolar fluid reabsorption are decreased. Dopamine (DA) and isoproterenol (ISO) have been shown to increase active Na+ transport in rat lungs by upregulating Na+-K+-ATPase in the alveolar epithelium. We studied the effects of DA and ISO in isolated rat lungs with increased left atrial pressure (Pla = 15 cmH2O) compared with control rats with normal Pla (Pla = 0). Alveolar fluid reabsorption decreased from control value of 0.51 ± 0.02 to 0.27 ± 0.02 ml/h when Pla was increased to 15 cmH2O (P < 0.001). DA and ISO increased the alveolar fluid reabsorption back to control levels. Treatment with the D1 antagonist SCH-23390 inhibited the stimulatory effects of DA (0.30 ± 0.02 ml/h), whereas fenoldopam, a specific D1-receptor agonist, increased alveolar fluid reabsorption in rats exposed to Pla of 15 cmH2O (0.47 ± 0.04 ml/h). Propranolol, a β-adrenergic-receptor antagonist, blocked the stimulatory effects of ISO; however, it did not affect alveolar fluid reabsorption in control or DA-treated rats. Amiloride (a Na+ channel blocker) and ouabain (a Na+-K+-ATPase inhibitor), either alone or together, inhibited the stimulatory effects of DA. Colchicine, which disrupts the cellular microtubular transport of ion-transporting proteins to the plasma membrane, inhibited the stimulatory effects of DA, whereas the isomer β-lumicolchicine did not block the stimulatory effects of DA. These data suggest that DA and ISO increase alveolar fluid reabsorption in a model of increased Pla by regulating active Na+ transport in rat alveolar epithelium. The effects of DA and ISO are mediated by the activation of dopaminergic D1 receptors and the β-adrenergic receptors, respectively.

METHODS AND MATERIALS

Isolated-perfused lung. The lungs and heart of rats anesthetized with 50 mg/kg body wt of intraperitoneal pentobarbital were removed en bloc after a 10-min ventilation with 100% O2 and anticoagulation with heparin as previously described (23, 28). The pulmonary artery and left atrium were catheterized and perfused continuously with a solution of 3% bovine serum albumin (BSA) in buffered physiological salt solution (135.5 mM Na+, 119.1 mM Cl−, 25 mM HCO3−, 4.1 mM K+, 2.8 mM Mg2+, 2.5 mM Ca2+, 0.8 mM SO42−, 8.3 mM glucose). Trace amounts of fluorescein-labeled (FITC) active sodium transport; cytoskeleton

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albumin were also added to the perfusate. The recirculating volume of the constant-pressure perfusion system was 90 ml; arterial and venous pressures were set at 15 and 0 cmH2O, respectively, in the group of rats with normal Pla and 20 and 15 cmH2O, respectively, in the increased Pla study groups. The vascular pressures were recorded every 10 s with a multichannel recorder (Cyber Sense, Nicholasville, KY). The lungs were immersed in a “pleural” bath (100 ml) filled with the same BSA solution. The entire system was maintained at 37°C in a water bath. Perfusion pH was maintained at 7.40 by bubbling with a gas mixture of 95% O2-5% CO2. The lungs were then instilled via tracheal cannula in two sequential phases with a total of 5-ml volume of the BSA solution containing 0.125 μCi/ml of 22Na+ (Du Pont-NEN, Boston, MA) and 0.025 μCi/ml of [3H]mannitol (Du Pont-NEN). Samples were taken from the instillate, perfusate, and bath solutions after an equilibrium time of 10 min from the instillation and 60 min later. To ensure a homogenous sampling of the instillate, a volume of 2 ml was aspirated and reintroduced into the air spaces three times before each sample was removed. All samples were centrifuged at 3,000 g for 10 min. Absorbance analysis of the supernatant or EBD albumin was performed at 620 nm in a Hitachi model U2000 spectrometer (Hitachi, San Jose, CA). Analysis of FITC-albumin (excitation 487 nm and emission 520 nm) was performed in a Perkin-Elmer fluorometer (model LS-3B, Perkin-Elmer, Oakbrook, IL). Scintillation counts (for 22Na+ and [3H]mannitol) were measured in a Beckman beta counter (model LS 6500, Beckman Instruments, Fullerton, CA).

Calculations. The mathematical calculation of lung liquid clearance, the movement of sodium, and the flux of mannitol were described elsewhere (23). Briefly, the amount of instilled EBD-albumin was measured at 620 nm in the increased Pla study groups.

\[
V_d[EBD]_0 = V_i[EBD]_l \
V_i = V_d[EBD]_0/[EBD]_l \
J = (V_i - V_o)/t
\]

where \(V_o\) is the initial known volume instilled into rat air spaces containing a known concentration of Evans blue dye-albumin ([EBD]0) and [EBD] are the alveolar fluid volume and EBD concentration in the instillate at time \(t\), respectively; and \(J\) is the volume flux during a time period \(t\).

The sodium concentration is equal and constant in all the compartments, and because 22Na+ is instilled only in the air space, the disappearance of the radioactive tracer from the air spaces reflects the total or unidirectional Na+ outflux from the air space \(J_{Na,out}\). The passive or bidirectional Na+ flux between the air space and the other compartments is the difference between the unidirectional \(J_{Na,out}\) and active Na+ outflux \(J_{Na,net} = [Na^+]J\). The passive sodium movement can be calculated by

\[
J_{Na,in} = [Na^+]d(\ln C_i - \ln C_o)/(\ln V_i - \ln V_o)
\]

where \(C_i\) and \(C_o\) are the concentrations of 22Na+ initially and at time \(t\), respectively, and \([Na^+]\) is the constant Na+ concentration in the buffered salt albumin solution.

Similarly, the mannitol flux (typically expressing the surface area permeability \(P_A\)) is given by

\[
P_A = J(\ln M_i - \ln M_o)/(\ln V_i - \ln V_o)
\]

where \(M_i\) and \(M_o\) are [3H]mannitol concentrations initially and at time \(t\), respectively.

The fraction of FITC-albumin that appears in the alveolar space during the experimental protocol was used to calculate the albumin flux from the pulmonary circulation into the alveolar space.

Study groups. One hundred nineteen specific pathogen-free male Sprague-Dawley rats (275-325 g) were acquired from Harlan Sprague Dawley (Indianapolis, IN). DA, ISO, SCH-23390, colchicine, and β-lymocolchicine were purchased from Sigma Chemical. Ouabain and propranolol were purchased from RBI (Natick, MA), and fenoldopam was generously provided by Neurex (Menlo Park, CA).

Control group of rats studied at Pla 0 cmH2O (n = 7) and Pla 15 cmH2O (n = 7). Rats were instilled with 10-4 M DA into the air space at Pla 0 cmH2O (n = 8) and Pla 15 cmH2O (n = 7). Rats were perfused with 10-6 M ISO through the pulmonary circulation at Pla 0 cmH2O (n = 6) and Pla 15 cmH2O (n = 6).

To evaluate the dopaminergic D1-receptor pathway, we studied alveolar fluid reabsorption by instilling 10-6 M fenoldopam, a specific D1-receptor agonist, into rat air spaces exposed to Pla 15 cmH2O (n = 6). We also studied the effect of dopaminergic receptor-1 antagonist SCH-23390 by instilling rat lungs with 10-4 M SCH-23390 into the air space at Pla 15 cmH2O either alone (n = 3) or with 10-4 M DA (n = 4).

To evaluate the β-adrenergic pathway, we instilled the β-adrenergic blocker 10-4 M propranolol into the rat air spaces at Pla 15 cmH2O either alone (n = 5) or in the presence of 10-4 M ISO (n = 5).

To examine the contributory role of the amiloride-sensitive Na+ pathways and basolateral Na+ and K+-ATPase on dopaminergic effects, we instilled 10-4 M amiloride (Na+ channel blocker) into the rat air spaces (n = 4) and perfused the lungs with 5 × 10-4 M ouabain (Na+-K+-ATPase blocker) either alone (n = 7) or both agents together (n = 6) in rat lungs exposed to Pla 15 cmH2O and in the presence of DA (amiloride + DA, n = 4, ouabain + DA, n = 6, and both antagonists with DA, n = 4) in lungs with Pla 15 cmH2O.

To evaluate the role of cell microtubular transport system on alveolar fluid reabsorption modulation by dopamine, we studied rats treated with colchicine (0.25 mg/100 g body wt) injected intraperitoneally 15 h before the experiments at Pla 15 cmH2O either alone (n = 5) or with 10-4 M DA (n = 6) instilled into the rat air spaces. We also studied the effects of 0.25 mg/100 g body wt of β-lymocolchicine injected intraperitoneally 15 h before the experiments at Pla 15 cmH2O either alone (n = 4) or with 10-4 M DA (n = 4) instilled into the rat air spaces. β-Lymocolchicine is an isomer of colchicine that does not bind tubulin and does not depolymerize microtubules; however, it shares other properties of colchicine, such as inhibition of protein synthesis (37).

Statistical analysis. Data are presented as means ± SE; n is the number of animals in each study group. One-way analysis of variance was used when multiple comparisons were made followed by a multiple comparison test (Tukey’s) when the F statistic indicated significance. Results were considered significant when \(P < 0.05\).

RESULTS

Alveolar fluid reabsorption. As depicted in Fig. 1, alveolar fluid reabsorption in rat lungs exposed to Pla of 15 cmH2O was decreased by ~45% compared with control rats with Pla of 0 cmH2O (from 0.51 ± 0.02 to 0.27 ± 0.02 ml/h, \(P < 0.001\)). Treatment with DA or ISO restored alveolar fluid reabsorption to control lev-
DOPAMINE INCREASES ALVEOLAR FLUID REABSORPTION

Fig. 1. Dopamine (DA) and isoproterenol (ISO) increased alveolar fluid reabsorption in rat lungs exposed to left atrial pressure (Pla) of 0 (left) and 15 cm H₂O (right). Bars represent means ± SE. *P < 0.001 vs. control (CT) group with Pla 0 cm H₂O; †P < 0.001 vs. control group with Pla 0 cm H₂O.

To determine whether the effects of DA in rat lungs exposed to increased Pla occurred via D₁ receptors, 10⁻⁶ M fenoldopam (a specific D₁ agonist) were instilled into the air spaces. Fenoldopam increased alveolar fluid reabsorption to control levels (0.47 ± 0.04 ml/h). As shown in Fig. 2A, instillation of the specific D₁ antagonist SCH-23390 in the rat air spaces prevented the DA-mediated increase in alveolar fluid reabsorption. As depicted in Fig. 2B, the instillation of the β-adrenergic-receptor antagonist 10⁻⁴ M propranolol inhibited the stimulatory effects of ISO; however, in DA-treated lungs, propranolol did not affect the rate of alveolar fluid reabsorption.

As shown in Fig. 3, instillation of 10⁻⁴ M amiloride into the air spaces and/or the perfusion of 5 × 10⁻⁴ M ouabain in the pulmonary circulation inhibited alveolar fluid reabsorption in rat lungs with Pla of 15 cm H₂O. These data suggest that DA increased alveolar fluid reabsorption in this model by regulating the amiloride-sensitive Na⁺ pathways and Na⁺-K⁺-ATPase in the alveolar epithelium.

As depicted in Fig. 4, alveolar fluid reabsorption was decreased in rats pretreated with either colchicine or β-lumicolchicine and then exposed to increased Pla.
compared with control rats (from 0.51 to 0.18 ± 0.07 to 0.34 ± 0.01 ml/h). Treatment with DA increased alveolar fluid reabsorption in β-lumicolchicine-treated rats to 0.50 ± 0.04 ml/h but had no effects in the colchicine-treated rats.

**Epithelial permeability.** As shown in Table 1, alveolar epithelial permeability to the small solutes, as measured by $^{22}$Na$^+$ and [3H]$^+$mannitol flux, was significantly increased in the rat lungs exposed to Pla of 15 cmH$_2$O compared with control rats. The movement of FITC-albumin from the pulmonary circulation into the air spaces was slightly increased in all rat lungs exposed to Pla 15 cmH$_2$O compared with control rats (P$_{la}$ 0 cmH$_2$O).

The pulmonary circulation flow rate did not change among the different study groups (Table 1). The Na$^+$ concentration was $\sim$135 meq/ml in all compartments: instillate, perfusate, and pleural bath.

**DISCUSSION**

During congestive heart failure with increased Pla, patients can develop cardiogenic pulmonary edema. Recently, it has been shown that, in animal models of increased pulmonary vascular pressures, there is not only increased edema formation but also impairment of alveolar fluid reabsorption (7, 22, Saldias et al., unpublished observations). Additionally, in some patients with hydrostatic pulmonary edema, the alveolar fluid reabsorption was preserved, whereas in other patients, the ability to clear edema was impaired (35). We reason that, in patients suffering from chronic heart failure, the neurohumoral axis is activated, which results in increased endogenous catecholamines (8, 10, 30). Thus it is possible that, in patients with increased levels of endogenous catecholamines, alveolar fluid reabsorp-
Table 1. Alveolar epithelial permeability to small solutes

<table>
<thead>
<tr>
<th>Left Atrial Pressure, cmH₂O</th>
<th>Sodium Flux, ml/h</th>
<th>Mannitol Flux, ml/h</th>
<th>Albumin Flux, ml/h</th>
<th>Perfusate Flow, ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>1.07 ± 0.16*</td>
<td>0.52 ± 0.03*</td>
<td>0.04 ± 0.01†</td>
</tr>
<tr>
<td>Dopamine</td>
<td>15</td>
<td>1.89 ± 0.27±</td>
<td>0.94 ± 0.21</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>Fenoldopam</td>
<td>15</td>
<td>2.01 ± 0.17±</td>
<td>0.84 ± 0.09</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>15</td>
<td>1.54 ± 0.06±</td>
<td>0.69 ± 0.09</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td>SCH-23390</td>
<td>15</td>
<td>2.50 ± 0.30±</td>
<td>1.05 ± 0.17</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>SCH-23390 + dopamine</td>
<td>15</td>
<td>1.89 ± 0.31±</td>
<td>1.04 ± 0.15</td>
<td>0.14 ± 0.01</td>
</tr>
<tr>
<td>Propranolol</td>
<td>15</td>
<td>2.57 ± 0.31±</td>
<td>0.97 ± 0.17</td>
<td>0.13 ± 0.03</td>
</tr>
<tr>
<td>Dopamine + propranolol</td>
<td>15</td>
<td>1.91 ± 0.35±</td>
<td>0.78 ± 0.09</td>
<td>0.13 ± 0.03</td>
</tr>
<tr>
<td>Isoproterenol + propranolol</td>
<td>15</td>
<td>2.19 ± 0.31±</td>
<td>1.08 ± 0.17</td>
<td>0.13 ± 0.03</td>
</tr>
<tr>
<td>Amiloride</td>
<td>15</td>
<td>1.87 ± 0.21±</td>
<td>0.88 ± 0.021</td>
<td>0.15 ± 0.03</td>
</tr>
<tr>
<td>Dopamine + amiloride</td>
<td>15</td>
<td>1.61 ± 0.13±</td>
<td>0.70 ± 0.08</td>
<td>0.10 ± 0.02</td>
</tr>
<tr>
<td>Ouabain</td>
<td>15</td>
<td>2.04 ± 0.31±</td>
<td>1.13 ± 0.19</td>
<td>0.18 ± 0.02</td>
</tr>
<tr>
<td>Dopamine + ouabain</td>
<td>15</td>
<td>1.76 ± 0.15±</td>
<td>0.74 ± 0.06</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>Ouabain + amiloride</td>
<td>15</td>
<td>1.58 ± 0.07±</td>
<td>0.58 ± 0.06</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>Dopamine + ouabain + amiloride</td>
<td>15</td>
<td>1.83 ± 0.08±</td>
<td>0.71 ± 0.12</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>Colchicine</td>
<td>15</td>
<td>2.12 ± 0.24±</td>
<td>0.94 ± 0.10</td>
<td>0.21 ± 0.02</td>
</tr>
<tr>
<td>Dopamine + colchicine</td>
<td>15</td>
<td>1.89 ± 0.26±</td>
<td>0.95 ± 0.23</td>
<td>0.19 ± 0.03</td>
</tr>
<tr>
<td>Lumicolchicine</td>
<td>15</td>
<td>1.71 ± 0.38±</td>
<td>0.69 ± 0.04</td>
<td>0.19 ± 0.03</td>
</tr>
<tr>
<td>Dopamine + lumicolchicine</td>
<td>15</td>
<td>1.84 ± 0.06±</td>
<td>0.70 ± 0.01</td>
<td>0.13 ± 0.04</td>
</tr>
</tbody>
</table>

Values are means ± SE. Passive movement of small solutes across the epithelial barrier of rat lungs exposed to left atrial pressure (Pla) of 15 cmH₂O did not change significantly in study groups vs. control rat lungs with increased Pla. However, they were increased significantly in control and dopamine (DA) groups with Pla 15 cmH₂O vs. rat lungs with normal pulmonary circulation pressures. Albumin movement increased significantly in control and DA groups with Pla 15 cmH₂O vs. rat lungs with Pla 0 cmH₂O. In rat lungs with Pla 15 cmH₂O, there were no significant differences in albumin flux (P > 0.05) among study groups. Perfusate flow did not change significantly in all study groups (P > 0.05). *P < 0.05 vs. rat lungs with Pla 15 cmH₂O. †P < 0.01 vs. rat lungs with Pla 15 cmH₂O.
alveolar fluid reabsorption. Our data agree with reports in sheep (7, 11) and rats (Saldias et al., unpublished observations) in which the rate of alveolar fluid reabsorption was impaired when hydrostatic pulmonary circulation pressures were increased.

The epithelial permeability to small solutes and to albumin was slightly increased compared with rat groups with normal Pla. These observations are similar to studies in which increased permeability of tracers across the alveolo-capillary barrier was reported when Pla increased (19, 34, 36). We reason that this is possibly due to capillary stress failure or stretch pore phenomena that may transiently affect the permeability of the alveolo-capillary barrier (2, 36).

This report demonstrates the beneficial effects of DA and ISO by improving alveolar fluid reabsorption in a model of increased hydrostatic pulmonary circulation pressures. Left heart failure with resultant decrease in effective arterial volume is characterized mainly by decreased cardiac output and left atrial enlargement (1, 14). Consequently, this may lead to increased levels of atrial natriuretic peptides, angiotensin II, endothelin, and possibly endogenous ouabain-like substances (12, 15, 24, 30). It has been reported that atrial natriuretic peptides instilled in the rat air spaces decreased the active Na\(^+\) transport and alveolar fluid reabsorption (18, 33). Also, endogenous ouabain-like substances may decrease alveolar fluid reabsorption by inhibiting alveolar Na\(^+\)-K\(^+\)-ATPase. We reason that DA and ISO can counteract these decreases in active Na\(^+\) transport and increase alveolar fluid reabsorption.

In summary, we report that DA and ISO increase alveolar fluid reabsorption in a model of increased Pla. The effects of DA are mediated by the activation of dopaminergic D\(_1\) receptors, whereas the effects of ISO are mediated by the activation of the β-adrenergic receptors that cause an upregulation of the amiloride-sensitive Na\(^+\) pathways and Na\(^+\)-K\(^+\)-ATPase. This new information is of potential clinical relevance in the treatment of patients with cardiogenic pulmonary edema.

This study was supported in part by National Heart, Lung, and Blood Institute Grants HL-48129 and HL-65161, National Research Service Award HL-09806, and Pontificia Universidad Catolica de Chile FONDECYT 1990515.

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