Dopamine Restores Lung Ability to Clear Edema in Rats Exposed to Hyperoxia

FERNANDO J. SALDÍAS, EMILIA LECUONA, ALEJANDRO P. COMELLAS, KAREN M. RIDGE, and JACOB I. SZNAJDER

Division of Pulmonary and Critical Care Medicine, Michael Reese Hospital, University of Illinois at Chicago, Chicago, Illinois; and Departamento de Enfermedades Respiratorias, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile

Exposure to hyperoxia causes lung injury, decreases active sodium transport and lung edema clearance in rats. Dopamine (DA) increases lung edema clearance by stimulating vectorial Na^+ flux and Na,K-ATPase function in rat alveolar epithelium. This study was designed to test whether DA (10^{-5} M) would increase lung edema clearance in rats exposed to 100% O_2 for 64 h. Active Na^+ transport and lung edema clearance decreased by approximately 44% in rats exposed to acute hyperoxia ($p < 0.001$). DA increased lung edema clearance in room air breathing rats (from 0.50 ± 0.02 to 0.75 ± 0.06 ml/h) and in rats exposed to 100% O_2 (from 0.28 ± 0.03 to 0.67 ± 0.03 ml/h). Disruption of cell microtubular transport system by colchicine blocked the stimulatory effect of DA on active Na^+ transport in control and hyperoxic rats, whereas the isomer β-lumicolchicine, which does not affect cell microtubular transport, did not inhibit the stimulatory effects of dopamine. The Na,K-ATPase α_1-subunit protein abundance increased in the basolateral membranes of alveolar type II (ATII) cells incubated with 10^{-5} M DA for 15 min, probably by recruiting Na^+ pumps from intracellular pools. Colchicine, but not β-lumicolchicine, prevented the recruitment of α_1 subunits to the plasma membrane by DA. Accordingly, DA restored lung ability to clear edema in hyperoxic-injured rat lungs. Conceivably, dopamine induces recruitment of Na^+ pumps from intracellular pools to the plasma membrane of alveolar epithelial cells and thus increases lung edema clearance. Saldías FJ, Lecuona E, Comellas AP, Ridge KM, Sznajder JI. Dopamine restores lung ability to clear edema in rats exposed to hyperoxia.

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The outcome of patients with acute hypoxemic respiratory failure improves when lung epithelial function is restored and pulmonary edema resolves (1–3). Pulmonary edema is cleared out of the alveoli by active Na^+ transport (4–6). Na^+ is transported across the alveolar epithelium predominantly by apical amiloride-sensitive sodium channels (7, 8) and basolaterally located Na,K-ATPases (9, 10).

Adult rats exposed to 100% oxygen develop severe lung injury after approximately 60 h and usually die after approximately 72 h owing to respiratory failure and pulmonary edema (11–14). It has been reported in this model that approximately 50% of capillary endothelial cells are damaged causing an increase in lung permeability, whereas the alveolar epithelium is more resistant to oxidant injury (11, 14). We have previously reported that rats exposed to 100% oxygen for 64 h had decreased ability to clear edema in the isolated-perfused lung model, in association with decreased Na,K-ATPase activity in alveolar epithelial type II (ATII) cells isolated from the same rats (15). Other studies have also shown a marked inhibition in the Na,K-ATPase function (16, 17) and alveolar epithelial Na^+ transport by oxygen free radicals (18, 19).

Dopamine (DA) has been reported to increase active sodium transport and lung edema clearance by stimulating the Na,K-ATPase function in the alveolar epithelium of normal rats (20). Also, it has been shown that β-adrenergic agonists stimulate lung edema clearance in rats exposed to acute hyperoxia for 40 and 60 h (21, 22).

This study was designed to evaluate whether DA increases lung edema clearance in rats exposed to 100% oxygen for 64 h. In the isolated-perfused rat lung model, we demonstrate that DA instilled into the airspaces restores lung ability to clear edema in hyperoxic-injured rat lungs. Additional studies with colchicine and β-lumicolchicine in isolated rat lungs and cultured ATII cells suggest that dopamine effects are probably mediated by recruitment of Na,K-ATPase proteins from intracellular pools to the plasma membrane of the alveolar epithelium.

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Correspondence and requests for reprints should be addressed to J. I. Sznajder, M.D., Department of Medicine, Michael Reese Hospital, and Medical Center, 2929 S. Ellis Avenue, Baum 101, Chicago, IL 60616.

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METHODS

A total of 128 rats were studied. Pathogen-free, male, Sprague-Dawley rats weighing 280 to 320 g were purchased from Harlan Sprague-Dawley, Inc. (Indianapolis, IN). The animals were provided food and water *ad libitum* and maintained on 12 h:12 h light–dark cycle. Dopamine, ouabain, colchicine, and β-lumicolchicine were purchased from Sigma Chemical Co. (St. Louis, MO).

Specific Protocols

Fifty-two room air breathing rats were studied in four groups:

Group A: control group in which 5 ml buffered salt albumin (BSA) solution was instilled into the airspaces (n = 10).

Group B: 10^{-5} M DA was added to BSA solution instilled into the airspaces (n = 6).

Group C: To evaluate the active Na^+ transport pathway, we studied the effect of the Na,K-ATPase antagonist ouabain (5×10^{-4} M) perfused through the pulmonary circulation alone or associated with 10^{-5} DA instilled into the airspace (n = 6 in each group).

Group D: We evaluated the possible role of intracellular microtubular transport system on active Na^+ transport stimulated by DA. We studied lung liquid clearance in rats treated with colchicine (0.25 mg/100 g body weight injected intraperitoneally approximately 15 h before the experiments) alone (n = 6) or associated with 10^{-5} M DA added into the airspace (n = 6). Finally, we studied the effects of β-lumicolchicine (0.25 mg/100 g body weight injected intraperitoneally approximately 15 h before experiments) with and without dopaminergic stimulation (n = 6 in each group). Lumicolchicine is an isomer of colchicine that does not bind tubulin and does not depolymerize microtubules (23). However, it shares other properties of colchicine, such as inhibition of protein synthesis, and it is therefore an appropriate control to demonstrate that the observed effects of colchicine are caused by microtubular disruption. The inhibitory effect of colchicine but not lumicolchicine on microtubular transport has been previously reported on bile secretion studies and lung edema clearance modulation by β-adrenergic agonists in rats (24, 25).

Fifty-two rats were exposed to 100% O_2 for 64 h, maintained in a $68 \times 99 \times 83$ cm forced air environmental chamber. Oxygen concentration in the chamber was continuously monitored with an Oxycheck Critikon (McNeil Laboratories, Irvine, CA). After 64 h of oxygen exposure, the rats were studied in four groups:

Group E: control group instilled with 5 ml BSA solution into the airspaces (n = 10).

Group F: 10^{-5} M DA was added to BSA solution instilled into the airspaces (n = 6).

Group G: We studied the effect of the Na,K-ATPase antagonist ouabain (5×10^{-4} M) perfused through the pulmonary circulation alone or associated with 10^{-5} M DA instilled into airspace (n = 6 in each group).

Group H: We examined the role of cell microtubular transport system on hyperoxic rats. We studied lung liquid clearance in rats treated with colchicine (0.25 mg/100 g body weight injected intraperitoneally approximately 15 h before the isolated-perfused rat lung experiments) alone or associated with 10^{-5} M DA added into rat airspace. Finally, we studied the effect of β-lumicolchicine in rat lungs with and without dopaminergic stimulation (n = 6 in each group).

Isolated Lungs

The isolated lung preparation was performed as previously described (6, 15, 20, 25). Briefly, rats were anesthetized with 50 mg/kg body weight of pentobarbital, tracheotomized, and mechanically ventilated with a tidal volume of 2.5 ml, peak airway pressure of 8 to 10 cm H_2O, and 100% oxygen for 5 min. The chest was opened via a median sternotomy, after which 400 U heparin sodium was injected into the right ventricle. After exsanguination, the heart and lungs were removed en bloc. The pulmonary artery and left atrium were catheterized, and the pulmonary circulation was flushed 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, 3% bovine albumin, and osmolality of 300 mOsm/kg H_2O. The solution was maintained 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 volume of the epithelial lining fluid (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 in the alveolar space. Finally, the lungs were immersed in a "pleural bath" reservoir containing 100 ml 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 fluorescein isothiocyanate–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 cm H_2O and recorded via a pressure transducer with a zero reference point at the level of the left atrium. Pulmonary artery and left atrial pressures were recorded continuously with a multichannel recorder (Gould 3000 Oscillograph Recorder; Gould Inc., Cleveland, OH). Pulmonary circulation pressures and flow rates were measured periodically during the experiments.

Samples were drawn from the three reservoirs: airspace instillate, "pleural bath," and perfusate at 10 and 70 min after starting the experimental protocol. To ensure homogeneous sampling from the airspaces, 2 ml of instillate were aspirated and reintroduced into the airspaces three times before removing each sample. This has been shown to provide a reproducibly mixed sample in our laboratory and in previous work (6, 15, 20, 25). All samples were centrifuged at $1{,}000 \times g$ for 15 min. Colorimetric analysis of the supernatant for EBD (absorbance at 620 nm) was performed in a Hitachi Model U2000 Spectrophotometer (Hitachi Inst., 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 betacounter (Packard Tricarb, Downers Grove, IL). Sodium concentration was measured in an automated microprocessor controlled analyzer employing the ion-selective electrode technique (Lytening 1; AMDEV, Danvers, MA).

Calculations

The alveolar lining fluid volume (V_{ELF}) was calculated by instilling 3 ml of fluid (V_0) containing a known concentration of albumin $(EBD)_0$, tagged by EBD into the airspace. After 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 $[V_0(EBD)_0]$ and in the lung after initial mixing $[(V_0 + V_{ELF})(EBD)_t]$. Equating the two yields:

$$V_0(EBD)_0 = (EBD)_t(V_0 + V_{ELF}) \quad (1)$$

or

$$V_{ELF} = V_0(EBD)_0/(EBD)_t - V_0. \quad (2)$$

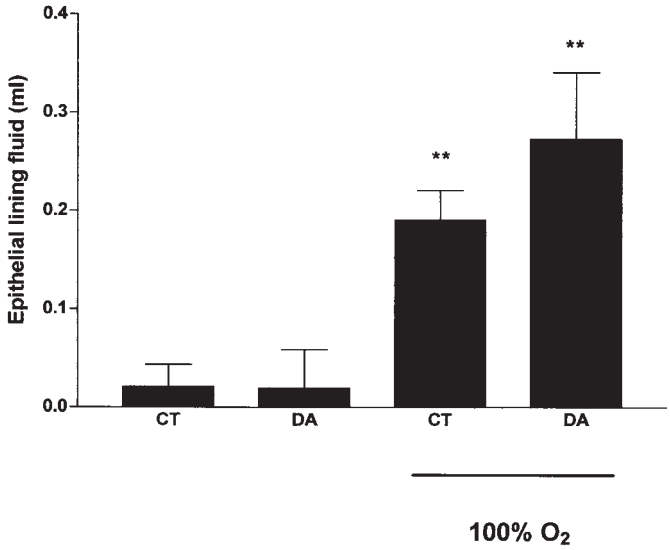

Figure 1. ELF volume increased significantly in rats exposed to 100% oxygen for 64 h. *Bars* represent means ± SEM. **p < 0.01 compared with room air breathing rats. CT: control group (n = 10); DA: 10^{-5} M dopamine instilled into airspace (n = 6).

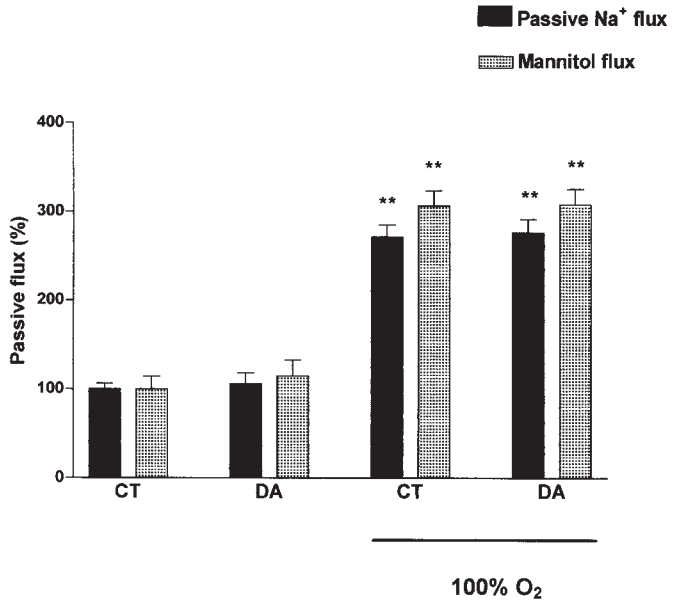

Figure 2. Passive $^{22}Na^+$ and [^{3}H]mannitol movement increased significantly after oxygen exposure. DA did not change lung permeability to small solutes. *Bars* represent means ± SEM. **p < 0.01 compared with room air breathing rats. CT: control group (n = 10); DA: 10^{-5} M dopamine instilled into airspace (n = 6).

Similarly, the alveolar fluid volume at time t is estimated by:

$$V_t = V_0(EBD)_0/(EBD)_t . \quad (3)$$

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 airspaces, and $J_{Na,in}$ is the passive bidirectional flux of Na^+ between the airspace 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:

$$J = (V_t - V_0)/t . \quad (4)$$

As described by Rutschman and coworkers (6), the passive movement of $^{22}Na^+$, $J_{Na,in}$, is given by:

$$J_{Na,in} = [Na^+]J(\ln C_{(t)} - \ln C_{(0)})/(\ln V_t - \ln V_0) \quad (5)$$

where C(x) is the $^{22}Na^+$ concentration at time x and $[Na^+]$ is the constant Na^+ concentration in the BSA solution.

Similarly, the volume flux of mannitol (typically expressed as PA, permeability of surface area) is given by:

$$PA = J(\ln M_{(t)} - \ln M_{(0)})/(\ln V_t - \ln V_0) \quad (6)$$

where M(x) is the [^{3}H]mannitol mass at time x.

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

ATII Cells Isolation, Culture, and Western Blot Analysis

ATII cells were isolated from adult rat lungs as previously described (15, 25). Briefly, the lungs were perfused via the pulmonary artery, lavaged, and digested with elastase (30 U/ml; Worthington Biochemical, Freehold, 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%). Cells were suspended in Dulbecco's modified Eagle medium (DMEM; Irvine Scientific, Santa Ana, CA) containing 10% fetal bovine serum with 2 mM L-glutamine, 40 μg/ml gentamicin, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were incubated in a humidified atmosphere of 5% CO_2/95% air at 37° C. On the second day, ATII cells were exposed to 10^{-5} M colchicine or 10^{-5} M β-lumicolchicine for 4 h at 37° C. At that time we studied the stimulatory effect of 10^{-5} M DA incubated for 15 min in cultured ATII cells. It has been previously reported that colchicine treatment, but not β-lumicolchicine, depolymerizes the intracellular microtubular transport system in cultured epithelial cells (26).

Preparation of basolateral membranes. ATII cells were homogenized in homogenization buffer (300 mM mannitol in 12 mM Hepes,

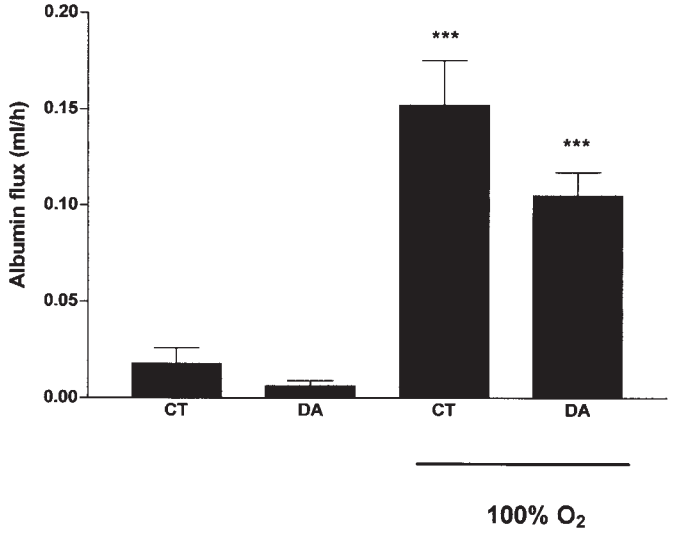

Figure 3. The movement of albumin from the pulmonary circulation into the airspace increased after acute exposure to hyperoxia. *Bars* represent means ± SEM. ***p < 0.001 compared with room air breathing rats. CT: control group (n = 10); DA: 10^{-5} M dopamine (n = 6).

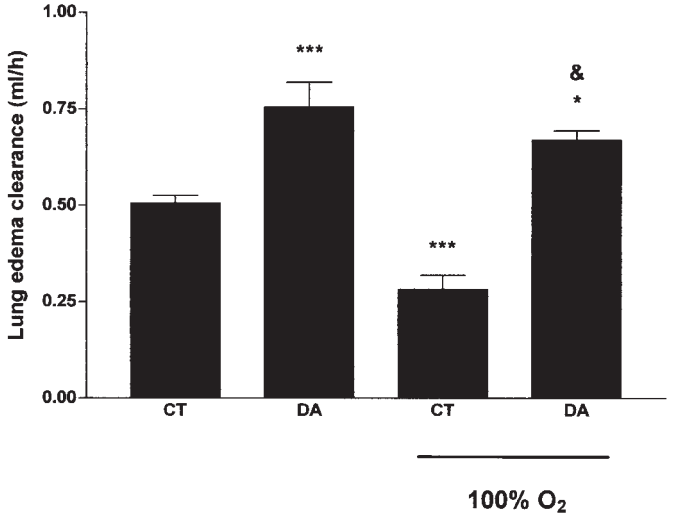

Figure 4. Exposure to hyperoxia decreased lung liquid clearance in adult rats. Dopamine increased lung edema clearance in room air breathing rats and after 64 h of exposure to 100% oxygen. *p < 0.05 and ***p < 0.001 compared with room air breathing control rats. &p < 0.001 compared with hyperoxia-injured control rat lungs. CT: control group (n = 10); DA: 10^{-5} M dopamine instilled into airspace (n = 6).

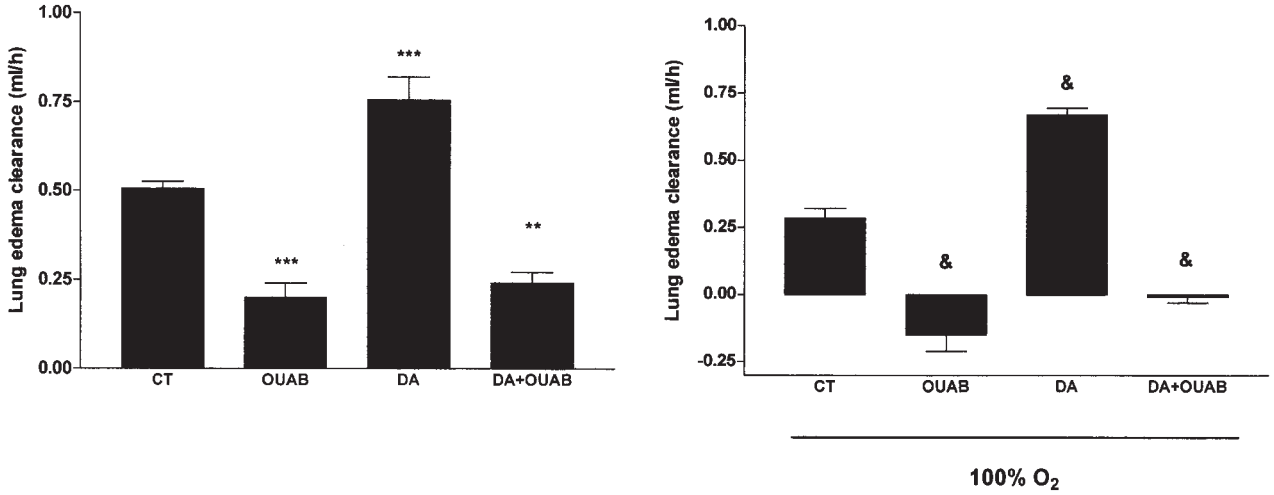

Figure 5. Ouabain perfused through the pulmonary circulation inhibited lung edema clearance in room air breathing rats (*left*) and rats exposed to acute hyperoxia (*right*). Dopamine did not increase lung liquid clearance in ouabain-perfused, control and hyperoxic rat lungs. *Bars* represent means ± SEM. **$p < 0.01$ and ***$p < 0.001$ compared with room air breathing control rats. $^{\&}p < 0.001$ compared with hyperoxia-injured control rat lungs. CT: control (n = 10); OUAB: 5×10^{-4} M ouabain (n = 6); DA: 10^{-5} M dopamine (n = 6).

pH 7.4) and basolateral membranes (BLM) were isolated using the technique described by Hammond and coworkers (27). After several centrifugations to discard the nuclear and mitochondrial pellet, the remaining supernatant was spun at 48,000 × *g* for 30 min. Finally, the BLM fraction was recovered after the membrane pellet was centrifuged in a Percoll gradient (16%) at 48,000 × *g* for 30 min.

Na,K-ATPase α_1-subunit abundance was determined by Western blot analysis in control and DA-stimulated ATII cells. Protein was quantified by Bradford assay and 5 μg of BLMs were loaded on each lane of a 10% polyacrylamide gel. Thereafter, they were transferred to nitrocellulose membranes (Optitran; Schleider & Schuell, Keene, NH) using a semidry transfer cell (Bio-Rad, Richmond, CA). Incubation with specific Na,K-ATPase monoclonal α_1 antibody (a generous gift from M. Caplan, Yale University, New Haven, CT) at 1:500 dilution was performed overnight at 4° C. Blots were developed as previously described with an enhanced chemiluminescence (ECL+; Amersham, Arlington Heights, IL) detection kit used as recommended by the manufacturer. The bands obtained were quantified by densitometric scan (Eagle Eye II; Stratagene, La Jolla, CA) and compared to the control group.

Data Analysis

Data are presented as mean values ± SEM; 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

Animals exposed to 100% oxygen for 64 h did not appear cyanotic in the environmental chamber. However, the transition to room air before the measurements resulted in cyanosis of the muzzle and paws. Approximately 10% of animals exposed to hyperoxia died in the oxygen chamber by 64 h. At autopsy, all of the 64-h oxygen-exposed animals had pleural effusion of variable magnitude (average volume: 9.3 ± 0.7 ml).

TABLE 1

ACTIVE AND PASSIVE MOVEMENT OF NA^+ IN ROOM AIR BREATHING RATS AND AFTER EXPOSURE TO 100% OXYGEN FOR 64 h*

	Unidirectional Na^+ Flux (*ml/h*)	Active Na^+ Flux (*ml/h*)	Passive Na^+ Flux (*ml/h*)
Control	1.58 ± 0.05	0.50 ± 0.02	0.96 ± 0.06
10^{-5} M DA (I)	1.78 ± 0.16	0.75 ± 0.06‡	1.02 ± 0.12
5×10^{-4} M OUAB (P)	1.22 ± 0.10	0.20 ± 0.04‡	1.00 ± 0.12
0.25 mg/100 g BW COL	1.32 ± 0.06	0.23 ± 0.03‡	1.09 ± 0.08
0.25 mg/100 g BW LUMIC	1.69 ± 0.21	0.51 ± 0.03	1.17 ± 0.20
Hyperoxia (O_2)	2.89 ± 0.35‡	0.28 ± 0.03‡	2.60 ± 0.34‡
O_2 + 10^{-5} M DA (I)	3.31 ± 0.21‡	0.67 ± 0.03§	2.65 ± 0.40‡
O_2 + OUAB (P)	1.90 ± 0.27	−0.11 ± 0.04‡§	2.02 ± 0.21†
O_2 + COL	3.95 ± 0.88‡	0.21 ± 0.02‡	3.74 ± 0.42‡
O_2 + LUMIC	3.05 ± 0.28‡	0.24 ± 0.07‡	2.80 ± 0.50‡

Definition of abbreviations: DA = dopamine; OUAB = ouabain; COL = colchicine; LUMIC = β-lumicolchicine; (I) = instillate; (P) = perfusate.

* Data are expressed as mean ± SEM.

† $p < 0.05$ compared with room air breathing control rats.

‡ $p < 0.01$ compared with room air breathing control rats.

§ $p < 0.01$ compared with hyperoxia-exposed control rats.

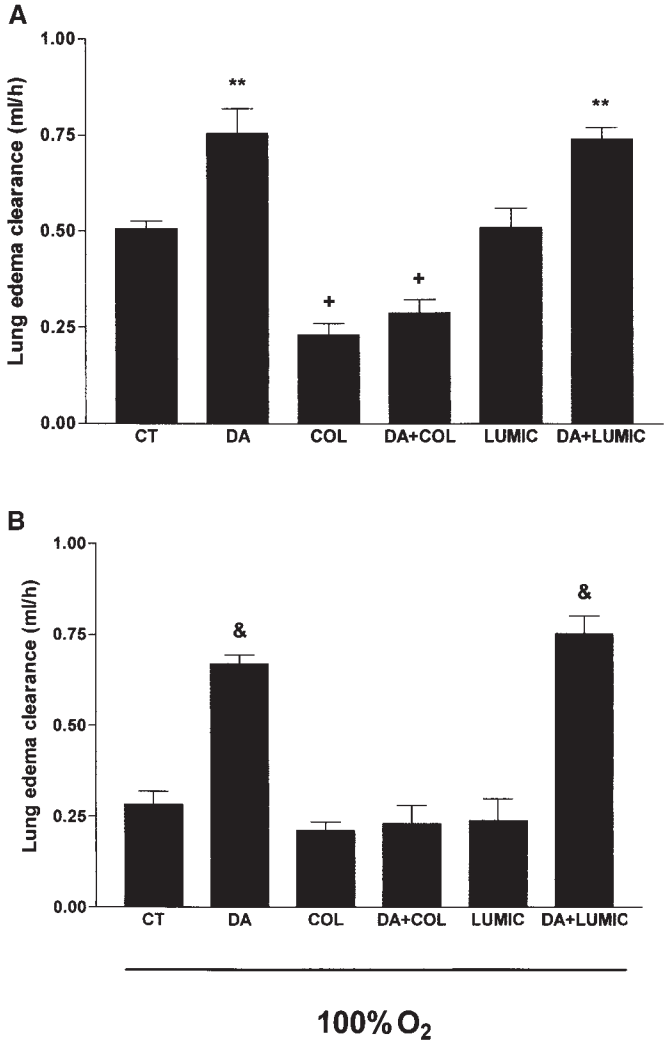

Figure 6. A-B. Effects of colchicine and β-lumicolchicine on lung edema clearance in control and hyperoxic rat lungs. Colchicine inhibited the stimulatory effect of DA on lung edema clearance after 100% oxygen exposure. *Bars* represent means ± SEM. **$p < 0.01$ compared with CT, COL, DA + COL, and LUMIC groups of room air breathing rats. $^{+}p < 0.01$ compared with CT, DA, LUMIC, and DA + LUMIC groups of normoxic rats. $^{\&}p < 0.001$ compared with CT, COL, DA + COL, and LUMIC hyperoxic rat lungs. CT: control (n = 10); DA: 10^{-5} M dopamine (n = 6); COL: 0.25 mg/100 g body weight colchicine (n = 6); LUMIC: 0.25 mg/100 g body weight β-lumicolchicine (n = 6).

Epithelial Permeability

The ELF volume measured in isolated-perfused lungs by the dilution of EBD in the first BAL increased in rats exposed to 100% oxygen for 64 h when compared with room air breathing rats, suggesting changes in alveolar permeability (Figure 1). As shown in Figure 2, exposure to acute hyperoxia increased significantly alveolar epithelial $^{22}Na^{+}$ and [^{3}H]mannitol permeability ($p < 0.01$). Dopamine did not change the passive flux of small solutes.

The movement of protein tracers across the alveolar epithelial barrier was similar to the previously reported rates in normal and injured rat lungs (6, 15, 20, 25). EBD-bound albumin instilled in the airspace was not detected in the perfusate or bath compartments in any of the experimental groups. However, the movement of FITC-albumin from the pulmonary vascular compartment into the airspaces increased in rat lungs after the hyperoxic exposure and it was not changed significantly by DA (Figure 3). The difference between the EBD-albumin and FITC-albumin measurements probably represents a higher sensitivity of FITC detection, which moves from a large space (90 ml) into a much smaller compartment (5 ml), whereas EBD-albumin is moving from a 5-ml compartment to an 18-fold larger compartment probably falling below the level of detection from the spectrophotometric assay.

Lung Edema Clearance

The unidirectional Na^{+} flux ($J_{Na,out}$), which includes both active and passive Na^{+} movement out of the alveolar space, increased in rats exposed to acute hyperoxia (Table 1). This was due to increased passive Na^{+} movement despite a reduction in active Na^{+} transport and lung edema clearance compared with control rats (Figure 2). The lungs of control rats instilled with 5 ml BSA solution cleared about 10% of the instillate in 1 h (0.50 ± 0.02 ml/h), whereas 10^{-5} M DA instilled into the airspaces increased lung liquid clearance by approximately 50% above control rats (Figure 4). Lung liquid clearance decreased by approximately 44% in rats exposed to acute hyperoxia compared with room air breathing rats ($p < 0.001$). DA restored lung edema clearance in rats exposed to 100% O_2 (from 0.28 ± 0.03 to 0.67 ± 0.03) (Figure 4). The Na,K-ATPase antagonist ouabain completely blocked the stimulatory effect of DA in hyperoxic and control rat lungs (Figure 5). Pulmonary circulation flow rates did not change with the administration of DA or ouabain in any experimental group.

Dopaminergic stimulation of active Na^{+} transport and lung edema clearance were inhibited as a result of colchicine-induced cell microtubular transport disruption in hyperoxic and control rats, whereas the isomer β-lumicolchicine did not affect the active Na^{+} transport pathway (Figure 6).

As shown in Figure 7, Na,K-ATPase α_1-subunit abundance was determined by Western blot analysis in BLM of ATII cells incubated with 10^{-5} M DA for 15 min. Dopamine increased the Na,K-ATPase α_1-subunit protein in the BLM of control and β-lumicolchicine-treated ATII cells, whereas disruption of intracellular microtubular transport by colchicine inhibited the recruitment of α subunits to the plasma membrane. A representative autoradiogram is shown for the α_1-subunit protein abundance in BLM of control and DA-stimulated ATII cells.

DISCUSSION

Exposure of adult rats to 100% O_2 causes severe lung injury and eventual death within 3 to 4 d of exposure (11, 14). Acute exposure to hyperoxia causes significant destruction of pulmonary capillaries, but initially only moderate damage to alveolar epithelial cells (11). The lung injury is characterized by damage to the alveolar capillary barrier resulting in accumulation of pulmonary edema (11, 13). As in normal lungs, after hyperoxic lung injury, edema is cleared via active Na^{+} transport out of the alveolar spaces, whereas water moves passively following Na^{+} isosmotically (4–6, 15).

It has been shown that DA and β-adrenergic agonists increase active Na^{+} transport and lung edema clearance in room air breathing mammals (5, 20, 25, 28). Considering that active Na^{+} transport and lung edema clearance are impaired during the exudative phase of hyperoxic lung injury (15), we evaluated whether DA could improve active Na^{+} transport and lung edema clearance in rats exposed to hyperoxia.

As a consequence of acute hyperoxic lung injury, the alve-

olar epithelium becomes more permeable to solutes and alveolar edema ensues. This was corroborated by a significant increase in the ELF volume in rats exposed to hyperoxia compared with control rats (*see* Figure 1). The passive movement of small solutes (Na^+, mannitol) and large solutes (albumin) across the alveolar epithelium increased after 64 h of 100% O_2 exposure. This was probably the result of oxygen free radicals damaging the alveolar epithelial tight junctions as it has been previously shown in epithelial cell monolayers (29). The exudative phase of oxygen toxicity is believed to represent a time period in which there is excess production of reactive oxygen species overwhelming the cell's normal antioxidant defense systems and eventually causing cell injury by oxidation of sulfhydryl-containing proteins, DNA damage, and peroxidation of lipids with resultant increases in membrane permeability (30, 31).

It has been reported that DA increases lung edema clearance by stimulating active sodium transport across the alveolar epithelium and upregulating the Na,K-ATPase function in ATII cells (20, 32). The stimulatory effect of DA in lung clearance appears to be mediated by D_1 receptor activation and not via the β-adrenergic pathway (32). It is known that DA can activate adrenergic and dopaminergic receptors at higher and lower doses, respectively. In the isolated-perfused rat lung model, we have shown that dopamine stimulation of alveolar epithelial Na^+ transport is mediated via D_1 receptor activation (32). Similarly to β-adrenergic agonist effects (21, 22), we have demonstrated that DA instilled into the airspaces could re-

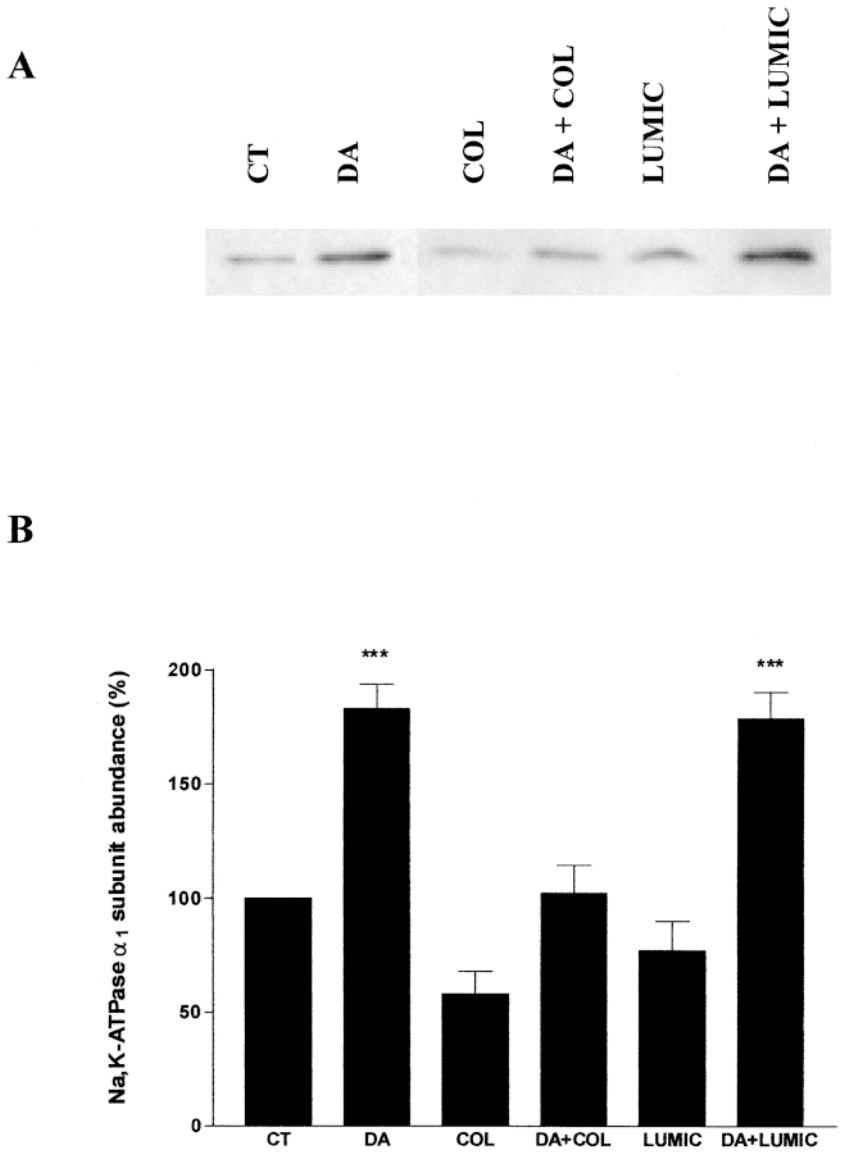

Figure 7. Na,K-ATPase α_1-subunit abundance in BLM of ATII cells exposed for 4 h to colchicine or β-lumicolchicine and after that incubated for 15 min with 10^{-5} M DA. Equal amounts of protein (5 μg) were loaded in each lane. The *top panel* (*A*) is a representative Western blot of the Na,K-ATPase α_1 subunit abundance and the *bottom panel* (*B*) shows the quantitative densitometric scans of four experiments. Colchicine inhibited the increase of the Na,K-ATPase α_1-subunit abundance in BLMs of ATII cells stimulated by DA. *Bars* represent means ± SEM. ***$p < 0.001$ compared with CT, COL, DA + COL, and LUMIC groups. CT: control; DA: 10^{-5} M dopamine; COL: 10^{-5} M colchicine; LUMIC: 10^{-5} M β-lumicolchicine.

store the lung's ability to clear edema in rats exposed to acute hyperoxia (*see* Figure 4). The stimulatory effect of DA was proportionally more accentuated in rats exposed to 100% O_2 compared with normoxic rats (increasing approximately 131% and 50% over basal lung clearance, respectively), and restored the ability of the lungs to clear edema to near-normal values.

Contrary to our results, Tibayan and colleagues (33) reported that dopamine did not affect lung edema clearance in anesthetized ventilated rats. We reason that the differences in the results are probably due to difference in experimental design. For example, Tibayan and colleagues instilled 1 to 2 ml into rat airspaces, about 60% less than the volume instilled in the lungs in our preparation (5 ml). Considering that lung liquid reabsorption is proportional to the instilled volume, it is probably more difficult to demonstrate changes in rates of edema clearance using smaller instilled volumes.

To evaluate the role of alveolar epithelial Na,K-ATPase on lung edema clearance stimulated by DA, we studied the effects of the Na,K-ATPase antagonist ouabain in hyperoxic rat lungs. As shown in Figure 5, dopaminergic effects on lung clearance were inhibited by ouabain, suggesting that DA upregulates Na,K-ATPase function in the alveolar epithelium as it has been previously reported in ATII cells isolated from healthy rat lungs.

There is evidence suggesting that the Na,K-ATPase exists in intracellular pools and in response to specific signals can be rapidly recruited via cell microtubular transport into the plasma membrane (34). This mechanism has been previously reported participating in lung edema clearance stimulation mediated by β-adrenergic agonists in normal rats (25). Therefore, we studied whether inhibition of cell microtubular transport of Na,K-ATPase from intracellular pools to the plasma membrane by colchicine could inhibit the stimulatory effects of DA on active Na^+ transport and lung edema clearance. Colchicine inhibited the stimulatory effect of DA in control lungs and hyperoxic rat lungs, whereas the isomer β-lumicolchicine, which shares many colchicine properties with the exception of inhibiting microtubular transport (23), did not inhibit dopaminergic stimulation of lung edema clearance (*see* Figure 6). We also observed that DA increased the Na,K-ATPase α_1-subunit abundance in BLM of ATII cells and that this effect was abolished by colchicine (*see* Figure 7). Our results suggest that DA stimulation of lung edema clearance is probably mediated by recruitment of ion-transporting proteins from inner pools to the plasma membrane in the alveolar epithelium. However, we did not examine the effect of DA and colchicine on other pathways involved in alveolar epithelial Na^+ transport and lung edema clearance such as the apical Na^+ channels, Na^+-glucose cotransporter and water channels. Therefore, similarly to β-adrenergic agonists, dopamine could modulate other mechanisms involved in lung edema clearance, but these were not explored in this study. Furthermore, disruption of intracellular microtubular transport by colchicine could affect the release of cytokines and growth factors by alveolar macrophages and monocytes that potentially could also affect lung edema clearance and alveolar epithelial Na,K-ATPase function.

Oxygen toxicity causes extensive damage to endothelial cells and increases lung capillary permeability, resulting in pulmonary edema (11, 13). Initially, there is little damage to alveolar epithelium despite being directly exposed to high levels of oxygen, possibly because of high levels of antioxidants present in the ELF (35). Our results lead us to speculate that alveolar epithelial Na,K-ATPases are internalized during acute oxygen exposure prior to developing an overwhelming injury to the epithelium. Conceivably, DA restores the lung's ability to clear edema by recruiting of Na,K-ATPase from intracellular pools to the BLM of the alveolar epithelium in rats exposed to acute hyperoxia. DA did not affect the passive movement of small solutes (Na^+, mannitol) and albumin flux in the hyperoxic lung injury model during the 1-h time period of our experimental protocol. However, we reason that DA, by recruiting back Na^+ pumps to cell plasma membrane and restoring the ability of the lung to clear edema, could improve alveolar epithelial permeability to solutes and thus promote lung healing.

In conclusion, we report that during acute hyperoxic lung injury DA restored the lung's ability to clear edema. Apparently, DA effects are mediated by recruitment and translocation of Na,K-ATPases from intracellular pools to the plasma membrane of the alveolar epithelium. Accordingly, DA enhances alveolar epithelial Na^+ transport, which could result in lung edema resolution and thus may be beneficial in the management of patients with acute hypoxemic respiratory failure.

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