Dibutyryl cAMP, aminophylline, and β-adrenergic agonists protect against pulmonary edema caused by phosgene

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KENNEDY, THOMAS P., JOHN R. MICHAEL, JOHN R. HOIDAL, DAVID HASTY, A. MARIO SCIUTO, CHISTOPHER HOPKINS, RANDE LAZAR, GOKUL K. BYSANI, ELIZABETH TOLLEY, AND GAIL H. GURTNER. *Dibutyryl cAMP, aminophylline, and β-adrenergic agonists protect against pulmonary edema caused by phosgene.* J. Appl. Physiol. 67(6): 2542–2552, 1989.—Phosgene is a toxic oxidant gas that causes the adult respiratory distress syndrome in exposed workers. Phosgene exposure markedly increased lung weight gain in buffer-perfused isolated rabbit lungs (31 ± 5 g over 60 min after phosgene vs. 7.7 ± 1.2 in control lungs, $P < 0.01$) and markedly increased the lung leak index for ^{125}I-albumin (0.28 ± 0.03 after phosgene vs. 0.02 ± 0.01 in control lungs, $P < 0.01$). Pretreatment with dibutyryl adenosine 3′,5′-cyclic monophosphate (DBcAMP), aminophylline, or terbutaline plus isoproterenol prevented the increase in lung weight caused by phosgene (31 ± 5 g phosgene, 11.7 ± 2.8 DBcAMP, 7.5 ± 2.5 aminophylline, 6.1 ± 1 terbutaline and isoproterenol, 6.1 ± 1.2 control + aminophylline, and 7.7 ± 1.2 control; all treatments were $P < 0.01$ vs. the untreated phosgene group and not significantly different from control lungs). Pretreatment with aminophylline prevented the increase in lung leak index for ^{125}I-albumin (0.28 ± 0.03 after phosgene vs. 0.06 ± 0.02 in aminophylline-treated lungs, $P < 0.01$). Posttreatment with aminophylline and terbutaline also prevented the increase in lung weight caused by phosgene. These results indicate that phosgene dramatically increases the movement of fluid and protein across the pulmonary vasculature and that treatment with DBcAMP, aminophylline, terbutaline, or isoproterenol markedly reduces the pulmonary edema caused by phosgene.

lung injury; oxidant lung injury; terbutaline; isoproterenol

LUNG INJURY from acute toxic gas inhalation with phosgene or N_2 mustard emerged into medical prominence during World War I, when chemical warfare casualities among US forces numbered 70,552 injured and 1,221 killed (17). Since World War I, the very agents that were so dreaded in the trenches of France and Belgium have become essential commodities in the peacetime synthetic chemical industry. As a result, acute toxic gas inhalation has become a significant hazard for workers exposed during industrial accidents or community bystanders caught by spreading gas clouds released during transportation or storage disasters. However, despite the passage of ~70 yr since the problem first arose, little more is known about therapy for victims of toxic gas inhalation than was known in 1918.

To investigate the pathophysiology and treatment of toxic gas inhalation, we developed an animal model of phosgene-induced lung injury in rabbits. Rabbits were exposed to phosgene and their isolated lungs were studied 0.5 h or 4 h after exposure. We assessed the effect of phosgene on lung weight gain at different levels of left atrial pressure and on the movement of ^{125}I-albumin from the vasculature into lung water and alveolar fluid. Drugs capable of elevating intracellular adenosine 3′,5′-cyclic monophosphate (cAMP), such as the cAMP analogue dibutyryl cAMP (DBcAMP), aminophylline, and isoproterenol, have been reported to reduce the pulmonary edema caused by endotoxin (13), air emboli (22), thrombin (28), acid aspiration (2, 29), and an oxidant lipid hydroperoxide (12). Consequently, we studied the effects of DBcAMP, aminophylline, terbutaline, and isoproterenol on phosgene-induced lung injury. We studied the effect of pretreatment and posttreatment beginning 10 min after phosgene exposure. We studied the effect of posttreatment beginning 10 min after exposure to simulate early treatment after industrial exposure. Pre- or posttreatment substantially reduced the pulmonary edema caused by phosgene.

METHODS

Phosgene Exposure

We exposed rabbits to phosgene by using a specially constructed facility. Phosgene was prepared by Matheson Gas Products (New York, NY) in 3Q cylinders containing 200 ppm phosgene in ultrapure air. Stability of the mixture was verified over 3 wk and periodically thereafter by using the method of Noweir and Pfitzer (31). Cylinder pressure (1,800 psi) was reduced to a delivery pressure of 10 psi with a Matheson model B15 regulator coupled to a Matheson model 206 rotameter for adjustment of flows from 0 to 25 l/min. Both regulator and rotameter were fitted with corrosion-resistant Monel valves. The cylinder and valve assembly were enclosed under a single high-velocity (1,200 l/min) laboratory exhaust hood to contain any accidental leakage of phosgene. Animal exposures were performed in a sealed Plex-

iglas box placed inside a second smaller laboratory exhaust hood. The Plexiglas box was connected to the valve and rotameter assembly by seamless ½ in stainless steel tubing. The effluent of the box and exposure hood was exhausted into a Mystaire HS-7 scrubber (Heat Systems Ultrasonics, Farmingdale, NY). This scrubber detoxifies phosgene by continuous exposure of the effluent to a 0.2% aqueous solution of potassium hydroxide (pH = 9). This detoxification is based on the principle that phosgene reacts rapidly with water to form HCl and CO_2. HCl is subsequently neutralized by the caustic in the mist. Investigators wore positive pressure self-contained breathing devices while in the exposure facility (ISI Ranger SCBA Apparatus, Direct Safety Co., Phoenix, AZ) and carried Monitox personal safety alarms (MDA Scientific, Lincolnshire, IL), which provide audio alert of phosgene concentrations in the atmosphere greater than the threshold limit value of 0.1 ppm.

Male New Zealand White rabbits (Myrtle Farms, Nashville, TN) weighing 2.5–3.0 kg were maintained on Carnation Rabbit Formula 18 and water ad libitum. On the day of the experiment, a protective water-based ointment was instilled into the rabbit's eyes to prevent conjunctival and corneal irritation. Rabbits were sealed into the Plexiglas exposure box and the phosgene mixture was delivered into the box at 5 l/min. The exposure dose of phosgene was the product of exposure time and the fixed (200 ppm) concentration of gas. Exposure dose was expressed as the concentration time product (ppm/min) (33). At the end of the exposure, phosgene was flushed from the box for 10 min by compressed air at a flow of 25 l/min before the animal was removed. Anesthesia was not required, as animals appeared to experience little distress during exposure to phosgene. Sham-exposed animals were treated as above except that the Plexiglas exposure box was ventilated with room air at 5 l/min.

Lung Perfusion Techniques

Rabbits were anesthetized with pentobarbital sodium (25 mg/kg) and given 3,000 U of heparin by ear vein. The chest was opened, and the animal was killed by rapid exsanguination from the left ventricle. Right and left parasternal incisions were made along the costal cartilages to remove the sternum and open the chest widely. Stainless steel cannulas were secured in the left atrium and pulmonary artery with umbilical tape. The ligature around the pulmonary artery was also passed around the aorta, preventing loss of perfusate into the systemic circulation. The pulmonary circulation was washed free of blood with ~500 ml of perfusate before recirculating flow was established at 50 ml/min. The perfusate medium was protein-free Krebs-Henseleit (KH) buffer maintained at a temperature of 37–38°C and pH of 7.35–7.40.

The lungs were ventilated with 5% CO_2 in air through a tracheostomy by using a Harvard animal respirator (Harvard Apparatus, Natick, MA) delivering a tidal volume of 7 ml/kg at 18 breaths/min with a 2 cmH_2O positive end-expiratory pressure. Lungs were perfused through a 250-ml circuit that included a perfusate reservoir, roller perfusion pump (Sarns, Ann Arbor, MI), filter (Swank transfusion filter, Extracorporeal Medical Specialities, King of Prussia, PA), and a heat exchanger connected by Tygon tubing. Except in the experiments in which left atrial pressure was increased, the perfusate reservoir was placed below the lowermost portion of the lung to keep left atrial pressure at zero.

Experimental Measurements

Physiological parameters measured. Pulmonary arterial and left atrial pressure were continuously measured by using Gould-Statham P23 ID pressure transducers (Gould-Statham Instruments, Hato Rey, Puerto Rico) connected to the inflow and outflow circuits. Pressure and force transducer measurements were continuously recorded on a Gould 2400S four-channel recorder. Lung weight changes were continuously recorded as the converse of the weight change of the perfusate reservoir, which was freely suspended from a force displacement strain-gauge transducer (Grass model FT10D, Grass Instrument, Quincy, MA). Wet-to-dry lung weight (W/D) ratio was determined by measuring the wet weight of the lungs and then drying the lungs in a vacuum oven at 85°C for 72 h or until dry lung weight was stable.

Measurement of Lung Malondialdehyde

To provide a biochemical parameter of lung injury, malondialdehyde was determined by the method of Buege and Aust (3). Portions of defrosted lungs (250 mg) were homogenized on ice with 2 ml of 1.15% KCl and mixed with 4 ml of thiobarbituric acid reagent (0.375% thiobarbituric acid in 0.25 N HCl to which 0.01% butylated hydroxytoluene was added just before use). After incubation at 100°C for 20 min and centrifugation at 1,000 *g* for 10 min, the absorbance of the supernatant was measured at 532 nm. An extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} and the W/D ratio were used to determine micromoles of malondialdehyde/g of dry lung.

Preparation of Lungs for Histological Study

To provide qualitative information about the histological effect of phosgene we studied a control lung perfused for 1 h with KH buffer beginning 30 min after sham exposure, a phosgene-exposed lung (2,000 ppm/min) perfused for 1 h with buffer beginning 30 min after exposure, and a phosgene-exposed lung (2,000 ppm/min) perfused for 10 min with buffer beginning 4 h after exposure. These protocols and perfusion times match those used in experiments in which lung weight gain was measured. Sections from the mid portion of the lung were fixed with modified Karnovsky fixative (3% paraformaldehyde and 3% glutaraldehyde) in tracheal distention at 25 cmH_2O pressure for 24 h, and rinsed in 0.1 M sodium cacodylate. Tissue for light microscopy was embedded in Epon-Araldite, and 1 μ thick sections were stained with 1% toluidine blue. Tissue for electron microscopy was postfixed in 1% osmium in 0.1 M sodium cacodylate, rinsed in 0.05 M maleic acid buffer, stained en bloc with 2% uranyl acetate in 0.05 M maleic acid buffer, dehydrated in a graded series of acetone, and infiltrated with

and embedded in Epon-Araldite. Thin sections of 60 nm were cut with a Reichert-Jung Ultracut E microtome and studied with a JEOL 1200FX electron microscope. The histological specimens were examined for evidence of alveolar-capillary injury, epithelial injury, or interstitial edema.

Experimental Protocols

Two sets of experiments were performed to determine the effects of phosgene and to investigate the effectiveness of therapy. In the first set of experiments we studied the lungs 30 min after exposure to phosgene. In these experiments we investigated the effect of pretreatment with DBcAMP, aminophylline, and the combination of terbutaline and isoproterenol. In the second set of experiments we studied the lungs 4 h after phosgene exposure. In these experiments we tested the effect of posttreatment beginning 10 min after phosgene exposure. We studied the effect of posttreatment with aminophylline, low-dose terbutaline, high-dose terbutaline, and aminophylline + high-dose terbutaline.

In preliminary dose-response experiments we found that exposure to 200 ppm phosgene for 10 min (2,000 ppm/min) consistently caused significant lung injury. In subsequent experiments this dose of phosgene was used except in the experiments performed 4 h after exposure in which fluid flux was measured at elevated left atrial pressures. In preliminary experiments the untreated lungs exposed to 2,000 ppm/min phosgene and studied 4 h later had such high rates of weight gain when left atrial pressure was increased that we were unable to obtain measurements at all four levels of left atrial pressure. Therefore, additional experiments were performed in which the dose of phosgene was lowered to 1,500 ppm/min. This dose allowed us to measure lung weight gain at all four levels of left atrial pressure in the untreated phosgene group studied 4 h after exposure.

Effect of Pretreatment on Lung Injury Caused by Phosgene

To determine if pretreatment with DBcAMP, aminophylline, and the combination of terbutaline and isoproterenol could attenuate phosgene-induced pulmonary edema, rabbits were pretreated 30 min before phosgene exposure. Six groups of rabbits ($n = 5$) were studied: a control group sham-exposed to air rather than phosgene; a control group pretreated with aminophylline; a group exposed to phosgene but untreated; and three pretreatment groups exposed to phosgene. The animals in the control plus aminophylline group were pretreated with aminophylline 16 mg/kg iv 30 min before sham exposure and with aminophylline (30 μg/ml) added to the perfusate reservoir at the beginning of lung perfusion. One treatment group was pretreated 30 min before phosgene exposure with DBcAMP 40 mg/kg iv and during lung perfusion DBcAMP (200 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was infused into the pulmonary artery. A second treatment group was pretreated 30 min before phosgene exposure with aminophylline (16 mg/kg iv) and at the start of lung perfusion aminophylline (30 μg/ml) was added to the perfusate reservoir. A third treatment group was pretreated 30 min before phosgene exposure with terbutaline (20 μg/kg sc) and during lung perfusion isoproterenol (0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was infused into the pulmonary artery. DBcAMP and isoproterenol were infused using a constant infusion pump (Sage Instruments, model 351, Cambridge, MA).

In these experiments the animals were killed 30 min after finishing phosgene exposure (2,000 ppm/min). Lungs were perfused for 60 min at a left atrial pressure of zero. Left atrial pressure was then increased sequentially to 5, 10 and 15 mmHg for 5 min at each level. When left atrial pressure was raised there was a transient increase in fluid flux followed by a steady increase. The steady increase in fluid flux throughout the 2nd to 5th minute at each level of left atrial pressure was measured. After lung perfusion, the W/D ratio was determined in one lung. The remaining lungs was frozen immediately in liquid N_2 and stored at -70°C until measurement of malondialdehyde content.

To assess the effect of phosgene and aminophylline pretreatment on albumin flux we measured the transvascular movement of ^{125}I-albumin in the isolated lung. Three groups ($n = 3$) were studied: a control group sham-exposed to room air; an untreated phosgene group; and a phosgene group pretreated with aminophylline. Aminophylline was given intravenously (16 mg/kg) 30 min before exposure and 30 μg/ml was added to the perfusate at the start of lung perfusion. Rabbits were exposed to 2,000 ppm/min phosgene, killed 30 min after finishing exposure to phosgene, and the lungs perfused. After 30 min of perfusion at a left atrial pressure of zero, 1.5 μCi of ^{125}I-human serum albumin (^{125}I-HSA, 8.3 mCi/g albumin, Syncor Corporation, Memphis, TN) was added to the reservoir, and left atrial pressure was increased to 10 mmHg. Ten minutes later, 1.0 ml of pulmonary venous effluent was obtained, weighed, and counted in a gamma counter to determine the number of counts in 1.0 g of circulating perfusate. The reservoir supplying perfusate to the lung was then changed to contain KH buffer without radioactivity. After lungs were perfused in a nonrecirculating manner for 5 min, 1.0 ml of pulmonary venous effluent was obtained and assessed for radioactivity to confirm that minimal ^{125}I-HSA counts remained in the vascular space. Perfusion was stopped and lungs were lavaged three times with 30 ml of normal saline. The lavage fluid was then thoroughly mixed and 1 ml of the fluid was weighed and counted to assess ^{125}I-HSA accumulation in the alveolar space. Lungs were then dissected free from the thorax and a portion of lung from the dorsal surface was weighed and counted to measure ^{125}I-HSA accumulation in lung tissue. ^{125}I-HSA counts per g of alveolar lavage fluid or lung tissue were then normalized by the number of counts present in 1.0 g of circulating perfusate to obtain lavage and lung leak indexes.

Effect of Posttreatment on Lung Injury Caused by Phosgene

Six groups of rabbits were exposed to phosgene (2,000 ppm/min) and then killed 4 h later: an untreated group;

a group treated with low-dose terbutaline; a group treated with high-dose terbutaline; a group treated with aminophylline; a group treated with high-dose terbutaline + aminophylline; and a group treated with propranolol + low-dose terbutaline. A control group of animals sham-exposed to air was also studied. Ten animals were studied in the untreated phosgene group and five in each of the other groups. Rabbits treated with low-dose terbutaline were injected subcutaneously with 10 μg/kg 10 min after exposure, and again at 2 and 4 h postexposure. Rabbits assigned to treatment with high-dose terbutaline were injected subcutaneously with 20 μg/kg 10 min after exposure and with 10 μg/kg every hour thereafter. Rabbits in the aminophylline treatment group were injected intravenously with 16 mg/kg 10 min after exposure and with 8 mg/kg ip 2 and 4 h after exposure. The group treated with high-dose terbutaline + aminophylline received the combination of the two regimens. Rabbits in the propranolol + low-dose terbutaline were given 0.5 mg of propranolol iv 5 min before beginning the phosgene exposure, exposed to phosgene, and then injected subcutaneously with terbutaline (10 μg/kg) 10 min after exposure, and again at 2 and 4 h postexposure. After the initial treatment, animals were returned to their cages and treatment was continued as outlined above. Lungs were perfused for 10 min beginning 4 h after exposure. At the end of the perfusion period the lungs were removed and the W/D ratio determined. Preliminary studies indicated that 10 min was the length of time that untreated lungs exposed to 2,000 ppm/min could be perfused before marked edema developed.

In separate experiments we studied transvascular fluid flux at elevated left atrial pressures 4 h after exposure to 1,500 ppm/min. This dose allowed us to measure fluid flux at all four levels of left atrial pressure in untreated lungs studied 4 h after phosgene exposure. Five groups of animals were studied ($n = 5$): a control group sham-exposed to air; a control group posttreated with aminophylline; an untreated phosgene group; a phosgene group posttreated with high-dose terbutaline; and a phosgene group posttreated with aminophylline. The high-dose terbutaline and aminophylline treatment protocols were as described above. At 4 h after exposure animals were killed and the lungs perfused. The lungs were perfused for 10 min at a left atrial pressure of 0 mmHg, and then left atrial pressure was sequentially increased to 5, 10, and 15 mmHg for 5 min at each level and the fluid flux at each left atrial pressure was measured as described above.

Reagents and Pharmaceuticals

Terbutaline (Brethine) was obtained from Geigy Pharmaceuticals, Ardsley, NY. Aminophylline and isoproterenol were obtained from Elkins-Sinn, Cherry Hill, NJ. Propranolol (Inderal) was obtained from Ayerst Laboratories, New York, NY. DBcAMP and all other reagents were purchased from Sigma Chemical, St. Louis, MO. DBcAMP, terbutaline, and isoproterenol were diluted with sterile 0.9% NaCl before injection.

Statistical analysis. Pulmonary arterial pressure, cumulative lung weight gain, and the rate of fluid flux at different left atrial pressures were analyzed by two-way analysis of variance with repeated measures or randomized split block design (37). Comparisons among groups were performed with Duncan's multiple range test (9). W/D ratio and lavage and lung leak indexes for ^{125}I-HSA were analyzed by one-way analysis of variance (38). Lung malondialdehyde content was analyzed with the Kruskal-Wallis test (4). Results are presented as means ± SE. Significance was assumed when $P < 0.05$.

RESULTS

Effects of Phosgene Exposure

One-half hour after exposure. Exposure to 2,000 ppm/min phosgene caused profound lung injury that was apparent even in lungs studied 0.5 h after exposure. Although phosgene-exposed lungs had a significantly lower pulmonary arterial pressure than did sham-exposed control lungs (Fig. 1), phosgene markedly increased lung weight gain during 1 h of perfusion (Fig. 2). Phosgene significantly increased the rate of transvascular fluid flux compared with control lungs at each level of left atrial pressure from 0 to 15 mmHg, $P < 0.001$ (Fig. 3*B*). We also measured the effect of phosgene on the movement of ^{125}I-albumin. In these experiments the left atrial pressure was maintained at 10 mmHg and the pulmonary arterial pressures were similar in the three groups (17 ± 1 mmHg control, 18 ± 2 phosgene, and 16 ± 1 phosgene + aminophylline). Phosgene increased by 30-fold the lavage fluid leak index for ^{125}I-albumin and by 14-fold the lung leak index for ^{125}I-albumin (Fig. 4). In addition, phosgene caused substantial peroxidation of lung lipids, doubling the content of malondialdehyde (Table 1). One-half an hour after phosgene exposure there was histological evidence of thickening of alveolar

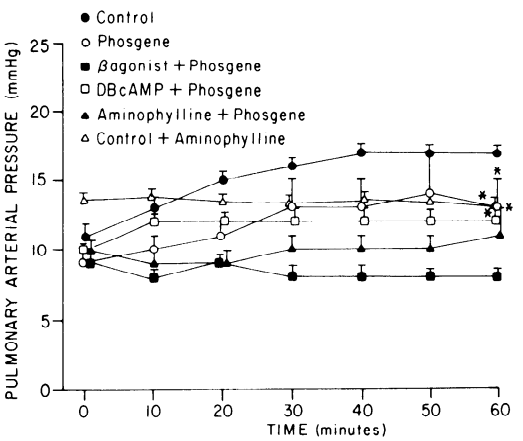

FIG. 1. Effect of pretreatment on pulmonary arterial pressure in phosgene- or sham-exposed animals. Lung perfusion was begun 30 min after sham or phosgene exposure (2,000 ppm/min). Phosgene decreased pulmonary arterial pressure compared with control lungs ($P < 0.01$). Pretreatment of phosgene-exposed lungs with dibutyryl adenosine 3′,5′-cyclic monophosphate (DBcAMP) or aminophylline did not significantly affect pulmonary arterial pressure compared with pressure in untreated phosgene group. Pretreatment with terbutaline followed by infusion of isoproterenol (β-agonists + phosgene) decreased pressure compared with untreated phosgene group ($P < 0.01$). * $P < 0.01$ compared with control group throughout 1 h of perfusion. Values are means ± SE; $n = 5$ in all groups.

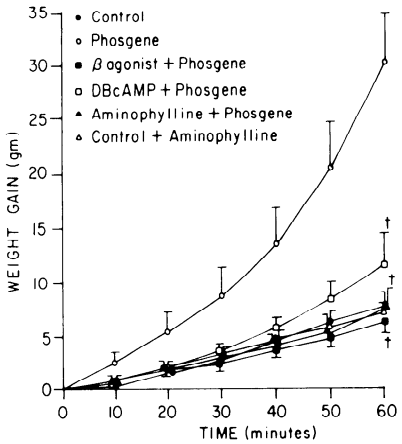

FIG. 2. Effect of pretreatment on lung weight gain. Lung perfusion was begun 30 min after sham or phosgene exposure (2,000 ppm/min). Phosgene significantly increased lung weight gain compared with control group ($P < 0.001$). Aminophylline treatment of control lungs did not affect lung weight gain compared with untreated control lungs. Pretreatment with dibutyryl adenosine 3′,5′-cyclic monophosphate (DBcAMP), aminophylline, or β-agonists markedly reduced the increase in weight gain caused by phosgene. † $P < 0.001$ compared with untreated phosgene lungs. Values are means ± SE; $n = 5$ in all groups.

septae and swelling of the interstitial space from edema (Fig. 5, *C* and *D*).

Four hours after exposure. When rabbits exposed to phosgene (2,000 ppm/min) were studied 4 h later, injury was so profound that untreated lungs could be perfused for only 10 min before edema fluid entered the tracheal cannula. Phosgene did not affect pulmonary arterial pressure (15 ± 1 mmHg phosgene vs. 14 ± 1 control). Phosgene markedly increased both lung weight gain and W/D ratio (Fig. 6). Four hours after phosgene exposure to 2,000 ppm/min histological sections showed disruption of alveolar septae and marked interstitial edema (Fig. 5, *E* and *F*). Lungs exposed to 1,500 ppm/min of phosgene were also studied 4 h after exposure. Exposure to this dose of phosgene also did not affect pulmonary arterial pressure, but strikingly increased fluid flux at all levels of left atrial pressure from 0 to 15 mmHg (Fig. 7).

Effect of Pretreatment on Lung Injury Caused by Phosgene

Aminophylline treatment of control lungs compared with untreated control lungs did not affect cumulative lung weight gain during 1 h of perfusion (Fig. 2). Pretreatment of phosgene-exposed lungs with DBcAMP or aminophylline did not significantly reduce pulmonary arterial pressure compared with the pressure in the untreated phosgene group (Fig. 1). Combined treatment with the β-adrenergic agonists terbutaline and isoproterenol reduced pulmonary arterial pressure compared with the untreated phosgene group ($P < 0.01$) (Fig. 1). DBcAMP, aminophylline, and the β-adrenergic agonists prevented the dramatic increase in lung weight caused by phosgene when the lungs were perfused for 1 h (Fig. 2). In fact, the weight gain in the three treatment groups was not significantly different from the weight gain in control lungs unexposed to phosgene (Fig. 2).

The effect of raising left atrial pressure on fluid flux

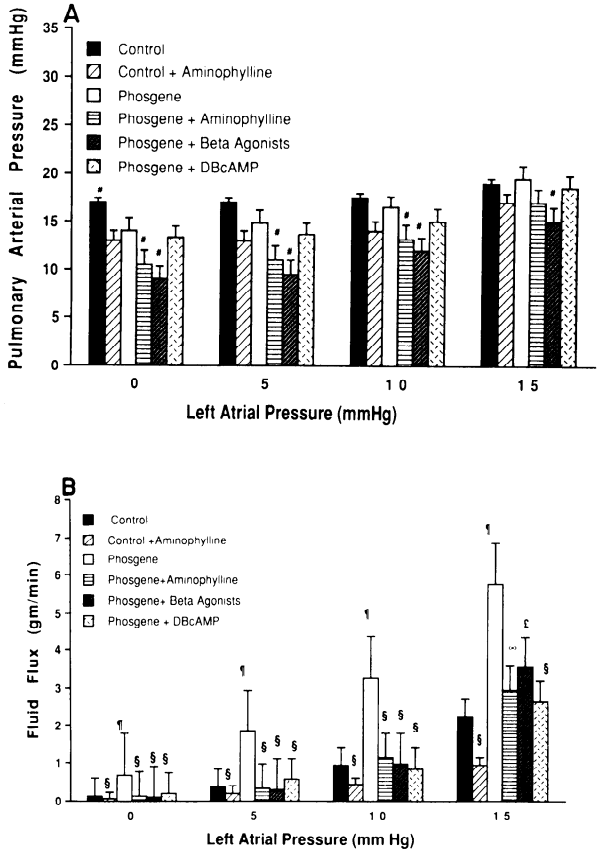

FIG. 3. Effect of pretreatment on pulmonary arterial pressure (*A*) and rate of transvascular fluid flux (*B*) at left atrial pressures of 0, 5, 10, and 15 mmHg. Lung perfusion was begun 30 min after sham or phosgene exposure (2,000 ppm/min). Pulmonary arterial pressure was similar in the groups except for a significantly lower pulmonary arterial pressure in the phosgene + aminophylline and phosgene + β-adrenergic agonists groups ($P < 0.05$). At each left atrial pressure phosgene significantly increased rate of fluid flux compared with rate in control lungs ($P < 0.001$). Aminophylline treatment of control lungs did not significantly affect fluid flux compared with control lungs, but the fluid flux in control + aminophylline group was less than in phosgene + aminophylline group ($P < 0.05$). Pretreatment with dibutyryl adenosine 3′,5′-cyclic monophosphate (DBcAMP), aminophylline, or β-agonists significantly reduced, at all levels of left atrial pressure, the increase in fluid flux caused by phosgene. Mean airway pressures when left atrial pressure was increased to 15 mmHg were as follows: control 4.2 ± 0.1, phosgene 6.7 ± 0.3, phosgene + β agonists 6.4 ± 0.3, phosgene + aminophylline 6.1 ± 0.2, and phosgene + DBcAMP 5.9 ± 0.5 mmHg (means ± SE). # $P < 0.05$ compared with untreated phosgene. ¶ $P < 0.001$ compared with control. § $P < 0.001$ compared with untreated phosgene. £ $P < 0.01$ compared with untreated phosgene. ∞ $P < 0.025$ compared with untreated phosgene. Values are means ± SE; $n = 5$ in all groups.

was also measured in these groups (Fig. 3). The pulmonary arterial pressure in the phosgene + aminophylline and phosgene + β-adrenergic agonists groups was significantly less than in the untreated phosgene group (Fig. 3*A*). Aminophylline treatment of control lungs did not significantly reduce fluid flux as left atrial pressure was increased compared with the response in untreated control lungs (Fig. 3*B*). The fluid flux in the control + aminophylline group, however, was significantly less than in the phosgene + aminophylline group ($P < 0.05$, Fig. 3*B*). At each level of left atrial pressure treatment with DBcAMP, aminophylline and the β-adrenergic agonists significantly reduced the increase in fluid flux

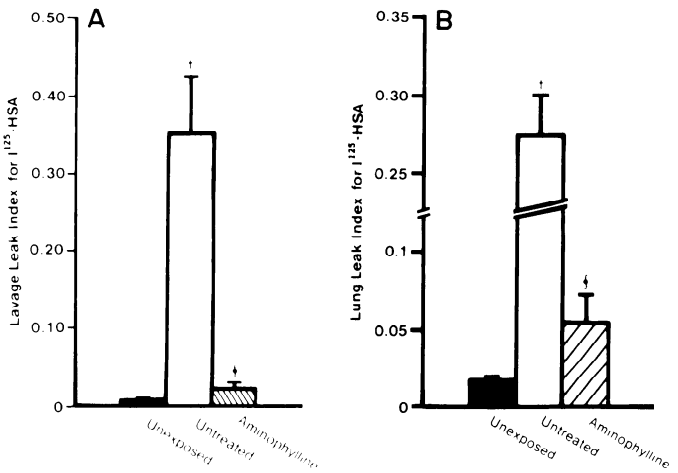

FIG. 4. Effect of pretreatment on lavage leak index (*A*) and lung leak index (*B*) for ^{125}I-human serum albumin (^{125}I-HSA). Lung perfusion was begun 30 min after sham or phosgene exposure (2,000 ppm/min). Lungs were perfused at a left atrial pressure of 10 mmHg. Phosgene markedly increased both lavage and lung leak indexes compared with control lungs ($P < 0.01$). Pretreatment with aminophylline prevented increase caused by phosgene in lavage and lung leak for ^{125}I-albumin. Lavage fluid leak index is expressed as cpm of ^{125}I-albumin/g of lavage fluid normalized to cpm/g of perfusate. Lung leak index is expressed as cpm of ^{125}I-albumin/g of lung normalized to cpm/g of perfusates. † $P < 0.01$ compared with control lungs; § $P < 0.01$ compared with untreated phosgene lungs. Values are means ± SE; $n = 3$ in each group.

TABLE 1. *Effect of phosgene and pretreatments on lung malondialdehyde*

Treatment Group	Malondialdehyde, μmol/g dry lung
Sham-exposed controls	24±1
Untreated phosgene	50±7*
Phosgene + DBcAMP	43±5
Phosgene + aminophylline	34±13†
Phosgene + β-adrenergic agonists	35±6

Values are means ± SE for 5 animals in each group. Sham-exposed controls, animals sham-exposed to air; untreated phosgene, untreated animals exposed to 2,000 ppm/min phosgene; DBcAMP, dibutyryl adenosine 3′,5′-cyclic monophosphate. Pretreatments were given as described in METHODS. Animals were killed 30 min after phosgene exposure and lungs were perfused with buffer for 1 h. * $P < 0.05$ compared with unexposed control lungs. † $P < 0.02$ compared with phosgene-exposed lungs.

caused by phosgene (Fig. 3*B*). Aminophylline pretreatment also prevented the dramatic increase in ^{125}I-albumin flux caused by phosgene (Fig. 4). Pretreatment with aminophylline, but not with DBcAMP or the β-adrenergic agonists, reduced the increase in malondialdehyde caused by phosgene (Table 1).

Effect of Posttreatment on Lung Injury Induced by Phosgene

Posttreatment with aminophylline or terbutaline significantly reduced the increase in lung weight gain and W/D ratio caused by 2,000 ppm/min of phosgene (Fig. 6). In these experiments the posttreatments reduced pulmonary arterial pressure compared with the pressure in the untreated phosgene group (15 ± 1 mmHg untreated phosgene, 12 ± 1 aminophylline, 13 ± 1 low-dose terbutaline, 13 ± 1 high-dose terbutaline, 13 ± 1 high-dose terbutaline + aminophylline, and 14 ± 1 unexposed controls, $P < 0.05$ for the treatments compared with untreated phosgene lungs, but the treatment groups were not different from the control group). To determine whether the protective effect of terbutaline was mediated by its effect on β-adrenergic receptors we studied the effect of combined treatment with propranolol and terbutaline. Pretreatment with propranolol before phosgene exposure abolished the beneficial effect of treatment with low-dose terbutaline [weight gain 2.5 ± 7 g/min in phosgene + propranolol + low-dose terbutaline group ($n = 5$) vs. 1.3 ± 0.4 in phosgene group and 0.3 ± 0.2 in phosgene + low-dose terbutaline group, $P < 0.01$ vs. phosgene or phosgene + low-dose terbutaline; W/D ratio = 19.9 ± 1.5 in phosgene + propranolol + low-dose terbutaline group vs. 14 ± 1 in phosgene group and 11 ± 0.2 in phosgene + low-dose terbutaline group, $P < 0.05$ compared with phosgene or phosgene + low dose terbutaline]. Propranolol by itself did not increase lung weight gain in two control lungs unexposed to phosgene (lung weight gain was 0.01 g/min in each).

In lungs exposed to 1,500 ppm/min of phosgene we studied the effect of posttreatment on fluid flux as left atrial pressure was increased from 0 to 15 mmHg. All groups had similar pulmonary arterial pressures at all four levels of left atrial pressure (Fig. 7*A*). Aminophylline treatment of control lungs did not significantly reduce fluid flux compared with untreated control lungs (Fig. 7*B*). Posttreatment with aminophylline or terbutaline blocked the increase in fluid flux caused by phosgene at all levels of left atrial pressure (Fig. 7). In fact, the rate of lung weight gain was the same in the aminophylline and terbutaline posttreated lungs as in the control or control + aminophylline groups (Fig. 7*B*).

DISCUSSION

Our objectives were to establish a model of lung injury from inhaled phosgene and determine if the injury could be favorably influenced by using pharmacological agents available to the practicing physician. The need for such research is obvious. In the wake of a civil disaster of the proportions of the recent catastrophe in Bhopal, the number of injured might greatly exceed the capacity of nearby medical facilities for handling the critically ill, even in the United States. Any combination of therapies that attenuate lung injury and can be administered to victims in the field might greatly decrease mortality and diminish the number requiring mechanical ventilation for survival.

Phosgene is a colorless oxidant gas, heavier than air, with a lethal exposure dose (LC_{50}) in humans of 500 ppm/min (LC_{50} in rabbits is 2,000 ppm/min) (6). Phosgene is 10 times more toxic than chlorine and is relatively water-insoluble like nitrogen dioxide (5). More than 1 million tons of phosgene are produced each year worldwide. Ten thousand people in the United States are estimated to be involved in its production or use. Most accidental exposures occur during the use of phosgene for synthesis of isocyanates, polyurethane, polycarbonate

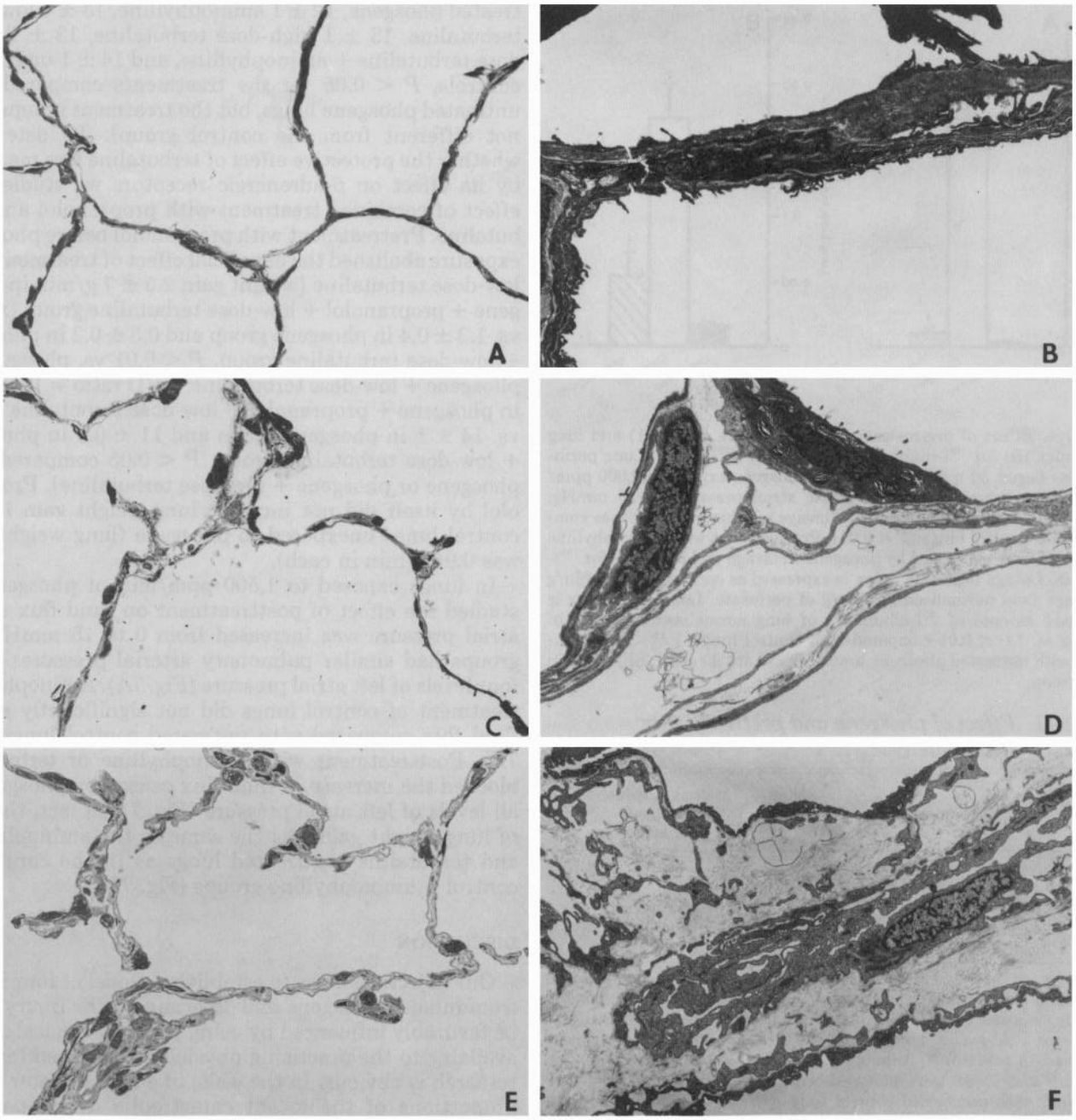

FIG. 5. Representative light and electron micrographs from a lung perfused for 1 h beginning 30 min after sham exposure (*A*, *B*), a lung perfused for 1 h beginning 30 min after phosgene exposure (2,000 ppm/min) (*C*, *D*), and a lung perfused for 10 min beginning 4 h after phosgene exposure (2,000 ppm/min) (*E*, *F*). In sham-exposed lung alveolar walls are of normal thickness. Thirty minutes after phosgene exposure alveolar walls were thickened. In the lung studied 4 h after phosgene exposure extensive swelling of alveolar walls was observed. Light micrographs ×620; electron micrographs ×10,000.

resins, dyes, and intermediary products used in the pharmaceutical industry. In addition, phosgene may be generated by the thermal decomposition of chlorinated hydrocarbons in fires or of the degreasing agent trichloroethylene that is used in welding.

Phosgene markedly increased weight gain in lungs studied 0.5 or 4 h after exposure (Figs. 2, 3, 6, and 7). Phosgene appeared to cause pulmonary edema by increasing pulmonary vascular permeability rather than by affecting vascular pressure. Phosgene lowered pulmonary arterial pressure and total pulmonary vascular resistance in lungs studied 30 min after exposure and did not affect pulmonary arterial pressure in lungs studied 4 h after exposure (Figs. 1, 3, and 7). Yet, phosgene caused a dramatic increase in fluid and albumin flux (Figs. 2–4, and 7).

The increase in fluid flux shown in Figs. 3 and 7 cannot be accounted for by an increase in downstream pressure. Raising left atrial pressure from 0 to 5 mmHg significantly increased fluid flux in the untreated phosgene group (30 min after exposure to 2,000 ppm/min fluid flux was 0.7 ± 0.2 g/min at 0 mmHg vs. 1.8 ± 0.4 at 5 mmHg,

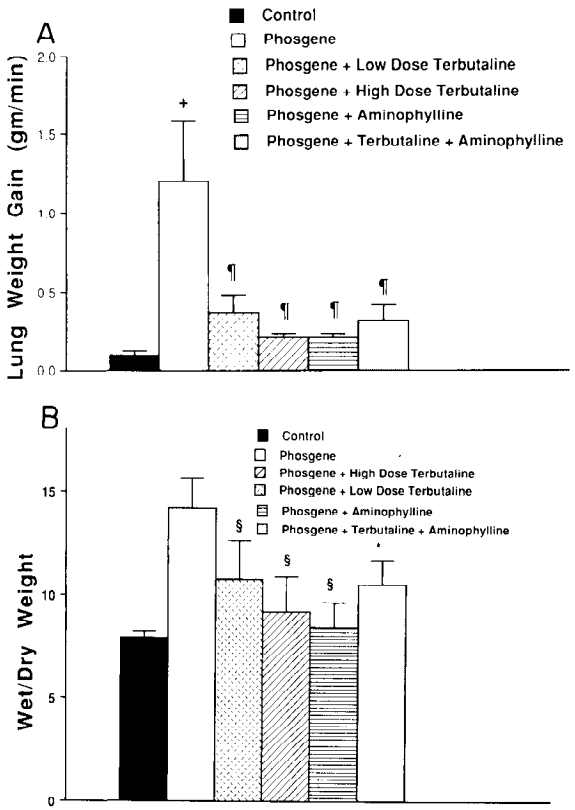

FIG. 6. Effect of posttreatment on lung weight gain (*A*) and the wet-to-dry lung weight (W/D) ratio (*B*). Lungs were perfused for 10 min beginning 4 h after sham or phosgene exposure (2,000 ppm/min). Posttreatment with terbutaline or aminophylline significantly reduced increase in weight gain and W/D caused by phosgene. † $P < 0.01$ compared with control lungs. ¶ $P < 0.01$ compared with untreated phosgene lungs. § $P < 0.05$ compared with untreated phosgene lungs. * $P = 0.06$ compared with untreated phosgene lungs. Values are means ± SE; $n = 10$ in untreated phosgene lungs and 5 in all other groups.

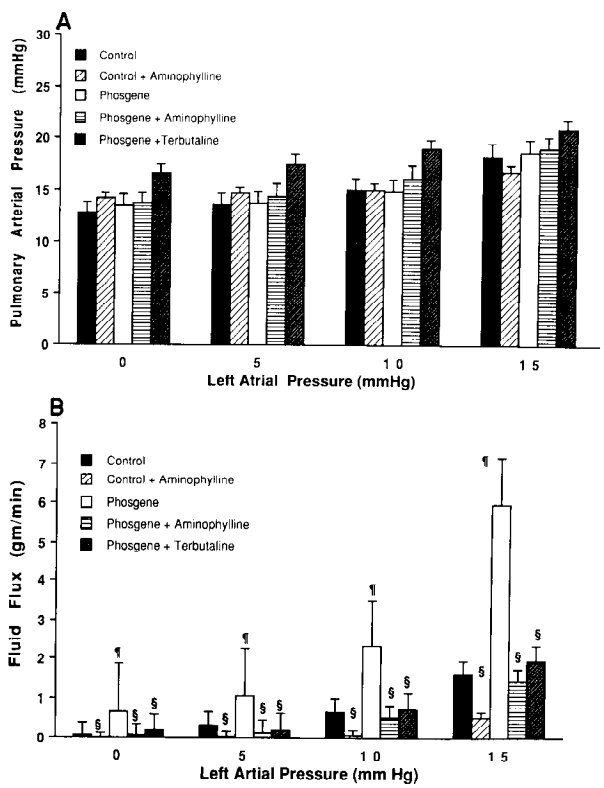

FIG. 7. Effect of posttreatment on pulmonary arterial pressure (*A*) and transvascular fluid flux (*B*) at left atrial pressures of 0, 5, 10, and 15 mmHg. Lung perfusion was begun 4 h after sham or phosgene exposure (1,500 ppm/min). Phosgene did not affect pulmonary arterial pressure but strikingly increased fluid flux at all 4 levels of left atrial pressure ($P < 0.001$). Pulmonary arterial pressure was similar in all groups. Aminophylline treatment of control lungs did not significantly reduce fluid flux compared with control lungs or phosgene + aminophylline lungs. Posttreatment with aminophylline or terbutaline prevented increase in fluid flux caused by phosgene. When left atrial pressure was 15 mmHg mean airway pressures were as follows: control 4.3 ± 0.1, phosgene 5.9 ± 0.4, phosgene + terbutaline 5.3 ± 0.4, and phosgene + aminophylline 5.8 ± 0.3 mmHg (means ± SE). ¶ $P < 0.001$ compared with control. § $P < 0.001$ compared with untreated phosgene lungs. Values are means ± SE; $n = 5$ in all groups.

$P < 0.05$, Fig. 3 and 4 h after exposure to 1,500 ppm/min fluid flux was 0.7 ± 0.1 g/min at 0 mmHg vs. 1.2 ± 0.2 at 5 mmHg, $P < 0.05$, Fig. 7). These results indicate that when the untreated phosgene groups were perfused at a left atrial pressure of 0 mmHg the downstream pressure was significantly <5 mmHg. Although the downstream pressures were likely similar in the untreated phosgene and control groups at all levels of left atrial pressure, this certainly should be the case when left atrial pressure was increased 10 or 15 mmHg. When left atrial pressure was increased to 10 or 15 mmHg, the pulmonary arterial pressures were similar in the control and untreated phosgene groups (Figs. 3 and 7). Yet, despite similar inflow and outflow pressures, the fluid flux at a left atrial pressure of 10 or 15 mmHg in the phosgene-untreated lungs was almost three times the flux in the control lungs (Figs. 3 and 7). These results indicate that the increase in fluid flux caused by phosgene cannot be explained by an increase in surface area or hydrostatic pressure. Because the lungs were perfused with a protein-free buffer, differences in osmotic pressure also cannot account for the striking increase in fluid movement caused by phosgene.

We also measured the effect of phosgene on the movement of ^{125}I-albumin from the vasculature into lung water and alveolar fluid. In these experiments the phosgene and control lungs were perfused at a left atrial pressure of 10 mmHg and both groups had similar pulmonary arterial pressures (18 ± 1 mmHg phosgene and 17 ± 1 control). Despite the similar vasculature pressures, phosgene dramatically increased the movement of ^{125}I-albumin into the lung and alveolar fluid, increasing the lung leak index by 14-fold and the lavage leak index by >30-fold (Fig. 4). These results along with the fluid flux measurements at elevated left atrial pressure provide strong evidence that phosgene increases pulmonary vascular permeability. This experimental evidence of an increase in vascular permeability is consistent with the occurrence of increased amounts of protein in the bronchoalveolar fluid of animals exposed to phosgene (5, 15) and the development of noncardiogenic pulmonary edema in humans exposed to phosgene (10, 34).

Phosgene exposure doubled lung malondialdehyde content, suggesting that phosgene caused oxidant injury that resulted in substantial peroxidation of lung lipids. Aminophylline pretreatment significantly reduced the increase in malondialdehyde caused by phosgene (Table 1). Pretreatment with DBcAMP and the β-adrenergic

agonists did not significantly reduce the increase in malondialdehyde.

The major finding of this study is that treatment with DBcAMP, aminophylline, and the β-adrenergic agonists terbutaline and isoproterenol markedly reduced the pulmonary edema caused by phosgene. Pretreatment with DBcAMP, aminophylline, and the combination of terbutaline and isoproterenol markedly reduced the increase in lung weight gain caused by phosgene exposure (Figs. 2 and 3). Pretreatment with DBcAMP and aminophylline did not significantly reduce pulmonary arterial pressure during 1 h of perfusion compared with the pressure in the untreated phosgene group (Fig. 1). Combined therapy with terbutaline and isoproterenol did, however, reduce pulmonary arterial pressure compared with the untreated phosgene group (Fig. 1). If these drugs solely reduced lung weight gain by lowering vascular pressure, then an increase in pulmonary venous pressure should completely eliminate their protective effect. We therefore determined the effect of elevating vascular pressure on the protective action of DBcAMP, aminophylline, and the β-adrenergic agonists by measuring fluid flux as left atrial pressure was increased from 0 to 15 mmHg (Fig. 3). At all levels of left atrial pressure, the treatments significantly reduced the dramatic increase in fluid flux caused by phosgene (Fig. 3). Thus elevating left atrial pressure did not eliminate the protective effect of pretreatment with DBcAMP, aminophylline, or the β-adrenergic agonists.

Posttreatment with aminophylline or terbutaline beginning 10 min after phosgene exposure also strikingly reduced the increase in lung weight caused by phosgene (Fig. 6). Measurement of fluid flux as left atrial pressure was increased demonstrated that the groups posttreated with aminophylline or terbutaline had similar pulmonary arterial pressures as in the untreated phosgene group. Aminophylline or terbutaline prevented at all levels of left atrial pressure the increase in fluid flux caused by phosgene (Fig. 7).

In addition to the beneficial effect on fluid flux, pretreatment with aminophylline markedly reduced the increase in ^{125}I-albumin flux caused by phosgene (Fig. 4). In these experiments the pulmonary arterial pressures were similar in the control, untreated phosgene, and phosgene + aminophylline groups (17 ± 1 mmHg control, 18 ± 2 phosgene, and 16 ± 1 phosgene + aminophylline). In addition, the lungs in all three groups were perfused with a left atrial pressure of 10 mmHg. Despite similar vascular pressures, phosgene dramatically increased the flux of ^{125}I-albumin; and aminophylline pretreatment prevented this striking increase in ^{125}I-albumin flux (Fig. 4). The results shown in Figs. 3, 4, and 7 indicate that a change in vascular pressure or surface area cannot explain the protective effects of the treatments. The experimental findings strongly suggest that the treatments prevented the increase in vascular permeability caused by phosgene.

Treatment with DBcAMP, aminophylline, or β-adrenergic agonists has been reported to reduce pulmonary edema in a variety of animal models of acute lung injury. Foy et al. (13) found that posttreatment with isoproterenol or aminophylline markedly reduced the increase in lung lymph flow caused by infusing *Pseudomonas aeruginosa* into sheep. Mizus and colleagues (29) demonstrated that isoproterenol and aminophylline reduced the increase in pulmonary vascular pressure and permeability caused by the intratracheal instillation of hydrochloric acid in rabbits. Isoproterenol has also been reported to decrease the pulmonary edema and increase in albumin flux caused by acid aspiration in isolated dog lungs (2). Minnear and coworkers (28) found that isoproterenol reduced the increase in vascular pressure and permeability caused by the intravenous infusion of thrombin into sheep. DBcAMP, aminophylline, isoproterenol, and prostaglandin E_1 prevented the pulmonary edema caused by infusion of an oxidant lipid hydroperoxide into rabbit lungs (12). In addition, Kobayashi et al. (22) have presented evidence that infusion of DBcAMP in sheep attenuates the increase in pulmonary vascular permeability arising from air emboli. Our findings indicate that pretreatment or early posttreatment with DBcAMP, aminophylline, or β-adrenergic agonists significantly reduces the pulmonary edema caused by another type of acute lung injury, phosgene inhalation.

Although it is conceivable that the compounds used in this study worked via another mechanism, the most likely explanation for their similar effect is an increase in intracellular cyclic AMP. DBcAMP, for example, is an analog of cAMP that passes easily through the cell membrane, undergoes deacylation to cAMP or N^6-monobutyryl cyclic AMP, which also augments intracellular cAMP by inhibiting the activity of the phosphodiesterase for cAMP (11, 26). Terbutaline and isoproterenol activate β-adrenergic receptors leading to an increase in intracellular cAMP via activation of adenylate cyclase (20, 32). The experiments demonstrating that propranolol pretreatment blocks the protective effect of terbutaline support the concept that the beneficial action of terbutaline is mediated via β-adrenergic receptors. Aminophylline has a number of reported effects, including inhibition of the phosphodiesterases for cAMP and cGMP (1, 24, 25), antagonism of adenosine receptors (14), and sequestration of calcium in mitochondria (23). Based on in vitro studies with the isolated phosphodiesterase enzyme the concept has arisen that aminophylline in the usual plasma therapeutic range (9 to 18 μg/ml or 50 to 100 μM) may not work via inhibition of phosphodiesterase. This concept, however, assumes that the plasma concentration is the same as the intracellular concentration and that the in vitro studies with an isolated enzyme accurately reflect the effect of aminophylline in intact cells. These assumptions, however, may be invalid as indicated by a recent report that 1 wk of oral theophylline therapy (average plasma concentration of 9 μg/ml or 50 μM) increased cAMP in the patients' polymorphonuclear cells by 160% (30). In our experiments we used a perfusate concentration of aminophylline (160 μM) that is slightly above the in vitro IC_{50} for the cAMP phosphodiesterase in human or guinea pig lung or porcine vascular tissue (1, 24, 25).

If these compounds increase intracellular cAMP, this would be expected to activate the cAMP-dependent pro-

tein kinase that via its regulatory effect on numerous intracellular systems might affect fluid or solute flux. An increase in cAMP in the endothelium of the exchanging vessels might, for example, reduce the increase in vascular permeability caused by phosgene. In systemic vessels, agents capable of increasing cAMP have been reported to prevent the increase in permeability caused by histamine, bradykinin, and fibrin degradation products (7, 16, 27, 39). These drugs appear to prevent contraction of actin and myosin filaments that is believed to play a key role in forming endothelial gaps and increasing vascular permeability (7, 27, 39). Compounds that increase pulmonary vascular permeability, such as cytochalasin B, ethchlorvynol, thrombin, and O_2 radicals, cause endothelial gaps to develop between cultured endothelial cells (35, 36, 42, 43). Generation of O_2 radicals via the reaction of xanthine oxidase and xanthine, for example, reversibly alters the configuration of actin filaments in pulmonary endothelial cells. This effect appears linked to the development of gaps between endothelial cells and an increase in albumin transit across the endothelial monolayer (35). If widening of endothelial junctions plays a role in the phosgene-induced increase in pulmonary vascular permeability, one speculative possibility for the protection offered by DBcAMP, aminophylline, terbutaline, and isoproterenol is prevention of cytoskeletal contraction and opening of endothelial junctions by increasing cAMP in microvascular endothelial cells. Isoproterenol, for example, has been shown to increase cAMP in pulmonary artery endothelial cells (18).

Because aminophylline and isoproterenol prevent the increase in pulmonary edema caused by the intratracheal instillation of hydrochloric acid (29), as well as the edema caused by inhalation of phosgene, these drugs may also affect epithelial permeability. Recent studies indicate that changes in the cytoskeleton may affect the permeability of epithelial cells (41). Hydrogen peroxide-induced oxidant injury to cultured epithelium, for example, increases transepithelial electrical conductance, permeability to [^{14}C]mannitol, and alters the normal pattern of the cytoskeleton, particularly at cell junctions, causing the cells to pull apart (41). In addition, changes in cAMP may regulate epithelial permeability; specifically, an increase in cAMP has been reported to decrease the permeability of epithelial cells by altering the structure of tight junctions (8). Because epithelial cells are the first cells exposed to phosgene at the alveolar level and junctions between epithelial cells are tighter than junctions between endothelial cells (40), prevention of cytoskeletal contraction and opening of the intracellular tight junctions provide another theoretical mechanism for how DBcAMP, aminophylline, and the β-adrenergic agonists might reduce the injury caused by phosgene. Because compounds capable of increasing cAMP have been reported to reduce systemic vascular permeability to the extent that phosgene may increase the permeability of bronchial vessels, these drugs might also reduce phosgene-induced injury by an effect on bronchial vessels.

Another possible site of action for DBcAMP, aminophylline, and β-adrenergic agonists is on alveolar macrophages, mast cells, or leukocytes. Currie et al. (5) have reported that low-dose phosgene exposure increases the influx into the lung of inflammatory cells, especially polymorphonuclear leukocytes. This influx begins 24 to 48 h after the low-dose exposure. It is unknown whether exposure to high-dose phosgene stimulates the acute influx of polymorphonuclear cells into the lung. If it does, these cells might magnify lung injury from phosgene by releasing lipid mediators, proteases, or additional oxidants. Aminophylline and isoproterenol attenuate leukotriene generation by lung mast cells and prevent lysosomal degranulation of neutrophils (19). In addition, therapeutic levels of aminophylline and β-adrenergic agonists inhibit eicosanoid production (21) and superoxide anion generation by polymorphonuclear leukocytes (30). Thus potential mechanisms exist by which DBcAMP, aminophylline, or the β-adrenergic agonists might reduce phosgene-induced lung injury by affecting inflammatory cells.

Our results indicate that DBcAMP, aminophylline, terbutaline, and isoproterenol significantly reduce the pulmonary edema and the likely increase in pulmonary vascular permeability caused by phosgene. Should clinical studies confirm our findings in phosgene-exposed rabbits, these drugs might eventually prove useful therapy for victims of phosgene inhalation if administered soon after exposure.

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