Effects of Cyclooxygenase Inhibitors on the Alterations in Lung Mechanics Caused by Endotoxemia in the Unanesthetized Sheep

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Abstract The effects of Escherichia coli endotoxin on lung mechanics, hemodynamics, gas exchange, and lung fluid and solute exchange were studied in 12 chronically instrumented unanesthetized sheep. A possible role for cyclooxygenase products of arachidonate metabolism as mediators of the endotoxin-induced alterations in lung mechanics was investigated by studying sheep before and after cyclooxygenase inhibition with sodium meclofenamate and ibuprofen. Sheep were studied three times in random order: (a) sodium meclofenamate (or ibuprofen) infusion alone; (b) E. coli endotoxin alone; and (c) meclofenamate (or ibuprofen) and endotoxin. Meclofenamate alone had no effect on any of the variables measured. Endotoxin alone caused early marked changes in lung mechanics: resistance to airflow across the lungs (R_L) increased 10-fold, dynamic lung compliance (Cdyn) decreased 80% and functional residual capacity (FRC) decreased by >30%. The alveolar-to-arterial oxygen difference (ΔAaPO_2) increased markedly following endotoxia. In the presence of sufficient meclofenamate to inhibit accumulation of thromboxane-B_2 and 6-keto-prostaglandin F_1α in lung lymph, endotoxin caused no increase in R_L, Cdyn decreased by <40%, and FRC decreased by only 6%. Meclofenamate significantly attenuated the hypoxemia and early pulmonary hypertension caused by endotoxemia but had no effect on the late increases in lung fluid and solute exchange. Ibuprofen had similar effects to those observed with meclofenamate. We conclude that both the pulmonary hypertension and changes in lung mechanics observed after endotoxemia may be mediated, at least in part, by constrictor prostaglandins or thromboxanes and that gas exchange may be improved by preventing endogenous synthesis of these mediators.

Introduction

Endotoxia has been used, in the unanesthetized sheep, as an animal model of the adult respiratory distress syndrome (ARDS) (1, 2). Although alterations in lung mechanics and hypoxemia are an integral part of ARDS (3), the effects of sublethal doses of endotoxin on lung mechanics have not been studied in the unanesthetized sheep. The hypoxemia associated with ARDS (4) and following endotoxemia in sheep (2), dogs, and baboons (5) is not simply the result of pulmonary edema. There is considerable evidence that cyclooxygenase products of arachidonate metabolism are important mediators of the pulmonary hypertension observed in the sheep within 1 h of endotoxemia (6, 7).

We hypothesized that sublethal doses of endotoxin would cause marked changes in lung mechanics as well as alterations in the pulmonary circulation. To test this hypothesis, 12 chronically instrumented unanesthetized sheep were studied in a specially constructed pressure-compensated integrated flow whole-body plethysmograph that allowed for the simultaneous measurement of lung mechanics, hemodynamics, and the collection of blood and lung lymph. We further hypothesized that cyclooxygenase products of arachidonate metabolism mediate alterations in lung mechanics as well as changes in hemodynamics following.


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endotoxemia in the unanesthetized sheep. To test this hypothesis, 10 sheep were studied three times in random order: (a) with the cyclooxygenase blocking agent sodium meclofenamate alone (5 mg/kg over 0.5 h + 3 mg/kg x h i.v. for 5.5 h); (b) Escherichia coli endotoxin infusion alone; and (c) the same dose of endotoxin begun 1 h after beginning the same dose and infusion pattern of meclofenamate. In an additional two sheep, sodium ibuprofen (10 mg/kg, i.v. bolus) was substituted for meclofenamate. The effects of endotoxin alone on lung lymph and plasma concentrations of thromboxane-B2 (TxB2) and 6-keto-prostaglandin F1a (6-keto-PGF1a) were studied in an additional 19 sheep and comparisons made between lymph and plasma concentrations of these arachidonate metabolites and concomitant changes in the differential variables measured.

We found that sublethal doses of endotoxin caused marked changes in lung mechanics. These alterations were most pronounced at 1 h following endotoxemia, concomitant with the pulmonary hypertension and accumulation of thromboxane and prostacyclin metabolites in lung lymph. Meclofenamate abolished the endotoxin-induced increases in resistance to airflow across the lungs (Rl) and inhibited the release of thromboxane and prostacyclin. The endotoxin-induced changes in dynamic compliance of the lungs (Cdyn), functional residual capacity (FRC), oxygenation, and the early pulmonary hypertension were significantly attenuated by meclofenamate. Ibuprofen had a similar effect to meclofenamate on the sheep's response to endotoxemia. We conclude that both the pulmonary hypertension and the changes in the lung mechanics observed early after endotoxemia may be mediated, at least in part, by constrictor prostaglandins or thromboxanes and that pulmonary gas exchange may be improved by preventing the endogenous synthesis of these mediators. Since similar changes in lung function occur in humans with gram-negative septicemia, these findings may be relevant to the pathogenesis and therapy of ARDS.

**GLOSSARY**

ΔAAPO₂ | alveolar-to-arterial oxygen difference
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ARDS | adult respiratory distress syndrome
BTFS | body temperature pressure standard
Cdyn | dynamic lung compliance
CtP | lymph protein clearance
CO | cardiac output
E | endotoxin infusion
FRC | functional residual capacity
6-keto-PGF₁a | 6-keto-prostaglandin F₁a
L/P ratio | protein concentration of lymph relative to plasma
M | meclofenamate infusion
Pao₂ | airway opening pressure
Pₐa | left atrial pressure
Ppa | pulmonary artery pressure
Ppl | pleural pressure
Psa | aortic pressure
Ptp | transpulmonary pressure
PVR | pulmonary vascular resistance
QLymph | lymph flow
Rl | resistance to airflow across the lungs
SGₖ | lung specific conductance
V | tidal volume
VAF | air flow

**METHODS**

**Experimental preparation.** Through a left thoracotomy, catheters were placed directly into the left atrium and pulmonary artery. Catheters were passed, from the neck, into the vena cava and the thoracic aorta. A thermal dilution Swan-Ganz catheter was passed through the external jugular vein into the pulmonary artery for measuring cardiac output (CO) using an Edwards model 9520A Thermodilution Cardiac Output Computer (Edwards High Vacuum, Inc., Grand Island, NY). Through a right thoracotomy, a silastic catheter was placed in the efferent lymph duct emerging from the caudal mediastinal lymph node. The tail of the caudal mediastinal lymph node was resected below the inferior pulmonary ligaments through a second right-sided thoracotomy. In the last six sheep, an additional left thoracotomy was performed to disrupt systemic lymph vessels running across the left hemidiaphragm and entering the caudal mediastinal lymph node causing systemic contamination of the lung lymph (B). A similar disruption was performed on the right hemidiaphragm when the tail of caudal mediastinal lymph node was resected. At the time of the right thoracotomy, a silastic envelope was placed in the pleural space for the measurement of pleural pressure. This rectangular envelope measured 4 X 5 cm and was constructed from silastic sheeting and a silastic catheter (0.157 cm i.d.) extending from within the silastic envelope. A tracheostomy was performed and a size 10 Shiley cuffed tracheostomy tube inserted. Sheep were allowed to recover for several days following surgery. These methods have been described in detail elsewhere (9).

**Measurements of lung mechanics.** Awake sheep were studied while standing in a specially constructed whole-body pressure-compensated integrated-flow plethysmograph. The plethysmograph was constructed on 1-cm thick clear plexiglas and has a volume of 285 liters. The sheep's tracheostomy tube was connected to external valve (which was used to obstruct the airway during determinations of FRC) via a flexible noncollapsible tubing. This permitted the animal to move without interfering with measurements. A loosely fitting sling was placed under the sheep to prevent it from lying while in the plethysmograph. A constant bias flow was used to reduce the effective deadspace of the tubing. Tidal volume (V) was measured by pressure compensating the integrated signal from the plethysmographic pressure transducer. Flow (V) was obtained by electrically differentiating the volume signal. Airway opening pressure (Pao₂) was measured by a multipurpose hole catheter positioned 0.5 cm past the distal end of the tracheostomy tube. Pleural pressure (Ppl) was measured directly from the silastic catheter and envelope in the pleural space. Transpulmonary pressure (Ptp) was the pressure difference between Pao₂ and Ppl. All pressure signals were measured using Validyne pressure transducers and Validyne amplification equipment (Validyne Engineering Corp., Northridge, CA). The signals from pressure trans-
ducers, catheters, and silastic envelope were tuned to eliminate phasic distortion to 6 Hz. These methods have been described in detail elsewhere (10).

Before each set of measurements of lung mechanics, the sheep's lungs were inflated to 40 cm H₂O airway opening pressure using the bias flow and an occluded airway. Simultaneous V/V and V/P_{a} curves were then recorded during spontaneous respiration on a Tektronix dual beam storage oscilloscope (Tektronix, Inc., Beaverton, OR) and photographed for calculation of Cdyn and Rl. Cdyn was calculated as V divided by P_{a} at points of zero flow and expressed in liter/cm H₂O at BTPS. Rl was calculated using the method of von Neergaard and Wirz (11) by dividing P_{a} by V at midtidal volume and was expressed as cm H₂O/liter x s at BTPS. Functional residual capacity was measured using the Boyle's law method of DuBois et al. (12). The airway was manually obstructed at end expiration. The sheep continued to make respiratory efforts against the obstruction for one to three breaths and a graph of the change in plethysmographic volume against the change in P_{a} was traced on the oscilloscope and photographed for calculation of FRC. Specific conductance, SGl, was calculated by dividing the reciprocal of Rl (conductance) by FRC and expressed as seconds per centimeter H₂O at BTPS.

Measurement of TxB₂ and prostacyclin metabolites. TxB₂ and 6-keto-PGF₁α were measured in lung lymph and plasma specimens collected during base line, at 30–60, 120–150, and 240–270 min after endotoxemia or after beginning the meclofenamate infusion in the meclofenamate alone experiments (M). Analyses were performed by radioimmunoassay, using rabbit anti-TxB₂ and anti-6-keto-PGF₁α antibodies obtained from Dr. J. Bryan Smith (Carderda Foundation, Philadelphia, PA). The anti-TxB₂ antibody cross-reacts <1% with PGE₂, PGF₂α, and 6-keto-PGF₁α, and <5% with PGD₂. The 6-keto-PGF₁α antibody cross-reacts <3% with PGF₂α, and <1% with PGE₂, PGD₂, and TxB₂. Authentic prostaglandins and TxB₂ were generously supplied by Dr. John Pike (Upjohn Co., Kalamazoo, MI). Radiolabeled [5, 6, 8, 9, 11, 12, 14, 15-³H]TxB₂ and [5, 6, 8, 9, 11, 12, 14, 15-³H]6-keto-PGF₁α were purchased from New England Nuclear (Boston, MA). Bovine γ-globulins, Trizma buffer, and ammonium sulfate were purchased from Sigma Chemical Co. (St. Louis, MO).

The radiolabeled ligand (≈2,000 cpm/tube) was first mixed with bovine γ-globulins (10 mg/ml in Trizma, pH 7.4). To 100-μl aliquots of this mixture were added 100-μl aliquots of sample or appropriate unlabeled standard dilutions. The binding reaction was initiated by addition of 100 μl of the appropriate antibody diluted to yield 60% binding of the label. The binding reaction continued for 60 min at 37°C and was terminated by precipitation of the immune complexes with ammonium sulfate at a final concentration of 50% of saturation. After centrifugation at 2,500 g at 4°C for 10 min, 300 μl of supernatant was counted in Aquasol (New England Nuclear). Each sample was assayed in duplicate, and duplicate determinations differed by <10%. The detection limit of both assays was <20 pg.

Experimental protocols. Three experiments were performed on each sheep: (a) meclofenamate infusion alone (M); (b) endotoxin infusion alone (E); and (c) meclofenamate and endotoxin (M + E). The order of the three experiments was randomized and a minimum of 3 days allowed between experiments. The entire experiment was performed with the sheep within the plethysmograph. Pulmonary artery pressure (Ppa), left atrial pressure (PLa), and aortic pressure (Pao) were continuously monitored using Hewlett-Packard model 1208C pressure transducers (Hewlett-Packard Co., Palo Alto, CA). Pulmonary vascular resistance (PVR) was calculated by subtracting Ppa from Pao and dividing by CO. Lung lymph was collected continuously with flow determined for each 15-min interval and samples of lymph and blood were collected every one-half hour for measurement of total proteins. Total protein concentrations in lung lymph and blood plasma were measured by a modified biuret method (13) with an automated system (AutoAnalyzer, Technicon Instruments Corp., Tarrytown, NY). Lymph protein clearance (CL) was calculated by multiplying the lymph flow by the lymph to plasma protein concentration ratio. Arterial blood samples were collected every half hour anaerobically during steady state baseline conditions and experimental periods and PO₂, Pco₂, and pH measured using an Instrumentation Laboratories model 513 blood gas analyzer (Instrumentation Laboratories, Inc., Lexington, MA). The alveolar-to-arterial oxygen difference (ΔAaPO₂) on room air was calculated using the alveolar gas equation with a fixed respiratory exchange ratio of 0.8 (oxygen consumption was not measured and the respiratory exchange ratio may change following endotoxemia in the unanesthetized sheep). Lung mechanics were measured every 15 min except during the first hour following endotoxemia when they were measured every 7.5 min.

In the meclofenamate alone experiments (M), after a 1-h control period, sodium meclofenamate powder (Parke-Davis Div. of Warner Lambert Inc., Morris Plains, NJ) was dissolved in sterile saline (10 mg/kg/ml) and infused intravenously at 5 mg/kg over one-half hour followed by 3 mg/kg/hr for an additional 5.5 h via a Harvard Apparatus constant infusion pump (Harvard Apparatus Co., Inc., S. Natick, MA). In the endotoxin alone experiments (E), after a 1-h control period, E. coli endotoxin (Difco Laboratories, Inc., Detroit, MI, prepared by the Westphal (14) method from E. coli 055:B5) was infused intravenously as 0.35±0.2 μg/kg·h over one-half hour. The animals were followed for 5 h following endotoxemia. In the meclofenamate and endotoxin experiments (M + E), after a 1-h control period, each animal received the identical dose and infusion pattern of meclofenamate as used in the meclofenamate alone experiments. The same dose of endotoxin as given in the endotoxin alone (E) experiments was given 1 h after beginning the meclofenamate infusion.

The effects of a second cyclooxygenase inhibitor of arachidonate metabolism, sodium ibuprofen (generously supplied by the Upjohn Co., Kalamazoo, MI), on the sheep's response to endotoxia was studied in an additional two animals. This protocol was identical to that described above except ibuprofen (10 mg/kg as a single intravenous bolus) was substituted for meclofenamate. The ibuprofen was given 1 h before beginning the endotoxin infusion in the ibuprofen and endotoxin (I + E) experiments. One sheep was studied first with endotoxin alone (E), then with ibuprofen and endotoxin (I + E), and then with ibuprofen alone (I). The second sheep was studied first with ibuprofen and endotoxin (I + E), then with endotoxin alone (E), and then with ibuprofen alone (I). As in the meclofenamate experiments, each sheep received the identical doses of both ibuprofen or endotoxin whether the agents were given singly or in combination.

Lung lymph and plasma TxB₂ and 6-keto-PGF₁α concentrations were obtained following endotoxin alone (E) from only five of the meclofenamate and endotoxin experiments (M + E) and from the two ibuprofen and endotoxin (I + E) experiments. To compare 1, 2.5, and 4.5 h TxB₂, 6-keto-PGF₁α, and the ratio of TxB₂ to 6-keto-PGF₁α concentrations in lung lymph and plasma to the various physiologic variables measured, data from an additional 19 endotoxin alone
(E) experiments were used increasing the n to from 16 to 26 for these comparisons. The dose of endotoxin and the experimental protocol for the total group of 19 additional sheep given endotoxin alone (E) was not different from that used in the endotoxin alone (E) experiments whether they were part of the meclofenamate and endotoxin (M + E) or ibuprofen and endotoxin (I + E) protocols. In these additional 19 endotoxin alone (E) experiments, the sheep had not been previously studied or received other interventions.

Statistics. The effects of meclofenamate alone over time were examined using two-way analysis of variance (15). The effects of meclofenamate alone, endotoxin alone, and meclofenamate and endotoxin were compared using the non-parametric Wilcoxon signed rank test and paired t test (16). Comparisons between TxB2 and 6-keto-PGF1α concentrations in lung lymph and concomitant changes in hemodynamics, lung mechanics, and measures of lung fluid and solute exchange were made using linear and exponential curve fitting techniques. A P value of <0.05 was considered significant. At least two of the three experiments were performed on all 10 sheep used in the meclofenamate and endotoxin series of experiments, 6 of these sheep had all three experiments completed. All three experiments were performed on the two sheep receiving ibuprofen and endotoxin. The data used for the figures is the mean ± SE of all experiments of a given type performed. Statistical analyses were performed using only paired data from the same animal. The n varies slightly for a given measurement but is always greater than seven except for the TxB2 and 6-keto-PGF1α concentrations in lung lymph where n was five. The n for the comparison of TxB2 and 6-keto-PGF1α concentration and the physiologic variables varied between 16 and 26.

RESULTS
Meclofenamate alone (M) had no significant effect on any of the physiologic variables measured (Figs. 1–4). The order in which the experiments were performed had no effect upon the results. For example, endotoxin alone (E) caused as large a change in Ppa and lung mechanics if it were the initial experiment or if it followed the meclofenamate alone experiment (M) or the meclofenamate and endotoxin experiment (M + E). No differences were found between the sheep that had the additional diaphragmatic stripplings to remove possible systemic contamination of lung lymph and the sheep that simply had the tail of caudal mediastinal lymph node ligated and resected.

Endotoxin alone (E) caused Ppa to increase dramatically in the first hour following endotoxia, increasing from a control value of 16 ± 1 cm H2O to 63 ± 4 cm H2O. Ppa rapidly returned towards normal but remained significantly greater than both the preendotoxin controls and the meclofenamate alone (M) values throughout the 5 h of observation (Fig. 1 A). Similarly, PVR increased from 2.7 ± 0.4 to 14.4 ± 2.0 cm H2O/liter × min at 1 h after endotoxia and then rapidly returned towards control values though remaining significantly greater than control throughout the 5 h of observation (Fig. 1 B). Endotoxin alone (E) caused oxygenation to worsen dramatically early following endotoxia and to remain markedly abnormal throughout the 5 h of observation (Fig. 1 C). The ΔAaPO2 increased from a control value of 25 ± 1 torr to 55 ± 3 torr at 1 h and 51 ± 4 torr at 4 h following endotoxia. PaO2 decreased by 42 ± 4 torr at 1 h and 31 ± 5 torr at 4 h following endotoxia. PCO2 decreased by 14 ± 5 torr at 1 h and 8 ± 2 torr at 4 h following endotoxia.

Lung lymph flow, (Q lymph) increased markedly during the early pulmonary hypertension from a control value of 2.0 ± 0.3 ml/15 min to 8.1 ± 0.9 ml/15 min at 1 h following endotoxin alone (E) (Fig. 2 A). The lung lymph at 1 h following endotoxia was relatively protein poor with the protein concentration of lymph relative to plasma (L/P ratio) decreasing from 0.66 ± 0.02 to 0.48 ± 0.02 (Fig. 2 B). Q lymph remained elevated through the period of observation but the lymph became relatively protein rich. At 5 h after endotoxin alone (E), Q lymph was 6.9 ± 1.1 ml/15 min and the L/P ratio 0.67 ± 0.03. When the data are expressed as lymph protein clearance (the product of Q lymph and L/P ratio), C LP rises throughout the 5 h following endotoxia increasing from a control value of 1.3 ± 0.2 ml/15 min to 4.8 ± 0.8 ml/15 min at 5 h after endotoxin alone (E) (Fig. 2 C).

Sublethal doses of endotoxin alone (E) caused dramatic changes in lung mechanics (Fig. 3). These changes were most marked at 1 h following endotoxia, concomitant with the most severe pulmonary hypertension, and then returned either rapidly or slowly depending upon the variable towards the preendotoxin control values. Cdyn decreased from a control value of 0.093 ± 0.010 liter/cm H2O to 0.020 ± 0.003 liter/cm H2O at 1 h after endotoxia. Cdyn slowly returned towards the preendotoxin control values (Fig. 3 A). FRC decreased, following endotoxin alone (E), from a control value of 1.222 ± 0.050 liter to 0.795 ± 0.47 liter at 1 h following endotoxia and then returned towards normal though remaining significantly lower than control for the first 4 h following endotoxia (Fig. 3 B). The sheep were systemically ill, drooping against their passive restraints, during the most marked changes in FRC. By 2 h following endotoxin alone (E), the sheep stood erect and subjectively appeared markedly improved.

Endotoxin alone (E) caused dramatic elevations in RL. RL increased from control values of 1.26 ± 0.39 cm H2O/liter × s to 10.64 ± 2.27 at 1 h following endotoxia and then rapidly returned towards normal (Fig. 3 C). These changes are temporally similar to the alterations that occur in Ppa following endotoxin alone (E) (Fig. 1 A). Since RL can change with lung volume, and since endotoxin alone (E) caused changes in FRC, the data are also expressed as SG L. SG L decreased from a control value of 1.10 ± 0.22 s/cm H2O to 0.17 ± 0.03 s/cm H2O at 1 h following endotoxin

alone (E) and then rapidly returned towards the pre-endotoxin control value (Fig. 3 D).

Endotoxin alone (E) caused lung lymph TxB2 to increase from 0.21±0.11 ng/ml to 10.89±2.37 ng/ml at 1 h following endotoxemia (Fig. 4 A). TxB2 returned fairly rapidly towards control but was still elevated 4 h after endotoxemia (3.07±0.52 ng/ml). Lung lymph 6-keto-PGF1α, a metabolite of prostacyclin, followed a similar pattern to that observed with TxB2 (Fig. 4 B). 6-keto-PGF1α increased from a control value of 0.05±0.01 ng/ml to 4.24±0.71 1 h following endotoxin alone (E). At 4 h after endotoxemia, lung lymph 6-keto-PGF1α returned to near control (0.20±0.08 ng/ml).

Meclofenamate markedly altered the response to endotoxemia (Figs. 1–4). In the combined meclofenamate and endotoxin experiments (M + E), Ppa at 1 h was only 27±2 cm H2O as opposed to 63±4 cm H2O following endotoxin alone (E) (P < 0.05). From 2 to 5 h after endotoxemia, Ppa was significantly greater in the meclofenamate and endotoxin experiments (M + E) than the preendotoxin control and the meclofenamate alone experiments (M) but was not significantly different from the endotoxin alone experiments (E) (Fig. 1 A). A similar pattern was observed for PVR. PVR in the meclofenamate and endotoxin experiments (M + E), at 1 h after endotoxemia, was significantly less than in the endotoxin alone experiments (E) (4.53±0.66 vs. 14.04±1.97 cm H2O/liter × min; Fig. 1 B). Meclofenamate significantly attenuated the hypoxemia caused by endotoxemia over the entire 5 h of observation (Fig. 1 C). Meclofenamate alone (M) had no effect on ΔAaPO2, PAO2, PCO2, or pH.

Meclofenamate markedly attenuated the early rise in Q lymph (Fig. 2 A) that follows endotoxemia. Q lymph was significantly lower in the meclofenamate and endotoxin experiments (M + E) than in the endotoxin alone (E) experiments for the first 3 h after endotoxemia. At 4 and 5 h after endotoxemia there was no difference between the two sets of experiments. Meclofenamate attenuated the drop in L/P ratio that

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**Figure 1** Effect of meclofenamate on endotoxin-induced changes in Ppa, PVR, and ΔAaPO2. Data are expressed as the mean±SE. The 1-h point is the mean of the data obtained from 30 to 60 min after beginning the endotoxin infusion. All other data points represent the mean of the data obtained over the previous 60 min. The data from the endotoxin alone experiments (E) are drawn as (A — — A); the data from the meclofenamate and endotoxin experiments (M + E) are drawn as (Δ — — Δ); the data from the meclofenamate alone experiments (M) are drawn as (O — — O). (●) indicates that the data point is significantly different (P < 0.05) from the time-matched meclofenamate alone (M) data point. (†) indicates that the data point is significantly different (P < 0.05) from the time-matched meclofenamate and endotoxin (M + E) data point. n = 7.

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A

\[ \dot{Q}_{LYMPH} \text{ (ml/15 min)} \]

B

PROTEIN CONCENTRATION

LYMPH
PLASMA

C

LYMPH PROTEIN CLEARANCE

(ml/15 min)

ENDOTOXIN

TIME (HOURS)
follows endotoxemia with the L/P ratio being significantly higher in the meclofenamate and endotoxin experiments \((M + E)\) than in the endotoxin alone experiments \((E)\) for the first 3 h. The L/P ratio following endotoxemia in the meclofenamate and endotoxin experiments \((M + E)\) was, at no point in time, significantly different from the meclofenamate alone experiments \((M)\). \(C_{dp}\) followed a pattern similar to that seen for \(Q\) lymph with there being no difference between meclofenamate and endotoxin experiments \((M + E)\) and the endotoxin alone experiments \((E)\) at 4 and 5 h following endotoxemia.

Meclofenamate markedly altered the changes in lung mechanics caused by endotoxemia in the unanesthetized sheep (Fig. 3). \(C_{dyn}\) decreased significantly only at 1 h following endotoxemia in the combined meclofenamate and endotoxin experiments \((M + E)\). The decrease in \(C_{dyn}\) was significantly less in the meclofenamate and endotoxin experiments \((M + E)\) than in the endotoxin alone experiments \((E)\) at both 1 and 2 h \((0.057\pm 0.009\) in the meclofenamate and endotoxin experiments \((M + E)\) vs. \(0.020\pm 0.003\) liter/cm H\(_2\)O in the endotoxin alone experiment \((E)\) at 1 h after endotoxemia; Fig. 3 A). At no point in time was FRC significantly different in the meclofenamate and endotoxin experiments \((M + E)\) from the control meclofenamate alone experiments \((M)\). The sheep did not droop against their passive restraints or become subjectively systematically ill in the meclofenamate and endotoxin experiments \((M + E)\). FRC was significantly lower following endotoxin alone \((E)\) than in meclofenamate and endotoxin experiments \((M + E)\) over the entire 5 h of observation (Fig. 3 B).

Meclofenamate completely blocked the increase in \(R_L\) observed after endotoxin alone (Fig. 3 C). In the meclofenamate and endotoxin experiments \((M + E)\), \(R_L\) was \(0.90\pm 0.24\) cm H\(_2\)O/liter \(\times\) s at 1 h following endotoxemia (slightly, but not significantly, less than the preendotoxin control of \(1.04\pm 0.18\) cm H\(_2\)O/liter \(\times\) s) as opposed to \(10.64\) cm H\(_2\)/L \(\times\) s in the endotoxin alone experiments \((E)\). \(R_L\) was significantly greater in the endotoxin alone experiments \((E)\) than in the meclofenamate and endotoxin experiments \((M + E)\) for the first 4 h following endotoxemia. \(R_L\) tended to increase slightly at 4 and 5 h following meclofenamate and endotoxin \((M + E)\) but these changes were small and not significant. SG\(_L\) increased slightly for the first 3 h following meclofenamate and endotoxin \((M + E)\) and then dropped at 4 and 5 h. These changes were not significantly different from the control meclofenamate alone experiments \((M)\). SG\(_L\) for the first 3 h following endotoxin alone \((E)\) was significantly lower than the SG\(_L\) observed following meclofenamate and endotoxin \((SG_L\) of \(0.17\pm 0.03\) s/cm H\(_2\)O following endotoxin alone \((E)\) as opposed to \(1.12\pm 0.22\) s/cm H\(_2\)O following meclofenamate and endotoxin \((M + E)\) at 1 h after endotoxemia; Fig. 3 D).

Meclofenamate inhibited the increases in lung lymph concentrations TxB\(_2\) and 6-keto-PGF\(_{1\alpha}\) observed following endotoxin alone \((E)\) (Fig. 4). At 1 h following endotoxemia, lung lymph TxB\(_2\) was \(2.96\pm 2.17\) ng/ml in the meclofenamate and endotoxin experiments \((M + E)\) as opposed to \(10.89\pm 2.37\) ng/ml in the endotoxin alone experiments \((E)\) (Fig. 4 A). TxB\(_2\) concentrations did not increase in three of the five sheep on which these measurements are available from the meclofenamate and endotoxin experiments \((M + E)\). In the other two sheep, TxB\(_2\) increased at 1 h following endotoxemia to \(3.39\) and \(11.25\) ng/ml in the meclofenamate and endotoxin experiments \((M + E)\). In the endotoxin alone \((E)\) studies, on these same two sheep, TxB\(_2\) concentrations reached \(11.65\) and \(15.24\) ng/ml, respectively. The alterations in lung mechanics were attenuated by a similar magnitude in all five sheep. Similarly, lung lymph 6-keto-PGF\(_{1\alpha}\) increased only slightly at 1 h following meclofenamate and endotoxin \((M + E)\) \((0.46\pm 0.38\) ng/ml) as opposed to the endotoxin alone experiments \((E)\) \((4.24\pm 0.71\) ng/ml). 6-keto-PGF\(_{1\alpha}\) concentrations increased slightly \((0.24\) and \(1.97\) ng/ml) in the same two sheep that increased TxB\(_2\) concentrations at 1 h following endotoxemia in the meclofenamate and endotoxin \((M + E)\) experiments. 6-keto-PGF\(_{1\alpha}\) concentrations reached \(4.67\) and \(11.90\) ng/ml respectively in these same two sheep in the endotoxin alone \((E)\) experiments. 6-keto-PGF\(_{1\alpha}\) levels had returned to base line by 2.5 h following endotoxemia in the meclofenamate and endotoxin experiments \((M + E)\) (Fig. 4 B). The results from the two sheep studied with ibuprofen and endotoxin are similar to those in the meclofenamate and endotoxin experiments. Ibuprofen inhibited the normal increase in lung lymph TxB\(_2\) and 6-keto-PGF\(_{1\alpha}\) observed following endotox-
emia. In these two sheep, average TxB$_2$ and 6-keto-PGF$_{1a}$ increased from preendotoxin control values of 0.033 and 0.170 ng/ml to 8.425 and 0.672 ng/ml 1 h following endotoxia in the endotoxin alone (E) experiments. In the ibuprofen and endotoxin experiments (I + E), average TxB$_2$ and 6-keto-PGF$_{1a}$ concentrations were 0.055 and 0.162 ng/ml 1 h following endotoxia. Average P$_{pa}$ pressure was 67 cm H$_2$O in the endotoxin alone (E) experiments and 32 cm H$_2$O in the ibuprofen and endotoxin (I + E) experiments 1 h following endotoxia. Ibuprofen alone (I) had no effect on any of the variables measured. Average ΔAaPO$_2$ was 43 torr 1 h following endotoxia in the endotoxin alone (E) experiments and 26 torr in the ibuprofen and endotoxin (I + E) experiments. 1 and 4 h following endotoxia average lung lymph flow was 4.0 and 4.2 ml/15 min in the endotoxin alone (E) experiment and 2.0 and 2.9 ml/15 min in the ibuprofen and endotoxin (I + E) experiments. In these two sheep, average C$_{dyn}$ decreased to 51.2% of the preendotoxin control in the endotoxin alone (E) experiment and to 79.7% in the ibuprofen and endotoxin (I + E) experiments 1 h following endotoxia. Average R$_L$ increased to 392.1% of the preendotoxin control value 1 h following endotoxia in the endotoxin alone (E) experiments but did not increase (103.7%) in the ibuprofen and endotoxin (I + E) experiments. Average FRC was 81.9% of preendotoxin control value 1 h following endotoxia in the endotoxin alone (E) experiments and 94.3% in the ibuprofen and endotoxin experiments (I + E).

No significant correlations were found between lung lymph or plasma 1 h TxB$_2$ concentrations, 6-keto-PGF$_{1a}$ concentrations or the ratio of TxB$_2$ to 6-keto-PGF$_{1a}$ concentrations and the 1, 2.5, or 4.5 h P$_{pa}$, PVR, ΔAaPO$_2$, Q lymph, L/P ratio, C$_{L,F}$, C$_{dyn}$, R$_L$, FRC, or S$_G$. Similarly, no significant correlations were found between 2.5 and 4.5 h TxB$_2$, 6-keto-PGF$_{1a}$ concentrations or the ratio of TxB$_2$ to 6-keto-PGF$_{1a}$ concentrations and the concomitant 2.5 or 4.5 h P$_{pa}$, PVR, ΔAaPO$_2$, Q lymph, L/P ratio, C$_{L,F}$, C$_{dyn}$, R$_L$, FRC, or S$_G$. The n for these comparisons varied between 16 and 26.

**DISCUSSION**

Endotoxia, in the unanesthetized sheep, has been used as an animal model of ARDS (1, 2). Since changes in lung mechanics are an important component of ARDS in man (3), we have adapted the sheep lung lymph preparation (9) for the simultaneous measurement of lung mechanics. Since the animals survive individual experiments, we were able to study the same animal on different days and thus examine the effects of meclofenamate and ibuprofen, both presumed selective inhibitors of the cyclooxygenase pathway of arachidonate metabolism, on the lung dysfunction caused by endotoxia using each animal as its own control.

We were able to demonstrate that sublethal doses of endotoxin cause dramatic changes in lung mechanics in the unanesthetized sheep (Fig. 3). These changes were most marked at 1 h following endotoxin, concomitant with the most severe pulmonary hypertension, and then returned, depending upon the variable, either rapidly or slowly towards preendotoxin control values. The rapid infusion of endotoxin or live *E. coli* into anesthetized dogs (5) and live *E. coli* into anesthetized baboons caused similar, but less marked, changes in lung mechanics. The magnitude of the changes in lung mechanics observed following endotoxia in unanesthetized sheep is far greater than has been previously reported in sheep with inhaled bronchoconstrictor agents (18, 19).

The dramatic increase in R$_L$ and S$_G$ in the sheep in the first hour following endotoxia is associated with a reproducible decrease in FRC. The sheep has been proposed as being more valuable than other large animals to study airway responsiveness since the unanesthetized sheep, like man, may increase its FRC following aerosol challenge (18). The argument that the increase in FRC observed in some sheep following aerosol bronchial challenge is secondary to air trapping (an increase in residual volume and FRC) in an animal with relatively few collateral channels (18) seems unlikely. If gas trapping was occurring, one would have expected an increase in FRC, rather than a decrease.

**Figure 3** Effect of meclofenamate on endotoxin-induced changes in C$_{dyn}$, FRC, R$_L$, and S$_G$. Data are expressed as the mean±SE. The 1-h point is the mean of the data obtained from 30 to 60 min after beginning the endotoxin infusion. All other data points represent the mean of the data obtained over the previous 60 min. The data from the endotoxin alone experiments (E) are drawn as (○——○); the data from the meclofenamate and endotoxin experiments (M + E) are drawn as (△——△); the data from the meclofenamate alone experiments (M) are drawn as (□——□). (*) indicates that the data point is significantly different ($P < 0.05$) from the time-matched meclofenamate alone (M) data point. (†) indicates that the data point is significantly different ($P < 0.05$) from the time-matched meclofenamate and endotoxin (M + E) data point. $n = 8$. 

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FIGURE 4 Effect of meclofenamate on endotoxin-induced changes in TxB₂ and 6-keto-PGF₁α. The 0-h point is the mean of the data obtained over the preceding 60 min. The 1-h point is the mean of the data obtained from 30 to 60 min after beginning the endotoxin infusion. The 2.5-h point is the mean of the data obtained from 120–150 min after beginning the endotoxin infusion while the 4.5 h point is the mean of the data obtained from 240 to 270 min after beginning the endotoxin infusion. The data from the endotoxin alone experiments (E) are drawn as (O —— O); the data from the meclofenamate and endotoxin infusion experiments (M + E) are drawn as (△ —— △); the data from the meclofenamate alone experiments (M) are drawn as (□ —— □). n = 5. (*) indicates that the data point is significantly different (P < 0.05) from the time-matched meclofenamate alone (M) data point. (†) indicates that the data point is not significantly different (P > 0.05) from the time-matched meclofenamate and endotoxin (M + E) data point. n = 8.
with the marked changes in $R_L$ observed following endotoxemia in these experiments. It seems more likely that the changes observed in FRC in the unanesthetized sheep following both bronchial challenge and endotoxemia are dynamic (possibly related to the postural changes following endotoxemia) rather than directly related to alterations in $R_L$.

The most severe alterations in lung mechanics occur within the first hour after endotoxemia (Fig. 3). These rapid changes in lung mechanics are not due simply to pulmonary edema formation. The changes occur before the development of pulmonary edema (2). If edema caused these changes, then lung mechanics should not improve at a time when changes in lung fluid and solute exchange are worsening (Fig. 2). It appears far more likely that the early alterations in lung mechanics are not related directly to the later changes in lung fluid and solute exchange. These conclusions are supported by the work of McCaffree et al. (5) with live E. coli in anesthetized dogs and baboons in which they concluded that the early changes in lung mechanics were not the result of pulmonary edema formation.

The temporal relationship between the early pulmonary hypertension (Fig. 1 A and B) and increased $R_L$ (Fig. 3 C) is striking and suggests a common factor causing both pulmonary vasoconstriction and bronchoconstriction. The early pulmonary hypertension presumably accounts for the early increase in $Q_L$ lymph (Fig. 2 A) and drop in L/P ratio (Fig. 2 B) since the production of large quantities of relatively protein-poor lung lymph is typical of increased hydrostatic driving pressures (20). The increased PVR (Fig. 1 B) must be, at least in part, postcapillary and not entirely secondary to hypoxic vasoconstriction (Fig. 1 C) since hypoxia, in the adult sheep, increases $P_{pa}$ and PVR but does not increase $Q_L$ lymph (21).

The hypoxia that follows endotoxemia, like the changes in lung mechanics, cannot simply be explained by the development of pulmonary edema. The temporal relationship is not consistent with this and, in experiments where lethal doses of endotoxin were given to sheep, there was no relationship between the severity of the hypoxemia and lung water measured postmortem (2). Similarly, in patients with ARDS, no relationship was found between measured lung water and the concomitant hypoxemia (4). It is far more likely that a common factor causes alterations in both lung mechanics and hemodynamics resulting in local ventilation-perfusion mismatching and hypoxemia.

The experiments with meclofenamate and ibuprofen were designed to test whether these presumed selective inhibitors of the cyclooxygenase pathway of arachidonate metabolism would alter the early changes in lung mechanics and pulmonary hypertension observed following endotoxemia. Meclofenamate and ibuprofen had no effect upon any of the physiologic variables (Figs. 1–4) and presumably had their effects on the sheep’s response to endotoxin through their actions as pharmacologic inhibitors of the cyclooxygenase pathway of arachidonate metabolism. The determinations of lung lymph concentrations of the stable thromboxane-A$_2$ metabolite (TxB$_2$) and the prostanclin metabolite (6-keto-PGF$_1\alpha$) show that meclofenamate and ibuprofen did inhibit cyclooxygenase, markedly attenuating the increases in TxB$_2$ and 6-keto-PGF$_1\alpha$ observed in sheep after endotoxemia (Fig. 4). These experiments do not rule out the possibility that the effects of both meclofenamate and ibuprofen on the sheep’s response to endotoxin were related to a pharmacologic property of these agents other than their effects as inhibitors of the cyclooxygenase pathway of arachidonate metabolism. They could, for example, also inhibit the 5-lipoxygenase pathway of arachidonate metabolism and thus the production of leukotrienes (including slow-reacting substance of anaphylaxis). This seems unlikely though since similar effects were observed with two different agents (meclofenamate and ibuprofen) and since a third cyclooxygenase inhibitor, indomethacin, has been shown to inhibit the pulmonary hypertension caused by endotoxemia (6, 7).

The order of the three separate experiments (endotoxin alone [E], meclofenamate alone [M], and meclofenamate and endotoxin [M + E] or endotoxin alone [E], ibuprofen alone [I], and ibuprofen and endotoxin [I + E]) was randomized in this series of experiments. The order of experiments did not affect the changes observed in lung mechanics, hemodynamics, or fluid and solute exchange. Additionally, the results from sheep that had bilateral diaphragmatic stripping as an additional precaution against potential systemic contamination of lung lymph (8) did not differ from results from sheep prepared in the traditional manner (9). Diaphragmatic contamination of lung lymph does not appear to contribute significantly to the alterations in fluid and solute exchange caused by endotoxemia.

Meclofenamate and ibuprofen markedly attenuated the fall in $C_{dyn}$ (Fig. 3 A) and FRC (Fig. 3 B) observed following endotoxemia in sheep. The changes were similar to those observed with $P_{pa}$ (Fig. 1 A) and PVR (Fig. 1 B). Meclofenamate and ibuprofen also attenuated the early rise in $Q_L$ (Fig. 2 A), fall in L/P ratio (Fig. 2 B) and rise in $C_{tp}$ (Fig. 2 C) that follows endotoxemia. Presumably this effect is the result of meclofenamate and ibuprofen attenuating the early pulmonary hypertension (Fig. 1 A) and thus the increase in microvascular pressure. Sheep receiving me-
clofenamate and ibuprofen did not appear systemically ill or droop against their passive restraints following endotoxia. This may partly account for the attenuation of the decrease in FRC observed following endotoxin.

The most dramatic effect of meclofenamate and ibuprofen, in this series of experiments, was their effect on the increase in $R_l$ observed following endotoxia in sheep. Meclofenamate and ibuprofen completely blocked the increase in $R_l$ and drop in $S_G$ following endotoxin (Fig. 3 C and D). This is, to our knowledge, some of the most direct evidence that cyclooxygenase products of arachidonate metabolism may be responsible for airway changes under pathophysiologic conditions. Ideally one would like to have observed a dose-response relationship between lung lymph or plasma concentrations of $TxB_2$ and 6-keto-PGF$_{1alpha}$ (or their ratio) and the magnitude of the early alterations in lung mechanics and hemodynamics observed following endotoxia. No such relationships were observed in this series of experiments. Although some have reported, in the sheep, a positive correlation between lung lymph $TxB_2$ concentrations and $P_{pa}$ following endotoxia (7). The lack of a correlation in the present series of experiments does not rule out a role for cyclooxygenase products in the altered lung mechanics or hemodynamics observed following endotoxia. It is possible that $TxB_2$ and 6-keto-PGF$_{1alpha}$ concentrations do not accurately reflect local concentrations of their short-lived parent compounds at the reactive site. It is also possible that supramaximal local concentrations of thromboxane-$A_2$ and/or prostacyclin are achieved, thus obscuring a quantitative relationship. Lung lymph concentrations of $TxB_2$ and 6-keto-PGF$_{1alpha}$ reflect mean changes occurring over time. This potentially could obscure a precise quantitative relationship. Other cyclooxygenase products than thromboxane-$A_2$ or prostacyclin may be responsible for the altered lung mechanics and pulmonary hypertension observed early after endotoxia in the unanesthetized sheep. Studies with infused arachidonate products demonstrate that prostaglandins $F_{2alpha}$, $D_2$, $B_2$, $H_2$, and $E_2$ as well as thromboxane can cause pulmonary vasoconstriction in the sheep (21). Infused leukotriene-$D_4$ also causes pulmonary vasoconstriction but these effects may be secondary to stimulation of the cyclooxygenase pathway of arachidonate metabolism (22). We feel that the observed effects of meclofenamate and ibuprofen on the sheep's response to endotoxin are most likely secondary to the pharmacologic inhibition of the cyclooxygenase pathway of arachidonate metabolism and that cyclooxygenase products do mediate the alterations in lung mechanics and hemodynamics observed following endotoxia.

Meclofenamate and ibuprofen also markedly attenuated the severity of the hypoxemia caused by endotoxin in sheep (Fig. 1 C). This effect was observed both early and late following endotoxia and, presumably, resulted from improved ventilation-perfusion matching. Loss of hypoxic vasoconstriction has been observed in dogs following endotoxia (24). Since meclofenamate in dogs prevented the loss of hypoxic vasoconstriction, Weir et al. (24) argued that a circulating dilator prostaglandin (possibly prostacyclin) blocked normal homeostatic hypoxic vasoconstriction after endotoxia. Meclofenamate and ibuprofen may improve oxygenation following endotoxia in sheep through a similar effect on hypoxic vasoconstriction.

Meclofenamate and ibuprofen did not block the late increase in $P_{pa}$ and PVR observed following endotoxia (Fig. 1 A and B). Similarly meclofenamate did not block the late increase in $Q$ lymph and $C_{lp}$ (Fig. 2 A and C). These late changes in fluid and solute exchange, characterized by the production of large quantities of protein-rich lung lymph in the presence of only mildly elevated pulmonary vascular pressures, have been interpreted as representing "increased permeability" of the pulmonary vascular endothelium (1). According to this analysis, meclofenamate does not block the increase in pulmonary vascular permeability observed late after endotoxia. The changes in $C_{dyn}$, $R_l$, and $S_G$ late in the sheep's response to endotoxia were not significantly attenuated by meclofenamate or ibuprofen.

From these experiments, it appears that there are two temporally and pathophysiologically separable components of the sheep's response to endotoxia. Products of the cyclooxygenase pathway of arachidonate metabolism may be important mediators of the early phase of marked alterations in lung mechanics and pulmonary hypertension but not of the late phase of "increased permeability" of pulmonary exchange vessels.

There is considerable evidence that granulocytes may be important in the late phase of the sheep's response to endotoxia (25, 26). There is no correlation between the severity of the early pulmonary hypertension and the late changes in fluid and solute exchange or the magnitude of the leukopenia caused by endotoxia (25). On the other hand, the more marked the leukopenia late in the sheep's response to endotoxia, the greater the increase in $C_{lp}$ (25). Meclofenamate has no effect on the leukopenia caused by endotoxia (25). Granulocyte depletion has no effect on the early pulmonary hypertension but markedly attenuates the late increases in fluid and solute exchange following endotoxia (26). Steroids attenuate both the early pulmonary hypertension and the late changes in fluid and solute exchange (27).

The unanesthetized sheep's response to endotoxia
is unquestionably complex and involves multiple interrelated pathologic and homeostatic mechanisms. This is well illustrated by the early alterations in lung mechanics and hemodynamics caused by endotoxemia. Granulocyte depletion not only attenuates the late changes in lung fluid and solute exchange, but also attenuates the early alterations in lung mechanics, while not effecting the early pulmonary hypertension (28). Therefore, if cyclooxygenase products mediate both the early changes in lung mechanics and the early pulmonary hypertension, then the cyclooxygenase products responsible for the airway changes may be of granulocyte origin, while those involved in the pulmonary hypertension are not. The mechanism through which granulocytes effect the late increases in fluid and solute exchange also remain unexplained through possible roles of oxidative injury through free radical generation or the release of 5-lipoxygenase products of arachidonate metabolism such as the leukotrienes (leukotriene-C4 can increase pulmonary capillary permeability [29]) are the subject of ongoing research. The results of these studies, hopefully, will prove directly applicable to man and suggest interventions of value in the therapy of the ARDS.

In summary, we have shown that endotoxemia not only causes changes in hemodynamics, fluid and solute exchange and oxygenation in the unanesthetized sheep but also causes marked alterations in lung mechanics. Evidence is presented that suggest that cyclooxygenase products of arachidonate metabolism may be pathophysiologically important in the early changes in lung mechanics (especially R5) and hemodynamics but not in the late changes in fluid and solute exchange caused by endotoxemia. Meclofenamate and ibuprofen attenuate the severity of the hypoxemia observed both early and late following endotoxemia. Since similar changes in lung function occur in humans with gram-negative endotoxemia, these findings may be relevant to the pathogenesis of the ARDS.

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