
DISCUSSION
A discussion ensued on the negative inotropic effects of the various calcium channel blockers. In patients with preserved ventricular function, nifedipine and diltiazem probably exert less negative inotropic effects than verapamil. In contrast, in patients with compromised right or left ventricular function, all of the three available calcium channel blockers can exert important negative inotropic effects, although preliminary evidence suggests that diltiazem may be the least cardiodepressant. This may explain why adverse hemodynamic and clinical reactions have been reported following the administration of nifedipine and verapamil to patients with primary pulmonary hypertension, whereas similar unfavorable events have not yet been observed with diltiazem.

Should patients who show no acute pulmonary vasodilator response to vasodilators receive long-term treatment? Many of those present felt that patients who failed to respond to IV prostacyclin were unlikely to respond favorably to other vasodilator drugs, given either acutely or chronically; similar findings have not been established, however, for other agents (ie, nitroglycerin, nitroprusside, or nifedipine). Estimates of the proportion of patients who might show long-term hemodynamic and symptomatic improvement with vasodilator therapy varied from 10 to 35 percent; this range probably reflects differences in the criteria used to select patients for treatment.

The discussants pointed out that these patients must be characterized both clinically and pathologically. From a clinical point of view, they all have unexplained pulmonary hypertension, but from a pathologic standpoint, it is not known how many have pleuriform lesions, and, thus, would not be expected to respond favorably to vasodilator therapy. One reason for the differing perceptions of the efficacy of vasodilators is that each investigator sees relatively few patients. Consequently, it is important that the experience of various centers be combined and analyzed, using a mechanism such as the NIH primary pulmonary hypertension registry.

Vasoconstriction and Remodeling in Pulmonary Hypertension
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In one group of sheep, *Escherichia coli* endotoxin was given intravenously three times per week for ten weeks, and in another group the cyclooxygenase inhibitor indomethacin was given subcutaneously two times per day for three weeks. Both groups developed the structural and functional changes of modest but sustained pulmonary hypertension and showed granulocyte sequestration in the peripheral lung. Indomethacin enhanced pulmonary vasoreactivity, but endotoxin depressed reactivity transiently. Prolonged inflammation of the lung may be associated with alterations in vasoreactivity and the development of chronic pulmonary hypertension.

Chronic pulmonary hypertension develops in several human diseases when chronic or repeated inflammation of the lung is seen, eg, cystic fibrosis and chronic bronchitis and emphysema. In addition, patients who die after a prolonged, acute course of the adult respiratory distress syndrome often have persistent pulmonary hypertension.

The purpose of the present study was to establish in awake sheep (1) whether repeated lung inflammation caused by *E. coli* endotoxemia leads to the functional and structural changes of chronic pulmonary hypertension, and (2) whether chronic inhibition of the cyclooxygenase pathway leads to maintained vasoconstriction, resulting in the structural changes of chronic pulmonary hypertension.

MATERIALS AND METHODS
Chronically catheterized sheep were prepared as previously described and measurements made twice weekly of baseline pulmonary and systemic artery pressure, left atrial pressure, cardiac output, arterial blood gases, pH, and number of circulating white blood cells. Pulmonary vasoreactivity in response to breathing 12 percent oxygen and to a 1-ml bolus injection (0.1 µg/ml) of an analog of PGH₂ (PGH₂-A; Upjohn Co, Kalamazoo, MI) was also tested twice weekly throughout the experiments.

In some sheep the caudal mediastinal lymph node was cannulated. In these sheep, measurements were made of the levels of the stable metabolites of prostacyclin and thromboxane A₂ in lung lymph and blood plasma.

Structural Studies
Baseline biopsy tissue was taken from each animal at the time of catheter insertion and at various times throughout the experiments. The biopsy technique has been previously described. Sections from the biopsy tissue were stained with hematoxylin and eosin and used to assess number of peripheral lung granulocytes. At the end of the experiments the sheep were killed by an overdose of pentobarbital and the lungs and heart removed intact. The pulmonary arterial circulation was distended with a barium sulfate gelatin mixture prior to fixation by means of airway distention with 10 percent of formal saline solution, and quantitative techniques were applied to assess the structural changes of chronic pulmonary hypertension.
Experimental Protocols

Repeated endotoxin: E. coli endotoxin (0.5 to 4 μg/kg) was infused IV into sheep three times a week over a ten-week period. Control sheep received saline infusions.

Chronic indomethacin treatment: Indomethacin (Sigma Chemical Co) was administered subcutaneously twice a day (5 mg/kg) over a three-week period. Control sheep received a similar volume (2 ml) of vehicle (phosphate buffer [150 mM] made alkaline with 2M sodium bicarbonate, pH 7.6).

RESULTS

Repeated Administration of Endotoxin

Thrice weekly IV infusions of endotoxin into awake sheep led to a significant 50 percent increase in pulmonary artery pressure by week 9 (n = 4) and, in some sheep, to an increase in pulmonary vascular resistance by week 7. Left atrial pressure increased gradually throughout the study (baseline = 1.0 ± 1.1 cm H2O, m ± SE: 10 weeks = 8.0 ± 3.2), but cardiac output remained unchanged.

Studies of pulmonary vasoreactivity to breathing 12 percent oxygen and to an IV bolus injection of PGH2-A revealed a gradual reduction in both their pressor responses, reaching nadirs at five and six weeks. Temporally, this reduction corresponded to increased levels in the metabolite of prostacyclin measured both in lung lymph and blood plasma.

In lung tissue taken at biopsy throughout these experiments, preliminary data have shown approximately a fourfold increase in the number of peripheral lung granulocytes at each time studied (baseline number per 100 alveoli = 9.4 ± 3.4). Preliminary quantitative light microscopic studies applied to the pulmonary arteries have revealed extension of muscle into smaller and more peripheral arteries than normal, increased medial thickness of the large normally muscular arteries, a reduction in number of arteries that fill with barium and right ventricular hypertrophy.

Thus, repeated infusions of endotoxin result in modest increases in pulmonary artery pressure and pulmonary vascular resistance and in transiently increased levels of prostacyclin that parallel a reduction in pulmonary vasoreactivity. Structural studies show peripheral lung granulocyte sequestration and changes of chronic pulmonary hypertension.

Repeated Administration of Indomethacin

In a recent study we have reported on the functional and structural effects of chronic inhibition of the cyclooxygenase pathway by indomethacin on the pulmonary circulation.3 Briefly, long-term indomethacin treatment led to a 50 percent increase in pulmonary artery pressure (Fig 1) and a doubling in pulmonary vascular resistance over the three weeks of the study. Left atrial pressure and cardiac output remained unchanged. That prostanoid production was blocked by indomethacin treatment was confirmed, since low levels of thromboxane B2 and 6-keto-PGF1α were found in both lung lymph and blood plasma. Additionally, these animals failed to respond to infusions of arachidonic acid.

Vasoreactivity to breathing 12 percent oxygen, in these same animals, was transiently increased at week 1, while vasoconstrictor responses to PGH2-A increased more gradually and were sustained.

Structural studies showed an approximately fourfold increase in number of peripheral lung granulocytes in the indomethacin treated sheep during the three-week study. Quantitative techniques applied to the autopsy specimens revealed a reduction in the number of barium-filled peripheral arteries and a reduction in their external diameter. A trend toward an increase in the proportion of muscular arteries at intra-acinar levels was also apparent. No evidence of either increased medial thickness or right ventricular hypertrophy was found.

Thus, repeated administration of indomethacin also leads to functional and structural changes in the lungs microcirculation consistent with the development of chronic pulmonary hypertension. These changes are accompanied by increased pulmonary vasoreactivity and granulocyte sequestration in peripheral lung.

SUMMARY AND CONCLUSIONS

This study examined (1) the effect of repeated or prolonged inflammation of the lung by E. coli endotoxin and (2) the effect of chronic inhibition of cyclooxygenase activity. Our findings suggest that chronic lung inflammation is associated with both structural and functional changes of chronic pulmonary hypertension. Somewhat paradoxically, our results following repeated injections of the anti-inflammatory agent indomethacin also show sequestration of granulocytes in peripheral lung and development of modest but sustained pulmonary hypertension. The structural changes seen in the pulmonary circulation following indomethacin may, however, reflect maintained vasoconstriction rather than structural remodeling of the pulmonary circulation.

Two different interventions, both of which sequestered leukocytes in the lung periphery, caused sustained pulmonary hypertension and structural alterations in the lung circulation but had opposite effects on pulmonary vasoreactivity. These studies may implicate interactions of inflam-
flammatory cells with lung vessels in the pathogenesis of chronic pulmonary hypertension and suggest that vasodilator prostanooids modulate pulmonary vasoreactivity when the lungs are chronically inflamed.

REFERENCES

DISCUSSION

Much of the discussion focused on the role of cyclooxygenase and lipoxygenase products in the normal lung and in models of pulmonary hypertension. Prostacyclin is the only pulmonary vasodilator in the cyclooxygenase pathway. Unlike prostaglandin E₃, it is a potent pulmonary vasodilator. It is liberated even by such benign stimuli as the deformation occurring during normal ventilation and can be found in the lung lymph. The chronic inhibition of cyclooxygenase in numerous patients ingesting nonsteroidal anti-inflammatory agents is not known to be associated with pulmonary hypertension. However, the possible effects of such inhibition on the pulmonary vasculature have not been studied. In the experiments reported here the dose of indomethacin used to cause chronic pulmonary hypertension in sheep was twice the largest dose usually given to humans. In one instance these findings were reproduced by ibuprofen. Because indomethacin caused some inflammatory cell infiltrate, the question was raised whether an effect on the leukocytes might be responsible for the development of pulmonary hypertension, rather than a direct action on the vessel wall. With the inhibition of cyclooxygenase by indomethacin, the possibility that increased lipoxygenase metabolites might cause pulmonary hypertension was also considered. For example, increased lung injury and leukotriene production (including LTB₄) has been observed when indomethacin is administered to rats treated with monocrotaline. In one sheep there was a large increase in 12-HETE, which suggested activation of the 12-lipoxygenase pathway, but the chemotactic leukotriene, LTB₄, has not been measured. The pulmonary hypertension in the sheep caused by repeated doses of endotoxin could be seen as a model of the pulmonary hypertension which occurs in cystic fibrosis, chronic bronchitis, and emphysema.

The question was raised whether the endothelial cells were altered by the endotoxin and whether the failure of small arteries to appear on barium injection was due to loss of vessels or merely lack of filling. When the biopsy was taken a day after endotoxin injection, the endothelium was normal. The decrease in vessels on the barium radiograph was thought to be due to lack of filling rather than loss. Although the demonstration of leukocytes in the lungs does not necessarily imply a pathologic role, the granulocytes are activated each time endotoxin is administered.