Pulmonary antioxidants, in hydryl pulmonary medial is to ically specific Hypoxic Reprint IlAssistant estrogen-treated taglandin female din reaction. Thus with observation intracellular sulfur/hydryl lung reduces pulmonary vasculature could theoretically explain the predominant effect of oxidants on the pulmonary vessels. It is likely that under normoxic conditions lung vessels are exposed to considerably higher levels of oxygen, and thus oxygen radicals, than systemic vessels. It is not known whether it is the smooth muscle cell or another cell in the lung which is primarily affected by sulphydryl oxidizing agents.

The question was raised whether the oxidant agents causing pulmonary vasodilation in the intact dog could be acting through an action on red or white blood cells. Because xanthine oxidase or glucose/glucose oxidase reduce hypoxic vasoconstriction in the isolated rat lung perfused with a salt solution, changes in the formed elements of the blood cannot wholly explain the vasoconstriction caused by oxidant stimuli. The presence of meclofenamate in the perfusate makes a role for dilator prostaglandins unlikely.

The point was raised that pulmonary vasoconstriction has been observed in the isolated rabbit lung under conditions of oxygen toxicity. In some experiments this has been prevented by cyclooxygenase inhibitors or thromboxane synthesis inhibitors. It is not clear whether this represents injury or a physiologic response. One discussant suggested that under different conditions oxygen radicals may ultimately be found to be involved in mechanisms of both vasoconstriction and vasodilatation. Oxygen radical production is increased during severe ischemia. If oxygen radicals can cause vasodilatation as suggested in this paper, the increased production of radicals might account for the roll-off of hypoxic pulmonary vasoconstriction. In the coronary bed the increased production of radicals associated with reperfusion might explain the mechanism of postocclusion hyperemia.

**Prostaglandins and Estradiol-Induced Attenuation of Hypoxic Pulmonary Vasoconstriction**


Pretreatment with estradiol (20 mg IM) attenuated vasoreactivity to decreases in inspired P\textsubscript{F}O\textsubscript{2}, lowered baseline resistance measured under conditions of maximal vasodilation (P\textsubscript{F}O\textsubscript{2} = 0 mm Hg), and appeared to increase prostaglandin release in isolated, blood-perfused lungs of juvenile female sheep. Indomethacin (40 μg/ml) inhibited prostaglandin release and restored hypoxic vasoreactivity in estrogen-treated lungs, but did not alter the estrogen-induced decrease in baseline resistance. These results suggest that estradiol enhanced the production of prostaglandins which secondarily attenuated hypoxic vasoactivity. The estradiol-induced decrease in baseline resistance, however, must have been mediated by some other mechanism.

We have previously found that isolated lungs from six-month-old male sheep had a greater vasoconstrictor response to acute hypoxia than females. Moreover, hypoxic responses measured in isolated lungs from six-month-old castrated within the first week of life, juvenile males, and juvenile females were not different from those measured in six-month-old, noncastrated males. These results suggested that the gender difference observed in isolated lungs of six-month-old sheep arose from attenuation of the hypoxic response in the female at the time of puberty, possibly because of enhanced release of female sex hormones. Consistent with this possibility, we found that estradiol pretreatment attenuated hypoxic pulmonary vasoconstriction in isolated lungs from juvenile female and six-month-old castrated male sheep.

Estrogens enhance prostaglandin production in uterine tissue. In addition, Chang and colleagues recently demonstrated that estradiol stimulated release of prostacyclin by rat aortic smooth muscle. Since prostacyclin, a potent pulmonary vasodilator, may modulate pulmonary vasoconstrictor responses to hypoxia, the present study was performed to assess the role of prostaglandins in the attenuation of hypoxic pulmonary vasoconstriction induced by estradiol. The purpose of this communication is to report our preliminary findings.

**METHODS**

Twenty-three juvenile female sheep between 53 and 90 days of age weighing 10 to 31 kg were used in the experiments. Three to five days before the experiments, 12 sheep were given 20 mg of a long-acting...
preparation of estradiol (Delestrogen, Squibb) intramuscularly. On the day of the experiment, the sheep were anesthetized with IM ketamine (40 to 50 mg/kg). Using methods previously described, their lungs were isolated in situ, ventilated with a respirator, and perfused with a mixture of autologous blood and 3 percent dextran-70 in Ringer's lactate by means of an extracorporeal perfusion circuit. Left atrial pressure was kept subatmospheric. Perfusion temperature was maintained between 39 and 40°C. Except during the measurement of pressure-flow relationships, perfuse flow was 50 ml/min/kg body weight. Perfusate glucose concentration was maintained above 90 mg/dl, pH between 7.35 and 7.45, and inspired CO₂ concentration at 5.4 percent. One hour was allowed for stabilization of the preparation before experiments were begun.

Four groups of lungs were studied. Indomethacin (40 μg/ml) was added to the perfuse of five of the 12 lungs from sheep pretreated with estradiol and five of the 11 lungs from control sheep. In all four groups of lungs, the steady-state relationship associated with inspired oxygen tension (PŌ₂) and pulmonary vasomotor tone was determined as described previously. Inspired PŌ₂ was decreased in a stepwise fashion from 200 to 0 mm Hg. At each level of PŌ₂ the relationship between pulmonary artery pressure and flow was measured at five-minute intervals until a steady state was achieved. The hypoxic stimulus-response relationship was quantified by determining the pressure in the pulmonary artery at a flow of 50 ml/min/kg (Ppapo) directly from the pressure-flow curve after a steady state was achieved at each level of PŌ₂. In addition, perfuse samples were obtained when a steady state had been achieved at a PŌ₂ of 200 mm Hg for determination of thromboxane B₂ (TXB₂) and 6-keto-prostaglandin F₆ (6-keto-PGF₆) concentrations by radioimmunoassay.

Statistical comparisons were made using analysis of variance and t tests. Differences were considered significant when p<0.05.

RESULTS

Perfusate prostaglandin concentrations in the four groups are shown in Table 1. Estradiol significantly increased the concentration of 6-keto-PGF₆. The estradiol-induced increase in TXB₂ approached statistical significance (.05 < p < .1). Addition of indomethacin to the perfuse markedly decreased the levels of these prostaglandins and also prevented the increase caused by estradiol.

The hypoxic stimulus-response relationships of the pulmonary circulation are shown in Figure 1. In lungs which did not receive indomethacin, estradiol markedly depressed the pulmonary vasoconstrictor response to hypoxia and in addition lowered the Ppapo measured under conditions of maximal vasodilation (PŌ₂ = 0 mm Hg). Indomethacin had no effect on the stimulus-response relationship in animals not receiving estradiol, but in estradiol-treated animals it restored the slope of the relationship to control values; however, indomethacin did not reverse the estradiol-induced decrease in Ppapo measured under conditions of maximal vasodilation.

DISCUSSION

The results shown in Figure 1 confirm our previous observations that estradiol attenuated the pulmonary pressor response to hypoxia in isolated sheep lungs. They are also consistent with the observations of Moore and Reeves in female dogs but not with those of Fuchs et al in isolated lungs of female rats. The reasons for these inconsistencies are unknown.

The purpose of this investigation was to assess the possibility that the estradiol-induced attenuation of the hypoxic response might be due to enhanced release of prostaglandins. There are at least four reasons why this possibility should be considered. First, estrogen treatment has been shown to enhance production of prostaglandins in uterine tissue and in vascular smooth muscle. In the latter, estradiol increased prostacyclin production apparently by stimulating the activity of prostaglandin cyclooxygenase and prostacyclin synthetase. The distribution of arachidonic acid, the endogenous fatty acid composition, and the phospholipase activity of the vascular smooth muscle cells were not affected. Second, several investigators found that the uterine vasodilator response to estrogen can be at least partially reversed by prior administration of cyclooxygenase inhibitors. Third, we previously found that perfusion of isolated sheep lungs with autologous blood caused release of cyclooxygenase products. Fourth, Voelkel and colleagues found that hypoxic pulmonary vasoconstriction in isolated rat lungs caused release of prostacyclin, which may act to modulate the response.

It is clear from Table 1 that administration of indomethacin inhibited prostaglandin production in the isolated sheep lung. As shown in Figure 1, this inhibition was associated with a significant alteration in the effects of estradiol on the hypoxic stimulus-response relationship. Indomethacin restored the "reactivity" (the slope of the stimulus-response relationship) to control values.

![Graph](image-url)

FIGURE 1. The steady-state relationships between pulmonary artery pressure measured at a flow of 50 ml/min/kg (Ppapo) and inspired O₂ tension (PŌ₂). Estradiol depressed this stimulus response relationship. Indomethacin reversed this depression in that it restored "reactivity" (the change in Ppapo induced by a change in PŌ₂); however, indomethacin did not affect the estradiol-induced depression of the baseline (Ppapo at PŌ₂ = 0 mm Hg).
relationship; however, it did not reverse the estradiol-induced decrease in "baseline" resistance (Ppa\textsubscript{O\textsubscript{2}} at P\textsubscript{O\textsubscript{2}} = 0 mm Hg).

These results suggest that estradiol attenuated the hypoxic stimulus-response relationship in at least two ways. First, it may have reduced the "reactivity" of the pulmonary vasculature to changes in P\textsubscript{O\textsubscript{2}} by enhancing the production and therefore the modulating activity of the prostaglandins. Consistent with this notion are the increases in prostaglandin release observed in estradiol-treated control sheep (Table 1). Second, estradiol decreased the minimum resistance of the pulmonary vasculature. The mechanism of this effect is unknown, but it apparently did not depend on cyclooxygenase products since it was not reversed by indomethacin. It may be that Ppa\textsubscript{O\textsubscript{2}} measured when P\textsubscript{O\textsubscript{2}} = 0 mm Hg was an index of the passive resistive properties of the bed. In this case, the decrease in Ppa\textsubscript{O\textsubscript{2}} at P\textsubscript{O\textsubscript{2}} = 0 mm Hg could reflect morphologic alterations of the pulmonary vessels. Wolinsky\textsuperscript{a} found that estrogen treatment prevented the medial hypertrophy observed in rats with renovascular hypertension. In addition, estrogens are thought to accelerate angiogenesis in fetal rabbit lungs\textsuperscript{b} and to influence the degree of vasodilatation determined histologically in neonatal rabbit lungs.\textsuperscript{c} Perhaps in our experiments estradiol decreased the baseline resistance by inducing morphologic changes in the lung, which increased vascular cross-sectional area. Whether or not these speculations are correct will require further investigation.

REFERENCES
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DISCUSSION
There was discussion concerning the importance of male and female hormones in clinical pulmonary hypertension. There is a higher incidence of primary pulmonary hypertension in women between adolescence and menopause. There appears to be a male preponderance in high altitude pulmonary edema and in chronic mountain sickness. While it is known that there are steroid receptors in the lungs and steroid metabolism occurs in the lungs, it is not known whether receptors are present on the pulmonary vascular smooth muscle. Therapeutic use of estrogens in pulmonary hypertension cannot be considered at present because very large doses were used in the current experiments and under some circumstances estrogens can actually enhance pulmonary vascular reactivity. The possibility was suggested that the large doses of estrogen used might have a nonspecific effect. There is considerable interest at present in compounds known as the dihydroxy-catechol-estrogens. These substances can inhibit catechol-o-methyltransferase.

The administration of testosterone has been reported to increase right ventricular hypertrophy and hematocrit during chronic hypoxia. In the isolated perfused lung no significant difference in the pressor response to hypoxia was observed between lungs from castrated and noncastrated male sheep. Although in the experiments reported there appears to be a protective effect of estrogens, the possibility that male hormones enhance reactivity has not been excluded.