acid, putative inhibitors of cyclooxygenase and lipoxygenase, but not by incubation with indomethacin or cycloheximide.

Separation of cell surface components of radio-iodinated monolayers revealed multiple bands ranging in molecular weight from 24 to 200 kD. Monolayers incubated under conditions of hypercarbia, but not hypoxia, displayed marked enhancement or appearance of bands of molecular weight about 26 and 52 kD. However, adherence of quiescent or activated PMN to endothelial monolayers was not enhanced by either hypoxia or hypercarbia.

**DISCUSSION**

These results demonstrate that cultured endothelial cells are capable of responding to environmental hypoxia through release of chemotaxtractant activity for PMN. Although the identity of this chemotaxtractant activity is not yet known, the results of inhibitor experiments suggest that it may be a metabolite of arachidonic acid. In addition, hypercarbia but not hypoxia altered endothelial cell surface proteins, with the appearance of bands of molecular weight 26 and 52 kD. Although endothelial cell surface substances may modulate PMN adherence, the adherence of PMN to hypoxic or hypercarbic monolayers was not changed. Thus, endothelial cells respond to ischemic conditions by both release of substances into media and cell surface changes.

We speculate that endothelial cells may contribute to inflammatory responses in ischemic tissues by release of chemotaxtractant activity for PMN. Our studies further suggest that endothelial cells from different vascular beds differ in sensitivity to ambient hypoxia and perhaps to subsequent ischemic vascular injury.

**REFERENCES**


**Subacute Hypoxic Exposure Increases Lung Transvascular Protein Escape in Rats**

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Pulmonary edema occasionally develops following rapid ascent to high altitude. Despite wide recognition of the clinical features and natural history of this condition, its pathophysiology remains poorly understood. Considerable debate exists concerning the relative roles of increases in hydrostatic pressure and vascular permeability in leading to the development of edema at altitude. Although pulmonary artery pressures are significantly elevated in this condition, several features suggest that increases in vascular permeability may play a role in high-altitude pulmonary edema. First, findings from autopsy studies and analysis of bronchoalveolar lavage demonstrate significant elevations of protein content, suggesting a breakdown in the normal vascular barrier to protein flux. In addition, pulmonary capillary wedge and left atrial pressures are consistently normal in this condition, suggesting by exclusion that a permeability defect is responsible for the development of the edema. However, direct attempts to measure hypoxia-induced increases in vascular permeability have produced conflicting results that may reflect differences in experimental design, animal species, or in the methods used to measure vascular permeability, which may not have been sensitive enough to detect mild degrees of lung injury. The purpose of this study was to determine whether abrupt exposure to simulated altitude in a hypobaric facility led to increases in pulmonary vascular permeability in rats using a technique capable of differentiating changes in surface area recruitment from changes in vascular barrier function.

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**Figure 1.** Bovine aortic, pulmonary arterial, coronary arterial, and human umbilical vein endothelial cells incubated with MEM for 4 h at 37°C in a sealed chamber gassed with either 21%, 10%, 3%, or 0% O2, 5% CO2, balance N2. No serum was added during the experimental period. After 4 h, culture media were collected and centrifuged at 5,000 rpm for 15 min. Supernatants frozen at −70°C until chemotaxis assays were performed; then, 25 µl of supernatant assessed for stimulation of PMN migration, the result expressed as percentage of neutrophil response to buffer (M-190) alone. Mean ± SEM; the number at the base of each bar represents the number of monolayers tested; *p < 0.05 compared to data at 21% O2 of the same endothelial cell type.
Sprague Dawley rats (200-300 g) were exposed to hypobaric hypoxia (Pb 450 mm Hg, ~14,500 ft) in a chamber evacuated by a roof-mounted vacuum pump. Vascular permeability was assessed as the extravascular accumulation of radiolabeled transferrin albumin given IV with 14Cr-tagged RBCs. Altitude exposures of 24 and 48 hours led to increases (31% and 36%, respectively) in lung transvascular protein escape and gravimetric estimates of lung water (bloodless wet lung weight/body weight) compared to normoxic control rats. Morphometric measurements of perivascular edema cuff areas confirmed the gravimetric estimates of lung water by demonstrating small but significant increases in edema cuff area in vessels 40-150 μm in maximal diameter in altitude-exposed animals. Briefer hypoxic exposures (1-13 h) were unassociated with such changes, while increases in transvascular protein escape comparable to hypobaric hypoxia were observed following 48 hours of normobaric hypoxia (15% O2).

Previous studies suggested that hypoxia has the potential to lead to a paradox increase in oxidant stress. To explore whether oxidant stress might mediate hypoxia-induced lung injury, we measured plasma oxidized glutathione (GSSG), a marker of oxidant stress, in normoxic and altitude-exposed animals. We found significant increases in plasma (but not lung) GSSG in altitude-exposed animals (Fig. 1). In addition, DMSO (50 mM/kg every 12 h), a hydroxyl radical scavenger, but not mannitol (10 mM/kg every 12 h) completely prevented the altitude-induced increases in transvascular protein escape (Fig 2), lung water, and plasma GSSG.

Dexamethasone has been used to both prevent and treat edematous disorders at altitude. We found that dexamethasone at doses comparable to those used in human trials completely blocked the altitude (Pb 380 mm Hg)-induced increases in transvascular protein escape and lung water. In addition, when hemodynamic measurements were made in altitude-exposed animals, no differences were noted in mean pulmonary artery pressure, cardiac output, or total pulmonary vascular resistance between dexamethasone-treated and untreated animals. Conversely, reduction of endogenous glucocorticoids via adrenalectomy led to significant augmentation of the altitude-induced increases in transvascular protein escape. Furthermore, glucocorticoid re-placement in adrenalectomized animals reversed the augmented protein leak.

We concluded that: (1) hypoxia led to increases in lung transvascular protein escape consistent with an increase in vascular permeability; (2) plasma GSSG is increased following 48 hours of hypoxia, and DMSO prevents the hypoxia-induced increase in plasma GSSG and transvascular protein escape, suggesting a possible role for oxidant stress in this syndrome, and (3) dexamethasone prevents the hypoxia-induced increase in lung transvascular protein escape, while glucocorticoid removal via adrenalectomy augments the altitude-induced changes, suggesting an important modulating role for glucocorticoids on hypoxia-induced vascular permeability.

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