Hypoxia-induced Increases in Pulmonary Transvascular Protein Escape in Rats

Modulation by Glucocorticoids

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Abstract

Pulmonary edema after ascent to altitude is well recognized but its pathogenesis is poorly understood. To determine whether altitude exposure increases lung vascular permeability, we exposed rats to a simulated altitude of ~ 14,500 feet (barometric pressure [Pb] 450 Torr) and measured the pulmonary transvascular escape of radiolabeled ^{125}I-albumin corrected for lung blood content with ^{51}Cr-tagged red blood cells (protein leak index = PLI). Exposures of 24 and 48 h caused significant increases in PLI (2.30±0.08 and 2.40±0.06) compared with normoxic controls (1.76±0.06), but brief hypoxic exposures of 1–13 h produced no increase in PLI, despite comparable increases in pulmonary artery pressure. There were associated increases in gravimetric estimates of lung water in the altitude-exposed groups and perivascular edema cuffs on histologic examination. Normobaric hypoxia (48 h; fractional inspired oxygen concentration [FIO_2] = 15%) also increased lung transvascular protein escape and lung water.

Dexamethasone has been used to prevent and treat altitude-induced illnesses, but its mechanism of action is unclear. Dexamethasone (0.5 or 0.05 mg/kg per 12 h) started 12 h before and continued during 48 h of altitude exposure prevented the hypoxia-induced increases in transvascular protein escape and lung water. Hemodynamic measurements (mean pulmonary artery pressure and cardiac output) were unaffected by dexamethasone, suggesting that its effect was not due to a reduction in pulmonary artery pressure or flow. The role of endogenous glucocorticoid activity was assessed in adrenalectomized rats that showed augmented hypoxia-induced increases in transvascular protein escape, which were prevented by exogenous glucocorticoid replacement. In summary, subacute hypoxic exposures increased pulmonary transvascular protein escape and lung water in rats. Dexamethasone prevented these changes independent of reductions of mean pulmonary artery pressure or flow, whereas adrenalectomy increased pulmonary vascular permeability and edema at altitude. Increases in vascular permeability in hypoxia could contribute to the development of high-altitude pulmonary edema and endogenous glucocorticoids may have an important influence on pulmonary vascular permeability in hypoxia.

Introduction

Rapid ascent to high altitude may be associated with the development of pulmonary edema (HAPE)[1] (1, 2). Despite wide recognition of the clinical features and natural history of this condition, its pathogenesis remains controversial. Central to this debate is the question of the relative contributions of increased hydrostatic pressure and increased vascular permeability to the development of HAPE. Pulmonary artery pressure is increased at altitude because of hypoxic vasoconstriction. If this vasoconstriction were uneven, it could lead to overperfusion of vascular beds in nonvasoconstricted lung zones, with subsequent increases in microvascular hydrostatic pressure (1). However, hemodynamic studies in subjects with HAPE suggest a noncardiogenic, high-permeability mechanism, in that pulmonary capillary wedge pressures are consistently normal in this condition (1). Autopsy findings in patients dying of HAPE also demonstrate protein-rich alveolar fluid (3) and bronchoalveolar lavage studies in subjects with HAPE (4) show significant elevations of protein content, suggesting that the vascular barrier to protein movement has been decreased. Increased vascular protein permeability thus may be an important pathophysiologic component of high-altitude pulmonary edema.

Despite this, attempts to demonstrate hypoxia-induced changes in permeability in the laboratory have produced conflicting results. Bland et al. (5) and Landolt et al. (6) failed to detect increases in lung lymph flow in adult sheep during hypoxia, even with the added stress of increased blood flow. In contrast, other studies have demonstrated increases in lymph flow in newborn lambs (7) and dogs (8–10) after hypoxia. Furthermore, studies in isolated perfused dog lungs have revealed increased filtration and reflection coefficients consistent with increased vascular permeability after brief exposures to anoxic gas (11, 12). Differences in both species and experimental design and in the sensitivity and specificity of the techniques used for the in vivo permeability measurements may partially explain these opposing results. Like humans, many animal species develop hypoxia-induced increases in pulmonary artery pressure, but most animal species fail to develop increases in lung water in response to hypoxia. Although gross alveolar flooding in humans exposed to hypoxia is uncommon, recent clinical and physiologic observations suggest that increases in lung interstitial edema may develop in a large percentage of individuals abruptly exposed to hypoxia (13). To study the mechanism of such hypoxia-induced increases in lung water, we felt that it was important to study the effects of hypoxia on the pulmonary circulation in an animal that has been found in previous reports to have a similar pulmonary vascular response to hypoxia. The rat (as opposed to the ovine species) has been shown to develop hypoxia-induced increases in lung water (14–17), but no assessment of the influence of hypoxia on pulmonary vascular permeability has been carried out in this species.

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1. *Abbreviations used in this paper:* DEX, dexamethasone; HAPE, high-altitude pulmonary edema; RBC, red blood cells.

We therefore sought to determine whether exposure to hypoxia of a degree encountered at moderate altitude increases vascular permeability in rats. We measured the transvascular flux of radiolabeled protein together with an intravascularly confined marker (radiolabeled red blood cells [RBC]) as our index of permeability. This technique appears to be capable of differentiating changes in surface area recruitment from changes in vascular barrier function (18). We also determined the time of onset and the relative effects of hypobaric and normobaric hypoxia on transvascular protein escape. Finally, because the synthetic glucocorticoid dexamethasone has been used for both prevention and therapy of altitude-associated illness in humans (19–22), we investigated the role of exogenous and endogenous glucocorticoids in the modulation of vascular permeability in hypoxia.

Methods

Experimental procedure. Male Sprague-Dawley rats (200–300 g) were used in all studies. Animals were exposed to reduced barometric pressure in a hypobaric chamber evacuated by a roof-mounted vacuum pump. The chamber was maintained at room temperature and continuously purged with a low flow of room air to maintain conditions of low humidity and CO_2. A passive CO_2 absorber system kept the ambient CO_2 at undetectable levels when gas samples were analyzed in a blood gas machine (model 165/2; Corning Medical, Corning Glass Works, Medfield, MA). The chamber barometric pressure in our initial studies was maintained at 450 Torr, whereas the studies involving dexamethasone were carried out at 380 Torr to enhance differences noted at 450 Torr. Control animals were maintained in Denver's ambient Pb of 630 Torr. The chamber was brought to ambient pressure each day for a few minutes to replenish food and water.

After altitude exposures of 1, 3, 6, 13, 24, and 48 h, the animals were removed from the hypobaric chamber and vascular permeability was assessed as described below. In separate experiments, animals treated with dexamethasone received intraperitoneal injections of dexamethasone sodium phosphate (0.5, 0.05, or 0.005 mg/kg; Elkins-Sinn, Inc., Cherry Hill, NJ). Control animals received normal saline in equal volumes 12 h and immediately before hypobaric exposure and then every 12 h during 48 h of subsequent exposure. Vascular permeability was then assessed (see below).

To assess the effects of normobaric hypoxia on lung vascular permeability, we used a chamber equipped with a continuous paramagnetic oxygen sensor linked to a solenoid valve that regulated the inflow of nitrogen from a liquid nitrogen tank to maintain a desired degree of ambient hypoxia in the chamber (15% O_2). CO_2 concentration was maintained at undetectable levels by continuous purging of the chamber together with a passive CO_2 absorber system. After 48 h of normobaric hypoxia, vascular permeability was assessed as in the hypobaric studies described above.

Determination of lung vascular permeability. Vascular permeability was measured using a modification of the method of Sugita et al. (23, 24). This technique measures the accumulation of an intravascularly administered radiolabeled protein molecule in the extravascular space of the lung over 1 hr. As the extravascular accumulation of this label depends not only on the permeability of the vascular barrier to protein movement, but also on the exchange surface area and the driving force (concentration) of the tracer protein, we corrected our calculations for these latter two variables to minimize their influence on our results.

Blood for radiolabeling was taken by direct cardiac puncture with heparinized syringes from donor rats (Sprague-Dawley) after brief ether anesthesia. The blood was spun at 800 *g* for 5 min and plasma removed. The cells were gently mixed with ^{51}Cr (New England Nuclear, Boston, MA) for 15 min and then washed twice with the previously decanted plasma to remove unbound Cr. We find that this procedure produces binding of > 85% of the originally administered chromium to the RBC, as judged by measurements of ^{51}Cr in serial washes. HSA labeled with ^{125}I (Nuclear Pharmacy, Denver, CO) was used as our protein tracer and found to be > 96% bound by measuring ^{125}I activity in the supernatant of protein precipitated samples. After hypoxic exposure, a mixture of the radiolabeled ^{125}I albumin (1 μCi/rat) and the ^{51}Cr-tagged RBC (5–10 μCi/rat) in a total volume of 0.5 cm^3 was given via tail vein injection. 1 h later, the chest was opened and a blood sample was obtained by cardiac puncture before removal of the heart and lungs *en bloc.* The lungs were dissected free from the hilar structures and external blood was removed by gentle blotting. The lungs and blood sample were subsequently weighed and then counted in a multichannel gamma scintillation counter with correction for background and Compton scatter. Extravascular ^{125}I protein accumulation was then calculated as described below: extravascular lung ^{125}I counts = whole lung ^{125}I counts − intravascular lung ^{125}I counts, where intravascular lung ^{125}I counts = blood ^{125}I activity × ^{51}Cr counts in lung/blood ^{51}Cr activity, blood ^{125}I activity = blood ^{125}I counts/g blood, blood ^{51}Cr activity = blood ^{51}Cr counts/g blood, and lung blood weight = ^{51}Cr counts in lung/blood ^{51}Cr activity. The extravascular lung ^{125}I counts are then divided by intravascular radiolabeled protein concentration (blood ^{125}I activity) and lung blood weight to derive the protein leak index. The former corrects for differences in radiolabeled protein concentrations between rats, and the latter is intended to minimize the effects of differences in vascular surface area and relative lung mass (18). Protein leak index = extravascular lung ^{125}I counts/blood ^{125}I activity/Lung blood weight.

Hemodynamic measurements. 1 d before hypoxic exposure animals were lightly anesthetized with 100 mg/kg intramuscular ketamine and 15 mg/kg rompun for catheter placement. A PE-50 catheter was inserted in the right carotid artery and a second polyvinyl catheter (i.d. 0.028 mm; o.d. 0.061 mm) with a shallow curve at its distal end was introduced into the main pulmonary artery to measure systemic and pulmonary vascular pressures. Location of the pulmonary artery catheter was determined from the shape of the pressure trace and confirmed at autopsy. Another PE-50 catheter was placed in the left jugular vein; the catheters were filled with heparinized saline, tied to prevent backflow, and tunneled under the skin to the back of the neck where they were secured and protected at the end of the procedure. An antibiotic (0.1 cm^3 Longicil) was given intramuscularly after surgery. After a 24-h recovery period rats were placed in the hypobaric chamber for 48 h and then immediately studied in the awake state after this exposure. Control animals were treated in a similar fashion but maintained in room air conditions over a comparable time frame. Pressure tracings were displayed on an oscilloscope. Mean pressures and heart rates were calculated by a computer (Nova; Data General Corp., South Boro, MA). Cardiac output was measured by a dye-dilution method using a closed-loop circuit that obviated the need for blood removal (25). Blood was pumped through a shunt from carotid artery to the left jugular vein catheter at 3 ml/min through a cuvette (Waters Associates, Milford, MA) and densitometer, which senses cardiogreen dye (0.05 ml containing 1 mg/ml) after injection into the pulmonary artery. Cardiac output was calculated by the Nova computer using an algorithm based on the standard Stewart-Hamilton method (26). Measurements were made both in ambient room air and in 12% oxygen to simulate hypoxic conditions at 380 Torr.

Gravimetric measurements of lung water. The lungs and blood samples were removed at the time of killing and weighed. The wet weight of the lung was corrected for the contribution of lung blood content, calculated by measurement of ^{51}Cr-labeled RBC activity, to derive the bloodless lung wet weight. This value was then divided by the prealtitude exposure body weight which gave the value for the bloodless wet lung weight/body weight ratio (grams/kilograms). Lungs and blood samples were then dried to constant weight in an oven (50°C) and calculations were also made of the bloodless wet lung weight/dry weight ratios (27).

Adrenalectomy. Animals were adrenalectomized after anesthesia with 15 mg/kg rompun and 100 mg/kg ketamine. Through small bilat-

eral retroperitoneal incisions, the adrenals were identified at the superior pole of each kidney, removed, and the skin sutured with 3-0 silk. Sham controls underwent similar surgery, except the adrenals were not removed. Rats drank 0.9% physiologic saline solution ad lib. after recovery. One group of adrenalectomized animals also received glucocorticoid replacement after adrenalectomy (0.05 mg/kg dexamethasone (DEX) every 12 h). The animals were allowed 4–5 d of recovery before hypoxic exposure. Adrenalectomies were confirmed at autopsy and by plasma corticosterone determinations (28) run by RIA at Hazelton Laboratories, Vienna, VA (Adrenal intact, Mean, 11.20±2.93 μg/dl; range, 4.20 to 23.20 μg/dl; adrenalectomized, Mean-0.51±0.04 μg/dl; range, 0.30 to 0.60 μg/dl.)

Histology. Hypoxic-(Pb 450) and normoxic rats (n = 6) were anesthetized as above and tracheostomized and the lungs fixed via the airway with 10% buffered formalin at 20 cm water pressure. Longitudinal sections of each lung lobe were prepared for each of our six experimental and control animals and stained with trichrome. Measurements of pulmonary vascular cuff area were made by planimetry in a blinded protocol (the observer did not know if the section came from control or hypoxic animals). A computer (MOP-3, Carl Zeiss, Inc., Thornwood, NY) was used to make calculations of maximal vessel diameter, vessel area, and cuff area. To minimize distortions in relative cuff area due to collapsed or oddly shaped vessels we also measured the form factor of each vessel. The form factor calculated as, $F = 4\pi A/P2$, describes the relationship of the area (A) to the perimeter (P) of an object. A perfect circle has a form factor of 1, whereas a substantial perimeter with little area would have a value close to zero. Only vessels with a form factor of 0.6 or greater were used. We attempted to measure the area and size of every vessel in a given specimen that was not contained in a bronchovascular bundle. These vessels were chosen due to difficulties in accurately measuring cuff area within the bronchovascular sheath. Three groups of vessel sizes were compared and included vessels with maximal diameters of 10–20, 30–40, and 50–140 μm. Approximately 30–50 vessels of all three size groups (with form factor > 0.6) were counted for each section. Thus, as five sections were prepared for each animal, between 150 and 250 vessels per rat were counted. Approximately equal numbers of each vessel size group were available for group comparisons.

Statistics. For all groups the mean values±SE are shown. Comparison of group differences was done by one way analysis of variance with multiple comparisons. Differences were considered significant when $P < 0.05$ by Student-Newman-Keuls test. Morphometric measurements were analyzed statistically by a one-tailed t test.

Results

Transvascular protein escape and lung water with hypoxia. Hypobaric hypoxia (Pb 450 Torr) produced increases in pulmonary transvascular protein escape (PLI) and gravimetric measurements of lung water (bloodless wet lung weight/body wt) after exposures of 24 and 48 h (Table I). Briefer hypoxic exposures for 1, 3, 6, and 13 h did not cause significant increases in the PLI or gravimetric lung water.

To determine whether hypobaria had caused these changes, measurements of transvascular protein escape were made in rats exposed to comparable degrees of normobaric hypoxia (FIO_2 = 15%, 48 h). PLI in the normobaric hypoxia group were significantly increased over values seen in normoxia (1.63±0.10, normoxia; 3.13±0.56, normobaric hypoxia; n = 8). Both hypobaric and normobaric hypoxia thus caused increases in lung transvascular protein escape.

Because our measurements of transvascular protein escape were performed in the room air environment of the laboratory, we wondered if the restoration of normoxia after hypoxic exposure could contribute to the development of increased permeability in the hypoxic animals. Accordingly, we performed additional experiments in a hypobaric facility of sufficient size to accommodate the investigators so that hypoxia was continuous from the beginning of the exposure throughout the measurement period. We again found increases in PLI in altitude exposed animals (n = 6) compared with normoxic controls (1.58±0.10, normoxic control; 2.71±0.01, continuous hypobaric hypoxia). Increases in lung water measured either by bloodless wet lung weight/body wt (W/B) or wet lung weight/dry wt (W/D) were also seen in the group maintained in continual hypobaric hypoxia compared with room air controls. (4.43±0.23, normoxic lung W/B; 5.16±0.22,* continuous hypobaric hypoxia lung W/B; 4.87±0.06, normoxic lung W/D; 5.09±0.03,* continuous hypobaric hypoxia lung W/D; * = $P < 0.05$ from normoxic control).

Table I. Effect of Hypobaric Hypoxia on Protein Leak Index and Gravimetric Measurements of Lung Water

Groups	n	PLI	Lung wet/ body	Lung wet/ dry
Normoxia	30	1.76±0.06	4.48±0.06	5.09±0.05
Hypobaric hypoxia (*24 h*)	20	2.30±0.08*	4.70±0.08*	5.20±0.05
Hypobaric hypoxia (*48 h*)	27	2.40±0.06*	5.00±0.06*	5.23±0.06

PLI, bloodless wet lung weight/body weight, and bloodless wet lung weight/dry weight ratios for normoxic rats and rats exposed to hypobaric hypoxia (Pb 450 Torr) for either 24 or 48 h.
* = $P < 0.05$ from normoxic control.

Hemodynamic effects on protein leak measurement. To determine whether increases in pulmonary artery pressure or surface area recruitment due to hypoxia had caused the increases in transvascular protein escape seen at 24 or 48 h independent of increases in vascular permeability, the effects of acute increases in pulmonary artery pressure on the PLI were assessed. Two groups of rats (animals identical to those used in prior studies) were studied 24 h after placement of catheters for hemodynamic measurements. A normoxic group was confined in a Plexiglas cage purged with normoxic gas while continuous hemodynamic measurements and protein leak measurements were made over 1 h. Arterial blood gases were performed to document normoxia before injection of radiolabeled RBC and albumin and measurement of the protein leak index 1 h later. Animals in the second group were exposed to hypoxic gas mixtures (FIO_2 8–15%) to maintain elevations of mean pulmonary artery pressures in the 27–30-mmHg range for 1 h during measurement of the PLI. Mean pulmonary artery pressures in the hypoxic animals (27.9±0.9) were similar to pressures observed in the 48-h hypoxia-exposed animals (see below) and exceeded values in normoxic controls (21.4±0.7; $P < 0.001$). No significant differences in cardiac output were noted between the two groups. Despite increases in pulmonary artery pressure comparable to those observed in the 48-h hypoxia group described below, no differences in PLI between the normal pressure and increased pressure groups were observed. (PLI normal pressure = 1.54±0.21; increased pressure = 1.32±0.11; P = NS.)

Histology. Although no gross alveolar fluid could be discerned, gravimetric increases in lung water in hypoxia-exposed animals were confirmed by small but significant increases in

average perivascular edema cuff areas in vessels 50–140 μm in greatest diameter compared with normoxic animals. (Hypoxia = $15.76 \pm 1.6 \times 10^3$ μm^2, control = $12.3 \pm 1.0 \times 10^3$ μm^2, $P = 0.03$.) In contrast, no consistent increases in edema cuff areas in smaller vessels (10–20 and 30–40 μm) from altitude exposed animals were noted.

Effect of dexamethasone on vascular permeability at altitude. Dexamethasone pretreatment at doses of 0.5 and 0.05 mg/kg reduced transvascular protein escape (PLI) and lung wet weight/body weight ratios compared with saline-pretreated altitude-exposed animals (Fig. 1).

Because the protective effect of DEX on lung vascular permeability at altitude might have been due to a reduction of pulmonary arterial pressure or blood flow, we measured mean pulmonary artery pressure, cardiac output, and total pulmonary vascular resistance in normoxic rats, and in rats exposed to 48 h of hypobaric hypoxia (Pb 380 Torr) who received intraperitoneal injections of either dexamethasone (0.05 mg/kg) or normal saline ($n = 6$ in each group). As measurements of lung vascular permeability were made in room air after removal from the hypobaric chamber, initial hemodynamic data was collected while the rats were breathing room air (15 min) to estimate the hemodynamics during this period. Mean pulmonary artery pressure was increased in altitude-exposed animals and this increase was unaffected by dexamethasone. Cardiac output and total pulmonary vascular resistance showed no significant differences between the groups tested (Fig. 2).

Subsequent hemodynamic measurements were made while rats were breathing 12% O_2 to estimate values present during the hypoxic exposure. Both hypoxic groups demonstrated further increases in mean pulmonary artery pressure, cardiac output, and total pulmonary vascular resistance, but no significant differences were noted between the dexamethasone-pretreated and untreated groups.

Effect of adrenalectomy on vascular permeability at altitude. To determine whether endogenous glucocorticoids might exert a protective effect on hypoxia-induced vascular permeability we measured PLI and lung water in adrenalectomized rats exposed to 48 h of altitude (Pb 380 Torr) and compared them with values found in altitude-exposed, sham-operated rats and to sham-operated rats maintained in normoxia. Adrenalectomy augmented the altitude-induced increase in PLI and gravimetric lung water over values seen in adrenal-intact

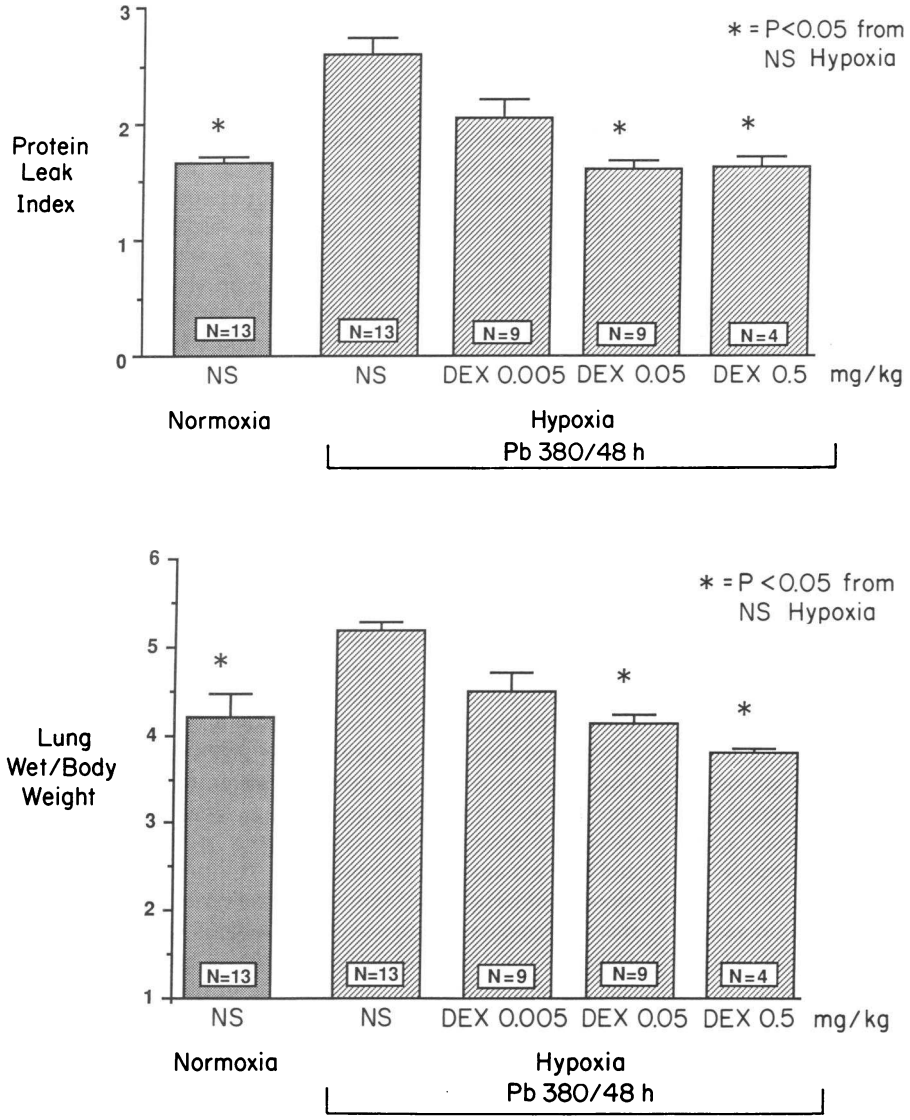

Figure 1. (*A*) PLI for normoxic control animals (*shaded bar*) and hypoxia-exposed animals (*cross-hatched bars*) pretreated with normal saline (NS) or with dexamethasone (0.005, 0.05, or 0.5 mg/kg) 12 h and immediately before hypoxic exposure (Pb 380 Torr for 48 h) and every 12 h during the subsequent 48 h of altitude exposure. * = $P < 0.05$ from Hypoxia/NS. (*B*) Lung water as estimated by bloodless wet lung weight/body weight ratios for the experimental groups described in *A*. * = $P < 0.05$ from Hypoxia/NS.

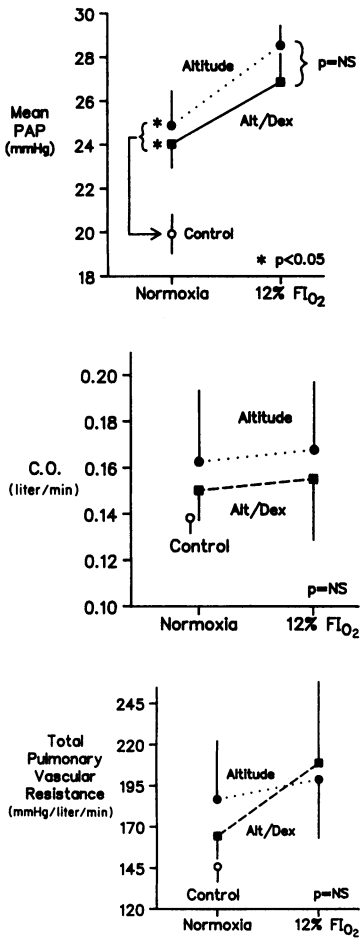

Figure 2. (*A–C*) Data from hemodynamic measurements of control and altitude exposed animals (48 h/Pb 380 Torr) pretreated with normal saline or 0.05 mg/kg DEX 12 h and immediately before altitude exposure and every 12 h during 48 h of subsequent altitude exposure. Measurements were made immediately after altitude exposure either in room air (normoxia) or under conditions of hypoxia induced with 12% O_2 in a small plexiglass enclosure. *A* shows measurements of mean pulmonary artery pressure, while *B* shows results of cardiac output and *C* shows the calculated total pulmonary vascular resistance for the three groups under both normoxic and hypoxic conditions. * = $P < 0.05$ from control normoxia ($n = 6$ each group).

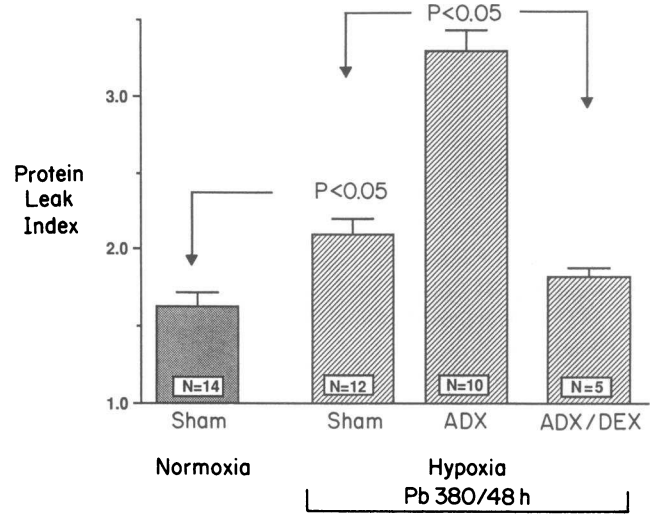

Figure 3. PLI for animals who had been adrenalectomized (ADX) or had undergone sham adrenalectomies (SHAM) 4–5 d before normoxic (*shaded bar*) or hypoxic exposure (*cross-hatched bars*) are shown. Some adrenalectomized animals received replacement glucocorticoids in the form of DEX (0.05 mg/kg) every 12 h after adrenalectomy (ADX/DEX) and all animals were allowed free access to water as well as to normal saline solutions after surgery. Arrows show statistical differences between groups.

animals exposed to hypobaric hypoxia (Fig. 3). In addition, the increase in PLI in adrenalectomized animals was prevented by administration of stress replacement doses of glucocorticoids (0.05 mg/kg per 12 h DEX), thereby further implicating endogenous glucocorticoids in the modulation of these hypoxia-induced vascular changes.

Discussion

These experiments demonstrate that subacute exposure to moderate hypoxia led to increases in transvascular protein escape and lung water in rats consistent with an increase in pulmonary vascular permeability. This hypoxia-induced increase in protein flux was inhibited by pretreatment with low doses of DEX in the absence of effects on hemodynamic parameters, and conversely was augmented by depleting endogenous glucocorticoids via adrenalectomy. Moreover, glucocorticoid replacement in adrenalectomized animals reversed the augmented protein leak, suggesting an important physiological role of both exogenous and endogenous glucocorticoids on vascular permeability in hypoxia.

Our conclusions regarding the effect of hypoxia on vascular permeability depend on the specificity and reliability of our protein leak measurement as an indicator of changes in vascular permeability. Previous studies from our laboratory have shown that measurement of the transvascular flux of radiolabeled protein is sensitive to low-level lung injury produced by relatively small doses of lung toxins such as thiourea (18) or endotoxin (29), in which there was little or no increase in lung water. This technique was able to clearly distinguish between lung edema due to increases in hydrostatic pressure (increased left atrial pressure to 23 mmHg) with attendant vascular recruitment and that due to increases in vascular permeability due to toxins (thiourea). We have used a modification of this technique in this study, which has been previously shown to be capable of detecting small increases in vascular permeability after low-dose thiourea-induced lung injury in the rat (24).

Because transvascular escape of radiolabeled protein depends not only on the permeability of the vascular barrier but also on the intravascular concentration of the tracer molecule and the size of the exchange surface area, our PLI contains terms intended to correct for alterations in the radiolabeled protein concentration and lung blood content (reflection of surface area) and thereby minimize their influence on transvascular protein escape (24). Significant increases in this corrected index of transvascular protein escape were noted in our hypoxic animals compared with controls implying increases in vascular permeability. It could be argued that the hypoxia-induced increases in protein escape we observed might reflect the corrections rather than a true increase in vascular permeability. Several lines of evidence suggest that this is not the case. First, the correction for radiolabeled protein concentration does not seem to explain our findings, since intravascular radiolabeled protein concentrations were similar in normoxic and hypoxic animals. Secondly, our PLI uses lung small vessel blood volume as a reflection of exchange surface area and lung mass. If hypoxia caused a decrease in blood volume, using this value as a correction for surface area could cause an increase in our protein leak index independent of changes in vascular permeability. However, we found that the volume of blood in the lungs of our hypoxic animals was no different from our control animals. This is consistent with findings by Bartlett and Remmers (14) who also found no increase in pulmonary

blood volume in rats exposed to altitude for 7 d. In addition, we also analyzed our data using body weight, bloodless lung wet weight, or bloodless lung dry weight as alternate corrections for lung size or surface area, and again found significant increases in transvascular protein escape in the hypoxic animals in each case (Fig. 4). The latter two corrections tended to minimize the differences between control and hypoxic animals, because both bloodless lung wet and lung dry weights increased in the hypoxic animals. The parallel increases in these two parameters in hypoxic animals is also responsible for the insensitivity of the lung wet weight/dry weight ratio as an estimate of lung water. These observations are consistent with previous reports by Bartlett and Remmers (14) who also noted increases in both lung wet and dry weights in rats after 7 d of altitude exposure, which they attributed to increases in extravascular lung water and plasma protein extravasation. Nevertheless, regardless of which correction for lung size was used, significant increases in transvascular protein escape were observed after hypoxic exposure.

The increases in gravimetric lung water and increased vascular edema cuff area in the hypoxic rats also suggest that the increases in PLI were not merely a reflection of increased surface area. Histologic evidence of lung edema in hypoxic rats has also been reported by Sobin (16), Whayne and Severinghaus (17), and Scott (15). Although no true alveolar flooding has been seen, interstitial edema and endothelial injury similar to that caused by toxic substances has been reported in hypoxic rats (2, 30) with both light and electron microscopic techniques.

Could our hypoxia-induced increases in transvascular protein escape simply be due to hemodynamic effects? To answer this question, we studied the consequences of acutely increasing pulmonary artery pressure on our protein leak measurement to determine whether increases in pulmonary artery pressure alone could increase the PLI. We chose hypoxia (1 h exposure) as the pressor stimulus to produce a longitudinal pattern of pulmonary vascular resistance across the microvasculature that might more closely mimic that seen with more prolonged hypoxia. We saw no effect of an acute increase in pressure on transvascular protein escape in these animals despite increases in pulmonary artery pressures over the 1-h exposure to levels similar to those noted in our 48-h hypoxic animals. As further evidence against hemodynamic-induced effects, the reduction in transvascular protein escape produced by glucocorticoids without associated reductions in pulmonary artery pressure also suggests that increased pressure per se does not explain the observed increases in transvascular protein escape. We considered the possibility that the hypoxia-induced increases in transvascular protein escape after 24- or 48-h exposures may have been due to effects of hypoxia on shifting the site of pulmonary vascular resistance downstream and thereby causing increases in microvascular pressure not seen with either acute hypoxia or our glucocorticoid-treated animals. However, although we have no true measure of microvascular pressure in our animals, such a mechanism seems unlikely, since previous studies indicate that increases in microvascular pressure caused by elevating left atrial pressure to levels accompanied by substantial increases in lung water had no effect on our protein leak measurements (18). Together, these lines of evidence suggest that our PLI measurements are valid and make hypoxia-induced increases in hydrostatic pressure an unlikely explanation for the increases in transvascular protein escape seen in our hypoxic animals. Alternatively, it is possible that the observed increase in pulmonary pressure with prolonged hypoxia, although mild, might have damaged the pulmonary vascular barrier. Previous investigators have suggested that increases in hemodynamic forces during hypoxia including high pulmonary artery pressures and perhaps sheer stress may lead to endothelial injury with resultant increases in vascular permeability and edema (31). It is possible that glucocorticoids act not by reducing the hemodynamic forces, but rather by maintaining the integrity of the endothelial barrier in the face of such injurious forces. DEX has been shown to prevent the disruption of the blood-brain barrier produced by drug-induced acute hypertension consistent with such an effect (32).

At first glance our findings seem to further complicate an already controversial topic. Previous reports by Bland et al. (5) and Landolt et al. (6) in adult sheep found no increase in vascular permeability as assessed by lung lymph flow after up to 48 h of significant alveolar hypoxia even when coupled with substantial increases in blood flow produced by partial lung resection. A subsequent report by Hansen et al. (7) reported increases in lung lymph flow in newborn lambs although the lymph/plasma protein ratio fell in these animals indicating a pressure induced increase in fluid filtration. Contrary to these reports, however, other investigators have reported reversible increases in lung lymph flow with an unchanged lymph/plasma ratio in dogs during hypoxic exposure (8–10% O_2), which could reflect increases in either vascular permeability or

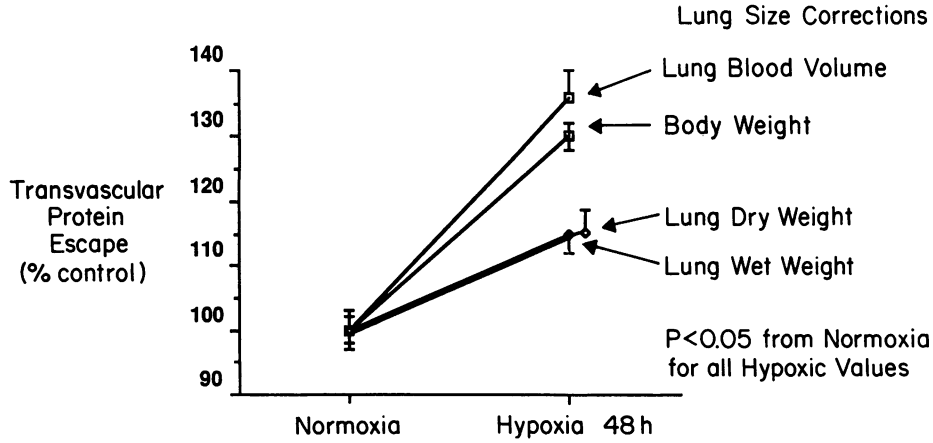

Figure 4. Transvascular protein escape is shown for normoxic animals (n = 30) and animals exposed to hypobaric hypoxia (PB 450 Torr) for 48 h (n = 27) to show the effect of substituting various lung size corrections on the PLI (expressed as percent control). Data are redrawn from experiments depicted in Table I. Lung blood volume correction is the term routinely used in calculating the PLI. All hypoxic values are statistically different from normoxia ($P < 0.05$).

exchange surface area (8–10). Furthermore when exchange surface area during severe hypoxia or anoxia was controlled in in vitro experiments in this same species (11, 12), increases in filtration and reflection coefficients were noted suggesting true increases in lung vascular permeability.

It seems likely that discrepancies between past studies and our own may in part reflect differences in experimental design (degree and duration of hypoxia) as well as in the methods used to measure vascular permeability which in some cases may not have been sufficiently sensitive to detect mild changes. As previously mentioned, measurement of the transvascular escape of radiolabeled protein seems to be relatively sensitive to low-level lung injury insufficient to cause significant changes in lung water or gas exchange and thus may have some advantages over past methods (18, 29). The increases in transvascular protein escape seen in our hypoxic rats is quite modest compared with changes that follow thiourea-induced injury (24) and thus may not have been appreciated with less sensitive techniques. Species differences may also contribute to reported discrepancies as past studies suggesting that hypoxia does not lead to an increase in lung vascular permeability have all been carried out in the ovine species. Considerable evidence exists that suggests that the pulmonary vascular response to hypoxia in sheep may differ from those of other mammalian species (2, 8, 33, 34). Specifically, histologic evidence of interstitial edema is lacking in the ovine species after hypoxia, and a report in newborn lambs actually showed hypoxia-induced decreases in extravascular lung water (7). In contrast, other species including the rat demonstrate endothelial injury and edema formation similar to findings reported in human cases of high altitude pulmonary edema (2, 3, 15, 16, 17, 30). The failure of hypoxia to increase vascular permeability in the sheep and lamb thus may not be applicable to other animals species or man.

Our demonstration of the suppressive effects of DEX on hypoxia-induced transvascular protein escape in rats is consistent with observations made in human altitude-induced illnesses. Johnson et al. (20) first demonstrated that DEX reduced symptoms of acute mountain sickness in man when given before altitude ascent. These results were subsequently confirmed by Ellsworth et al. (21) who demonstrated the superiority of DEX over acetazolamide in preventing symptoms during rapid altitude ascent. Subsequently, Hackett et al. (19) and Oelz et al. (22) also demonstrated the effectiveness of DEX when given after the development of symptoms of acute mountain sickness. In addition to improvement in symptoms, dexamethasone improved oxygen saturation and measurements of airflow in these subjects, consistent with objective improvements in pulmonary function. The mechanism of these effects, however, was unclear. Our results in the rat suggest that DEX might act in part by preventing the rise in vascular permeability in hypoxia, which could explain its beneficial effects on altitude-induced cerebral and pulmonary symptoms.

Our studies also suggest an important role for endogenous glucocorticoids in the prevention of hypoxia-induced pulmonary vascular permeability. Cortisol levels are known to increase significantly after exposure to hypoxia in both man and in animals (35). Our results demonstrating that adrenalectomy augments hypoxia-induced vascular permeability, and that exogenous glucocorticoid replacement reverses this process, point to the potential importance of this hormonal response in protecting the host from the direct adverse effects of hypoxia or from the consequences of hypoxia on activation of endogenous injurious forces. Our results, however, do not permit further delineation of the cause of the increase in vascular permeability in hypoxia nor of the means by which glucocorticoids suppress this process.

Our observations suggest several parallels between the human condition of HAPE and the hypoxia-induced increases in protein escape and lung water seen in the rat. First, evidence suggests that, as in the rat, a significant percentage of humans may develop increases in lung water after subacute hypoxia. Rales on chest examination have been detected in 30–50% of asymptomatic individuals after hypoxic exposure along with mild abnormalities in lung mechanics and gas exchange that together have been ascribed to the presence of interstitial lung edema (12). Secondly, the temporal development of increases in lung water in the rat, first seen after a latency of 24 h, is quite similar to the development of HAPE in man, which also tends to occur after 12–24 h of hypoxic exposure. Finally, the known beneficial effects of DEX on the prevention of altitude-induced respiratory difficulties (19–22) also parallel our observations in the rat. Thus, the tendency of the rat to develop increases in lung water after hypoxic exposure, the latency of the onset of the increases in lung water after hypoxia, and the beneficial effects of glucocorticoids on this response all parallel features seen during the development of the human condition of HAPE and may have relevance to the understanding of this process.

In conclusion, we have shown that moderate levels of hypoxia led to increases in lung transvascular protein escape in rats, suggesting an increase in pulmonary vascular permeability to protein. This increase in lung transvascular protein escape was inhibited by glucocorticoid pretreatment and augmented by adrenalectomy. The glucocorticoid effect was independent of any measurable hemodynamic effects, but the mechanism of this suppressive action was otherwise unclear. These effects of hypoxia on transvascular protein escape could contribute to the development of HAPE and also conceivably to other altitude-associated edematous conditions.

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