Chronic hypercapnia inhibits hypoxic pulmonary vascular remodeling

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Chronic hypercapnia inhibits hypoxic pulmonary vascular remodeling. Am. J. Physiol. Heart Circ. Physiol. 278: H331–H338, 2000.—Chronic hypercapnia is commonly found in patients with severe hypoxic lung disease and is associated with a greater elevation of pulmonary arterial pressure than that due to hypoxia alone. We hypothesized that hypercapnia worsens hypoxic pulmonary hypertension by augmenting pulmonary vascular remodeling and hypoxic pulmonary vasoconstriction (HPV). Rats were exposed to chronic hypoxia (inspiratory O2 fraction \( F_{I, O_2} \) = 0.10), chronic hypercapnia (inspiratory CO2 fraction \( F_{I, CO_2} \) = 0.10), hypoxia-hypercapnia (\( F_{I, CO_2} \) = 0.10, inspiratory CO2 fraction = 0.10), or room air. After 1 and 3 wk of exposure, muscularization of resistance blood vessels and hypoxia-induced hematocrit elevation were significantly inhibited in hypoxia-hypercapnia compared with hypoxia alone (\( P < 0.001, \) ANOVA). Right ventricular hypertrophy was reduced in hypoxia-hypercapnia compared with hypoxia at 3 wk (\( P < 0.001, \) ANOVA). In isolated, ventilated, blood-perfused lungs, basal pulmonary arterial pressure after 1 wk of exposure to hypoxia (20.1 ± 1.8 mmHg) was significantly (\( P < 0.01, \) ANOVA) elevated compared with control conditions (12.1 ± 0.1 mmHg) but was not altered in hypoxia-hypercapnia (13.5 ± 0.9 mmHg) or hypercapnia (11.8 ± 1.3 mmHg). HPV (\( F_{I, CO_2} \) = 0.03) was attenuated in hypoxia, hypoxia-hypercapnia, and hypercapnia compared with control (\( P < 0.05, \) ANOVA). Addition of \( N\)-nitro-L-arginine methyl ester (10^{-5} M), which augmented HPV in control, hypoxia, and hypercapnia, significantly reduced HPV in hypoxia-hypercapnia. Chronic hypoxia caused impaired endothelium-dependent relaxation in isolated pulmonary arteries, but coexistent hypercapnia partially protected against this effect. These findings suggest that coexistent hypercapnia inhibits hypoxia-induced pulmonary vascular remodeling and right ventricular hypertrophy, reduces HPV, and protects against hypoxia-induced impairment of endothelial function.

increased morbidity and is an independent predictor of reduced survival (22). The major mechanisms that are believed to contribute to the pathogenesis of pulmonary hypertension in lung disease are hypoxic pulmonary vasoconstriction (HPV) and pulmonary vascular remodeling (1, 22, 27). In the normal lung the pulmonary vascular endothelium plays a pivotal role in the modulation of pulmonary vascular tone and HPV through the release of vasoactive agents, including nitric oxide (NO). In chronically hypoxic rat lungs, impaired endothelium-dependent relaxation has been demonstrated (1, 20), and a similar impairment has been demonstrated in patients with chronic hypoxic lung disease (8), suggesting that impaired endothelium-dependent relaxation may contribute to the increased pulmonary vascular resistance of chronic hypoxia.

In addition to its effects on pulmonary vascular tone, NO has been demonstrated to be critical in the modulation of hypoxic pulmonary vascular remodeling. Hypoxic vascular remodeling and pulmonary arterial pressure (PAP) are increased in endothelial NO synthase (NOS) knockout mice (10, 30), whereas chronic administration of inhaled NO attenuates hypoxic vascular remodeling (18, 28). Stimulation of endogenous NO production by dietary supplementation with L-arginine also protects against hypoxia-induced vascular remodeling (9, 24). These data suggest that impaired endothelial function may contribute to the development of chronic hypoxic pulmonary hypertension through the loss of an endothelium-derived basal antiproliferative activity (11).

Chronic hypercapnia is a common finding in patients with progressive hypoxic lung disease, and it is a common clinical observation that significant pulmonary hypertension does not develop in the presence of hypoxic lung disease unless hypercapnia is also present (22). A close correlation has been noted between the arterial PCO2 and the PAP in this setting (4, 17). These observations suggest that elevated CO2 may contribute importantly to the development of pulmonary hypertension in chronic lung disease, although the potential mechanism of this effect is unknown. Alternatively, the association between elevated arterial PCO2 and increased PAP may simply reflect the presence of more severe lung disease in hypercapnic patients. The potential independent effect of elevated CO2 on hypoxic pulmonary hypertension has not been previously examined.
We hypothesized that chronic hypercapnia augments the increase in PAP seen after exposure to chronic hypoxia by augmenting pulmonary vascular remodeling and increasing the pulmonary vascular responsiveness to hypoxia. Furthermore, we hypothesized that chronic hypercapnia produces this effect, at least in part, by increasing the endothelial dysfunction caused by chronic hypoxia alone. Rats were exposed to chronic hypoxia, chronic hypercapnia, or chronic hypoxia-hypercapnia in an environmental chamber. Morphometric analysis was used to assess pulmonary vascular remodeling in these animals. Baseline PAP and response of the lungs to acute hypoxic challenge were assessed using an isolated blood-perfused lung preparation. Finally, an isolated pulmonary arterial preparation was used to examine endothelium-dependent relaxation.

**MATERIALS AND METHODS**

**Animal Model**

Male, specific pathogen-free Sprague-Dawley rats (Harlan, Bicester, UK) were maintained in a normobaric opaque Perspex environmental chamber (volume 325 liters). Animals were maintained in room air (inspiratory O2 fraction \( F_{O_2} = 0.21 \), inspiratory CO2 fraction \( F_{CO_2} < 0.01 \), hypoxia \( F_{O_2} = 0.10, F_{CO_2} < 0.01 \), hypercapnia \( F_{O_2} = 0.21, F_{CO_2} = 0.10 \), or hypoxia-hypercapnia \( F_{O_2} = 0.10, F_{CO_2} = 0.10 \)). Chamber gases were monitored continuously with O2 and CO2 analyzers (models 1175 and 1505, respectively, Servomex, Crowborough, UK). Excess CO2 was removed by recirculating gases through the tracheal catheter by use of the same fixative at a flow rate of 520 ml/min. Animals were anesthetized with pentobarbital sodium (60 mg/kg ip) and anticoagulated by an intracardiac injection of heparin (1,000 IU/kg iv) was administered, and the animals were killed by exsanguination. The thoracic contents were removed through a midline sternotomy, and cannulas were inserted into the main pulmonary artery and left atrium and tied in place. The thoracic contents were removed en bloc and suspended in a humidified chamber maintained at 37°C. A warmed (37°C), humidified gas mixture of 5% CO2-95% air was used to ventilate control and hypoxic animals. A warmed humidified gas mix containing 10% CO2-90% air was used to ventilate hypercapnic and hypoxic-hypercapnic animals. Airway pressure was monitored continuously, and the lungs were briefly hyperinflated to an airway pressure of 16 cmH2O every 5 min to prevent the development of progressive atelectasis.

Blood from the experimental animal and from one or two donor animals, exposed to the same experimental condition, was reserved and mixed in a ratio of 2:1 with physiological saline solution (PSS) to prime the perfusion circuit. In the case of animals exposed to zero CO2 inspirate, the composition of the PSS was 119 mM NaCl, 24 mM NaHCO3, 4.7 mM KCl, 0.9 mM MgSO4, 1.2 mM KH2PO4, 2.5 mM CaCl2, 5.5 mM glucose, and 40 g/l BSA, so that when the perfusate was ventilated with gas mixtures containing 5% CO2, the pH was ~7.40. In the case of animals maintained in hypercapnic conditions, the PSS was modified by isomotic substitution of NaHCO3 for NaCl so that the perfusate pH was again maintained at ~7.40. The circuit consisted of, in order, the left atrial cannula, a venous outflow pressure transducer (Sensor Nor 840, Horten), a warmed, thermoregulated perfusate reservoir, a peristaltic roller pump (Stockert Instrumente, Munich, Germany), connecting tubing, a bubble trap, an arterial pressure transducer (Sensor Nor 840, Horten), and the pulmonary artery cannula.

Perfusion through the circuit was maintained at a constant flow rate (0.04 ml min⁻¹ g⁻¹) so that changes in arterial perfusion pressure reflected changes in total pulmonary vascular resistance. The perfusate was warmed to 37°C in a reservoir before entering the pulmonary artery. Venous outflow pressure was maintained at 2.6 cmH2O to ensure zone 3 conditions at end expiration, when measurements of vascular pressure were made. Arterial, venous, and airway pressures were continuously recorded using an analog-to-digital system (model MP100 WS, Biopac Systems, Santa Barbara, CA) connected to a desktop computer (Power Macintosh 7100/80, 0.10, FICO2 0.21, inspiratory CO2 fraction (FICO2)) 5 0.10, or hypoxia-hypercapnia (FICO2, 0.10). Chamber gases were monitored continuously with O2 and CO2 analyzers (models 1175 and 1505, respectively, Servomex, Crowborough, UK). Excess CO2 was removed by recirculating gases through the tracheal catheter by use of the same fixative at a flow rate of 520 ml/min. Animals were anesthetized with pentobarbital sodium (60 mg/kg ip) and anticoagulated by an intracardiac injection of heparin (1,000 IU/kg iv) was administered, and the animals were killed by exsanguination. The thoracic contents were removed through a midline sternotomy, and cannulas were inserted into the main pulmonary artery and left atrium and tied in place. The thoracic contents were removed en bloc and suspended in a humidified chamber maintained at 37°C. A warmed (37°C), humidified gas mixture of 5% CO2-95% air was used to ventilate control and hypoxic animals. A warmed humidified gas mix containing 10% CO2-90% air was used to ventilate hypercapnic and hypoxic-hypercapnic animals. Airway pressure was monitored continuously, and the lungs were briefly hyperinflated to an airway pressure of 16 cmH2O every 5 min to prevent the development of progressive atelectasis.

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were initially bathed in control PSS (in mM: 122.6 NaCl, 5.4
previously described (31). Rings from control and hypoxic rats
a tissue bath (50 ml) for recording of isometric wall tension, as
from each rat was cleared of adherent tissue and mounted in
were isolated postmortem. A 2- to 3-mm-long ring segment
were subjected to three sequential depolarization-
induced contractions by exposure to solutions containing 80
rings were subjected to three sequential depolarization-
iso-osmolal, 70-90 mm Hg; 2% CO2-98% N2; 3% CO2-10% O2-97% N2. The perfusion pressure was recorded and allowed to return to baseline values. The specific NOS inhibitor N-nitro-l-arginine methyl ester (l-NAME) was added to the reservoir to give a final concentration of 10^{-5} M in the perfusate. Administration of l-NAME produced a slow-rising but sustained pressor response, which was allowed to stabilize. A second hypoxic challenge was then presented. Finally, sodium nitroprusside was added to the perfusate to a final concentration of 10^{-5} M, which resulted in vasodilation and a fall in PAP. Perfusion pH was monitored at regular intervals with a blood gas analyzer (model 278, Ciba-Corning Diagnostics, Halstead, UK) and corrected to pH 7.35–7.45, if necessary, by the addition of aliquots of NaHCO_3 (0.1 M).

Isolated Pulmonary Artery Ring Preparation

Rats were exposed to the four study conditions outlined above for a period of 1 wk, then the rat hearts were dissected free and weighed as described previously. The extrapulmonary branches of the left and right pulmonary arteries were isolated postmortem. A 2- to 3-mm-long ring segment from each rat was cleared of adherent tissue and mounted in a tissue bath (50 ml) for recording of isometric wall tension, as previously described (31). Rings from control and hypoxic rats were initially bathed in control PSS (in mM: 122.6 NaCl, 5.4 KCl, 20 NaHCO_3, 0.9 NaH_2PO_4, 0.8 MgSO_4, 2.4 CaCl_2, and 5.5 glucose) equilibrated with 5% CO_2-95% air (pH 7.39 ± 0.00). Rings from hypercapnic and hypoxic-hypercapnic rats were maintained in modified PSS (NaHCO_3 isosmotically substituted for NaCl) with 10% CO_2-95% air, so that extracellular pH was maintained at control values (pH 7.38 ± 0.01). Isometric tension was recorded as a function of time by use of an analog-to-digital system identical to that described for the isolated lung studies.

The rings were left for an equilibration period of 1 h and then set, for a further 30 min, at a pretension of 1 g (31). The rings were subjected to three sequential depolarization-induced contractions by exposure to solutions containing 80 mM KCl (isosmotically substituted for NaCl). Rings were relaxed after each contraction by rinsing with PSS. A first cumulative concentration-response curve to the α_1-adrenergic agonist phenylephrine (10^{-10}–10^{-5} M) was then recorded, the bath was flushed with control solution, and the preparation was allowed to return to resting tension. On the basis of this cumulative concentration-response curve, the concentration of phenylephrine required to produce a submaximal contraction of the ring segment (70% of maximal, EC_{50}) was determined and added to the bath. The concentration-dependent relaxations in response to ACh (10^{-10}–10^{-4} M) were then determined. Bath pH and PcO_2 were sampled at regular intervals with a blood gas analyzer (model 278, Ciba-Corning Diagnostics).

Reagents

All salts, phenylephrine, ACh, and l-NAME (Sigma, Poole, UK) were made up to stock (10^{-1} M) solutions in distilled water and subsequently diluted in PSS to achieve the desired bath concentration. Heparin (mucous) was obtained from Leo Laboratories (Princes Risborough, Poole, UK) and pentobarbital sodium from Rhône Pâtioux (Harlow, UK).

Data Analysis

Isolated lungs. Increase in total pulmonary vascular resistance in response to hypoxic challenge was expressed as the change in mean perfusion pressure during the 5th min of the hypoxic challenge. Similarly, responses to inhibition of NOS were expressed as the change in perfusion pressure from the stable baseline before the addition of L-NAME compared with that after the addition of L-NAME to the perfusate and the development of a stable response.

Isolated pulmonary rings. Relaxations in response to ACh were expressed as a percentage of the submaximal contraction produced by the EC_{50} of phenylephrine (%EC_{50}).

Values are means ± SE. Paired t-test was used for comparison of paired values. For multiple comparisons of means across experimental groups, ANOVA was carried out, and where a significant F value was found, Student-Newman-Keuls post hoc test was used to assess the significance of the differences between means. Differences were considered statistically significant at P < 0.05.

RESULTS

Growth of Animals

Table 1 also shows the mean hematocrit values for animals exposed to the experimental conditions for 1 wk (299.0 ± 3.7 g) or 3 wk (300.4 ± 4.4 g) were not significantly different. Table 1 shows the mean changes in body weight in groups of animals after exposure to the experimental conditions for 1 or 3 wk. Exposure to hypoxic, hypercapnic, or hypoxic-hypercapnic conditions for 1 wk resulted in a loss of body weight, which was most prominent in hypoxia-hypercapnia. After 3 wk of exposure, hypoxic and hypercapnic animals showed a small weight gain compared with initial weights, which was considerably less than the gain in control animals. Hypoxic-hypercapnic animals at 3 wk experienced a weight loss similar to that seen after 1 wk of exposure, suggesting that the weight loss experienced during hypoxia-hypercapnia stabilized after the 1st wk.

Hematocrit

Table 1 also shows the mean hematocrit values for animals exposed to the experimental conditions for 1 and 3 wk. Exposure to hypoxia for 1 wk resulted in an elevated blood hematocrit compared with control animals (P < 0.001, ANOVA). Hypoxia-hypercapnia caused a small elevation in hematocrit over control conditions (P < 0.05, ANOVA), which was significantly (P < 0.001, ANOVA) less than that seen with hypoxia alone. Hematocrit in hypercapnic rats was not significantly different from that in control animals. The hematocrit values in the 3-wk groups were similar to those in the 1-wk groups in all conditions.

RV Hypertrophy

Figure 1 shows the mean RV/LV + S from animals exposed to the experimental conditions for 1 and 3 wk.
Exposure to hypoxic conditions for 1 wk resulted in RV hypertrophy, which was further increased after 3 wk of exposure. Significant RV hypertrophy was observed at 1 wk ($P < 0.001$, ANOVA) in hypoxia-hypercapnia, but this hypertrophy was not significantly altered after 3 wk in this condition ($P = 0.15$, ANOVA). The hypertrophy observed with hypoxia-hypercapnia was significantly less than that observed with hypoxia alone at 1 wk ($P < 0.001$, ANOVA) and 3 wk ($P < 0.001$, ANOVA) of exposure.

Morphometry

Figure 2 shows the effects of the experimental conditions on muscularization of peripheral vessels. After 1 wk of exposure to experimental conditions, hypoxic lungs showed an increased percentage of muscularized intra-acinar blood vessels compared with control lungs. Hypoxic-hypercapnic lungs showed significantly ($P < 0.001$, ANOVA) less muscularization of intra-acinar vessels than hypoxic lungs after 1 wk of exposure. The degree of muscularization in hypoxic-hypercapnic lungs was not significantly greater than in control lungs ($P = 0.06$, ANOVA). Hypercapnia alone did not cause increased muscularization compared with control conditions.

A similar pattern was observed in lungs exposed to experimental conditions for 3 wk. Exposure to 3 wk of hypoxia or hypoxia-hypercapnia produced a greater degree of muscularization of peripheral blood vessels than 1 wk of exposure to each condition. The degree of muscularization remained significantly ($P < 0.001$, ANOVA) less in the hypoxic-hypercapnic group than in the hypoxic group. However, the degree of muscularization was now significantly ($P < 0.05$, ANOVA) greater in the hypoxic-hypercapnic group than in control lungs. No significant difference was noted between the control and hypercapnic groups.

Baseline PAP. Baseline perfusion pressure was higher ($P < 0.01$, ANOVA) in the hypoxic group (20.1 ± 1.9 mmHg, $n = 7$) than in the control (12.7 ± 0.5 mmHg, $n = 14$), hypcapnic (11.5 ± 1.6 mmHg, $n = 7$), and hypoxic-hypcapnic groups (13.7 ± 0.9 mmHg, $n = 7$). Baseline pressures in the hypercapnic and hypoxic-hypercapnic groups were not significantly different from control values. Addition of L-NAME to the perfusate increased the baseline PAP in all four experimental groups. The increase in PAP was significantly ($P < 0.01$, ANOVA) greater in the hypoxic group (10.7 ± 2.6 mmHg, $n = 7$) than in the control (4.2 ± 0.7 mmHg, $n = 14$), hypcapnic (2.4 ± 0.7 mmHg, $n = 7$), or hypoxic-hypercapnic (2.9 ± 0.7 mmHg, $n = 7$) group. There was no significant difference between the in-
creases in PAP in the control, hypercapnic, and hypoxic-hypercapnic groups. Addition of the NO donor sodium nitroprusside to the perfusate at the end of the protocol returned perfusion pressure to the initial baseline value, indicating that the L-NAME-induced changes were reversed by exogenous NO.

HPV response. Figure 3 shows the increase in PAP in response to hypoxia in each of the experimental conditions before and after L-NAME. The pre-L-NAME pressor response to an acute hypoxic challenge was attenuated in the hypoxic (P < 0.01, ANOVA), hypercapnic (P < 0.05, ANOVA), and hypoxic-hypercapnic (P < 0.01, ANOVA) groups compared with control. HPV responses in the hypoxic and hypoxic-hypercapnic groups were not significantly different. After the addition of L-NAME, the HPV response was significantly augmented in the control and hypercapnic groups compared with the HPV response before L-NAME (Fig. 3). In contrast, the HPV response in the hypoxic-hypercapnic group was reduced after L-NAME and was then significantly less (P < 0.001, ANOVA) than the response in all other groups after L-NAME.

The mean hematocrit of the isolated lung perfusate in the hypoxic group (33.7 ± 0.7) was significantly (P < 0.001, ANOVA) greater than in the control (23.5 ± 0.3), hypercapnic (23.6 ± 0.5), and hypoxic-hypercapnic (24.8 ± 0.4) groups.

DISCUSSION

The results of the present study demonstrate that, in the rat, coexistent hypercapnia markedly reduces the extent of hypoxia-induced pulmonary vascular remodeling, attenuates hypoxia-induced RV hypertrophy, and almost completely abolishes the increase in pulmonary vascular resistance observed after chronic hypoxia.
alone. The elevation of hematocrit produced by chronic hypoxia was also largely abolished. Pressor responses to acute hypoxia were similarly impaired in chronically hypoxic and hypoxic-hypercapnic lungs. Our data also suggest that coexistent hypercapnia may partially protect against the impairment of endothelium-dependent relaxation produced by chronic hypoxia alone.

There is an extensive body of experimental literature reporting the acute effects of hypercapnia on pulmonary vascular resistance, although the results are conflicting: some suggest a vasoconstrictor action and others a vasodilator effect (see Ref. 31 for review). In contrast, investigations of the chronic effects of hypercapnia are sparse. Clinical studies demonstrate that, in COPD, chronic hypercapnia and hypoxia together cause greater increases in PAP than a similar degree of chronic hypoxia acting alone, although the mechanism underlying this phenomenon is largely unexplored (22). We had initially hypothesized that one of the mechanisms leading to greater pulmonary hypertension in hypoxia-hypercapnia was augmented pulmonary vascular remodeling. The vascular remodeling that we observed in the chronically hypoxic animals at 1 and 3 wk was similar to that previously reported in rats (5, 16, 18, 19, 24, 27, 28, 30). Our demonstration that chronic hypoxia-hypercapnia produced less remodeling than was induced by chronic hypoxia alone, at 1 and 3 wk of exposure, refutes the initial hypothesis. The data from the isolated perfused lung experiments support this interpretation. Chronic hypoxia alone led to an increased baseline pulmonary vascular resistance. When animals were simultaneously exposed to hypercapnia and hypoxia, no elevation of pulmonary vascular resistance was observed after 1 wk of exposure. Exposure to hypercapnia alone did not cause vascular remodeling or changes in resistance compared with control conditions. Taken together, these data indicate that hypercapnia in isolation does not lead to structural alterations in the pulmonary circulation, but when it coexists with hypoxia, hypercapnia acts to inhibit hypoxic pulmonary vascular remodeling.

Our finding that the chronically hypoxic animals developed RV hypertrophy was as expected (1, 3, 5, 9, 16, 18, 19, 24, 27, 28, 30, 32) and indicates that the elevated pulmonary vascular resistance observed in isolated lungs was also present in vivo. Similarly, the observation that RV hypertrophy was less in the hypoxic-hypercapnic than in the hypoxic group at 3 wk suggests that this condition led to reduced vascular resistance in vivo, consistent with the finding of a normal baseline pulmonary vascular resistance in the isolated lungs from these animals.

We found that, in hypoxia-hypercapnia, hematocrit was markedly reduced compared with hypoxia alone. Hypercapnia in the absence of hypoxia had no effect on hematocrit, indicating that elevated CO2 did not alter red cell production under normal circumstances. However, in the presence of hypoxia, hypercapnia prevented the normal compensatory polycythemic response, suggesting that hypercapnia acted by inhibiting a hypoxia transduction mechanism. In this context, it is interesting to note that Dhillon et al. (7) previously reported that hypercapnia inhibits the carotid body hypertrophic response to chronic hypoxia. Taken together, these data suggest that elevated CO2 may exert a general inhibitory effect on hypoxia-induced protein synthesis and cell proliferation.

A second component to our initial hypothesis was that chronic hypercapnia might augment hypoxic pulmonary hypertension by enhancing the vasoconstrictor response of the pulmonary vasculature to hypoxia. We found that chronic hypoxia inhibited the acute hypoxic vasoconstrictor response in isolated lungs, a finding in agreement with previous reports (13, 14, 21), although others have reported that the acute hypoxic vasoconstrictor response in isolated lungs is unchanged in these conditions (3, 29). Chronic hypercapnia alone also significantly reduced the acute hypoxic vasoconstrictor response. The hypoxic-hypercapnic group demonstrated a response that was not significantly different from that of the hypoxic group, a finding that is not compatible with the initial hypothesis that coexistent chronic hypercapnia would augment acute hypoxic vasoconstriction in chronically hypoxic lungs.
To examine the effect of hypoxia and hypercapnia on pulmonary vascular resistance, we used an isolated lung preparation, which avoided the direct, reflex-mediated attenuation of hypoxic vasoconstriction that is observed in intact animals (25). This preparation also removed the influence of other in vivo responses to the experimental conditions, such as hyperventilation or changes in blood volume and cardiac output, that might have caused secondary effects on PAP. In order that the perfusate conditions would be similar to those in vivo, lungs were perfused with blood from animals that had chronically adapted to the same experimental condition mixed with some PSS used to prime the perfusion circuit. The dilution produced by this PSS meant that in all groups the hematocrit was substantially $<0.40$. Below this value, blood viscosity is almost constant and changes little with further reductions in hematocrit (2, 33), indicating that the increased perfusion pressure that we observed in the hypoxic group was not a consequence of the higher hematocrit of the perfusate used in that group. In support of this interpretation, Hakim and Macek (12) demonstrated that when normal lungs were perfused with blood from chronically hypoxic animals, no increase in pulmonary vascular resistance was observed and, conversely, when chronically hypoxic lungs were perfused with blood from control animals, no reduction in resistance was noted. Furthermore, the acute hypoxic vasoconstrictor responses of control and chronically hypoxic lungs were unaffected by the perfusate used. Thus the elevated hematocrit of the perfusates used in the hypoxic condition does not account for the increased pulmonary vascular resistance observed in those lungs.

Chronic hypoxia causes impaired endothelium-dependent relaxation in the pulmonary circulation in chronic hypoxic lung disease (8) and in hypoxic animals (1, 20). This effect may contribute to an increased baseline pulmonary vascular tone and remove endothelium-derived inhibitory influences on vascular remodeling present in the normal vessels (11). In the present experiments, endothelium-dependent relaxation was impaired in vessels isolated from chronically hypoxic rats, in keeping with previous reports (20). We observed that, in isolated, preconstricted vessels, chronically hypoxia-hypercapnia led to an endothelium-dependent relaxation that was significantly greater than that in the hypoxic group and closer to that in control animals. These data suggest that hypoxia acted to preserve endothelial function in chronic hypoxia, an action that may have contributed to the lesser remodeling seen in those conditions.

There is evidence that upregulation of basal NOS activity acts to attenuate hypoxic pulmonary vasoconstriction and, in chronically hypoxic lungs, acts to limit vascular remodeling (9, 15, 24). This suggested to us that the attenuation of hypoxic remodeling and the reduction of hypoxia-induced increases in pulmonary vascular resistance by coexistent chronic hypcapnia may have been brought about by an increased influence of endogenously produced NO beyond that produced by hypoxia alone. As expected, NOS inhibition increased the hypoxic vasoconstrictor response in control lungs, confirming previous reports that NO normally acts to attenuate acute hypoxic vasoconstriction (3, 6, 13, 26). The increase in acute hypoxic vasoconstriction produced by NOS inhibition in chronically hypoxic lungs in the present experiments was small, a finding in agreement with that of Isaacson and co-workers (14), although others have found that NOS inhibition significantly increases the hypoxic vasoconstrictor response in chronically hypoxic lungs (3, 26, 29). Addition of L-NAME restored acute hypoxic vasoconstriction to control values in chronically hypercapnic lungs, suggesting that enhanced NO influence caused the impaired response in this condition. However, in the hypoxic-hypercapnic group, NOS inhibition led to a small further reduction in the acute hypoxic vasoconstrictor response, indicating that augmented NOS activity could not account for the reduced hypoxic response. One potential explanation of this finding is that hypoxia-hypercapnia caused the production of an additional, unidentified vasodilator.

The previously reported positive correlation between arterial $Paco_2$ and PAP in patients with COPD seems at variance with our results. However, it is important to note that our investigations focused on the potential effect of chronic hypercapnia on hypoxia-induced increases in pulmonary vascular resistance. In the setting of COPD, other factors may interact with arterial hypercapnia and contribute importantly to elevation of PAP. Hypercapnia may act to increase cardiac output, thus causing an increase in PAP. This effect may be particularly important in COPD, since it has been demonstrated that, in such patients, resting PAP may be normal or minimally elevated, whereas increased cardiac output during exercise leads to abnormally large increases in PAP (22). A factor contributing to increased sensitivity to elevated cardiac output in diseased lungs may be reduction in the volume of the pulmonary microcirculation due to parenchymal loss caused by the underlying disease process. A second factor augmenting the changes in PAP caused by hypercapnia in diseased patients may be abnormal airway mechanics. Elevated alveolar pressure augments the change in PAP in response to increased pulmonary blood flow in normal subjects (22). The abnormal airway mechanics in patients with COPD cause increased alveolar pressure, which may interact with high pulmonary blood flows induced by hypercapnia, leading to augmented pulmonary hypertension. In the absence of preexisting parenchymal lung disease, as in the present study, these mechanisms would not act. Direct experimental testing of these possibilities is required. Finally, the greater pulmonary hypertension observed in hypercapnic patients may be, in whole or in part, due to more extensive lung damage in such patients and not to an independent effect of hypercapnia.

In summary, we have demonstrated that chronic hypercapnia inhibits the development of hypoxic pulmonary vascular remodeling and RV hypertrophy, attenuates the polycythemic response to hypoxia, inhibits the pulmonary vasoconstrictor response to acute hypoxia...
by an NOS-independent mechanism, and partially protects against the impairment of endothelium-dependent relaxation caused by chronic hypoxia. These findings imply that, in hypoxic COPD, the augmentation of pulmonary arterial hypertension observed in the presence of CO₂ retention is not caused by a hypocapnia-induced augmentation of elevation in pulmonary vascular resistance or hypoxic responsiveness.

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