Respiratory system responsiveness in rabbits in vivo is reduced by prolonged continuous positive airway pressure

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Submitted 9 February 2005; accepted in final form 1 April 2005

Xue, Z., L. Zhang, R. Ramchandani, Y. Liu, V. B. Antony, S. J. Gunst, and R. S. Tepper. Respiratory system responsiveness in rabbits in vivo is reduced by prolonged continuous positive airway pressure. J Appl Physiol 99: 677–682, 2005. First published April 7, 2005; doi:10.1152/japplphysiol.00165.2005.—Active, nonanesthetized, tracheotomized rabbits were subjected to continuous positive airway pressure (CPAP) for 4 days to determine the effects of chronic mechanical strain on lung and airway function. Rabbits were maintained for 4 days at a CPAP of 6 cmH2O (high CPAP), at a CPAP of 0 cmH2O (low CPAP), or without tracheostomy (no CPAP). After treatment with CPAP, changes in respiratory resistance in response to increasing concentrations of inhaled ACh were measured during mechanical ventilation to evaluate respiratory system responsiveness in vivo. Intraparenchymal bronchial segments were isolated from the lungs of all animals to evaluate airway smooth muscle responsiveness and bronchial compliance in vitro. Rabbits maintained for 4 days at high CPAP demonstrated significantly lower responsiveness to ACh compared with rabbits that were maintained at low CPAP or with no CPAP. Airways isolated from the lungs of animals subjected to the chronic application of high CPAP were also less responsive to ACh in vitro than the airways isolated from animals subjected to low CPAP or no CPAP. The persistence of the decreased responsiveness in the excised airway tissues suggests that the decreased respiratory system responsiveness observed in vivo results primarily from direct effects on the airways. The results demonstrate that the application of prolonged mechanical strain in vivo can reduce airway reactivity.

mechanical strain; airway reactivity; isolated bronchial segments; intraparenchymal airways

THE MECHANICAL STRAIN IMPOSED on the lungs during breathing is an important modulator of airway responsiveness in vivo (1). Deep inspirations and tidal breathing decrease airway responsiveness in healthy adults and animals, whereas the absence of a deep inspiration or tidal breathing increases airway responsiveness (4, 7, 10, 13, 16, 22, 23). Analogous findings have been reported in vitro in isolated airway segments and trachealis smooth muscle strips, in which the acute application of mechanical stretch or length oscillation reduces airway contractility and pharmacological responsiveness (2, 3, 5, 15, 19, 20).

The application of chronic mechanical strain to airway tissues in vitro has also been shown to affect their physiological properties. Our laboratory has previously demonstrated that isolated, excised rabbit bronchial segments maintained for a period of 1–2 days at a transmural pressure of 7 cmH2O exhibit an increase in volume, a higher compliance, and a decrease in contractile responsiveness to ACh (17). The application of chronic mechanical strain to isolated strips of trachealis smooth muscle for 1–2 days also induces changes in their active and passive physiological properties (11, 12, 21). These in vitro observations suggest that chronic alterations in the mechanical strain imposed on airway tissues can result in the modulation of their physiological properties. However, there is currently no evidence as to whether alterations in mechanical strain imposed in vivo under physiological conditions can induce alterations in respiratory system reactivity in vivo.

There is evidence from a previous study that the application of chronic mechanical strain to the lungs in vivo can result in changes in lung volume (6). Zhang and coworkers (25) applied continuous positive airway pressure (CPAP) to nonanesthetized, tracheotomized ferrets and found that animals maintained with a CPAP of 6 cmH2O for 2 wk had higher lung volumes. Their study indicates that the application of mechanical strain in vivo for prolonged periods can alter lung volume; however, its effect on airway responsiveness was not evaluated.

In the present study, we used CPAP to apply a mechanical strain to the lungs of nonanesthetized, tracheotomized rabbits for 4 days to evaluate the effect of strain on airway function. We found that the application of chronic mechanical strain in vivo resulted in lower respiratory system responsiveness to challenge with ACh in vivo. Airway segments that were excised from the lungs of these animals and studied in vitro also exhibited reduced contractility. We conclude that the chronic application of mechanical strain in vivo at physiological levels can reduce airway reactivity.

METHODS

Animal Preparation

New Zealand White rabbits (8–9 wk, 2.0–2.2 kg) were anesthetized (isoflurane) and tracheotomized. A custom-made 3.5-mm-ID tracheostomy cannula (model CL27585, Bivona Medical Technologies) was sutured into the trachea. The standard respiratory connection port to the cannula was modified; flexible tubing was inserted into the walls to create a T connection for the inspiratory and expiratory circuit with minimal dead space and minimal protrusion of the cannula from the neck. In addition, the standard opening of the tracheostomy cannula was fitted with a cap that could be removed to suction the airway. The flexible tubing from the tracheostomy connection was fitted with a cap that could be removed to suction the airway. The flexible tubing from the tracheostomy connection was fitted with a cap that could be removed to suction the airway.

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CPAP REDUCES AIRWAY RESPONSIVENESS IN VIVO

In Vivo Measurements

Lung volume. Lung volume was measured in sedated animals after placement of the tracheostomy tube and again after 4 days of CPAP. After the animals were subjected to mild hyperventilation to induce a brief apnea, the respiratory system was inflated with a syringe to an airway pressure of 30 cmH2O; the inflation lung volumes at pressures above atmospheric pressure were recorded. Respiratory system responsiveness. After 4 days of CPAP, respiratory resistance was measured by forced oscillation during challenge with an inhaled aerosolized ACh dissolved in normal saline. Rabbits were anesthetized with thiopental sodium (50 mg/kg) and mechanically ventilated using a computer-controlled volume ventilator (Flexivent, SCIREQ, Montreal, PQ, Canada) with a tidal volume of 10 mL/kg and a respiratory rate of 60 breaths/min at a positive end-expiratory pressure of 4–5 cmH2O. Before each measurement of resistance, the lungs were inflated four times to 30 cmH2O to establish a standard volume history. Respiratory system resistance was calculated using a multiple linear regression of the pressure, flow, and volume signals recorded during a 1-Hz volume oscillation signal (15 mL/kg) that interrupted mechanical ventilation for one cycle. Resistance was measured every 3 s for 60 s after the aerosol challenge to ensure that the maximal response was recorded. Measurements were obtained at baseline before ACh challenge and then approximately every 8 min after each stepwise change in the concentration of ACh (1.0, 3.3, 10, 20, 33, 50, and 100 mg/ml). The aerosol, which was produced with an ultrasonic nebulizer (model NE-U03V, OMRON Healthcare), was delivered into the inspiratory circuit and inhaled along the main axial pathway were isolated. All of the airway branches were tied with silk sutures to obtain a leak-free bronchial segment. Each end of the bronchial segment was slowly cycled between airway transmural pressures of −25 and +10 cmH2O until a constant pressure-volume curve was obtained; this usually required three to five cycles. Airway volumes at transmural pressures of −25 and +10 cmH2O were defined as zero volume and maximal volume, respectively. The next inflation and deflation cycle was then used for pressure-volume measurements. Bronchial volumes were measured at pressures of −20, −10, −5, −2, 0, 2, 5, 8, and 10 cmH2O, and the deflation pressure-volume curve for each airway segment was normalized by expressing bronchial volume as a fraction of maximum volume.

ISOVOLUME CONTRACTION TO ACh. The sensitivity of the excised airways to ACh and to maximal pressure generation was evaluated by using isovolumetric contractions to increasing concentrations of ACh. We assessed the contractile response of the bronchi at 40% of maximal volume. The ACh concentration in the tissue bath was increased sequentially from 10−9 M to 10−3 M to obtain a cumulative dose-response curve. At each concentration of ACh, pressure generation at constant volume was recorded until a plateau in pressure was achieved.

Lung parenchyma. VOLUME OF FIXED LUNG. The right lung was excised, degassed, and inflated with 10% formalin to a distending pressure of 20 cmH2O for fixation. The volume of the formalin-fixed, inflated lung was determined by liquid displacement (24).

ALVEOLAR SIZE. The right upper lobe was divided into three, equally spaced pieces of tissue that were embedded in paraffin, sectioned, and stained. Sections were visualized under a light microscope, and a digital picture was obtained. Alveolar size was quantified by measuring the mean linear intercept, which is the number of alveolar walls intersecting a line of known length (18).

In Vivo Measurements

Airway segments. After the in vivo measurements of lung function, the left lung from each animal was excised and placed in physiological saline solution [PSS; which contains (in mM) 110 NaCl, 3.4 KCl, 2.4 CaCl2, 0.82 MgCl2, 25.8 NaHCO3, 1.2 KH2PO4, and 5.6 glucose] and aerated with 95% O2 and 5% CO2. Using a dissecting stereomicroscope, the parenchymal tissue was removed and airway generations 5–6 along the main axial pathway were isolated. All of the airway branches were tied with silk sutures to obtain a leak-free bronchial segment. Each end of the bronchial segment was mounted and securely tied to a stainless steel cannula with a diameter comparable to that of the end of the bronchial segment. The bronchial segment with the inserted cannula was mounted in a 16-mL tissue bath, which is a Plexiglas chamber filled with PSS solution at 37°C. The bronchial segment was flushed and filled with PSS with the use of a stopcock and syringe connected to the cannula on the distal, smaller end of the segment. The cannula on the proximal end of the bronchial segment was connected to a differential pressure transducer (model 1566PC05GW, Honeywell Sensing), which measured transmural pressure, and a microsyringe, which measured changes in bronchial volume. The syringe was attached to a servo-controlled motor that could adjust bronchial volume and pressure. The pressure and volume signals were amplified, digitized (model DT 2801-A, Data Translation, Marlborough, MA), and recorded on a personal computer for real-time visualization of signals, as well as for subsequent data analysis with the use of commercial software (RHT Infodat, Montreal, PQ, Canada). The equipment and methodology were previously described (11).

PASSIVE DEFLEXION PRESSURE-VOLUME CURVES. The passive deflection pressure-volume curves of the rabbit bronchi were measured. Each bronchial segment was slowly cycled between airway transmural pressures of −25 and +10 cmH2O until a constant pressure-volume curve was obtained; this usually required three to five cycles. Airway volumes at transmural pressures of −25 and +10 cmH2O were defined as zero volume and maximal volume, respectively. The next inflation and deflation cycle was then used for pressure-volume measurements. Bronchial volumes were measured at pressures of −20, −10, −5, −2, 0, 2, 5, 8, and 10 cmH2O, and the deflation pressure-volume curve for each airway segment was normalized by expressing bronchial volume as a fraction of maximum volume.

Statistical Analysis

In vivo airway responsiveness from High-CPAP, Low-CPAP, and No-CPAP animals were compared by Kruskal-Wallis rank sum test. In vivo lung volumes from High- and Low-CPAP animals were compared by t-tests. The parameters calculated from the deflation pressure-volume curves, maximal pressure generation and sensitivity to ACh for airway segments, as well as lung volume and alveolar size obtained from High-, Low-, and No-CPAP rabbits, were compared by ANOVA. P values ≤0.05 were considered statistically significant.

RESULTS

In Vivo

Dose-response curves for the percent increase in respiratory system resistance above baseline for increasing concentrations of inhaled ACh are illustrated in Fig. 1. At the three highest concentrations of ACh (10, 20, and 33 mg/ml), animals exposed to high CPAP for 4 days had a significantly smaller increase in resistance than either animals exposed to low CPAP or no CPAP (P < 0.05). There was no significant difference in the responses of the Low- and No-CPAP groups. In addition, the baseline resistances were not significantly different for the three groups.

Lung volume at 30 cmH2O was measured before the application of CPAP (pre) and again after 4 days of CPAP (post). There were no significant differences in pre-CPAP lung volumes between the rabbits exposed to high and low CPAP (Fig.

J Appl Physiol • VOL 99 • AUGUST 2005 • www.jap.org
2). Lung volume increased significantly between the pre- and post-CPAP measurements for the High-CPAP animals ($P < 0.01$), whereas there was no significant change in lung volume for the Low-CPAP animals. In addition, post-CPAP lung volumes were significantly larger for High-CPAP animals than Low-CPAP animals ($P < 0.05$).

In Vitro

Intraparenchymal bronchial segments were isolated from animals maintained at high, low, and no CPAP and pressure generation to increasing concentrations of ACh was measured during isovolumetric contraction at 40% of maximal volume. Bronchi from High-CPAP animals generated significantly less pressure than bronchi from Low- and No-CPAP animals at ACh concentrations of $10^{-6}$, $10^{-5}$, $10^{-4}$, and $10^{-3}$ M ($P < 0.05$; Fig. 3). There was no difference in the pressure generation for the bronchi from Low- and No-CPAP animals. When the dose-response curves were normalized to the maximal pressure generated, the bronchi from High-CPAP animals also demonstrated less sensitivity to ACh. The dose-response curve for the High-CPAP animals was shifted to the right; at $10^{-6}$ M ACh, bronchi from High-CPAP animals generated a smaller percentage of maximal pressure than bronchi from Low-CPAP animals ($60 \pm 6$ vs. $78 \pm 4\%$; $P < 0.05$).

The deflation pressure-volume curves of isolated airway segments from High-, Low-, and No-CPAP animals were compared. The maximal volumes of the airway segments from High-CPAP animals were greater than the maximal volumes of the airway segments from Low- or No-CPAP animals; however, the differences did not achieve statistical significance (Fig. 4). With the pressure-volume curves normalized to the maximal volume of the airway segment, the compliance of the bronchi between 0 and 2 cmH2O was not significantly different for the groups of animals.

The volume of the right lung measured by water displacement after fixation at a 20 cmH2O was significantly greater for High-CPAP animals than the volumes from Low- or No-CPAP animals ($P < 0.05$; Fig. 5). There was no significant difference in right lung volume for the Low- and No-CPAP animals. Alveolar diameters, which were measured from the fixed right lobe, were also significantly greater in the High-
CPAP animals than Low- and No-CPAP animals ($P < 0.05$; Fig. 6). There was no significant difference in alveolar size between the Low- and No-CPAP animals.

**DISCUSSION**

The results of this study demonstrate that alterations in the mechanical forces on the lungs that occur in vivo may cause significant changes in its physiological function. Our study is the first to demonstrate that the prolonged application of mechanical strain to the lungs in vivo results in lower respiratory system responsiveness in vivo. In this study, the lungs of conscious, active, tracheotomized rabbits were distended for a period of 4 days by the application of 6-cmH$_2$O CPAP. The persistence of the lower responsiveness of the excised airways that were subjected to high transmural pressure in vitro also exhibited a higher compliance than airways excised from animals subjected to 0-cmH$_2$O CPAP. The persistence of the lower responsiveness in the excised airway tissues suggests that the decreased respiratory system responsiveness observed in vivo is a direct result of changes in the airways per se.

The effect of high CPAP on the responsiveness of airway segments in the present study is similar to the effects of imposing a chronic elevation in airway transmural pressure on isolated bronchial segments in vitro, that our laboratory reported previously (17). Isolated bronchial segments that are incubated in vitro at an elevated transmural pressure for a period of 2 days exhibit a lower level of responsiveness to ACh than airways incubated for 2 days in vitro at 0-cmH$_2$O transmural pressure (17). Our present findings demonstrate that the changes in airway properties elicited by mechanical strain in vitro can be elicited by physiological interventions imposed under in vivo conditions that alter the mechanical forces on the lungs.

We found that maintaining lung distention with 6-cmH$_2$O CPAP in rabbits for 4 days also resulted in an increase in lung volume and alveolar size, indicating that the application of prolonged CPAP resulted in changes in the structure of the lung parenchymal tissue. However, we found that the specific compliance of the respiratory system did not differ for the rabbits maintained at high and low CPAP. Similarly, Zhang and colleagues (25) found that ferrets treated with high CPAP for 2 wk had increased lung volumes. These investigators also found that there was no difference in the pressure-volume curves of air- or saline-filled isolated lungs from animals treated with high or low CPAP.

Theoretically, a change in the structure of the lung parenchyma might contribute to the lower responsiveness of the airways observed in vivo in animals treated with high CPAP by altering airway-parenchymal interdependence. However, we would expect that higher lung volumes might be associated with lower elastic recoil of the lung parenchyma, which should decrease the elastic load that limits airways smooth muscle shortening and result in greater airway narrowing in the High-CPAP group (1). Furthermore, the absence of differences in the specific compliance between the High- and Low-CPAP groups suggests that there may not be substantive differences in the forces of interdependence between the High- and low-CPAP groups. Therefore, there is little evidence that alterations in the lung parenchyma can account for the lower airway responsiveness that we observed in the rabbits maintained at high CPAP.

A higher mean airway volume was also obtained in the excised bronchial segments from animals exposed to high CPAP. On the basis of LaPlace’s law for thin-walled cylindrical vessels, a larger airway diameter in the airway segments from animals maintained at high CPAP might have contributed to the lower maximal pressure generation of the segments from these animals. The CPAP may have caused structural changes in the airways that resulted in higher volumes. This finding would be consistent with our laboratory’s recent report that isolated rabbit bronchial segments incubated in vitro for 48 h at a transmural pressure of 7 cmH$_2$O had larger volumes than segments incubated in vitro at a transmural pressure of 0 cmH$_2$O for the same time period (17).

The excised airways that were subjected to high transmural pressure in vitro also exhibited a higher compliance than airways incubated at 0-cmH$_2$O transmural pressure. The compliance of the airway segments removed from High-CPAP animals in the present study was not statistically different; however, the tidal breathing of the rabbits in vivo would...
provide an additional mechanical stretch on the airways that is not present in the airways incubated under static conditions in vitro. This additional stretch applied to the airways in vivo could have lessened the effect of the chronic transmural pressure difference experienced by the airways as a result of the CPAP. An increased rate of contraction of bronchial rings excised from sheep subjected to chronic lung volume restriction has also been reported (9). This would also be consistent with a mechanically induced decrease in airway compliance.

We used a computational model of rabbit airway narrowing to evaluate whether the differences in transmural pressure generation in response to ACh that we observed in the excised airway segments could account for the effects of CPAP on airway responsiveness in vivo. This computational model, which our laboratory has previously described (8), predicts airways resistance on the basis of a static balance between force generation by the airway smooth muscle and the elastic loads from the airway wall and lung parenchyma that limit smooth muscle shortening. The maximal isovolumetric pressure generation of the ACh-stimulated airway segments from High-CPAP group animals was 25% less than the maximal pressure generation from the airway segments from No-CPAP animals. We therefore incorporated a 25% decrease in force generation by the airway smooth muscle into the model of the High-CPAP animals. When other parameters in the model were maintained the same, the maximal increase in airway resistance predicted for the High-CPAP model was 50% lower than the maximal increase in resistance for the No-CPAP model. The difference predicted by the model is similar in magnitude to our in vivo observations of respiratory system responsiveness (Fig. 1). Our analysis suggests that a 25% lower level of pressure generation by the airways of the High-CPAP animals could account for the lower respiratory system responsiveness observed in these rabbits in vivo.

We also evaluated respiratory system responsiveness in a third group of animals that were not tracheotomized or exposed to CPAP (No-CPAP group) to determine whether the procedures required to maintain the animals under chronic CPAP had any effect on lung function. The presence of the chronic tracheotomy tube might result in inflammation of the lung and thereby alter airway responsiveness. We found no differences in the in vivo or the in vitro measurements of lung function between the Low-CPAP animals and the No-CPAP animals. Thus the procedures required to administer chronic CPAP to these animals did not appear to have a significant effect on lung function or to significantly contribute to the observed differences between the High- and Low-CPAP animals.

In conclusion, our results demonstrate that the application of chronic CPAP for a period of 4 days can reduce respiratory system responsiveness to ACh in rabbits and that this effect is likely to result from a direct effect of mechanical strain on the airways. This level of CPAP elicits increases in lung volume and transpulmonary pressure that are within a physiological range and that should not result in lung injury. In the rabbit lung, a CPAP level of 6 cmH2O will maintain the lung at ~50% total lung capacity (14). In human patients, levels of CPAP of 8 cmH2O or higher are routinely used clinically for outpatients with obstructive sleep apnea, as well as for infants with tracheomalacia or chronic lung disease of infancy. Our results in rabbits suggest that the administration of chronic CPAP might reduce airway responsiveness in human subjects. If so, this approach has the potential to provide a useful therapeutic intervention for patients with airway hyperresponsiveness.

ACKNOWLEDGMENTS

We thank of Debra Pearson, Adam Williams, and Nicole Tepper for technical assistance.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grants HL-48522 and HL-29269.

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