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*Am J Physiol Lung Cell Mol Physiol* 275:567-573, 1998.

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# Prolonged high intermittent positive-pressure ventilation induces airway remodeling and reactivity in young rats

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**Fukunaga, Tetsu, Paul Davies, Leilei Zhang, Yoshie Hashida, and Etsuro K. Motoyama.** Prolonged high intermittent positive-pressure ventilation induces airway remodeling and reactivity in young rats. *Am. J. Physiol. Cell. Mol. Physiol.* 275 (Lung Cell. Mol. Physiol. 19): L567–L573, 1998.—We postulated that prolonged exposure to intermittent positive-pressure ventilation (IPPV) with high pressure (HIPPV) alone without hyperoxia promotes the development of airway hyperresponsiveness and remodeling. To test this hypothesis, young rats were ventilated under halothane anesthesia with HIPPV (maximum inspiratory pressure at 32–35 cmH<sub>2</sub>O in 70% nitrous oxide and 30% O<sub>2</sub>) for 3.5–4 h daily for 6 days. Control rats were ventilated with low IPPV (maximum inspiratory pressure < 13 cmH<sub>2</sub>O) during the same time period with the same gas mixture. With the use of tracheal rings isolated from these rats and a setup in tissue baths, contractile responses to carbachol (10<sup>-6</sup> to 10<sup>-2</sup> mM), 5-hydroxytryptamine (5-HT; 10<sup>-9</sup> to 10<sup>-5</sup> mM) and KCl (1–100 mM) were examined isometrically. In tracheal rings from HIPPV rats compared with low-pressure IPPV rats, the concentration tension curves showed a significantly enhanced response to all agonists ( $P < 0.005$ ). Sensitivity to carbachol, 5-HT, and KCl was also significantly increased ( $P < 0.05$ ) compared with control rats as evidenced by decreases in EC<sub>50</sub>. Maximum tension (reactivity) to 5-HT and KCl in the HIPPV group increased significantly ( $P < 0.05$ ), and there was a trend ( $P = 0.07$ ) toward increased reactivity to carbachol in this group as well. Histological examinations of tracheal rings demonstrated epithelial squamous metaplasia in the HIPPV group. Morphometric studies demonstrated tracheal smooth muscle thickening ( $P < 0.05$ ) without changes in the thickness of the mucosa or the lamina propria. When contractile responses were normalized for the smooth muscle cross-sectional area (i.e., stress), reactivity to all contractile agents was reduced, whereas reactivity to 5-HT still demonstrated significant increase ( $P < 0.005$ ). Sensitivity of tracheal segments to all three agents was not affected by this normalization. These findings suggest that prolonged exposure to HIPPV without hyperoxia and the resultant overdistension of lung tissues (volutrauma) induced airway remodeling and airway hyperreactivity.

airway hyperresponsiveness; volutrauma; carbachol; 5-hydroxytryptamine

BRONCHOPULMONARY DYSPLASIA (BPD) is a chronic obstructive pulmonary disease that occurs in prematurely born infants after prolonged intermittent positive-pressure ventilation (IPPV) and O<sub>2</sub> therapy (1, 22). Histological features of infants dying from BPD include submucosal edema, chronic inflammation, squamous metaplasia of epithelial cells in large and small airways, thickening of airway smooth muscles, and peri-

bronchiolar fibrosis (28). Airway hyperresponsiveness (a positive response to bronchodilators) becomes evident within the first weeks of life, independent of the degree of prematurity or familial disposition to asthma (20). Infants born with severe congenital diaphragmatic hernia who are treated with mechanical ventilation also develop airway hyperresponsiveness during the first few weeks of life, a condition that becomes even more pronounced by 1–4 mo of age (2). Similar airway hyperresponsiveness and lower airway obstruction have been reported in infants following meconium aspiration and respiratory failure after IPPV and extracorporeal membrane oxygenation (16). In prematurely born infants with chronic lung disease, airway hyperreactivity (in response to histamine challenge) persists into adolescence, again independent of familial predisposition to asthma (15).

Likely causes of airway remodeling and hyperresponsiveness in BPD include pulmonary hyperoxia, hyperdistension (barotrauma or, more appropriately, "volutrauma"), and inflammation (7). To date, most investigations into probable causes of BPD have focused on the contribution of hyperoxia. Animal studies have shown that subchronic (7–8 days) exposure to hyperoxia (80–85%) alone in immature and adult animals can induce morphological changes such as airway smooth muscle thickening and airway hyperresponsiveness (29–31). Hershenson and colleagues (9, 10) demonstrated a correlation between increased airway responsiveness and smooth muscle and epithelial thickness after subchronic hyperoxia. They postulated that, after hyperoxia, airway remodeling contributes to the development of airway hyperresponsiveness.

In patients with respiratory failure, it is often necessary to maintain IPPV with relatively high pressures, together with a high inhaled O<sub>2</sub> concentration, to maintain adequate oxygenation. Several investigators (6, 14) have observed that mechanical ventilation with high maximum inspiratory pressure (30–45 cmH<sub>2</sub>O) for short durations (5 min to 48 h) can produce stress disruptions of the capillary endothelium, alveolar and airway epithelia, and basement membranes. These microscopic injuries increase microvascular permeability and edema formation. High-pressure IPPV (HIPPV) can produce acute lung injury as a function of both peak pressure and duration (6). The effects of prolonged HIPPV on airway morphology and airway responsiveness, however, have not been investigated.

Specific aims of this study were to test the hypothesis that HIPPV, without hyperoxia or infection, can induce

airway remodeling and airway hyperresponsiveness and, with the use of a young rat model, to see if there is a relationship between airway remodeling and hyperresponsiveness. The results of the present study suggest that prolonged HIPPV per se induces airway hyperresponsiveness associated with airway remodeling.

## METHODS

### *Animal and HIPPV Designs*

Pathogen-free 7-wk-old male Wistar rats (230–270 g; Hill-top Lab Animals, Scottsdale, PA) were used in this study. The rats were anesthetized over 6 consecutive mornings with 3–4% halothane in O<sub>2</sub> and intubated endotracheally with a 14-gauge intravascular catheter. Special care was taken not to injure the tracheal mucosa by carefully attaching the catheter to the maxilla. After endotracheal intubation, the rats were ventilated with a time-cycled, pressure-limited infant ventilator (Baby Bird; Bird, Palm Springs, CA) with high or low maximum inspiratory pressure (P<sub>max</sub>). The rats in the HIPPV group were ventilated with a P<sub>max</sub> of 32–35 cmH<sub>2</sub>O [tidal volume (V<sub>T</sub>) ~30 ml/kg; respiratory rate (f) 25 breaths/min], and the rats in the low-pressure IPPV (LIPPV) or control group were ventilated with a P<sub>max</sub> of <13 cmH<sub>2</sub>O (V<sub>T</sub> ~10 ml/kg; f 40 breaths/min) for 3.5–4 h daily for 6 days. Positive end-expiratory pressure was not added. During IPPV, the rats were sedated with 70% nitrous oxide (N<sub>2</sub>O) in O<sub>2</sub> and paralyzed with pancuronium bromide (1.2–1.6 mg·kg<sup>-1</sup>·h<sup>-1</sup> im). Five percent dextrose in 0.25% saline solution (3 ml·kg<sup>-1</sup>·h<sup>-1</sup>) was administered via an orogastric tube for hydration during the period of anesthesia and IPPV. After IPPV, atropine sulfate (0.2 mg/kg) and neostigmine methyl sulfate (0.6 mg/kg) were administered intraperitoneally to reverse residual effects of neuromuscular blockade by pancuronium bromide. To prevent respiratory infection, penicillin G (400,000 IU·kg<sup>-1</sup>·2 days<sup>-1</sup> im) and gentamicin (16 mg·kg<sup>-1</sup>·day<sup>-1</sup> im) were administered to both groups. When the rats resumed sufficient spontaneous breathing and body movement, they were extubated and returned to their cages for recovery and feeding overnight. All procedures were approved by the Institutional Animal Care and Use Committee.

### *Preparation of Tracheal Segments for Contractility Studies*

At the end of a 6-day period, the rats were killed with a large dose of thiopental sodium (200 mg/kg ip). The tracheae were excised and immediately placed in a Krebs solution of the following composition (in mM): 118 NaCl, 3.4 KCl, 25.6 NaHCO<sub>3</sub>, 1.2 KH<sub>2</sub>PO<sub>4</sub>, 0.8 MgSO<sub>4</sub>, 2.5 CaCl<sub>2</sub>, and 11 glucose. Each trachea was then cleansed of connective tissues under a dissecting microscope. Two cylinders or rings, each three cartilages wide, were cut from the distal end of each trachea at a point proximal to the carina. This region was distal to that reached by the endotracheal tube. The distal tracheal ring from each animal was suspended vertically across two stainless steel wires in a 30-ml organ bath at 37°C and aerated continuously with a 95% O<sub>2</sub>-5% CO<sub>2</sub> mixture. Special care was taken to ensure that the membranous portion of the tracheal ring was not mounted on the stainless steel wire. The lower wire was tethered to the bottom of the water bath, and the upper wire was connected to an isometric force transducer. The transducer was connected to an amplifier and a recording system (Grass Instruments, Quincy, MA). After a 60-min equilibration period, during which time the Krebs solution was changed every 10–15 min, the optimal resting tension of rings was determined by generating a

length-tension curve. The tracheal rings were set at their optimal length and then exposed to 60 mM KCl (the minimum concentration that induced the maximum contraction from preliminary experiments) to stabilize contractile responses and to demonstrate responsiveness and viability. All subsequent experiments were performed at the optimal length. This procedure has been described as a standard technique that produces consistent concentration-response data in organ bath experiments (12, 31).

### *Concentration-Response Curves*

Concentration-response curves to carbachol (10<sup>-6</sup> to 10<sup>-2</sup> mM), 5-hydroxytryptamine (5-HT; 10<sup>-9</sup> to 10<sup>-5</sup> mM), and KCl (1–80 mM) were generated in a noncumulative manner. Tissues were washed twice with a Krebs-Henseleit solution after each contraction and rested for 10 min to restabilize; the tissues were also rested for 30 min between different agonist experiments. All drugs were purchased from Sigma (St. Louis, MO), and all solutions were freshly prepared on the day of the experiment and stored on ice.

Tension was calculated as force relative to the axial length of the ring (in g/mm), and stress was calculated as force relative to the cross-sectional area of smooth muscle (in g/mm<sup>2</sup>). Force generation was determined in response to carbachol, 5-HT, and KCl at a range of concentrations. The maximum response (reactivity) was determined, and the concentration corresponding to the midpoint of the concentration-response curve (EC<sub>50</sub>) was calculated using a nonlinear regression.

### *Percent Tissue Water of the Trachea*

The proximal tracheal rings, not utilized for the dose-response curves, were used to obtain percent tissue water of the tracheal ring, an index of tissue edema. This segment of the lower trachea was distal to the segment reached by the tip of the endotracheal cannula. After the tracheal rings were blotted, wet tissue weight was determined. To determine dry weight, the same tracheal rings were weighed again after 24 h in a drying oven set at 68°C. The percent tissue water was calculated as follows (31)

$$\% \text{tissue water} = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \times 100$$

### *Airway Histology*

At the conclusion of the experiment, the tracheal rings were maintained at their resting length for 30 min and their axial length was measured. The rings were then fixed overnight in 4% paraformaldehyde in phosphate-buffered saline while being suspended in the tissue bath. The rings were removed from the tissue bath and embedded in paraffin, and sections 6 μm thick were cut in a plane perpendicular to the tracheal axis. The sections were stained with hematoxylin and eosin and then evaluated by two independent examiners (T. Fukunaga and Y. Hashida) for the following histological features: 1) epithelial squamous metaplasia, 2) mucosal necrosis, 3) inflammation in the lamina propria (submucosa), and 4) fibrosis in the lamina propria. These features have previously been described in postmortem examinations of infants with BPD (28). The degree of change in each histological category was rated using scores of 0 (no change), 1 (mild), 2 (moderate), and 3 (severe). For epithelial squamous metaplasia, loss of cilia or cell polarity was considered mild, focal metaplasia was moderate, and diffuse full-thickness metaplasia was severe. The types of submucosal inflammation pres-

Table 1. Effects of HIPPV on airway histology

	LIPPV				HIPPV				P Value
	None	Mild	Mod	Severe	None	Mild	Mod	Severe	
Squamous metaplasia	9	0	0	0	0	2	3	4	<0.05
Mucosal necrosis	9	0	0	0	6	1	2	0	0.07
L. propria fibrosis	9	0	0	0	6	2	1	0	0.07
L. propria inflammation	6	3	0	0	3	6	0	0	0.17

Type of inflammation	LIPPV				HIPPV			
	None	Acute	Mix	Chronic	None	Acute	Mix	Chronic
Type of inflammation	6	2	1	0	3	4	2	0

Values are no. of individual tracheal sections. LIPPV and HIPPV, low and high intermittent positive-pressure ventilation, respectively; L, lamina; Mod, moderate; Mix, mixed acute and chronic changes.

ent (acute, chronic, or mixed) were also evaluated on the basis of infiltrated cell types.

*Airway Morphometry*

Tracheal rings in transverse sections (3–6 sections/ring) were viewed in a light microscope (Olympus BH-2, Olympus Optical, Lombard, IL) fitted with a camera lucida, which enabled each image to be superimposed onto a digitizing plate (Summasketch Plus, Summagraphics, Fairfield, CT) and traced with a cursor online to a computer (IBM 386). The sectional areas of epithelium, lamina propria, and smooth muscle were determined with morphometric software (BQ system IV; R&M Biometrics, Nashville, TN). The mean thickness of the epithelium and lamina propria was computed by dividing the respective area by the perimeter length of the epithelial basement membrane. The mean thickness of the smooth muscle bundle was determined by dividing its sectional area by its perimeter length. Values obtained from three to six slices from the same tracheal ring were averaged.

*Statistical Analysis*

Contractile responses to carbachol, 5-HT, and KCl between HIPPV and LIPPV were compared by ANOVA for repeated measures to evaluate changes in response to the effects of HIPPV. Maximum tension was compared between HIPPV and LIPPV animals using an unpaired *t*-test. A comparison of EC<sub>50</sub> between the HIPPV and LIPPV rats was made by comparing log EC<sub>50</sub> values with an unpaired *t*-test. Histological data of the HIPPV vs. LIPPV groups were compared with a Mann-Whitney *U*-test; morphometric data and the tracheal percent tissue water of the HIPPV vs. LIPPV groups were compared with an unpaired *t*-test. Values are expressed as means ± SE, and *P* < 0.05 was considered significant.

**RESULTS**

Three of the fifteen rats in the HIPPV group died, all during mechanical ventilation, within the first 2 days. The deaths were associated with a decrease in chest movement (decrease in compliance), severe pulmonary edema, and alveolar flooding at autopsy. In contrast, all 12 control (LIPPV) animals survived. In 3 of the 12 HIPPV rats, the planned experiments could not be completed due to the damage to the trachea incurred during the process of harvesting tracheal rings or because the tracheal rings became unresponsive before the completion of the dose-response curves for three agonists. Consequently, data from nine rats from each group were used for data analysis.

*Percent Tissue Water of the Trachea*

There was no significant difference between the percent tissue water of the tracheal rings of the HIPPV and LIPPV groups (HIPPV, 75.7 ± 2.3%; LIPPV, 73.9 ± 1.3%; *P* > 0.50).

*Histology and Morphometry of Tracheal Rings*

*Epithelial layer:* A significantly increased incidence of squamous epithelial metaplasia was observed in all tracheal sections from the HIPPV group but not in the LIPPV group (Table 1). The degree of epithelial squamous change showed no significant correlation with contractile responses to all three contractile agonists. Mucosal necrosis was observed in three of nine animals in the HIPPV group, whereas no necrosis was seen in the LIPPV group; the difference in the mucosal necrotic changes between the two groups approached significance (Table 1). Mean epithelial thickness was similar in both groups (Fig. 1).

*Lamina propria.* In the HIPPV group, inflammation in the lamina propria was observed in six of the nine tracheal sections as evidenced by either an infiltrate

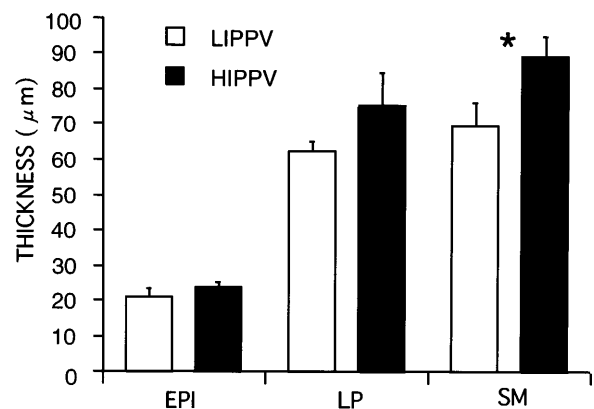


Fig. 1. Effects of high intermittent positive-pressure ventilation (HIPPV) on airway epithelial (EPI), lamina propria (LP), and smooth muscle (SM) layer thickness. Values are means ± SE. HIPPV increased airway SM layer thickness significantly compared with that of low IPPV (LIPPV; 89.0 ± 5.98 vs. 69.9 ± 6.57 µm, respectively; *P* < 0.05). Mean epithelial thickness was similar between HIPPV and LIPPV groups (24.1 ± 1.07 vs. 21.3 ± 2.22 µm; *P* > 0.20). There was no significant difference in LP thickness between the 2 groups (75.3 ± 0.91 vs. 62.2 ± 0.31 µm; *P* = 0.19). \**P* < 0.05 compared with LIPPV.

consisting exclusively of neutrophils or a mixed infiltrate with neutrophils and lymphocytes. Although the incidence and degree of inflammation tended to be less in the LIPPV group, the difference was not significant (Table 1). The difference between the two groups in incidence and degree of fibrosis in the lamina propria approached statistical significance (Table 1). There was no difference between the mean layer thickness of the lamina propria of the tracheal sections of the HIPPV and LIPPV groups (Fig. 1).

**Smooth muscle layer:** A significant increase in the mean thickness of the smooth muscle in the membranous portion of the trachea was evident in the HIPPV group compared with the LIPPV group (Fig. 1).

#### Effect of HIPPV on Isometric Force Generation

The tensions developed by the tracheal segments in response to each of the three contractile agonists (carbachol, 5-HT, and KCl) at a range of concentrations are shown in Fig. 2. The concentration-tension curves of tracheal segments from the HIPPV rats compared with those from the LIPPV rats showed significantly enhanced responses to carbachol, 5-HT, and KCl. Maximum tensions (reactivity) in response to 5-HT and KCl were also significantly elevated in the HIPPV group compared with the LIPPV group (Table 2, Fig. 2, B and C). The mean reactivity to carbachol also increased but did not reach significance ( $P = 0.07$ ) (Table 2, Fig. 2A). The  $EC_{50}$  values for all three agents decreased significantly in the HIPPV group (Table 3), indicating an enhanced sensitivity.

To compare the stress (in  $g/mm^2$ ) generated by a unit of tracheal smooth muscle mass between the HIPPV and LIPPV groups, force generated by tracheal rings was normalized by dividing the tension by the cross-sectional area of tracheal smooth muscles and was plotted against agonist concentrations (Fig. 3). These concentration-stress curves also showed that responses with the HIPPV group were still greater compared with the LIPPV group in all three contractile agonists. Maximum stress in the HIPPV group, however, was significantly elevated only in response to 5-HT (Fig. 3B; Table 4).  $EC_{50}$  values, on the other hand, were unaffected by the normalization and remained significantly lower in the HIPPV group for all three agents.

#### DISCUSSION

Infants on prolonged mechanical ventilation who subsequently develop BPD exhibit an early onset of airway hyperresponsiveness (20). Radiographic and histological findings in the lungs of infants with BPD showed lower airway obstruction with bronchiolar smooth muscle thickening and pulmonary fibrosis (17). Pathological changes in the respiratory systems of patients with BPD are not limited to the lung parenchyma but extend from the trachea to the most distal airways and alveoli. Dynamic compression of central airways is also a manifestation of BPD (19). In patients with lower airway obstruction, airway smooth muscle tone may be important in maintaining the patency of airways and pulmonary gas exchange (4).

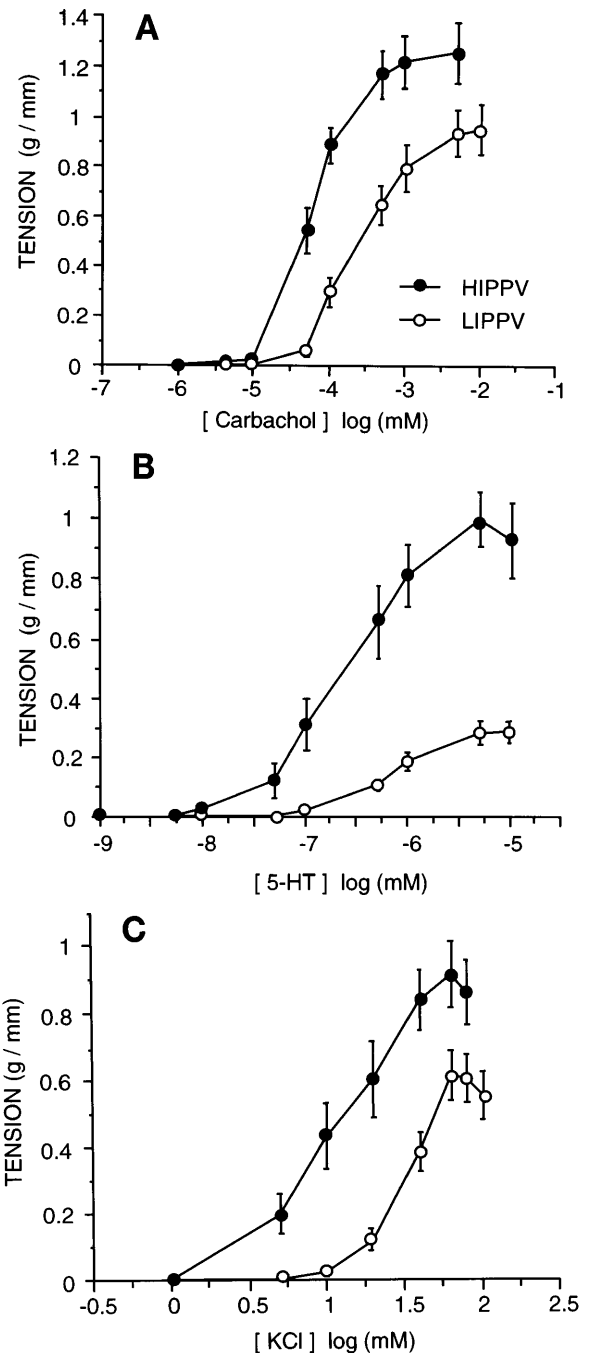


Fig. 2. Concentration-tension curves generated to carbachol (A), 5-hydroxytryptamine (5-HT; B), and KCl (C) in lower tracheal segments obtained from LIPPV and HIPPV groups. Values are means  $\pm$  SE. Tracheal rings from HIPPV group exhibited increased responses to carbachol ( $P < 0.0005$ ), 5-HT ( $P < 0.0005$ ), and KCl ( $P < 0.005$ ) compared with those from LIPPV group.

Infants with severe congenital diaphragmatic hernia and those with meconium aspiration syndrome treated with prolonged mechanical ventilation with added  $O_2$  also develop an early onset of airway hyperresponsiveness preceding airway obstruction (2, 16). These clinical studies indicate a possible link between early postnatal intensive care with exposure to hyperoxia and barotrauma or, more appropriately, volutrauma due to pulmonary hyperdistension (7) and the onset of

Table 2. Effects of HIPPV on maximum tension

	LIPPV	HIPPV	PValue
Carbachol	0.95 ± 0.105	1.26 ± 0.210	0.07
5-HT	0.29 ± 0.043	1.00 ± 0.093	<0.0001
KCl	0.63 ± 0.077	0.98 ± 0.124	<0.05

Values are means ± SE in g/mm. 5-HT, 5-hydroxytryptamine.

airway hyperresponsiveness (2, 20). Effects of both hyperoxia and volutrauma on developing lungs have been considered important in the development of BPD (5, 18).

Recent studies (9, 10, 30) have demonstrated that hyperoxia alone can induce airway remodeling and hyperresponsiveness in newborn and adult laboratory animals. The effects of prolonged repetitious hyperdistension of the lungs with HIPPV, however, have not been reported.

The results of the present study indicate that HIPPV without hyperoxia or infection for 4 h daily for 6 days in young adult rats can induce airway remodeling, characterized by squamous metaplasia of epithelial cells and thickening of the smooth muscle layer. These histological findings are similar to those seen in the airways of infants with BPD (28). The HIPPV regimen also induced airway hyperreactivity as evidenced by marked increases in contractile force generated by tracheal segments (rings or cylinders) in the tissue bath in response to carbachol, 5-HT, and KCl. More specifically, airway hyperreactivity was characterized by 1) a significant increase in maximum tension in response to 5-HT and KCl and a borderline increase in response to carbachol, 2) an increase in maximum stress generation in response to 5-HT when contractile responses were normalized by smooth muscle mass, and 3) a decrease in the EC<sub>50</sub> for carbachol, 5-HT, and KCl with or without normalization of generated tension by smooth muscle mass, an indication of enhanced sensitivity. These changes in smooth muscle mass and contractilities in the tracheal segments were not observed after 3 days of HIPPV (data not included). When HIPPV was continuous for 13 days, however, airway and vascular remodeling with smooth muscle thickening was observed both in the airways and in small pulmonary arteries of the lung parenchyma (21).

Thickening of the airway smooth muscle layer has been observed in the autopsies of patients with BPD and asthma and has been attributed to hyperplasia (23). Szarek et al. (30) found that adult rats exposed to hyperoxia for 5–7 days also demonstrated increased airway smooth muscle thickness. Hershenson et al. (11), using bromodeoxyuridine labeling of DNA, reported that prolonged exposure of rats to hyperoxia was

Table 3. Effects of HIPPV on EC<sub>50</sub>

	LIPPV	HIPPV	PValue
Carbachol, ×10 <sup>-5</sup>	26.6 ± 3.93	5.4 ± 0.64	<0.0001
5-HT, ×10 <sup>-7</sup>	7.4 ± 0.81	3.5 ± 1.25	<0.035
KCl	36.0 ± 2.75	14.2 ± 2.85	<0.0001

Values are means ± SE of EC<sub>50</sub> in mM.

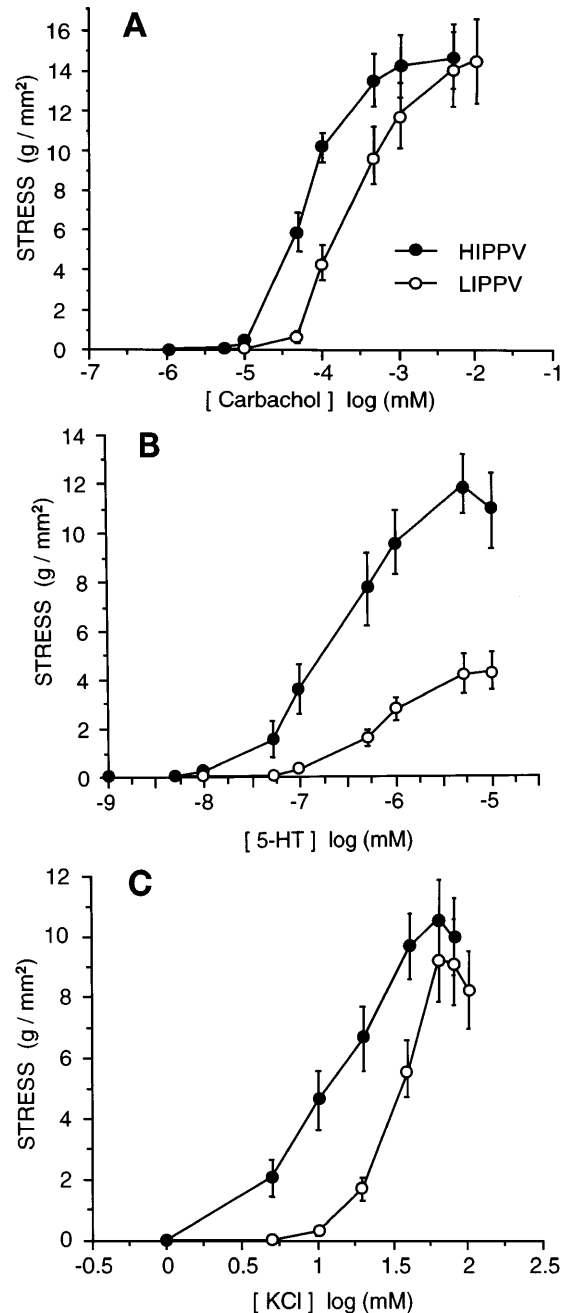


Fig. 3. Concentration-stress curves generated to carbachol (A), 5-HT (B), and KCl (C) in lower tracheal segments obtained from LIPPV and HIPPV groups. Values are means ± SE. Tracheal rings from HIPPV group exhibited increased responses to carbachol ( $P < 0.05$ ), 5-HT ( $P < 0.001$ ), and KCl ( $P < 0.02$ ) compared with airways from LIPPV group.

Table 4. Effects of HIPPV on the maximum stress after normalization for tracheal smooth muscle mass

	LIPPV	HIPPV	PValue
Carbachol	14.4 ± 2.09	14.6 ± 1.63	NS
5-HT	4.3 ± 0.82	11.9 ± 1.19	<0.005
KCl	9.4 ± 1.42	10.8 ± 1.25	NS

Values are means ± SE of stress in g/mm<sup>2</sup>. NS, no significant difference.

associated with an increased incidence of DNA synthesis in airway smooth muscle. On the basis of this finding, they suggested that the increased thickness of smooth muscle was due to hyperplasia. Whether the increased tracheal smooth muscle thickness in the current study, after 6 days of exposure to volutrauma without hyperoxia, was due to smooth muscle hyperplasia, hypertrophy, or both is yet to be determined. The mechanisms for the observed airway smooth muscle thickness are at present unclear, but subsection of airway mucosa and surrounding tissues to repetitive stretch is a likely possibility. Smith et al. (25) presented evidence that cyclic mechanical stretch of airway smooth muscle cells in culture for 3 days increased DNA synthesis and proliferation of smooth muscle cells. Recently, Siedman et al. (24) also found that repetitive mechanical stretch of cultured airway and pulmonary arterial smooth muscle cells in vitro for 3 days increased DNA synthesis, protein synthesis, and glucose utilization.

Previous studies (9, 12, 29–31) on rats exposed to hyperoxia have shown that the increase in the thickness of airway epithelium and smooth muscle is correlated with increased airway reactivity in vivo and in vitro in both immature and adult animals. Hay (8) suggested that, in patients with asthma, increased smooth muscle mass and epithelial cell damage are responsible for airway hyperresponsiveness. Although the results from our in vitro studies must be interpreted with caution when comparing them with in vivo determinations of airway reactivity, they nevertheless support the notion that increased smooth muscle mass in part explains increased reactivity.

When generated force was normalized to smooth muscle cross-sectional area to examine the generation of stress per unit of smooth muscle mass, the difference in the maximum stress generated in response to KCl in the HIPPV and LIPPV groups was no longer evident. Because KCl-elicited depolarization is independent of receptor-based signaling, this suggests that the contractile function of the phenotypically altered smooth muscle was not changed. This finding is in contrast to previous studies (3, 13) in which the maximum active force generated per unit smooth muscle mass by pathologically increased vascular smooth muscles in experimental hypertension decreased. Nevertheless, in the present study, the stress response to 5-HT increased, suggesting a change in an agonist-specific manner. Furthermore, the sensitivity of smooth muscles to all three agonists was still increased even after normalization for the smooth muscle area, suggesting that a common positive transduction mechanism is potentiated or that a negative regulatory mechanism is compromised by the HIPPV regimen.

With respect to negative regulation, another study (27) has shown that the epithelium can affect airway sensitivity to contractile agonists by synthesizing a variety of mediators. In the present study, 6 days of HIPPV produced moderate to severe squamous metaplasia. Whereas these changes may have affected the normal ability of the epithelium to control airway tone,

we could not demonstrate a positive correlation between the incidence of epithelial histological changes and airway reactivity. One possible explanation for the absence of this correlation may be that the present tracheal ring setup in a tissue bath is not as well suited to evaluate the effect of the epithelium on sensitivity as are the airway tubular preparations or in vivo experiments (27). In a tracheal ring preparation, three different surfaces (luminal, adventitial, and cut) are exposed to agonists simultaneously. Responses to agonists acting at the adventitial boundary are characteristically much greater (sensitivity is higher) than responses to agonists added at the luminal boundary. A 10- to 100-fold difference in sensitivity to contractile agents is observed in tubular segment preparations in which luminal and adventitial sides are circulated separately (27). The decreased responses through the epithelial surface are due to either decreased permeability through the epithelial boundary, the presence or release of epithelium-derived relaxing factors, or both (26).

In summary, the results of this study indicate that repetitive exposure of young rats to HIPPV in the absence of hyperoxia for a relatively short period of 6 days produces histological changes and hyperresponsiveness in the trachea that resemble those seen in BPD. This model is therefore a useful one with which to investigate mechanisms and delineate changes in more distal airways.

We thank Michael Young for help in setting up the organ bath system and Dr. Margaret McLaughlin for advice on the experimental protocol for passive and active length tension curves of the tracheal rings. We also thank Thanita Adams and David Chasey for editorial assistance.

This work was supported in part by National Heart, Lung, and Blood Institute Grant RO1-HL-41811; Grants-in-Aid from the Pennsylvania Chapter of the American Heart Association; and Seed Grants from the Department of Anesthesiology and Critical Care Medicine, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania.

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Received 5 November 1997; accepted in final form 1 May 1998.

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