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Respiratory mechanics and lung histology in normal rats anesthetized with sevoflurane

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Correa, Fatima C. F., Patricia B. Ciminelli, Haroldo Falcão, Bruno J. C. Alcântara, Renata S. Contador, Aline S. Medeiros, Walter A. Zin, and Patricia R. M. Rocco. Respiratory mechanics and lung histology in normal rats anesthetized with sevoflurane. *J Appl Physiol* 91: 803–810, 2001.—Respiratory system, lung, and chest wall mechanical properties were subdivided into their resistive, elastic, and viscoelastic/inhomogeneous components in normal rats, to define the sites of action of sevoflurane. In addition, we aimed to determine the extent to which pretreatment with atropine modified these parameters. Twenty-four rats were divided into four groups of six animals each: in the P group, rats were sedated (diazepam) and anesthetized with pentobarbital sodium; in the S group, sevoflurane was administered; in the AP and AS groups, atropine was injected 20 min before sedation/anesthesia with pentobarbital and sevoflurane, respectively. Sevoflurane increased lung viscoelastic/inhomogeneous pressures and static elastance compared with rats belonging to the P group. In AS rats, lung static elastance increased in relation to the AP group. In conclusion, sevoflurane anesthesia acted not at the airway level but at the lung periphery, stiffening lung tissues and increasing mechanical inhomogeneities. These findings were supported by the histological demonstration of increased areas of alveolar collapse and hyperinflation. The pretreatment with atropine reduced central and peripheral airway secretion, thus lessening lung inhomogeneities.

tissue resistance; viscoelasticity; lung morphometry; elastance

SEVOFLURANE IS A VOLATILE anesthetic agent that provides rapid induction of anesthesia and control of anesthetic depth and recovery due to its low solubility (12). In addition, sevoflurane causes less airway irritation than other inhaled anesthetics (13, 32, 34) and depresses ventilatory function (12, 13, 17, 26), as shown by a moderate increase in arterial P_{CO_2} and lower minute ventilation ($\dot{V}_E$). Sevoflurane has been reported to attenuate bronchoconstriction associated with anaphylaxis in a canine model (31) and in the presence of constrictor agonists (20, 25). It is believed that this attenuation is caused by a bronchodilating action of sevoflurane. However, the effect of this anesthetic agent on tissue resistance cannot be discounted, because airway stimulation not only decreases airway caliber but also increases pressure-volume hysteresis of lung tissue (25, 41).

Although there are many studies analyzing the effects of sevoflurane on respiratory mechanics in the absence of active smooth muscle tone, the results are controversial. There are some reports describing that pentobarbital sodium, sevoflurane, halothane, and isoflurane did not alter respiratory mechanics (17, 20, 26, 31), whereas others reported that sevoflurane is a potent bronchodilator (16, 19). The diversity of methods used for determining lung resistance, the variability in lung volume and respiratory frequency, and the differences in lung preparations (isolated vs. intact) could determine discrepant findings.

Hence, the aim of this study was to define the effects of sevoflurane in the respiratory system in rats without preexisting airway tone. For this purpose, the individual contributions of lung and/or chest wall elastic, resistive, viscoelastic, and other mechanical unevennesses to modify the respiratory system mechanical profile were evaluated. Functional residual capacity (FRC) was also determined. We also aimed to determine the extent to which pretreatment with atropine modified these parameters. In addition to measuring physiological parameters, we studied lung morphometry to determine whether the physiological changes reflected underlying morphological changes defining the sites of action of sevoflurane.

MATERIALS AND METHODS

Animal preparation. The experiments were performed on four groups of isogenic adult male Wistar rats. In the control group (P) [n = 6 (200–210 g)], the rats were sedated with diazepam (5 mg ip) and anesthetized with pentobarbital sodium (20 mg/kg ip). In the second group (S) [n = 6 (190–210 g)], the animals were anesthetized with sevoflurane (1 min-

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imal alveolar concentration). Sevoflurane was administered via a calibrated sevoflurane vaporizer (HB, Rio de Janeiro, Brazil) through which a flow of air was passed. In the atropine-pentobarbital (AP) [n = 6 (210–215 g)] and atropine-sevoflurane (AS) [n = 6 (190–210 g)] groups, atropine (0.05 mg/kg iv) was injected 20 min before sedation/anesthesia with pentobarbital sodium and sevoflurane, respectively. The rats were tracheotomized, and a snugly fitting cannula (1.5 mm ID) was inserted into the trachea. Sevoflurane was delivered to the animal through a tracheal cannula by means of a T-piece system, which did not cause any appreciable change in tracheal pressure (Ptr). Anesthesia was maintained throughout the experiment in stage III in the four groups. At the first moments of the experiments, with the animal breathing spontaneously, the level of anesthesia was assessed by evaluating the size and position of the pupil, its response to light, the position of the nictitating membrane, and the tone of the jaw muscles. After muscle relaxation, adequate depth of anesthesia was assessed by evaluating pupil size and light reactivity. The animals rested in the supine position on a surgical table.

Airflow ($\dot{V}$) was measured with a pneumotachograph (1.5 mm ID, length = 4.2 cm, distance between side ports = 2.1 cm) constructed according to Mortola and Noworaj (33) connected to the tracheal cannula. The pressure gradient across the pneumotachograph was determined by means of a Validyne MP45-2 differential pressure transducer (Northridge, CA). Volume (V) was obtained by integration of the flow signal. The flow resistance of the equipment (Req) (tracheal cannula included) was constant up to flow rates of 26 ml/s and amounted to 0.14 $cmH_2O \cdot ml^{-1} \cdot s$. Equipment resistive pressure (= Req·$\dot{V}$) was subtracted from respiratory system and pulmonary resistive pressures so that the results reported reflect intrinsic mechanical properties. Because abrupt changes of diameter were not present in our circuit, errors of measurement of flow resistance were avoided (10, 30). The equipment dead space was 0.4 ml. Ptr was measured at the side port of the tracheal cannula with a second differential pressure transducer (MP45-2 Validyne). Changes in esophageal pressures (Pes), which reflect chest wall pressure (Pw), were measured with a 30-cm-long water-filled catheter (PE205) with side holes at the tip connected to a PR23–2D-300 Statham differential pressure transducer (Hato Rey, Puerto Rico). The catheter was passed into the stomach and then slowly returned into the esophagus; its proper positioning was assessed by using the occlusion test (8). This consisted of comparisons of ΔPes and ΔPtr during spontaneous inspiratory efforts subsequent to airway occlusion at end expiration. In all instances, ΔPes was close to ΔPtr, the difference not exceeding 3%. The frequency responses of Ptr and Pes measurement systems were flat up to 20 Hz, without appreciable phase shift between the signals. All signals were conditioned and amplified in a Beckman type R dynograph (Schiller Park, IL). Flow and pressure signals were then passed through eight-pole Bessel filters (902LPF, Frequency Devices, Haverhill, MA) with the corner frequency set at 100 Hz, sampled at 200 Hz with a 12-bit analog-to-digital converter (DT-2801A, Data Translation, Marlboro, MA), and stored on a computer. All data were collected using LABDAT software (RHT-InfoData, Montreal, Quebec, Canada).

Ventilatory variables. During spontaneous breathing, durations of inspiration (T_I) and expiration and the respiratory cycle time (Ttot) were measured from flow signal. Using these variables, we calculated mean inspiratory flow rate [tidal volume (V_T)/T_I], duty ratio (T_I/Ttot), respiratory frequency, and $\dot{V}_E$. Respiratory system elastance and resistance were also computed by multiple linear regression using the signals of the Ptr, flow, and changes in lung volume.

Measurement of respiratory mechanics. Respiratory mechanics were measured from end-inspiratory occlusions after constant flow inflation (3, 4, 6, 7, 27, 28, 39). Initially, muscle relaxation was achieved with gallamine triethyliodide (2 mg/kg iv), and artificial ventilation was provided by a Salziner constant-flow ventilator (Instituto do Coração-USP, São Paulo, Brazil). During the test breaths, a 5-s end-inspiratory pause could be generated by adjusting the ventilator settings, whereas during baseline ventilation no pause was used. To avoid the effects of different flows and V_T (11, 27, 28), and thence inspiratory duration (39), on the measured variables, special care was taken to keep V_T (V = 2 ml) and flow ($\dot{V}$ = 10 ml/s) constant in all animals. Breathing frequency remained constant and equal to 100 breaths/min during the experiment. The T_I was set at 0.2 s, and the duty cycle (T_I/Ttot) amounted to 0.33.

Respiratory mechanics were measured by occluding the airway at end inspiration. Thereafter, there is an initial fast drop in Ptr (ΔP1,rs) from the preocclusion value (Pmax,rs) down to an inflection point (Pi,rs). The values of Pi,rs were obtained by back-extrapolation to the time corresponding to Pmax,rs by using computer-fitted curves, as described by Jackson et al. (23). A slow pressure decay (ΔP2,rs) ensues, until a plateau is reached. This plateau corresponds to the elastic recoil pressure of the respiratory system (Pel,rs). ΔP1,rs selectively reflects the pressure required to overcome the combination of pulmonary and chest wall resistances in normal animals (4, 6, 27, 28, 39) and humans (11), and ΔP2,rs reflects the pressure spent on viscoelastic properties or stress relaxation of lung and chest wall tissues, together with a small contribution of pendelluft in normal situations (6, 11, 27). The same procedures apply to the Pw, yielding the values of ΔP1,w; Pi,w; ΔP2,w; and Pel,w; respectively. Transpulmonary pressures (ΔP1,L; Pi,L; ΔP2,L; and Pel,L) were calculated by subtracting the chest wall data from the corresponding values pertaining to the respiratory system. Total pressure drop (ΔPtot) is equal to the sum of ΔP1 and ΔP2, yielding the values of ΔPtot,rs; ΔPtot,L; and ΔPtot,w. Respiratory system, lung, and chest wall static elastances (Est,rs; Est,L; and Est,w; respectively) were calculated by dividing Pel,rs; Pel,L; and Pel,w, respectively, by V_T. Dynamic elastances of the respiratory system, lung, and chest wall (Edyn,rs; Edyn,L; and Edyn,w, respectively) were obtained by dividing Pi,rs; Pi,L; and Pi,w, respectively, by V_T. ΔE was calculated as the difference Edyn − Est, yielding the values of ΔE,rs; ΔE,L; and ΔE,w. The data concerning respiratory system, lung, and chest wall elastances were presented in terms of static elastance and ΔE instead of dynamic elastance because the former represent, respectively, the elastic and viscoelastic properties of the respiratory system. Respiratory mechanics measurements were performed six to eight times in each animal in all groups. Immediately before the sampling period, the airways were aspirated to remove possible mucus collection, and the respiratory system was inflated three times to total lung capacity (Ptr = +30 cmH_2O) to keep volume history constant. The experiments did not last more than 30 min.

The delay between the beginning and the end of the valve closure (10 ms) was allowed for by back-extrapolation of the pressure records to the actual time of occlusion, and the corrections in pressure, although very minute, were performed as previously described (5).

All mechanical data were analyzed by use of ANADAT software (RHT-InfoData).

A continuous record of transcutaneous carbon dioxide level (Ptc_{CO_2}) and arterial blood oxygen saturation (Sa_{O_2}) was performed with a SensorMedics FasTrac (Yorba Linda, CA), and ranged between 37 and 42 Torr and 95–98%, respectively.

FRC measurement. Immediately after the determination of respiratory mechanics, with the animal still alive, the trachea was clamped at end expiration, and the abdominal aorta and vena cava were sectioned, yielding a massive hemorrhage that quickly killed the animals. FRC was determined in the following way (2): the lungs were rapidly surgically removed (on average, it took 90 s to remove the lungs) and submerged into warm (37°C) 0.9% NaCl solution (saline), the volume displaced was annotated, and the lungs were weighed. FRC corresponds to the difference between the saline displaced (in ml) and the lung weight (in g), assuming that the tissue and saline have identical densities and equal to 1.0 g/ml (2).

Lung histology. After the measurements of FRC, the lungs were quick-frozen by immersion in liquid nitrogen, to perform the morphometric study (43). Fixation was made with Carnoy's solution (ethanol-chloroform-acetic acid, 70:20:10 by volume) at −70°C. After 24 h, the concentration of ethanol was progressively increased (70%, 80%, 90%, 100%, respectively, 1 h each solution, at −20°C). The lungs were then kept in 100% ethanol for 24 h at 4°C. After fixation, the tissue blocks obtained from midsagittal slices of the lungs at the level of the axial bronchus were embedded in paraffin. Blocks were cut 4 μm thick by means of a microtome. Slides were stained with hematoxylin-eosin. Each slide had a code. Microscopic examination was performed by two investigators who were unaware of the origin of the material during scoring. Morphometric analysis was performed with an integrating eyepiece with a coherent system made of a 100-point grid consisting of 50 lines of known length, coupled to a conventional light microscope. The volume fraction of collapsed and normal pulmonary areas and the fraction of the lung occupied by large-volume gas-exchanging air spaces (hyperinflation structures with a morphology distinct from that of alveoli and wider than 120 μm) were determined by the point-counting technique (43), made at a magnification of ×40 across 10 random, noncoincident microscopic fields. The internal diameter of the central and peripheral airways was computed by counting the points falling on the airway lumen and those falling on airway smooth muscle and on the epithelium. The perimeter of the airways was estimated by counting the intercepts of the lines of the integrating eyepiece with the epithelial basal membrane. This procedure was repeated four times for each airway. The areas of smooth muscle and airway epithelium were corrected in terms of airway perimeter by dividing their values by the number of intercepts of the line system with the epithelial basal membrane of the corresponding airway. Because the number of intercepts (NI) of the lines with the epithelial basal membrane is proportional to the airway perimeter, and the number of points (NP) falling on airway lumen is proportional to airway area, the magnitude of bronchoconstriction [contraction index (CI)] was computed by the relationship $CI = NI/\sqrt{NP}$ (37).

Supplementary experiments. To rule out the increase in smooth muscle tone or airway secretions caused by acetylcholine release by gallamine, another group of rats ($n = 6$, 210–230 g) anesthetized with sevoflurane but paralyzed with vecuronium bromide (SV, 0.005 mg/kg intravenously) was studied. The animals were ventilated and prepared as previously described, and respiratory mechanics were measured.

All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guiding Principles in the Care and Use of Animals" approved by the council of the American Physiological Society.

Statistical analysis. To compare the results gathered from the C, S, SV, AS, and AP groups, first, the normality of the data (Kolmogorov-Smirnov test with Lilliefors' correction), and the homogeneity of variances (Levene median test) were tested. If both conditions were satisfied, one-way ANOVA was used; in the nonparametric case, Kruskal-Wallis ANOVA was selected instead. If multiple comparisons were then required, the Student-Newman-Keuls test was applied. We considered comparisons between P and S, P and AP, S and SV, S and AS, and AS and AP groups. To correlate the functional with the morphometric parameters, Spearman correlation was used. The significance level was always set at 5%.

RESULTS

Ventilatory variables and the values of respiratory system elastance and resistance during spontaneous breathing obtained in each group are shown in Table 1.

Table 1. *Ventilatory variables and Ers and Rrs in spontaneously breathing rats anesthetized with pentobarbital sodium or sevoflurane and after the injection of atropine 20 min before anesthesia with pentobarbital sodium or sevoflurane*

	P	S	AP	AS
V_T, ml	1.27 ± 0.07	2.02 ± 0.22*	1.41 ± 0.15	2.02 ± 0.05†
T_I, s	0.31 ± 0.03	0.45 ± 0.03*	0.32 ± 0.05	0.42 ± 0.03†
T_E, s	0.52 ± 0.08	0.82 ± 0.03*	0.50 ± 0.08	0.80 ± 0.09†
Ttot, s	0.83 ± 0.11	1.26 ± 0.04*	0.83 ± 0.11	1.22 ± 0.09†
f, cpm	73 ± 9	48 ± 1*	74 ± 9	50 ± 4†
$\dot{V}_E$, ml/min	94 ± 13	96 ± 11	100 ± 8	105 ± 24
T_I/Ttot	0.38 ± 0.02	0.36 ± 0.02	0.39 ± 0.03	0.35 ± 0.03
V_T/T_I, ml/s	4.11 ± 0.51	4.52 ± 0.56	4.48 ± 1.01	4.86 ± 0.41
Ers, cmH_2O/ml	3.87 ± 0.46	4.68 ± 0.53*	3.29 ± 0.55	4.13 ± 0.55†
Rrs, $cmH_2O \cdot ml^{-1} \cdot s$	0.28 ± 0.05	0.39 ± 0.09*	0.31 ± 0.05	0.29 ± 0.06†

Values are means ± SD of 6 animals (6–8 determinations/rat) anesthetized with pentobarbital sodium (P), sevoflurane (S), and after the use of atropine 20 minutes before pentobarbital (AP) or sevoflurane anesthesia (AS). V_T, tidal volume; T_I, duration of inspiration; T_E, duration of expiration; Ttot, respiratory cycle time; f, respiratory frequency; $\dot{V}_E$, minute ventilation; V_T/T_I, mean inspiratory flow rate; T_I/Ttot, duty ratio; Ers, respiratory system elastance; Rrs, respiratory system resistance. *Significantly different from P group ($P < 0.05$); †significantly different from AP group ($P < 0.05$).

The administration of sevoflurane was associated with significantly longer inspiratory and expiratory times than those gathered during pentobarbital sodium anesthesia, whereas T_I/T_{tot} was the same. Sevoflurane anesthesia increased V_T and diminished breathing frequency, yielding a constant $\dot{V}_E$. V_T/T_I was similar in all groups. Atropine did not modify the ventilatory behavior of the anesthesia. Respiratory system resistance and elastance increased after sevoflurane anesthesia compared with the P group. In addition, respiratory system resistance was reduced in AS compared with the S group.

The mean constant inspiratory flows and volumes did not present statistically significant differences among the five groups (Table 2). FRC was similar in the P (1.93 ± 0.33 ml), S (1.72 ± 0.33 ml), AP (1.74 ± 0.35 ml), and AS (2.02 ± 0.15 ml) groups.

Table 2 shows the mean ± SD values of respiratory system, lung, and chest wall ΔP, static elastance, and ΔE obtained in the P, S, AP, AS, and SV groups. Rats anesthetized with sevoflurane (S) had a significantly larger $\Delta P2_{,rs}$ than those anesthetized with pentobarbital sodium (P) because of a higher $\Delta P2_{,L}$. In addition, $\Delta P_{tot,rs}$ and $\Delta P_{tot,L}$ were significantly higher in the S group than in the P group. Sevoflurane anesthesia yielded $Est_{,rs}$; $Est_{,L}$; $\Delta E_{,rs}$; and $\Delta E_{,L}$ values greater than those in the P group. $\Delta P1_{,rs}$; $\Delta P1_{,L}$; $\Delta P1_{,w}$; $\Delta P2_{,w}$; $\Delta P_{tot,w}$; $Est_{,w}$; and $\Delta E_{,w}$ were similar among the five groups. In AS rats, only $Est_{,rs}$ and $Est_{,L}$ increased in relation to the AP group. Furthermore, $\Delta P2_{,rs}$; $\Delta P2_{,L}$; $\Delta E_{,rs}$; and $\Delta E_{,L}$ were less in the AS compared with the S group. All mechanical parameters were similar in the P and AP groups (Table 2). In addition, animals anesthetized with sevoflurane and paralyzed with vecuronium (SV) presented respiratory mechanical data identical to those anesthetized with sevoflurane and paralyzed with gallamine (Table 2).

Table 3. *Morphometric data in P, S, AP, and AS rats*

Group	Normal	Alveolar Collapse	Alveolar Hyperinflation	Contraction Index
P	91.65 ± 1.7	5.97 ± 0.34	1.28 ± 1.01	0.10 ± 0.01
S	79.13 ± 5.87*	14.57 ± 3.12*	3.70 ± 1.52*	0.12 ± 0.04
AP	88.17 ± 3.37	4.58 ± 1.68	2.61 ± 1.10	0.10 ± 0.01
AS	85.33 ± 4.62‡	8.87 ± 1.42†‡	2.47 ± 1.12	0.10 ± 0.01

Values are means ± SD of 6 animals each in *groups P, S, AP,* and *AS.* All values are percentage of normal, collapsed, and hyperinflated areas in 10 random, noncoincident fields per rat. *Significantly different from *group P* ($P < 0.05$); †significantly different from *group AP* ($P < 0.05$); and ‡significantly different from *group S* ($P < 0.05$).

The mean ± SD percentages of normal, collapsed, and hyperinflated areas and CI in the P, S, AP, and AS groups are depicted in Table 3. It can be seen that sevoflurane anesthesia yielded higher degrees of collapse and hyperinflation than those found in the P group (Fig. 1, *A–D*). In addition, the previous use of atropine in animals anesthetized with sevoflurane reduced alveolar collapse and hyperinflation, although they remained higher than in the P group (Fig. 1, *E* and *F*). The internal diameter of the central airways was similar in the four groups (Table 3). Central and peripheral airway secretion was present only in the S group (Fig. 1*D*).

Considering the P and S groups together, $\Delta P2_{,L}$ and $Est_{,L}$ were well correlated with the fraction of alveolar collapse ($P = 0.005$, $r = 0.74$ and $P < 0.0001$, $r = 0.81$, respectively).

DISCUSSION

The main findings of this study were as follows: sevoflurane anesthesia increased the tissue component of resistance (determined by viscoelastic elements and lung inhomogeneity) and lung Est in rats without pre-

Table 2. *Respiratory data in rats anesthetized with pentobarbital sodium or sevoflurane, after the injection of atropine 20 min before anesthesia with pentobarbital sodium or sevoflurane, and in anesthetized with sevoflurane but paralyzed with vecuronium*

	P	S	AP	AS	SV
Flow, ml/s	10.06 ± 0.03	10.04 ± 0.07	10.02 ± 0.01	10.02 ± 0.03	10.05 ± 0.03
Volume, ml/s	2.02 ± 0.46	2.02 ± 0.05	2.03 ± 0.03	2.00 ± 0.04	2.00 ± 0.03
$\Delta P_{tot,rs}$, cmH_2O	3.54 ± 0.46	4.82 ± 0.39*	3.40 ± 0.55	4.53 ± 0.72	3.40 ± 0.55
$\Delta P1_{,rs}$, cmH_2O	1.64 ± 0.23	1.93 ± 0.43	1.34 ± 0.28	2.26 ± 0.69	1.81 ± 0.46
$\Delta P2_{,rs}$, cmH_2O	1.89 ± 0.41	2.89 ± 0.26*	2.06 ± 0.30	2.27 ± 0.53‡	2.60 ± 0.56
$\Delta P_{tot,L}$, cmH_2O	2.45 ± 0.49	3.83 ± 0.46*	2.49 ± 0.50	3.53 ± 0.57	3.31 ± 0.65
$\Delta P1_{,L}$, cmH_2O	1.24 ± 0.25	1.55 ± 0.43	1.07 ± 0.27	1.91 ± 0.68	1.37 ± 0.41
$\Delta P2_{,L}$, cmH_2O	1.22 ± 0.44	2.29 ± 0.25*	1.42 ± 0.24	1.62 ± 0.47‡	1.95 ± 0.48
$\Delta P_{tot,w}$, cmH_2O	1.05 ± 0.18	0.99 ± 0.09	0.91 ± 0.24	1.00 ± 0.32	1.09 ± 0.20
$\Delta P1_{,w}$, cmH_2O	0.38 ± 0.14	0.38 ± 0.06	0.27 ± 0.08	0.35 ± 0.10	0.43 ± 0.08
$\Delta P2_{,w}$, cmH_2O	0.66 ± 0.18	0.61 ± 0.08	0.64 ± 0.21	0.65 ± 0.24	0.65 ± 0.15
$Est_{,rs}$, cmH_2O/ml	3.58 ± 0.52	4.76 ± 0.58*	3.66 ± 0.28	4.54 ± 0.56†	4.30 ± 0.50
$Est_{,L}$, cmH_2O/ml	3.00 ± 0.58	4.25 ± 0.64*	3.18 ± 0.37	3.93 ± 0.68†	3.73 ± 0.52
$Est_{,w}$, cmH_2O/ml	0.58 ± 0.10	0.51 ± 0.08	0.48 ± 0.19	0.60 ± 0.18	0.57 ± 0.10
$\Delta E_{,rs}$, cmH_2O/ml	0.94 ± 0.20	1.43 ± 0.13*	1.01 ± 0.14	1.14 ± 0.25‡	1.30 ± 0.27
$\Delta E_{,L}$, cmH_2O/ml	0.59 ± 0.23	1.13 ± 0.12*	0.70 ± 0.11	0.81 ± 0.22‡	0.98 ± 0.24
$\Delta E_{,w}$, cmH_2O/ml	0.35 ± 0.09	0.30 ± 0.04	0.32 ± 0.10	0.33 ± 0.12	0.32 ± 0.07

Values are means ± SD of 6 animals (6–8 determinations/rat) from *groups P, S, AP,* and *AS* and anesthetized with sevoflurane but paralyzed with vecuronium (SV). rs, Respiratory system; L, lung, w, chest wall; ΔPtot, ΔP1, and ΔP2, total, resistive, and viscoelastic/inhomogeneous pressures, respectively; Est, static elastance; ΔE, difference between dynamic and static elastances. *Significantly different from *group P* ($P < 0.05$); †significantly different from *group AP* ($P < 0.05$); and ‡significantly different from *group S* ($P < 0.05$).

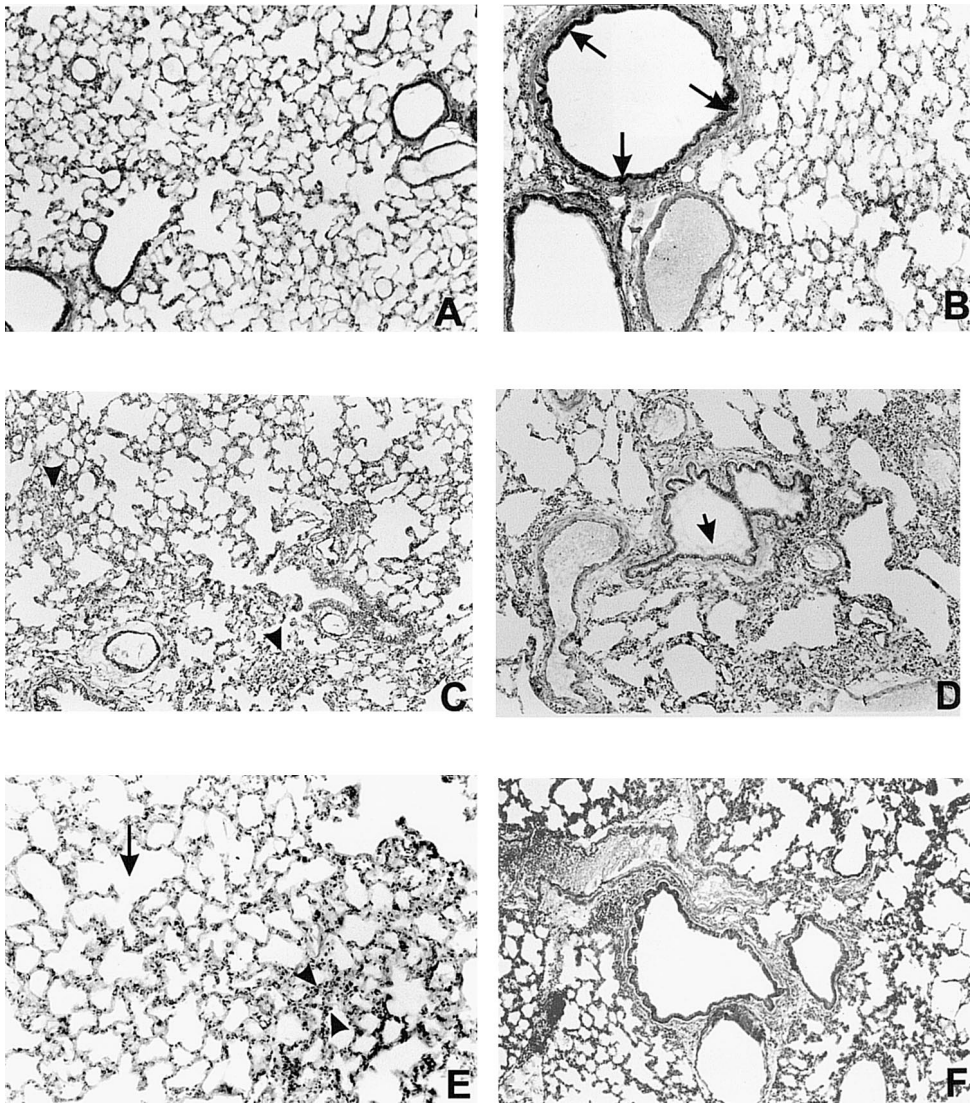

Fig. 1. Representative panel illustrating the histopathological patterns of distal pulmonary parenchyma (*A, C,* and *E*) and airways (*B, D,* and *F*) from rats anesthetized with pentobarbital sodium (*A* and *B*), sevoflurane (*C* and *D*), and anesthetized with sevoflurane but pretreated with atropine (*E* and *F*). In *B*, arrows indicate the absence of airway secretion in the lumen, whereas in *D* arrow indicates the presence of bronchial secretion. Arrowheads show regions of atelectasis (*C* and *E*). In *E*, note that lung parenchyma remains altered, with areas of alveolar collapse (arrowheads) mixed with hyperinflation (arrow). There is no secretion into the airway lumen (*F*). Hematoxylin-eosin stain; magnification ×200.

existing airway constriction. These findings were supported by the histological demonstration of increased areas of alveolar collapse and hyperinflation and the presence of secretion in the central and peripheral airways. Pretreatment with atropine reduced airway secretion, thus lessening but not eliminating lung inhomogeneities.

Sevoflurane has been reported to attenuate bronchoconstriction associated with anaphylaxis in dogs (31) and in the presence of constrictor agonists (20, 25). Mitsuhata et al. (31) demonstrated that sevoflurane can be an useful alternative to halothane, enflurane, or isoflurane in the treatment of bronchospasm in asthma. However, Katoh and Ikeda (25) described that sevoflurane was less effective than halothane but equivalent to isoflurane in preventing increases in lung resistance and decreases in dynamic compliance yielded by histamine. In addition, there was no difference in the effects of sevoflurane and isoflurane on lung resistance and dynamic compliance. The studies performed by Mitsuhata et al. and Katoh and Ikeda did not partition pulmonary resistance into its airway and parenchymal components. On the other hand, Habre and colleagues (20) applied alveolar capsules to piglets' pleural surfaces under sevoflurane anesthesia and observed that sevoflurane prevented the methacholine-induced rise in lung resistance by avoiding an increase in tissue resistance. However, the effects of sevoflurane are controversial considering baseline smooth muscle tone. Some authors report that neither sevoflurane nor halothane affected unstimulated resistances or compliances of the lungs (20, 25, 26, 31). However, there are other published articles that show that halothane (21, 42) and sevoflurane (16, 19) decrease resting baseline tone in animals.

Gallamine is a neuromuscular blocking agent that binds to M_2 muscarinic receptor. M_2 receptors in the airways are located presynaptically on postganglionic parasympathetic nerves regulating acetylcholine release. Thus antagonism of M_2 function can actually lead to an increase in actions mediated by the M_3 receptor, such as bronchoconstriction and increased mucus production (15, 18, 22). To rule out the possible consequences of gallamine itself increasing smooth

muscle tone or airway secretions, another group of rats (SV) was anesthetized with sevoflurane but paralyzed with vecuronium. Vecuronium was used instead of other muscle relaxants because it does not appear to have either M_2- or M_3-blocking properties (40). Sevoflurane plus vecuronium presented respiratory mechanical parameters similar to those resulting from sevoflurane and gallamine. In addition, we observed in the SV group the same increase in central and peripheral airway secretion as that resulting from the use of sevoflurane plus gallamine. Thus, although gallamine could have determined an increase in airway secretion, its effect was actually similar to that of vecuronium in normal rats. To eliminate the possible consequences of gallamine or vecuronium increasing smooth muscle tone or airway secretion, respiratory system resistance and elastance were computed in spontaneously breathing rats, and independently of the method used to compute respiratory mechanics we observed the same behavior (Tables 1 and 2). Thus we are analyzing only the effects of the anesthetic agent instead of the muscle relaxant.

Barbiturates can inhibit vagal reflexes (9) and directly contract or relax airway smooth muscle, depending on the dose (29) and on the species studied. Fletcher et al. (14) found that pentobarbital sodium has no effect on airway baseline tone. In addition, Reta et al. (35) demonstrated that pentobarbital sodium causes no modification in either respiratory mechanics or airway morphometry, i.e., it represents an ideal control drug.

Ptc_{CO_2} and Sa_{O_2} ranged between 37 and 42 Torr, and 95–98%, respectively. Consequently, the mechanical changes could not be attributed to either hyper- or hypocapnia nor to hypoxia.

The concentration of sevoflurane used in the present study ranged between 2.7 and 2.8%. These data are in accordance with those of Kashimoto and colleagues (24), who determined the minimal alveolar concentration value for sevoflurane to be 2.68 ± 0.19% in young rats. Anesthesia was maintained throughout the experiment in stage III in the five groups.

Sevoflurane and pentobarbital sodium exert a similar degree of ventilatory depression, as assessed by $\dot{V}_E$ and Ptc_{CO_2}. On the other hand, some authors report that sevoflurane depresses ventilatory function (12, 13, 17, 26). This difference could be attributed to the time at which this parameter was measured (15 min after the induction of anesthesia). Mechanical variables of the respiratory system, respiratory timing, and depth of breathing were different between the anesthetics (Table 1).

As shown in Table 2, sevoflurane anesthesia did not alter pulmonary resistive pressure dissipation ($\Delta P1,L$). As previously reported, $\Delta P1,L$ is directly related to airway resistance (38). There is no difference in the magnitude of bronchoconstriction (contraction index) between the P and S groups (Table 3), supporting the absence of changes in airway resistance. This finding is consistent with previous measurements of respiratory mechanics in unstimulated airways, in which airway resistance was identical in animals anesthetized with pentobarbital sodium or sevoflurane (20, 25, 31). The amount of central airway secretion was not high enough to increase $\Delta P1,L$.

Volatile anesthetics are traditionally considered to be potent bronchodilators and are even used to treat status asthmaticus. However, in the present study there is no functional or histological evidence of bronchodilation in rats anesthetized with sevoflurane and with no preexisting airway tone. Thus the effect of sevoflurane on airways probably could be determined by different airway smooth muscle tone. Some authors (19, 36) reported that, after tracheal intubation in persons without asthma, sevoflurane decreased respiratory system resistance. Our data cannot be compared with theirs, not only because of species differences but because they computed respiratory system resistance after tracheal intubation, which is a common way of generating bronchoconstriction during anesthesia. In the current study, respiratory mechanics were computed ~15–20 min after intubation and induction of anesthesia, and the measurements did not last longer than 30 min.

In the present study, $\Delta P2,L$ increased significantly (Table 2) during sevoflurane anesthesia. $\Delta P2,L$ can reflect pressure losses due to viscoelastic properties and/or mechanical inhomogeneities of the lung. Lung histology showed an increase in the percent values of alveolar hyperinflation and collapse in the S group (Table 3). The presence of secretion in the peripheral airways could affect the distribution of ventilation, thus increasing mechanical inhomogeneities. However, a certain amount of change in the contractile tone in distal parenchymal elements cannot be discarded. In fact, Park et al. (34) demonstrated in 5-hydroxytryptamine-preconstricted rat distal bronchial segments that sevoflurane has a direct bronchodilatory effect. Sevoflurane could also act on the mechanical properties of the lung tissues. The precise element that accounts for the viscous dissipation of energy at the tissue level is not known, but there are some possibilities. For example, if the contractile elements in the mouth of the alveolar duct dilate or constrict, then the geometry of the alveolar sac will be altered and the rheological properties of the air-liquid interface (surfactant) could be affected. Alternatively, collapse could pull open alveolar ducts. During ventilation, air would be shifted in and out of ducts and might affect pressure change measured at the alveolar level. Another possibility is that collapse or atelectasis in one subsegmental region of the lung might distort the parenchyma in an adjacent subsegment thereby affecting local tissue mechanics (1). Habre et al. (20) showed that tissue resistance was similar in animals anesthetized with pentobarbital sodium or sevoflurane. The discrepancy between our data and those of Habre et al. could be attributed to the different species and techniques (alveolar capsule) used, dose of pentobarbital sodium (10 mg/kg), and/or the simultaneous administration of fentanyl.

As shown in Table 2, the overall respiratory system and lung pressures (ΔPtot,rs and ΔPtot,L) used to overcome resistive and viscoelastic (central and peripheral mechanical components) elements increased (36% and 53%, respectively) with the use of sevoflurane. These findings are not consistent with previous measurements of pulmonary resistance in unstimulated airways (20). Because Pw values were not altered by sevoflurane (Table 2), the respiratory system mechanical profile reflects solely its pulmonary component.

Sevoflurane yielded higher Est,L, which led to increased Est,rs (Table 2), thus indicating that the pulmonary and respiratory system elastic components of the respiratory impedance were augmented under the present experimental conditions. The increase in Est,L could be attributed to atelectasis (Table 3, Fig. 1). In our study FRC did not change. However, the percentage of collapsed and hyperinflated areas increased by 144 and 189%, respectively (Table 3). In addition, the percentage of normal areas decreased by 15%. The overall effect of these changes might result in no change in FRC.

ΔE,rs and ΔE,L increased significantly during sevoflurane anesthesia, whereas ΔE,w remained unaltered (Table 2), suggesting that lung (and thus respiratory system) viscoelasticity/inhomogeneity became more prominent. This finding is confirmed by the increase in ΔP2,L (Table 2), as discussed above. The present study demonstrated that both pulmonary static (Est,L) and viscoelastic (ΔE,L) components contribute to increase Edyn,L. A fall in Edyn,L has also been reported, but the experiments were done in previously constricted lungs (20, 25, 31).

To elucidate the influence of airway secretion on respiratory mechanical changes due to sevoflurane anesthesia, atropine was injected before anesthesia. Atropine was also injected before pentobarbital sodium anesthesia to analyze the effect of atropine on bronchomotor tonus. Indeed, respiratory mechanics and lung histology were similar in the P and AP groups. In AS rats, only Est,rs (24%) and Est,L (23.5%) increased in relation to AP rats (Table 2). Thus atropine attenuated the increment of viscoelastic/inhomogeneous pressure induced by sevoflurane anesthesia. These changes could be possibly attributed to the decrease in the amount of bronchial secretion, yielding reduced mechanical inhomogeneities. Consequently, alveolar collapse was less important but remained higher than in the AP group (Table 3).

In conclusion, the present experiments disclosed that sevoflurane anesthesia in rats without preexisting airway constriction increased pulmonary viscoelastic/inhomogeneous and elastic pressures, reflecting stiffening of lung tissues and increased mechanical inhomogeneity. These findings were supported by the histological demonstration of increased areas of alveolar collapse and hyperinflation and by the greater amount of airway secretion. Indeed, we cannot discard the possibility that sevoflurane acts on the contractile tone in distal parenchymal elements that could also affect elastances and viscoelastic/inhomogeneous parameters. The pretreatment with atropine reduced the amount of central and peripheral airway secretion, thus lessening but not eliminating lung inhomogeneities.

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