Modulation of airway and lung tissue mechanics by different intrapulmonary resident gases

PhD Thesis

Enikő Erzsébet Lele MD

Department of Medical Physics and Informatics
and
Department of Anaesthesiology and Intensive Therapy
University of Szeged, Hungary

Szeged
2012

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List of papers included in this thesis

I. Lele E, Petak F, Fontao F, Morel DR, Habre W. Protective effects of volatile agents against acetylcholine-induced bronchoconstriction in isolated perfused rat lungs. *Acta Anaesthesiologica Scandinavica.* 2006; 50: 1145-1151.

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I. Habre W, Peták F, Ruchonnet-Metrailler I, Donati Y, Tolsa JF, Lele E, Albu G, Beghetti M, Barazzone-Argiroffo C. The role of endothelin-1 in hyperoxia-induced lung injury in mice. *Respiratory Research.* 2006; 7: 45.

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Glossary of terms

α	exponent of angular frequency in constant-phase tissue model
γ	complex propagation wave number of wave-tube
η	tissue hysteresivity
φ	phase angle
ω	angular frequency
ACh	acetylcholine
CO_2	carbon dioxide
CPB	cardiopulmonary bypass
f	frequency
FOT	forced oscillation technique
Fi_{CO_2}	fraction of inspired carbon dioxide
G	constant-phase tissue damping
H	constant-phase tissue elastance
I_{aw}	airway inertance
ID	internal diameter
ip	intraperitoneal(ly)
iv	intravenous(ly)
j	imaginary unit
K_{ATP}	ischaemic K^+ channel
L	length of wave-tube
MCh	methacholine
MAC	minimum alveolar concentration
P_1	pressure at loudspeaker end of wave-tube
P_2	pressure at distal end of wave-tube
P_{aw}	airway pressure
Pa_{O_2}	arterial partial pressure of oxygen
Pa_{CO_2}	arterial partial pressure of carbon dioxide
PEEP	positive end-expiratory pressure
$P_{ET_{CO_2}}$	end-tidal partial pressure of carbon dioxide
P_{la}	left atrial pressure
P_{pa}	pulmonary arterial pressure
P_{tr}	transrespiratory pressure
Q_p	pulmonary blood flow
R_{aw}	airway resistance
R_{int}	pulmonary interrupter resistance
R_L	pulmonary resistance
R_{rs}	respiratory resistance
R_v	pulmonary vascular resistance
SE	standard error
V'	airflow
X_L	pulmonary reactance
X_{rs}	respiratory reactance
Z_0	characteristic impedance of wave-tube
Z_L	pulmonary input impedance
Z_{rs}	respiratory input impedance

1. Introduction

Patients under general anaesthesia are subjected to continuous physiological monitoring to allow the control of safe induction, the maintenance of general anaesthesia and the prevention and management of emergency situations. To ensure safety, anaesthesia practitioners must possess a solid theoretical knowledge of the physiological and pathophysiological mechanisms of the neurohumoral regulation of the respiratory system and the bronchial motor tone. They must acquire a comprehensive and intricate knowledge of the use of various medical gases, anaesthetic agents and vapours, and medical breathing circuits. The measurement and monitoring of anaesthetic gases, such as oxygen, nitrous oxide, carbon dioxide (CO_2) and inhalational anaesthetics are mandatory for day-to-day anaesthesia practice.

1.1. Physiological control of the bronchial tone

Along the tracheobronchial tree, the zone including the bronchioles has the strongest muscular bands and consequently these regions are the main target of physiological regulatory mechanisms or regional lung ventilation[1]. Contraction of these helical bands can decrease the diameter of the bronchioles, wrinkle the mucosa into varying numbers of longitudinal folds and result in an elevated airway resistance.

1.1.1. Neural pathways

The physiological bronchial tone is regulated in part by neurohumoral mechanisms. The innervations of the airways are supplied by the autonomic nervous system. The parasympathetic cholinergic vagus nerve constitutes the predominant neural bronchoconstrictor system that serves an important role in regulation of the airway tone. Afferent and efferent fibres travel in the vagus nerve with efferent ganglia in the walls of the small bronchi. There are two classes of afferent nerve fibres: myelinated, rapidly adapting strech receptors and non-myelinated C-fibres. Efferent postganglionic cholinergic fibres, from the vagal motor nuclei of the brainstem via the vagus nerve to the parasympathetic ganglia (located within the bronchial wall)[2,3] and over the peribronchial plexus (all the way down to the level of small bronchi), innervate the airway smooth muscle and the submucosal glands. The density of the innervations diminishes from the trachea to the terminal bronchioles, there

is no further innervation of the airway epithelium and the alveoli in humans[4,5]. The acetylcholine (ACh) released from the preganglionic and postganglionic nerve fibres acts on the target cells through three types of muscarinic receptor: M_1, M_2, and M_3, which modulate the bronchial airway and vascular smooth muscle tone[6]. Direct physical stimulation, such as a suction manoeuvre, laryngoscopy, inhalation of dust or cold air, or direct chemical stimuli, such as gastric acids at low pH, can activate parasympathetic reflexes, causing bronchoconstriction.

Ultrastructural studies on humans have revealed adrenergic postganglionic sympathetic nerve fibres in the pulmonary vascular smooth muscle, in close proximity to submucosal glands, and airway ganglia, but they are not present in airway smooth muscle[7]. However, despite the lack of sympathetic innervation, the diffusion of the sympathetic postganglionic neurotransmitter norepinephrine to the parasympathetic ganglia or directly to the airway smooth muscle can inhibit airway constriction.

The airways are also regulated by a third form of autonomic control that is neither adrenergic nor cholinergic (NANC system). This NANC nervous system can exert either an excitatory function with the neurotransmitter substance P and neurokinin A, causing bronchoconstriction (e-NANC system), or an inhibitory function with the neurotransmitter neuropeptide Y, resulting in bronchodilatation (i-NANC system)[8].

1.1.2. Humoral control

Despite the lack of real anatomic sympathetic innervation, the bronchial smooth muscle possesses a high number of β_2-receptors that are sensitive to circulating adrenaline. Activation of the β_2-receptors increases the cAMP level, thereby lowering the Ca^{2+} influx into the airway smooth muscle cells, which results in bronchodilation. Under physiological circumstances, the basal levels of adrenaline do not modify the bronchial muscle tone, but this mechanism is of significance in the presence of an elevated muscle tone, such as that which develops during exercise or stress.

α-adrenoreceptors are situated in pulmonary and bronchial blood vessels, the bronchial epithelial cell membrane, and also on submucosal glands, in parasympathetic ganglia, and on cholinergic and C afferent nerve fibres[3]. *In vitro* studies have demonstrated that α_2-adrenergic stimulation increases mucus secretion and induces smooth muscle contraction[9]. However, this

effect can be detected only in preconstricted healthy airway smooth muscle, in humans and dogs, or in tissue samples originating from diseased human lungs[9]. *In vivo*, α_2-adrenergic stimulation has no effect on the bronchomotor tone in healthy individuals, but might induce bronchoconstriction in asthmatic patients[10,11].

1.1.3. Direct smooth muscle effects

Some substances are able to affect the tone of the airway smooth muscle directly. Decreased levels of the arterial partial pressure of carbon dioxide (Pa_{CO_2}), histamine, ACh, thromboxane A_2 and leukotrienes C_4 tend to cause direct airway smooth muscle contraction.

Besides these regulatory mechanisms, varying concentrations of physiological or anaesthesiological resident gases in the pulmonary system may also affect the different neurohumoral mechanisms that regulate and modulate the bronchial tone; these pathways, however, have not been fully characterized.

1.2. Role of carbon dioxide in regulation of the bronchial tone and in local ventilation distribution

CO_2 plays an important role in the regulation of the small airway smooth muscle tone. The most important feature of this regulation is the direct effect of the low alveolar CO_2 concentration on the smooth muscle cells of the lower airways. CO_2 can readily penetrate the cell membrane, and the local hypocapnia around the smooth muscle cells is associated with a prompt intracellular lowering of the H^+ concentration. Intracellular alkalosis leads to an elevated muscle tone and a consequently increased airway resistance[12,13].

Earlier investigations furnished evidence of a decreased ventilation to large lung regions made hypocapnic by unilateral main or lobar pulmonary artery occlusion, which could be prevented by adding CO_2 to the inspired air[14-16]. Experiments performed with a multiple breath washout technique with helium and nitrogen in animal models suggest that a decrease in the level of alveolar CO_2 increases the heterogeneity of the ventilation distribution between regions on the scale of acini and larger airway regions[17], and even the global matching of alveolar ventilation to perfusion can decrease[18,19].

This direct, rapid and local regulation of the airway tone drives the air towards lung regions or alveoli with lower input impedance because of retained perfusion. Thereby, the

local CO_2 concentration contributes to maintenance of the normal ventilation perfusion ratio of the lung parenchyma because the local ventilation depends strongly on the local CO_2 concentration, as a natural consequence of local perfusion. Modification of the airway calibre under physiological and pathophysiological circumstances plays an important role in preventing wasted ventilation and normalizing the ventilation perfusion relationship subsequent to temporary changes in upright or supine body position or mucus deposition[12,13,20].

The direct relaxation potential of CO_2 on the bronchial smooth muscle has been demonstrated against the bronchoconstriction induced by constrictor drugs[19]. Further, bronchoconstriction resulting from temporary regional pulmonary arterial occlusions is reversed by normalizing the partial tension of alveolar CO_2[14,21-23].

There is another, indirect mechanism via which the CO_2 concentration can modify the airway muscle tone. The systemic CO_2 level lowers the vagal withdrawal[24], and the consequent enhanced vagal tone may induce airway resistance through an indirect vagal nerve-mediated regulatory pathway[25,26]. In addition to these indirect effects, bilateral vagotomy or cooling of the vagus nerve precludes the development of bronchoconstriction subsequent to systemic hypercapnia, demonstrating that CO_2 also alters the airway calibre via indirect mechanisms mediated through the vagal reflexes[25,26].

The two constrictor mechanisms mentioned above can theoretically augment each other in certain diseases, e.g. pulmonary embolism.

Clarification of the subsequent changes in the intra-alveolar CO_2 concentration and the systemic blood CO_2 level may facilitate an understanding of the physiological and pathophysiological alterations in various clinical conditions. The effects of intra-alveolar CO_2 on airway calibre have frequently been investigated in isolated lung models by altering the fraction of inspired CO_2 (Fi_{CO_2})[27-29]. However, in the absence of physiological neural-humoral control of the airways, these earlier results did not allow a complete description of the mechanisms involved in a lung with intact nerves. In more comprehensive *in vivo* investigations, the alveolar CO_2 concentrations have been altered by changing the breathing pattern[29-31]. However, respiratory resistance and elastance change with varying lung volume and/or ventilation frequency[32]. Thus, an altered ventilatory pattern itself may affect the lung mechanics so that the active effects of CO_2 in the lungs are biased. Alternatively, regional

hypocapnia studied via unilateral occlusion of a pulmonary artery has been shown to elevate the airway tone[14,21-23,28]. Nevertheless, a compromised pulmonary perfusion also increases the systemic CO_2 level, which may lead to vagally-mediated bronchoconstriction[31]. This phenomenon may have a synergistic constrictor effect on the affected airways, which becomes indistinguishable from the direct effects of altered intra-alveolar CO_2 in the lungs. Moreover, the pulmonary arterial occlusion approach does not allow the investigation of hypercapnia and it cannot eliminate the vagal reflexes. Accordingly, none of these *in vivo* approaches permit a detailed characterization of how the different CO_2 concentrations affect the lung mechanics, i.e. the establishment of a dose-response curve. Despite the fact that the presence of a cardiopulmonary bypass (CPB) offers ideal conditions for altering both the alveolar and circulatory CO_2 levels precisely *in vivo*, without involvement of the biasing effects of an altered ventilation pattern or pulmonary ischaemia, the advantages of this approach have not been utilized for a systematic exploration of the effects of an altered CO_2 level on the airway tone.

1.3. Effects of volatile anaesthetic agents on lung mechanics

Bronchoconstriction under anaesthesia can occur because of direct stimulation of the laryngeal and tracheal areas, subsequent to the administration of adjuvant drugs with a potential to induce histamine release, and from noxious stimuli activating the vagal afferent nerves. The reflex response to these stimuli may be enhanced in patients with lung diseases involving airway hyperresponsiveness, such as asthma, upper respiratory tract infection and chronic obstructive pulmonary diseases.

Early investigations reported the beneficial bronchodilator properties of halothane in preventing and treating bronchospastic episodes in humans[33,34], animals[35] or isolated tracheal smooth muscle[36]. Besides the well-known halothane, enflurane and isoflurane, the introduction of sevoflurane and desflurane during recent decades has offered new perspectives to anaesthesiologists. Routine procedures performed during the induction and maintenance of general anaesthesia achieved with volatile agents may also alter both the airway and the pulmonary parenchymal properties[37-40].

Apart from the previous studies in which the bronchoactive properties of volatile agents were examined under physiological conditions, numerous earlier investigations

assessed their abilities to alter the elevated airway tone in the presence of a lung disease[41] or through the administration of constrictor agonists. A recent study of children with susceptible airways demonstrated beneficial properties of sevoflurane, whereas desflurane exhibited irritative properties even at 1 minimum alveolar concentration (MAC)[41]. In a previous experimental study, the protective effects of all commonly applied volatile anaesthetic agents were observed against the bronchoconstriction induced by an intravenous (iv) infusion of methacholine (MCh) under *in vivo* conditions[37], in which the autonomic nervous system was intact. The results of that study revealed that isoflurane, sevoflurane and desflurane were as effective as halothane in protecting against MCh-induced airway constriction in healthy lungs. In allergically sensitized animals, however, isoflurane improved the lung function more efficiently than did sevoflurane or halothane during sustained MCh-induced bronchoconstriction, whereas desflurane enhanced the airway smooth muscle tone even further[42].

The complex mechanisms of the effects of volatile anaesthetic agents on the lung mechanics involve multiple modes of action, which may contribute to these variable results. Isoflurane and sevoflurane have been shown to exert their effects through opening ischaemic K^+ (K_{ATP}) channels. The activation of K_{ATP} channels can hyperpolarize the smooth muscle cell membrane, leading to inhibition of the Ca^{2+} influx into the cell, and to subsequent relaxation of the airway smooth muscle[43-45]. Moreover, volatile anaesthetics appeared to inhibit the store-operated Ca^{2+} influx into airway smooth muscle cells in an animal model[46]. Conversely, desflurane stimulates the parasympathetic nervous system, resulting in the constriction of bronchial smooth muscle, mainly via a reflex mechanism[43]. Finally, the mechanical properties of the lungs may also be affected by the altered pulmonary haemodynamics observed following the administration of inhalation agents[47]. Although all of these previous reports had the aim of characterizing the bronchoactive properties of the volatile agents, the contributions of the direct and indirect pathways to these results have not yet been fully clarified.

While the beneficial properties of volatile anaesthetics are well established, they may also irritate the airways and initiate breath-holding, coughing and/or laryngospasm. Volatile anaesthetics differ in the concentration at which they irritate the airways, particularly during the induction of anaesthesia. Halothane and sevoflurane have minimal or no pungency at all

concentrations applied clinically, whereas isoflurane can irritate the airways at a concentration of 1.5 MAC (approximately 1.8%) or above[48]. In allergically sensitized animals, however, isoflurane improves the lung function better than do sevoflurane and halothane during sustained MCh-induced bronchoconstriction, while desflurane further enhances the airway smooth muscle tone[42]. Similar results have been reported in humans: at 1 MAC, isoflurane, sevoflurane and desflurane led to decreases in peak inspiratory pressure and respiratory resistance and to increases in dynamic compliance. At 2 MAC, these effects were preserved with isoflurane and sevoflurane, whereas desflurane developed bronchoconstrictive potential[49].

1.4. Characterization of respiratory mechanics

In clinical practice, pulmonary function tests are mainly of importance as concerns diagnoses, the monitoring of disease progression, and assessment of the effectiveness of therapies for asthma, chronic obstructive pulmonary disease[50] and interstitial lung disease. The identification of the respiratory mechanical parameters specific to the airway and lung tissue compartments played a key role in the exploration of the regulatory mechanisms exerted by the neurohumoral system and by the different resident gases in the lungs.

The most comprehensive description of the mechanical properties of the respiratory system can be supplied by the forced oscillation technique (FOT), first developed in the 1950s by DuBois *et al.*[51], which involves the generation of sinusoidal oscillations around the body surface and measurement of the impedance of the respiratory system through the application of small pressure oscillations at the mouth during normal breathing. In this pioneering work, the experiment was repeated at a number of different oscillation frequencies and the fundamental observation was established that respiratory impedance varies with frequency in a characteristic manner. Since those measurements, numerous variants in instrumentation, signal processing, and modelling relevant to FOT measurements have been developed for the assessment of various mechanical phenomena in healthy lungs and in respiratory diseases (asthma, chronic obstructive pulmonary disease and acute lung injury). Early investigations of the mechanical behaviour of the respiratory system with the FOT were also made by applying sequentially varying frequencies in mechanically ventilated humans[52], and around the range of normal breathing frequencies[53,54].

The principal concept of forced oscillatory respiratory mechanics is based on the measurement of "impedance" (Z), the spectral (frequency domain) relationship between the pressure (P) and airflow (V') which corresponds to the total pressure drop required to oscillate, i.e. to develop a flow in the respiratory system ($Z_{rs} = P/V'$). Generally, depending on the mechanical characteristics of the measured system, the P and V' signals are not in phase; with a phase shift between the two signals is φ. Z_{rs}, the respiratory input impedance, can be characterized by describing its "real" (respiratory resistance, R_{rs}) and "imaginary" (respiratory reactance, X_{rs}) components:

$$R_{rs} = Z_{rs} \cos \varphi$$

$$X_{rs} = Z_{rs} \sin \varphi$$

R_{rs} describes the dissipative mechanical properties of the respiratory system (lung and thoracic tissues). X_{rs} is related to the energy storage capacity at low oscillation frequencies and is therefore determined by the elastic properties of the respiratory tissues (the relationship between P and volume), whereas the inertive properties progressively become more important with increasing oscillation frequency (the relationship between P and volume acceleration)[55].

The impedance data obtained at the airway opening reflect mainly the overall central conducting airway geometry. However, the mechanical properties of the lung periphery are the major determinants of the overall lung function, and can change dramatically in disease. The investigations of the peripheral airways (called the "silent zone" of the lung), which are relatively inaccessible at the level of the whole organ, were based on application of the alveolar capsule technique, which allowed direct measurements of alveolar pressure in open-chest preparations[56]. To determinate the mechanical properties of the individual components of the pulmonary system and to partition the pulmonary impedance into airway and tissue components, the alveolar capsule technique was combined with the FOT[57] in experimental animals, including dogs[57,58], rabbits[59], rats[60] and mice[61]. Simultaneous measurements from multiple capsules have shown that the alveolar pressure becomes increasingly heterogeneous as bronchoconstriction develops[58], which may provide important physiological information. This heterogeneity apparently demonstrates the inherent limitation of this technique due to the inevitable undersampling of alveolar pressures in consequence of the finite size of the capsule and the fact that it can only access subpleural alveoli[61].

Since the airway and tissue compartments exhibit distinctly different frequency-domain behaviour, a model-based evaluation of the pulmonary and respiratory impedance data offers a valuable alternative to the capsule-based separation of the airway and tissue mechanics. To achieve this comprehensive description, the lung viscoelasticity was extensively evaluated in the early 1970s by Hildebrandt and co-workers. In their studies, the linear viscoelastic phenomena of the pulmonary system were resolved. Furthermore, the derived linear model in the time domain exhibited characteristics such as stress relaxation and dynamic hysteresis ascribed to viscoelasticity[62,63]. If these properties are examined in the frequency domain, the airways and respiratory system tissues reveal characteristically different dependences on frequency that allow separation of their contributions to impedance on the basis of mathematical models[58,64]. An important aspect of the coupling of resistive and elastic properties in the respiratory tissues was highlighted by Fredberg and Stamenovic, who demonstrated that the relationship between the frictional and elastic stresses in the lung tissue is nearly invariant; the frictional stress is invariable between 10% and 20% of the elastic stress. The ratio of the viscous and elastic stresses is referred to as the structural damping coefficient, or "hysteresivity"[65].

In routine clinical applications of FOT, it is recommended to use oscillation frequencies higher than 2-4 Hz if the oscillatory signal is superimposed on the spontaneous breathing[55]. In this medium-frequency range, the imposed oscillations start roughly 1 decade above the spontaneous breathing rate and extend up to a few times 10 Hz. The measured impedance spectra in the healthy respiratory system exhibit a largely frequency-independent R_{rs}, whose major component is the airway resistance (R_{aw}).

1.4.1. Low-frequency forced oscillation technique

Over the low-frequency range (below 2 Hz), the respiratory tissues predominate and the steep increase in reactance with frequency is mirrored by a marked decrease in resistance. In the medium-frequency range (2-40 Hz), the resistance expresses no more than mild changes, while at the first resonance (reactance = 0) the reactance undergoes a transition from dominance by the tissue elastic properties to dominance by the inertial properties of the gas in the airways[66].

The characteristic rheology of the structures of the respiratory tissues below 2 Hz can be established by investigation during voluntary apnoea, as has been demonstrated between 0.25 and 5 Hz in healthy humans by using the oesophageal balloon technique[54] or between 0.25 and 32 Hz in anaesthetized and paralysed patients[67]. Through utilization of these characteristic frequency dependences, a separate assessment of the airway and pulmonary parenchymal mechanics is possible by the application of forced oscillations at low frequencies[58]. A loudspeaker-in-box measurement system generating the pseudorandom broadband, low-frequency oscillatory pressure signals was specially designed to provide the excitatory pressure signal necessary for measurement of the input impedance spectra of the pulmonary system (Z_L) during short intervals of suspended mechanical ventilation at end-expiration.

Several investigations have indicated that the airways can be described by a frequency-independent R_{aw} and inertance (I_{aw})[58]. Conversely, both the parenchymal resistance and reactance have been reported to decrease roughly in inverse proportion to increasing frequency. Thus, to separate the airway and lung parenchymal mechanical properties, a model containing a frequency-independent R_{aw} and I_{aw} in series with a constant-phase tissue model[58,68], including parenchymal damping (G) and elastance (H), can be fitted to the Z_L spectra by minimizing the relative differences between the measured and modelled impedance values:

$$Z_L = R_{aw} + j\omega I_{aw} + (G - jH)/\omega^{\alpha}$$

where *j* is the imaginary unit, ω is the angular frequency ($2\pi f$), and $\alpha = 2/\pi \arctan (H/G)$.

The parameters R_{aw} and I_{aw} can be attributed to the airways, while G and H represent the viscous (damping or resistive component) and elastic properties, respectively, of the lung parenchyma. The lung tissue hysteresivity (η) is calculated as $\eta = G/H$ [65]. The optimization procedure is used with a relative (weighted) fitting criterion in most cases to give equal weight to the low and high-frequency components: the differences between the measured and modelled impedance values are normalized by the impedance magnitude at each frequency point[69].

2. Aims of the studies included in the present thesis

The primary purpose of the present thesis is to characterize the effects of an altered resident gas on the pulmonary mechanics, with particular focus on achieving a better understanding of the potencies of CO_2 and volatile anaesthetic agents on the pulmonary system by separating the airway and tissue mechanical responses occurring in routine clinical anaesthetic practice. Various studies included in the present thesis were therefore designed:

a) To establish a dose-response curve relating to a wide range of alveolar CO_2 levels without affecting the ventilation pattern. To achieve this aim, measurements were made in open-chest animal models subjected to a CPB.
b) To characterize the airway and pulmonary tissue consequences of systemic hypercapnia and acidosis.
c) To clarify the role of vagal activity in the regulation of the airway tone related to altered systemic CO_2 concentrations.
d) To characterize and, more specifically, to compare the bronchoactive properties of the volatile agents used routinely in clinical practice in the absence of an airway tone.
e) To clarify the relaxation properties of the volatile agents against the airway constriction induced by administration of a bronchoactive drug in the absence of neural control of the tracheobronchial tree.

3. Materials and methods

3.1. Animal models and materials

All of the animal models were chosen with regard to their suitability for the experiments in question and for measurement of the pulmonary mechanics. Accordingly, the investigation of the effects of CO_2 required the application of a CPB; a dog model was chosen for these experiments since the complicated surgical procedure can be carried out successfully in this species. Isolated perfused rat lungs were selected for investigations of the protective effects of volatile agents against lung constriction in the absence of a neural control, since the rat is the animal most commonly applied in studies of the respiratory consequences of MCh and volatile agents[37], and our research group has acquired extensive experience in performing such experiments. The animal experimental protocols were approved by the Institutional

Animal Care Committee of the Canton of Geneva in Switzerland (rat experiments) and the Institutional Animal Care and Use Committee of the University of Szeged in Hungary (dog experiments).

3.1.1. Open-chest dogs with a cardiopulmonary bypass

Eight adult mongrel dogs [mean body weight ± standard error (SE), 23.7 ± 5.0 kg] were anaesthetized (30 mg/kg pentobarbital, iv). Analgesia was provided by iv injections of fentanyl (5-10 µg/kg). Muscle relaxation was achieved with an iv bolus of pipecuronium bromide (0.1 mg/kg). The dogs were then intubated with an 8-9-mm-internal diameter (ID) cuffed endotracheal tube (Portex, Hythe, UK) and ventilated with a Siemens Servo 900C Ventilator (Solna, Sweden) in volume-controlled mode. A tidal volume of 10 ml/kg and a positive end-expiratory pressure (PEEP) of 5 cmH_2O were applied, and the frequency was set to maintain a normal arterial CO_2 level (40 mmHg) in the pre-bypass period. The anaesthesia was maintained by continuous iv infusion of propofol (50 µg/kg/min) and the muscle relaxant was administered as needed. After opening of the chest by a midline sternotomy, anticoagulant (heparin, 3 mg/kg, iv) was administered. The ascending aorta and the inferior and superior vena cava were then cannulated, and the CPB was achieved by means of a roller pump (Pemco, Inc., Cleveland, OH, USA) with non-pulsatile blood flow at 100 ml/kg/min and use of a membrane oxygenator (Spiral Gold Buxter Healthcare Irvine, CA, USA). A left vent was introduced into the left ventricle through the right upper pulmonary vein. During total CPB, the pulmonary circulation was ceased and the lungs were ventilated with a gas mixture of 50% O_2 in air with a controlled concentration of CO_2 added to this gas mixture from a cylinder attached to the low-pressure gas input of the respirator. The end-tidal partial pressure of CO_2 ($P_{ET_{CO_2}}$) and Fi_{CO_2} were monitored (Datex, Oscar Helsinki, Finland). Arterial blood gas samples were analysed (model 505, Acid Base Laboratory, Copenhagen, Denmark).

3.1.2. Isolated perfused rat lung model

Thirty-five adult male Sprague-Dawley rats (373 ± 4.17 g) were anaesthetized with 50 mg/kg pentobarbital (Nembutal) intraperitoneally (ip). The rats were then tracheotomized with a polyethylene cannula (14-gauge, Braun, Melsungen, Germany) using sterile techniques, and normoventilated mechanically with a tidal volume of 7 ml/kg body weight. A

PEEP of 2.5 cmH_2O was applied, together with a respiratory rate of 70–80 breaths/min with a constant volume-cycled rodent ventilator (model 683, Harvard Apparatus Co. Inc., South Natick, MA, USA). After induction, anaesthesia was maintained with iv injections of pentobarbital when required (10 mg/kg). Analgesia was provided by the continuous infusion of fentanyl at 2 µg/kg/h. In one group of rats, the induction and maintenance of anaesthesia were achieved with Nembutal (50 mg/kg ip for induction and 10 mg/kg iv for maintenance); these animals served as a control group without the application of volatile anaesthetics. Respiratory gases were monitored continuously with a Datex monitor (Ultima^{TM}, Datex/Instrumentarium, Helsinki, Finland), and the airway pressure (P_{aw}) was also measured continuously with a calibrated pressure transducer (Validyne DP 45 and Validyne model 2D15 carrier demodulator, Northridge, CA, USA). The femoral vessels were cannulated (28-gauge catheter, Portex, Hythe, Kent, UK) for blood sampling and continuous arterial blood pressure monitoring, using a calibrated pressure transducer (Honeywell, model 156-PC 06-GW2, Zurich, Switzerland). The rats were fully anticoagulated with 1.5 IU/g body weight heparin injected iv. Twenty-five millilitres of blood was then gently withdrawn over 5 min via the arterial cannula, the collected blood being replaced continuously by the iv infusion of a 6% colloid solution of hydroxyethyl-starch at a constant rate in order to maintain a constant intravascular and systemic blood pressure above 50 mmHg, so as to minimize lung ischaemic lesions during this normovolaemic haemodilution procedure. The collected diluted blood was centrifuged (1800*g* for 10 min), and 7 ml of plasma was extracted. The resulting concentrated blood, with a haematocrit of around 30%, served as the priming volume for the isolated perfusion circuit. A midline sternotomy was next performed; the chest was widely retracted, and a heart-lung block was prepared, as described in detail previously[70].

Procedure and continuous monitoring of the isolated lung model: The heart-lung block was placed in a thermostatically controlled and humidified Plexiglas chamber (Figure 1)[70], as detailed previously[70].

The lungs were ventilated with room air mixed with 5% CO_2, and a respiratory rate of 50/min, a tidal volume of 7 ml/kg, and a PEEP of 2.5 cmH_2O were maintained. A series of hyperinflations (peak pressure, 25-30 cmH_2O) were applied in order to standardize the lung history by eliminating the atelectatic areas. The perfusion circuit was primed with the rat's own blood after filtration (standard 200-mm filter) to remove possible debris. Lung perfusion

was performed from a perfusion reservoir initially at a pulmonary artery perfusion pressure (P_{pa}) of 15 mmHg. The distal extremity of the left ventricular outflow cannula was placed at a sufficient height to obtain a left atrial pressure (P_{la}) of 7.5 ± 2 mmHg at the beginning of reperfusion, which resulted in West zone 3 conditions ($P_{pa} > P_{la} >$ mean P_{aw}). The blood dripping from this cannula was collected in a 5-ml collection cylinder, and aspirated from this reservoir with polyethylene tubing passing through a roller pump (Ismatec Pump, Glattburg, Zurich, Switzerland). The priming volume of the tubing and reservoirs was 18 ml. A transit-time flowmeter (T-201 CDS, Transonic Systems Inc., Ithaca, NY, USA) was placed between the perfusion reservoir and the catheter cannulating the main pulmonary artery for continuous monitoring of the pulmonary blood flow (Q_p). The mean P_{pa} and P_{la} values were measured continuously, using calibrated pressure transducers (Honeywell, model 156-PC 06-GW2) zeroed at the level of the lung hilus. The pulmonary vascular resistance (R_v) was calculated by dividing the pressure drop on the pulmonary vasculature by Q_p.

The airflow, tidal volume, pressures and circuit flow were recorded and stored at a sampling rate of 50 Hz via an analogue-to-digital interface converter (Biopac, Santa Barbara, CA, USA). The perfusate temperature and pH were measured with a pH-meter (691 pH-meter, Metrohm, Herisau, Switzerland), or the pH was determined on repeated blood gas samples. It was maintained between pH 7.35 and 7.45 and, if necessary, was corrected with sodium bicarbonate or a change in inspired CO_2 as required by the blood gas analysis (model 505, Acid Base Laboratory, Copenhagen, Denmark). Steady-state gas exchange was confirmed by stable Pa_{O_2}, Pa_{CO_2} and haematocrit levels during the experiments.

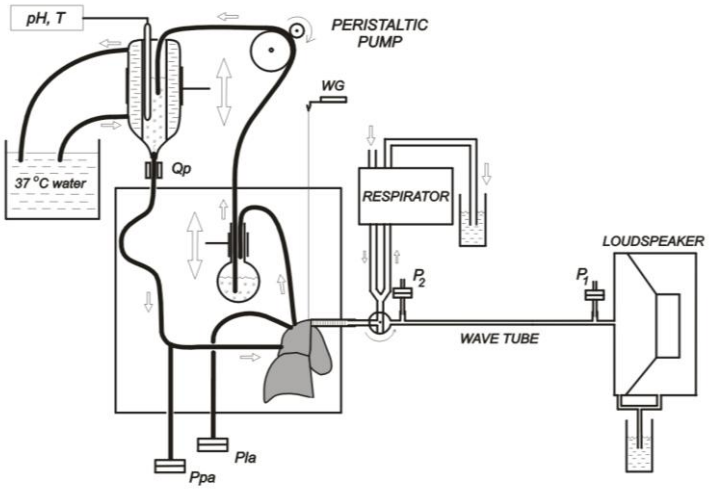

Figure 1. *Schematic illustration of the isolated perfused lung experimental set-up. pH: blood pH; T: blood temperature; WG: weight gain; P_{la}: left atrial pressure; P_{pa}: pulmonary arterial pressure; Q_p: pulmonary blood flow; P_1 and P_2:lateral pressures.*

3.2. Measurement of lung mechanics

3.2.1. Impedance measurements with the classical set-up in open-chest dogs during a cardiopulmonary bypass

The measurement system for the collection of the forced oscillatory input impedance spectra of Z_L was similar to that described in detail previously[58]. The set-up used for impedance measurements during short intervals of suspended mechanical ventilation is shown schematically in Figure 2.

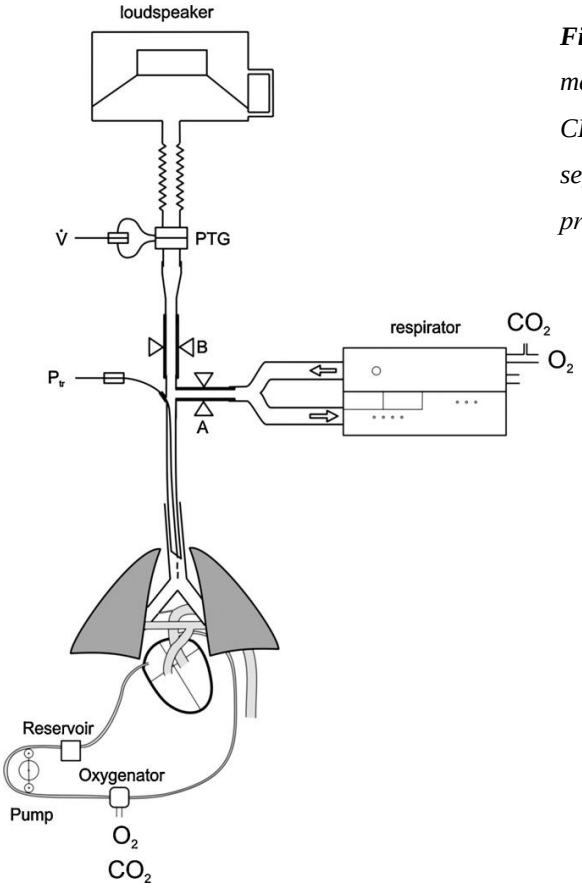

Figure 2. *Schematic illustration of the measurement set-up for open-chest dogs during a CPB. A and B denote collapsible latex tube segments. PTG: pneumotachograph; P_{tr}: tracheal pressure; V': tracheal flow.*

Two collapsible latex tube segments (A and B) were clamped alternately to switch the endotracheal tube from the respirator to the oscillatory device and back, as follows. During mechanical ventilation, segment A was open and segment B was closed. Following a few ventilatory cycles, the respirator was stopped at end-expiration and its tubing was detached from segment A. Segment B was then opened and segment A was clamped. In this apnoeic period, small-amplitude (1.5 cmH_2O peak-to-peak) pseudorandom pressure excitations were delivered by the loudspeaker into the trachea. The forcing signal contained 30 integer-multiple frequency components between 0.2 Hz and 6 Hz; the 15-s long recordings included 3

complete cycles of the periodic forcing signal. Tracheal flow (V') was measured with a 28-mm ID screen pneumotachograph connected to a differential pressure transducer (ICS Model 33NA002D; ICSensors, Miltipas, CA, USA). To exclude endotracheal tube impedance from the measurements, tracheal pressure (P_{tr}) was measured with an identical pressure transducer through a 1.5-mm-outer diameter polyethylene catheter, the tip of which, containing several lateral holes, was positioned 1.5-2 cm over the distal end of the endotracheal tube. The cross-power spectra between the electric signal driving the loudspeaker and the measured signals of P_{tr} and V' were computed by fast Fourier transformation with 10-s time windows and 95% overlapping ($Z_L = P_{tr}/V'$).

3.2.2. Wave-tube technique in isolated perfused rat lungs

The respective contributions of the airway and lung parenchymal mechanical properties to the total lung resistance were estimated by measuring the forced oscillatory Z_L *ex vivo* (isolated perfused rat lung) with the wave-tube technique, as described in detail previously[70] and demonstrated schematically in Figure 1[70]. Briefly, in this set-up, a three-way tap was used to switch the tracheal cannula from the respirator to a loudspeaker-in-box system at end-expiration. Before each measurement, the pressure in the box chambers was adjusted to 2.5 cmH_2O to keep the transpulmonary pressure constant during the measurements. The loudspeaker delivered a computer-generated, small-amplitude pseudorandom signal with frequency components between 0.5 and 20.75 Hz through a polyethylene wave-tube with known geometry (polyethylene tube: length (L), 100 cm; ID: 2 mm). The wave-tube was equipped with side-arms and miniature identical transducers (ICS model 33NA002D, ICSensors, Malpitas, CA, USA) to measure the lateral pressures at the loudspeaker end (P_1) and the cannula end (P_2).

The pressure transfer functions (i.e. P_1/P_2) were computed by fast Fourier transformation from the 6-s recordings, using 4-s time windows and 95% overlapping. According to transmission line theory, Z_L can be calculated from the P_1/P_2 spectra as the load impedance of the wave-tube[71]:

$$Z_L = Z_0 \sinh(\gamma L)/[P_1/P_2) - \cosh(\gamma L)]$$

where L is the length, Z_0 is the characteristic impedance and γ is the complex propagation wave number of the wave-tube. The last two parameters were determined by the geometrical

data and the material constants of the tube wall and the air. The load impedance of the endotracheal tube and the connecting tubing was also determined, and the R_{aw} and I_{aw} values were corrected by subtracting the instrumental resistance and inertance values from them.

3.3. Study designs

3.3.1. Lung mechanical changes during alveolar hypocapnia and hypercapnia in dogs under a cardiopulmonary bypass

In the *in vivo* open-chest dog experiments, after surgical preparation, alveolar hypocapnia was induced by applying a total bypass with cessation of the pulmonary blood flow. This manoeuvre allowed the decrease of $P_{ET_{CO_2}}$ to approximately 0.1-0.3% (~0.8-2.3 mmHg). A set of Z_L data was collected under these conditions. The alveolar CO_2 concentration was then increased to 7% (~53 mmHg) by applying stepwise elevations of Fi_{CO_2}, accomplished by altering the CO_2 flow from the cylinder attached to the low-pressure input of the respirator. After a 2-3-min period for the animal to reach equilibrium, other sets of Z_L data, including 3-5 data epochs, were collected at each $P_{ET_{CO_2}}$ level.

3.3.2. Changes in lung mechanics by systemic hypercapnia and acidosis during a cardiopulmonary bypass in an open-chest dog model

In a subgroup of 5 dogs, the effects of systemic CO_2 changes were also investigated. After the recording of the dose-response curve to alveolar CO_2, Fi_{CO_2} was set to zero. Ten min later, CO_2 was added to the oxygenator to achieve a Pa_{CO_2} of 60 mmHg and an arterial pH of approximately 7.2, and a set of Z_L spectra were recorded after these target values had been established. To assess the role of the vagal control of the lungs under these conditions, a bolus of atropine (0.5 mg) was injected into the iv port of the blood reservoir in order to eliminate the vagal tone, and the Z_L measurements were then repeated.

3.3.3. Effects of volatile anaesthetic agents against acetylcholine-induced bronchoconstriction in isolated perfused rat lungs

In the isolated perfused lung set-up, rats were randomly assigned to one or other of the following five protocol groups: group C (control group, n = 6), no volatile anaesthetic was

administered; group H (n = 6), halothane group; group I (n = 9), isoflurane group; group S (n = 6), sevoflurane group; and group D (n = 8), desflurane group. As illustrated in Figure 3, after the start of perfusion of the isolated rat lung, a period of 20-30 min was allowed for the pulmonary and haemodynamic variables to reach steady-state conditions and for the preparation to become isogravimetric. Before the administration of ACh, as constrictor agent, the lungs were hyperinflated by superimposing two inspiratory cycles to standardize the volume history. After 4-6 successive baseline Z_L recordings, the concentration of ACh in the blood container supporting the pulmonary artery was doubled from 100 to 200 μg/kg, and Z_L was then recorded at 15-s.

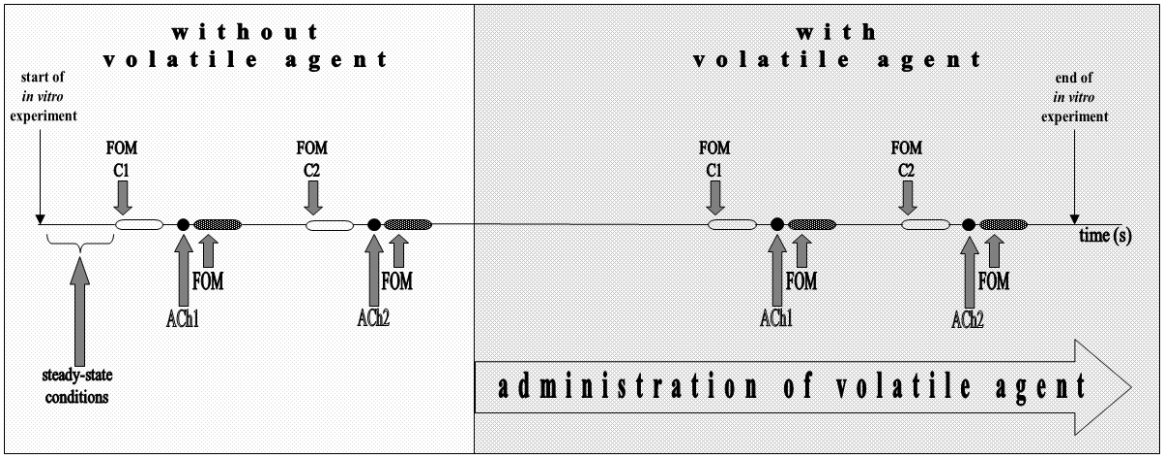

Figure 3. *Schematic illustration of the experimental protocol employed in isolated perfused rat lungs. FOM: forced oscillatory measurements. C1 and C2: control conditions before the first and second ACh doses, respectively. ACh1: acetylcholine dose of 100 μg/kg; ACh2: acetylcholine dose of 200 μg/kg.*

When the end-tidal concentration of the volatile agent reached 1 MAC [1% for halothane, 1.4% for isoflurane, 2.4% for sevoflurane and 6.9% for desflurane] and stable haemodynamic conditions had been established, Z_L measurements were performed to obtain new baseline data, and the ACh challenge was repeated. The concentrations of O_2, CO_2 and the volatile agent were monitored throughout the study (Ultima^{TM}, Datex/Instrumentarium).

3.4. Statistical evaluations

Scatters in parameters are expressed as SE values. The Kolmogorov-Smirnov test was used to test data for normality. One-way analysis of variance (ANOVA) was applied to compare the measured parameters between the independent protocol groups. Within the

protocol groups, repeated measures one-way ANOVA was performed to compare the relative changes in the measured parameters. The Student-Newman-Keuls multiple comparison procedure based on the means was applied to compare the data obtained in the different protocol groups and/or under the different conditions. Paired *t*-tests were applied to assess statistical significance under different conditions in the same groups. Statistical tests were performed with significance level set at $p < 0.05$.

4. Results

4.1. Alterations in lung mechanics by alveolar hypocapnia and hypercapnia

Representative pulmonary impedance data obtained under normocapnia and alveolar hypocapnia are demonstrated in Figure 4. The impedance data show the characteristic frequency dependence, with a sharp decrease in the real part at low frequencies mirrored by a similar increase in the imaginary part. The real part reaches a plateau at higher frequencies representing R_{aw}, while the imaginary part exhibits a fairly linear increase due to the inertive properties.

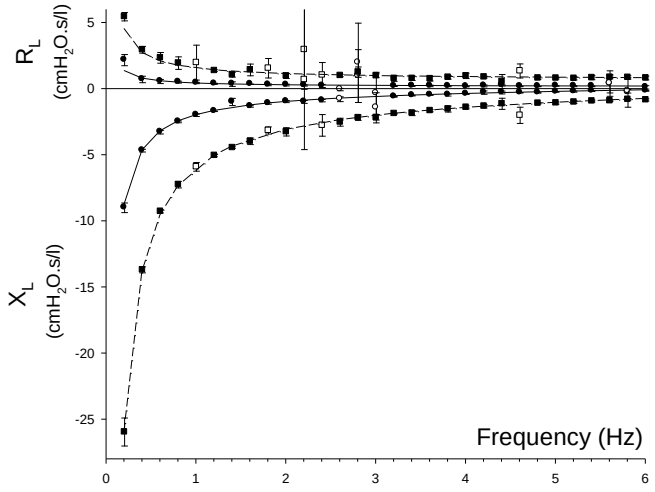

Figure 4*. Frequency dependence of pulmonary input impedance. Representative measurements of R_L and X_L as functions of frequency in open-chest dogs during CPB under normocapnia (circles) and during alveolar hypocapnia (squares). Lines denote model fits.*

The effects of Fi_{CO_2} on the airway and lung tissue mechanical parameters are demonstrated in Figure 5 for individual animals. A tendency to a decrease in R_{aw} is obvious at low Fi_{CO_2} levels (below ~2%), while G exhibits milder increases only in the presence of more severe hypocapnia (below ~1%). The changes in I_{aw} and H were rather variable, with some

animals exhibiting mild decreases, and others small increases with decreasing Fi_{CO_2}. Alveolar hypercapnia (Fi_{CO_2} > 6%) had no effect on any respiratory mechanical parameter.

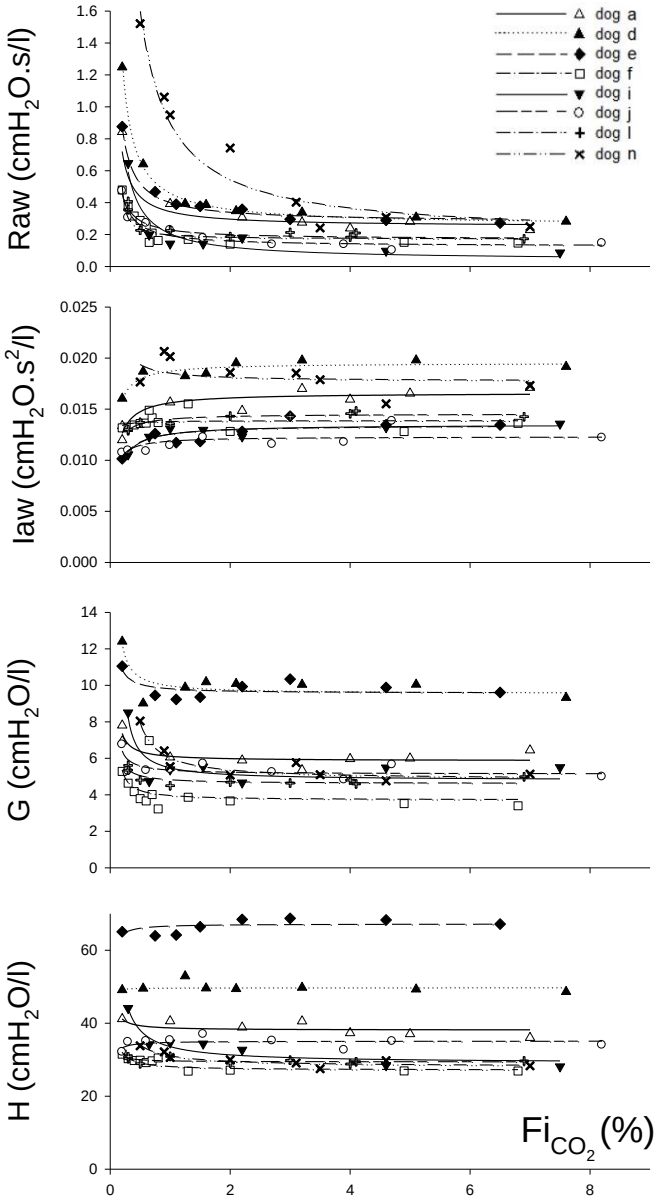

Figure 5. *The effects of Fi_{CO_2} on the airway and lung tissue mechanical parameters of individual animals during a CPB. Values were fitted to hyperbolas. R_{aw}: airway resistance, I_{aw}: airway inertance, G: lung parenchymal damping, H: lung parenchymal elastance, Fi_{CO_2}: fraction of inspired carbon dioxide.*

For a precise statistical evaluation of the changes in the respiratory mechanics in response to the alterations of Fi_{CO_2} in the dogs *in vivo*, the values of the model parameters

were obtained at discrete Fi_{CO_2} levels of 0.2% (1.5 mmHg), 0.3% (2.3 mmHg), 0.5% (3.8 mmHg) and 1 - 7% (7.6 - 53 mmHg) by reading their interpolated values from fitted hyperbolas to Fi_{CO_2} vs. R_{aw}, I_{aw}, G, H and η data in each individual animal. Figure 6 depicts the group means of these values for the airway and lung parenchymal mechanical parameters.

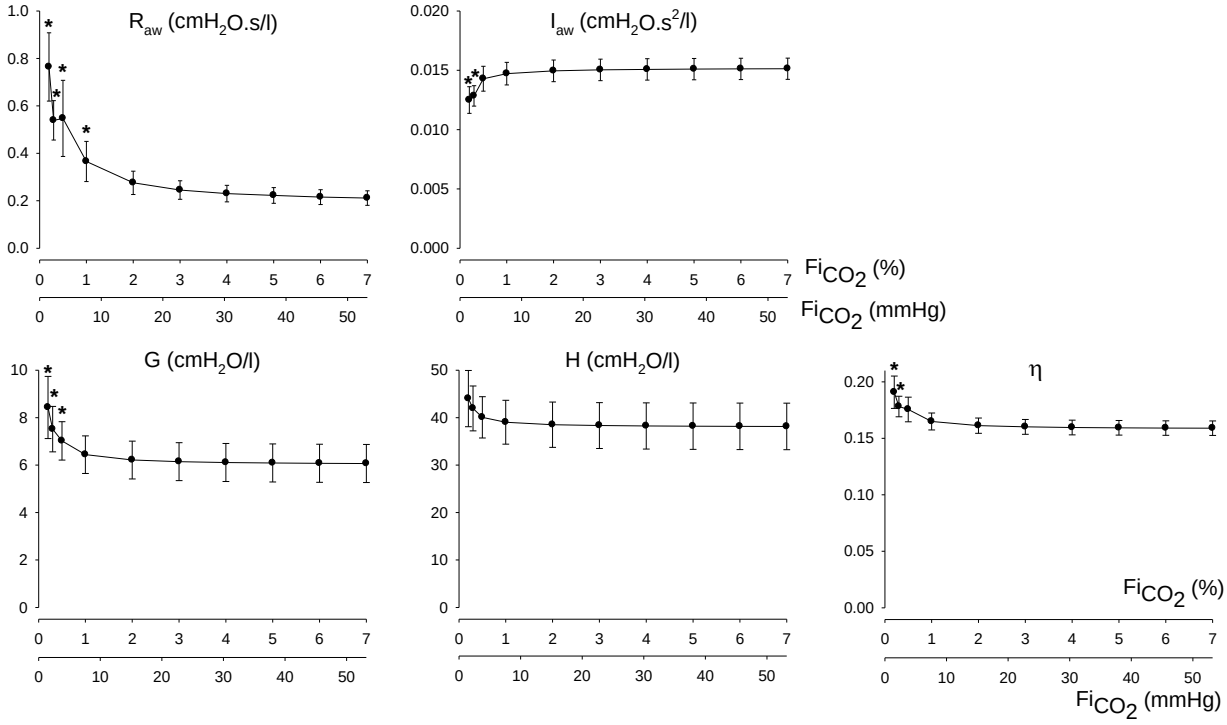

Figure 6. *Group means ± SE (n = 8) for the airway and lung parenchymal mechanical parameters at different levels of Fi_{CO_2}. R_{aw}: airway resistance; I_{aw}: airway inertance; G: lung parenchymal damping; H: lung parenchymal elastance; η: tissue hysteresivity; Fi_{CO_2}: fraction of inspired carbon dioxide. *p < 0.05 vs. the values obtained under normocapnia (Fi_{CO_2}= 4%, 30.4 mmHg).*

This analysis revealed a markedly elevated R_{aw} (243.2 ± 334.7%, $p < 0.05$ vs. normocapnia defined as 5% or 38 mmHg) at Fi_{CO_2} levels < 1% (7.6 mmHg), associated with a smaller decrease in I_{aw} (-17.2 ± 25.0%, $p < 0.05$) at lower Fi_{CO_2} levels. As regards the lung parenchymal parameters, G and η were moderately elevated (38.4 ± 63% and 22.5 ± 2.7%, respectively; $p < 0.05$ for both) at low Fi_{CO_2}, whereas no significant changes occurred in H (15.2 ± 21.5%; NS) throughout the study protocol. There were no detectable alterations in R_{aw}, I_{aw}, G, H and η in the presence of alveolar hypercapnia.

4.2. Effects of systemic hypercapnia and acidosis on the mechanics of the pulmonary system

The airway and lung tissue mechanical parameters determined before and after systemic acidosis, and the subsequent effects of atropine, are demonstrated in Figure 7 in the experiments performed on dogs *in vivo*. R_{aw} and I_{aw} were markedly and statistically significantly increased ($p < 0.001$ for both) in the presence of systemic hypercapnia and acidosis. Further, the administration of atropine counteracted this rise in R_{aw} statistically significantly, whereas atropine had no effect on the elevated I_{aw}. Systemic acidosis induced mild, but statistically significant increases in G ($p = 0.02$) and H ($p < 0.001$), which were not affected by the administration of atropine.

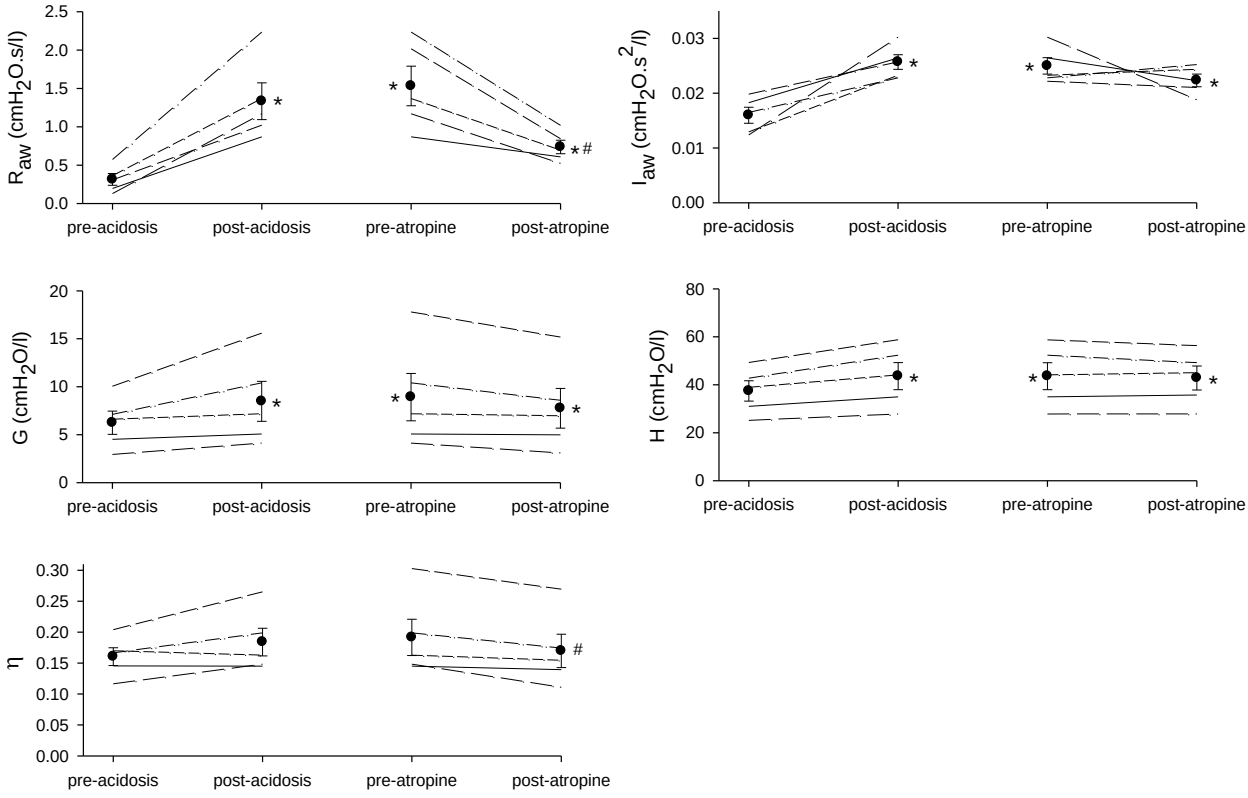

Figure 7. *Airway and lung tissue mechanical parameters before and after systemic acidosis, and before and after administration of atropine, for the individual animals (n = 5, lines), and as group means ± SE (symbols). R_{aw}: airway resistance; I_{aw}: airway inertance; G: lung parenchymal damping; H: lung parenchymal elastance; η: tissue hysteresivity. *p < 0.05 vs. the values obtained before acidosis, #p < 0.05 vs. pre- and post-atropine.*

4.3. Effects of volatile anaesthetic agents on the airway and pulmonary parenchymal mechanics

Figure 8 shows the percentage changes in the basic airway and lung tissue mechanical parameters in all groups of isolated perfused rat lungs. The basic airway tone was markedly and statistically significantly decreased by desflurane (-31.2 ± 3.8% change in R_{aw}) and sevoflurane (-18.0 ± 4.5% change in R_{aw}) administration, whereas halothane and isoflurane did not have a statistically significant effect on R_{aw} (-3.3 ± 5.1% and -8.6 ± 2.4%, respectively). I_{aw} was significantly higher during the administration of desflurane, while the other volatile agents had no effect on this parameter. None of the volatile agents exerted a significant effect on the baseline levels of the parenchymal mechanical parameters G and H, and η.

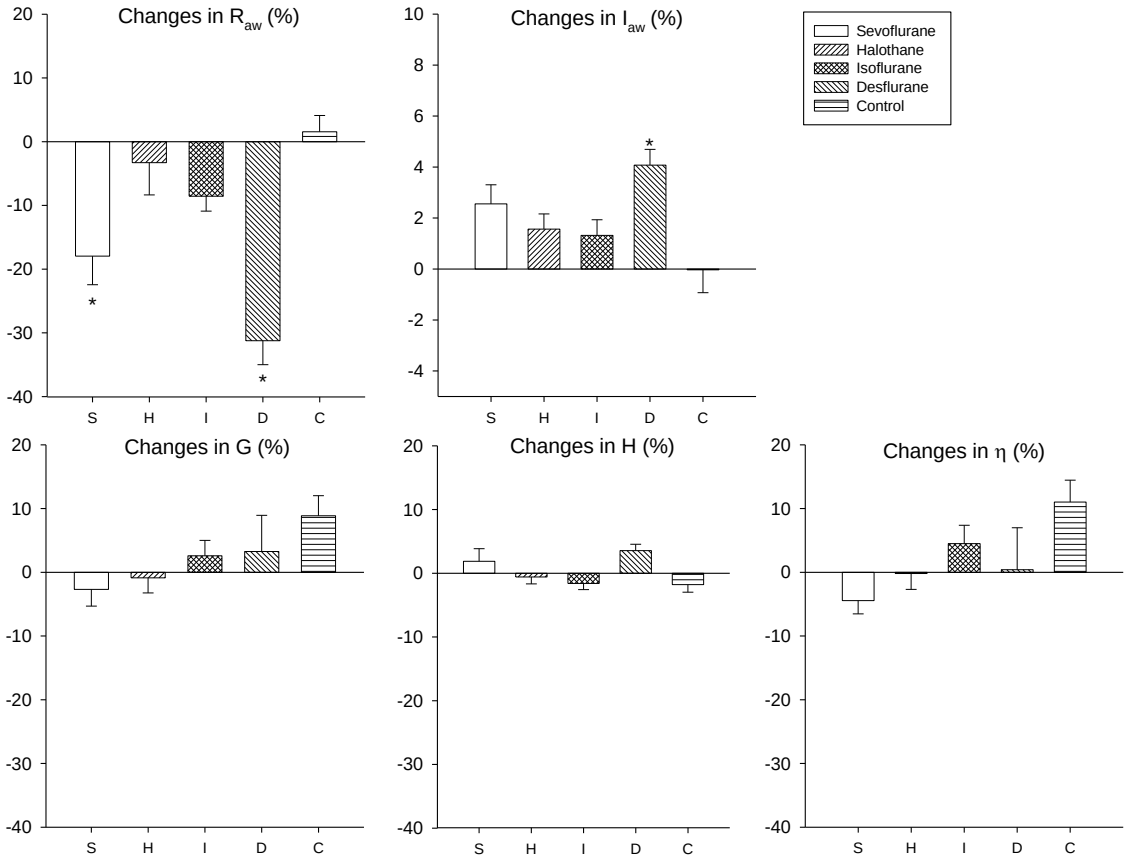

Figure 8. *Changes in the baseline values of airway resistance (R_{aw}), airway inertance (I_{aw},), tissue damping coefficient (G), tissue elastance (H) and tissue hysteresivity (η) following volatile agent administration. Values (mean and SE) are represented as relative changes from the control condition. C: control group; D: desflurane group; H: halothane group; I: isoflurane group; S: sevoflurane group. *p < 0.05 vs. the control group.*

4.4. Prevention of acetylcholine-induced changes in lung mechanics by volatile anaesthetic agents

Figure 9 illustrates the results of airway and parenchymal mechanical parameters under the control conditions (C1 and C2) and following the administration of the two different concentrations of ACh. In all groups, ACh induced statistically significant increases in R_{aw} and G as compared with the control conditions. Further, comparison of the ACh-induced increases in R_{aw} in the protocol groups revealed that sevoflurane and desflurane markedly and significantly inhibited the ACh-induced bronchoconstriction. Desflurane displayed the strongest effect; sevoflurane afforded less, but still significant, protection; the protective potentials of isoflurane and halothane did not reach the level of statistical significance. With regard to the lung parenchymal parameters, the ACh-induced elevations in G were slightly attenuated by all of the volatile anaesthetics; these minor effects were not statistically significant. In agreement with previous findings following the iv administration of MCh *in vivo*[72], the values of H remained at the baseline level throughout the study protocol.

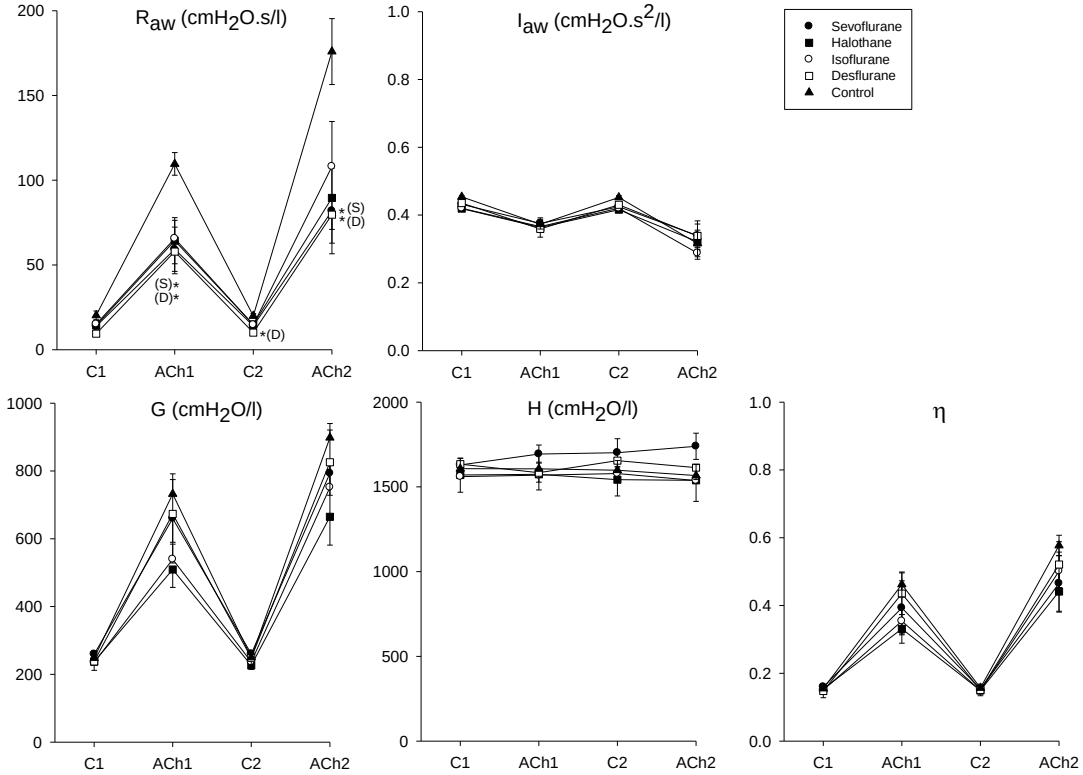

Figure 9. *Parameters of resistance (R_{aw}), airway inertance (I_{aw},), tissue damping coefficient (G), tissue elastance (H) and tissue hysteresivity (η) under baseline conditions (C1 and C2) and following the administration of two different concentrations of acetylcholine (ACh) (ACh1: acetylcholine dose of 100 μg/kg; ACh2: acetylcholine dose of 200 μg/kg) in all experimental groups. C: control group; D: desflurane group; H: halothane group; I: isoflurane group; S: sevoflurane group.*p < 0.05 vs. the control group.*

To further characterize the effects of the volatile agents on the enhanced airway tone obtained at different doses of ACh administration, we calculated the ratio of the changes in the mechanical parameters in the presence of the volatile agent and in the absence of the volatile agent. These relationships, expressed as percentage differences between the conditions (i.e. with and without volatile agent) are outlined in Figures 10 and 11. Noteworthy and statistically significant degrees of inhibition of the ACh-induced bronchoconstriction were observed following sevoflurane, desflurane and isoflurane administration, whereas the effects of halothane were considerably smaller. The presence of the volatile agents did not have a significant effect on the ACh-induced changes in G and H.

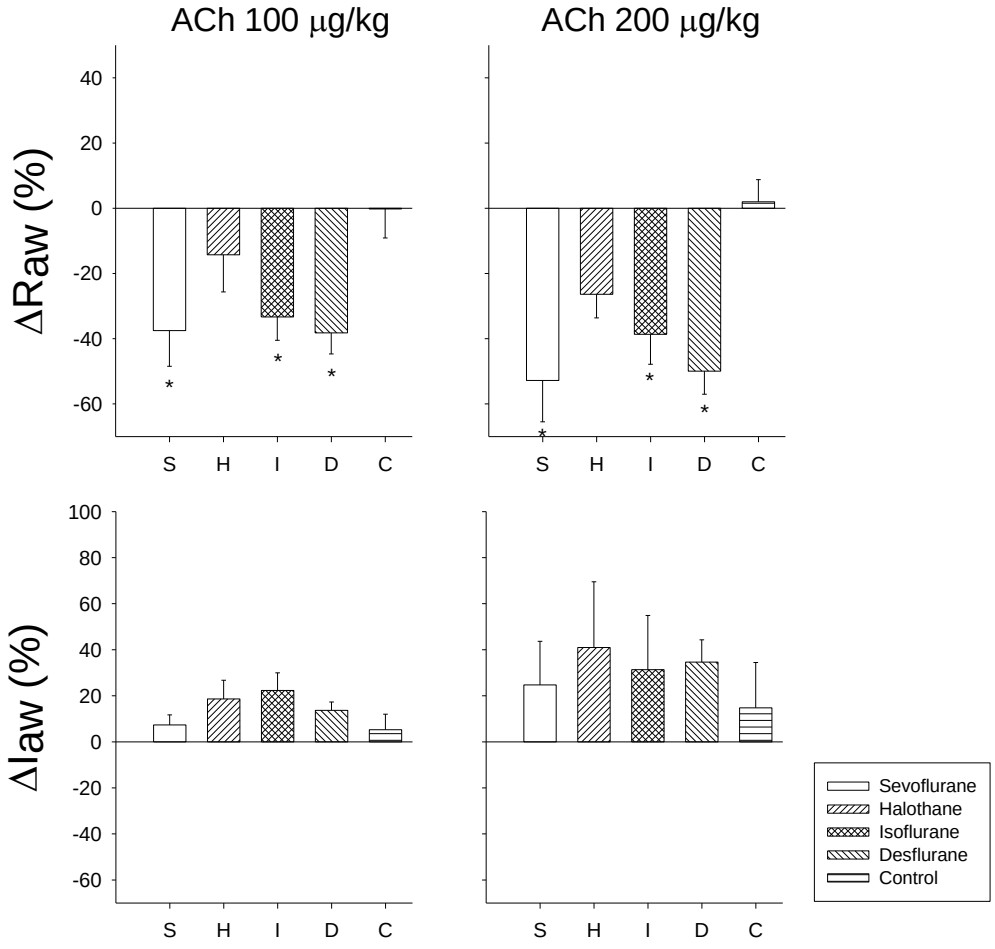

Figure 10. *Relative changes in airway resistance (R_{aw}) and airway inertance (I_{aw},) between the conditions (i.e. with and without volatile anaesthetics) following the administration of two different concentrations of ACh (ACh1: acetylcholine dose of 100 μg/kg; ACh2: acetylcholine dose of 200 μg/kg) in all experimental groups. S: sevoflurane group; H: halothane group; I: isoflurane group; D: desflurane group; C: control group. Values (mean and SE) are represented as relative changes from the control condition. *p < 0.05 vs. the control group.*

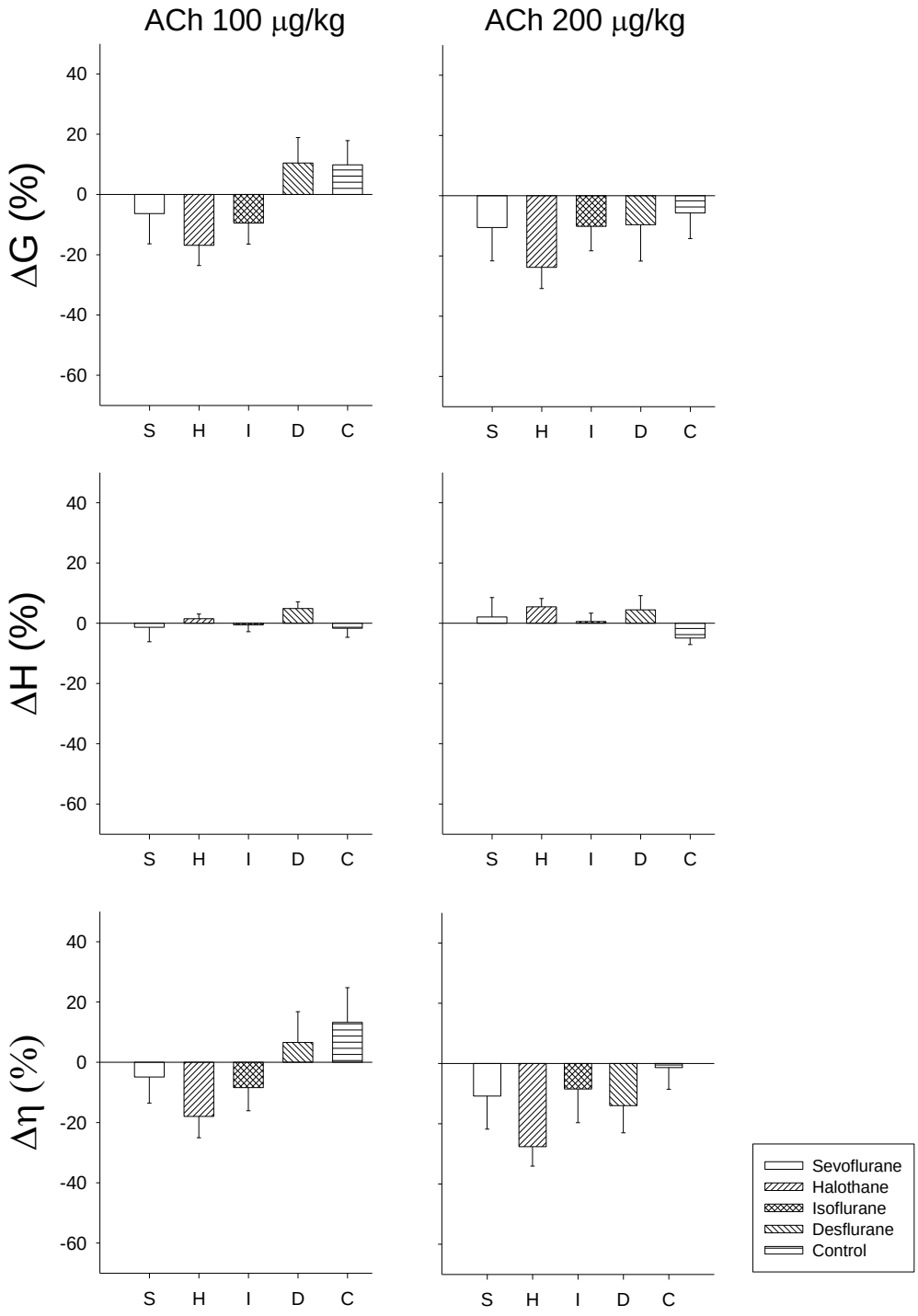

Figure 11. *Relative changes in tissue damping (G), tissue elastance (H) and tissue hysteresivity (η); between the conditions (i.e. with and without volatile anaesthetics) following the administration of two different concentrations of ACh (ACh1: acetylcholine dose of 100 μg/kg; ACh2: acetylcholine dose of 200 μg/kg) in all experimental groups. S: sevoflurane group; H: halothane group; I: isoflurane group; D: desflurane group; C: control group. Values (mean and SE) are represented as relative changes from the control condition. *p < 0.05 vs. the control group.*

5. Discussion

Besides the neural, humoral mechanisms participating in the regulation of global lung ventilation, the experimental models included in the present thesis revealed that CO_2 plays an important role in altering regional lung ventilation, and volatile anaesthetics decrease the elevated smooth muscle tone of the airways. The results also demonstrated that alterations of the endogenous CO_2 level (intra-alveolar or systemic) or the presence of an exogenous gas, such as a volatile anaesthetic agent, have the potential to modulate the pulmonary mechanical properties.

The results of the studies included in the present thesis revealed that *a)* the different levels of intra-alveolar CO_2 and the induction of systemic hypercapnia and acidosis alter the lung mechanical parameters under *in vivo* conditions during a CPB, and *b)* shed light on the protective abilities of the currently used volatile agents against ACh-induced bronchoconstriction at an organ level under *ex vivo* conditions.

5.1. Effects of alveolar hypocapnia and hypercapnia on the pulmonary mechanics

We investigated the alterations in the airway and lung parenchymal mechanical properties when different levels of CO_2 were maintained in the alveoli and in the systemic circulation. The application of extracorporeal circulation in the experimental design in open-chest dogs allowed the manipulation of intra-alveolar CO_2 levels in a wide range to establish a dose-response curve. The current experiments revealed the potential of alveolar hypocapnia to increase the airway tone markedly, with minor alterations in the pulmonary tissue parameters. The dose-response curve of R_{aw} to CO_2 revealed that, instead of a gradual increase in the bronchial tone, there was a sharp elevation in R_{aw} at very low alveolar CO_2 levels ($\leq 1\%$ or $\leq$ 7.6 mmHg).

Our study was designed to characterize the effects of CO_2 through wide-ranging changes of its concentration in the alveolar gas or in the blood in the systemic circulation, while the neurohormonal control was maintained intact. Utilization of a CPB was ideal for these purposes, since this approach permits not only the investigation of severe alveolar hypocapnia, but also independent manipulation of the alveolar and systemic CO_2 levels.

While the airway parameters R_{aw} and I_{aw} determined in our observations during normocapnia agree well with those reported previously in open-chest dogs, the current lung tissue parameters G and H appear to be somewhat larger[58,69]. This discrepancy is most probably due to the lack of pulmonary circulation in the present experiments, which compromises the lung tissue mechanics via loss of the tethering effect exerted by the filled pulmonary capillaries[73]. However, this bias was independent of the alveolar or systemic CO_2 levels, and thus the main findings of the present study are not affected by this phenomenon.

A number of previous *in vivo* or *ex vivo* studies have revealed the constrictor response of the lungs to alveolar hypocapnia. The bronchoconstrictive effect of a moderately low airway CO_2 concentration on the airway smooth muscle has been well established under *in vivo* conditions by manipulating the ventilation pattern or by occluding the pulmonary artery in various experimental models[14,28,30,74]. Study of the influence of severe hypocapnia (< 0.3% or < 2.3 mmHg CO_2), which is feasible only under *in vitro* conditions[31,75,76], further confirmed the development of severe airway narrowing while extremely low alveolar CO_2 levels were maintained. Similarly to those earlier findings, our investigation demonstrated significant increases in R_{aw} in response to decreases of the level of alveolar CO_2. These changes were associated with small elevations in G and η, and mild decreases in I_{aw}. Since this pattern of change in the lung mechanical parameters was manifested during airway constriction with marked ventilation heterogeneities[77], it may be concluded that alveolar hypocapnia exerts constrictions on both the central (leading to marked elevations in R_{aw}) and the peripheral airways (leading to ventilation heterogeneities reflected by apparent increases in η and decreases in I_{aw}).

The results relating to the effects of alveolar hypercapnia on the lung mechanics are more conflicting. Alveolar hypercapnia has been reported to elevate[78-80], decrease[31,81,82], or cause no changes[29] in the total lung resistance (R_L). The results of our study, obtained under well-controlled conditions, corroborate the latter earlier findings by demonstrating neutral effects of alveolar hypercapnia on the lung mechanics.

As far as we are aware, there has been only one previous *in vivo* study in which the changes in lung mechanical parameters were evaluated by measuring lung interrupter resistance (R_{int}) during wide-ranging alterations in the level of intra-alveolar CO_2[30]. Our observations indicating an increase in R_{aw} correspond well with those obtained previously in

the R_{int} range where the intra-alveolar and intra-arterial CO_2 overlap. Nevertheless, in consequence of the presence of a pulmonary circulation in the previous experiments, the minimum alveolar CO_2 partial pressure attained was ~20 mmHg (which corresponds to 2.6% in the present study). Thus, the noteworthy feature of the dose-response curve in R_{aw} to the altered intra-alveolar CO_2, the sharp increase in this parameter when Fi_{CO_2} decreased to 1% (7.6 mmHg), remained undetected in that previous study. This highly non-linear feature of the dose-response curve may be of importance in the mechanisms of adaptation of the lungs to altered conditions via regulation of the ventilation distribution.

Hyperventilation initiated regularly by the central nervous system to compensate hypoxaemia may reduce the intra-alveolar CO_2, but this decrease cannot reach a concentration of < 2% (15.2 mmHg)[30]. Our data indicate no detectable bronchoconstriction under these conditions (Figure 6), which is a sensible physiological response as the lung function remains normal to maintain optimum gas exchange. Intra-alveolar CO_2 concentrations of < 1% (7.6 mmHg) can develop in lung regions with no or only a severely diminished pulmonary perfusion, such as those observed following pulmonary embolism. Acute blockade of pulmonary perfusion by pulmonary embolism (clots, gas, etc. embolism) results in increased alveolar dead space ventilation of the affected non-perfused pulmonary region. Our findings demonstrate that the pulmonary embolism and the subsequent local alveolar hypocapnia also result in the activation of a local reflex mechanism leading to the redirection of the alveolar ventilation from the non-perfused regions to the units with normal perfusion[83]. Such a regional bronchoconstriction that develops due to alveolar hypocapnia[14,84-88] is therefore a compensatory physiological response, which can decrease the mismatch between ventilation and perfusion. Thus, the acute pulmonary embolism can decrease not only the pulmonary capillary, but additionally the alveolar surface. On the other hand, this parallel decline in the pulmonary perfusion and ventilation can protect against hypoxaemia, but can be accompanied by an elevated systemic CO_2 level. Our findings further demonstrate that this systemic hypercapnia contributes to the localized bronchoconstriction in order to prevent the affected lung regions from “reopening”, and hence to protect from the aggravation of the ventilation-perfusion mismatch. This phenomenon may be regarded as a synergistic pathophysiological constrictor ability of the affected non-perfused airways initiated by the direct effects of the reduced intra-alveolar and the indirect effects of the elevated systemic CO_2 concentration.

This mechanism is expected to be most effective if it affects the small airways in the lung periphery. Indeed, the involvement of peripheral airways in hypocapnia-induced bronchoconstriction is substantiated by the proportionally greater increases in G than in H leading to elevations in η, a hallmark feature of the presence of heterogeneous peripheral airway constriction with the development of ventilation heterogeneities[43].

5.2. Changes in the mechanics of the pulmonary system during systemic hypercapnia and acidosis

In contrast with the neutral effects of alveolar hypercapnia on the lung mechanics observed in dogs *in vivo*, hypercapnia induced in the systemic circulation generated significant elevations in both the resistive and inertive airway parameters and the parenchymal resistance and elastance values. The adverse changes in the airway mechanics under these conditions were inhibited by elimination of the vagal activity with atropine. However, vagal blockade with atropine did not reverse the deteriorated lung parenchymal mechanics in the presence of systemic hypercapnia and acidosis.

Systemic acidosis and hypercapnia with alveolar normocapnia can exist only in the presence of a bronchial circulation without pulmonary blood flow (as is the case during a CPB), for otherwise the elevated systemic CO_2 would consequently appear in the alveoli. Vice versa, the elevated Fi_{CO_2} during a CPB has no direct systemic effects, as the interrupted pulmonary circulation cannot transmit the CO_2 induced into the systemic circulation. We elevated $P_{ET_{CO_2}}$ levels in order to minimize the time necessary to reach a new equilibrium. There were no obvious temporal changes in the lung mechanical parameters during the experiments, and sufficient time was allowed for the animals to reach equilibrium in the lungs and the systemic circulation after establishing each CO_2 level. Although the randomized order of the CO_2 levels would have decreased the potential temporal effects in the experiments, the increasing order of $P_{ET_{CO_2}}$ was not likely to affect our findings significantly. While the bronchoprotecting potential of propofol might have blunted the constrictor effects of CO_2[89], our dose was much lower than that proved to be effective against MCh-induced bronchoconstriction.

In the present study, systemic acidosis via systemic hypercapnia was produced by supplying CO_2 into the extracorporeal circulation (into the oxygenator). An elevated level of

systemic CO_2 may exert its pulmonary effects via direct and indirect pathways. As concerns the direct effects of excess systemic CO_2, it most probably reaches the cells of the tracheobronchial tree in the terminal bronchioles, via the bronchial circulation, and then gains direct access to the proximal airway smooth muscle cells. Previous studies on denervated bronchi indicated a relaxation of the airway smooth muscle, suggesting the presence of direct CO_2-mediated bronchodilatation[78]. This discrepancy suggests that, in our observations, the direct bronchodilation activity of CO_2 was overwhelmed by vagally controlled indirect effects of systemic hypercapnia. Indeed, unlike the conflicting results concerning the pulmonary effects of alveolar hypercapnia, there is a consensus in the literature on the bronchoconstrictor potential of systemic hypercapnia when the neural control of the lungs remains intact[29,31,78,79]. The inhibition of ACh release from the vagal efferent nerve endings on the airways by the administration of atropine almost fully reversed the increases in R_{aw}, while the elevations in the lung tissue parameters were essentially unaffected. This points to a more pronounced role of the vagus nerve in regulating the central airways following systemic CO_2 changes, which accords well with earlier results demonstrating the primary site of vagal control in the central conducting airways[90].

5.3. Effects of volatile anaesthetic agents on the mechanical properties of the lung

Bronchospasm is a potential complication in any patient undergoing general anaesthesia. Tracheal intubation or any other manipulation in the airways, e.g. a suction manoeuvre, may induce severe bronchoconstriction in patients with asthma or chronic obstructive pulmonary disease. Administration of volatile anaesthetics prior to physical airway stimulation may be valuable in patients with airway susceptibilities.

A number of previous *in vivo* experimental or clinical studies have described the influence of the commonly used volatile anaesthetics on the bronchial smooth muscle tone. Conflicting data have been reported concerning the ability of desflurane to affect the airway tone. Most of the clinical studies have indicated a high incidence of airway irritation during the administration of desflurane (coughing, breath-holding, excessive secretion and laryngospasm)[91,92] and a similar potency was observed under experimental conditions[42]**.** In contrast, in a few clinical[93] and experimental studies[94], desflurane exhibited a marked

bronchodilator potential. The reasons for these controversial findings have not been fully elucidated. It is noteworthy, however, that *in vitro* studies have consistently demonstrated the bronchodilator effect of desflurane[95-99], as for other volatile anaesthetics[95-101]. Analogously to our findings, desflurane and sevoflurane have been shown to exert more potent bronchodilatator effects than those of halothane in isolated airway smooth muscles[95,99] and in isolated tracheal rings[98]. In contrast, other *ex vivo* studies have indicated that halothane has a greater relaxing effect on the airway smooth muscle than sevoflurane, isoflurane or desflurane[96]. Although the reasons for these conflicting results are not totally clear, all of these previous *in vitro* studies consistently demonstrated the bronchodilator potential of desflurane.

The relaxation properties of the volatile anaesthetic agents applied commonly in clinical practice were compared in our study in an isolated perfused rat lung model. This experimental setting, combined with a low-frequency forced oscillation technique, allows a separate assessment of the airway and parenchymal mechanics in the absence of neural control of the lungs and without the confounding influence of the alterations in pulmonary haemodynamics induced by bronchoactive drug administration. The current experiments revealed the potential of desflurane and sevoflurane to decrease the basal airway tone, whereas isoflurane and halothane were ineffective in this regard.

Many previous studies have focused on the cellular mechanisms of action of volatile anaesthetics on airway smooth muscle cell cultures[44,45] or isolated tracheal and/or bronchial rings[95-100]. The structural integrity of the lungs has been lost in such investigations, which makes it difficult to extrapolate these findings to an organ level. The results obtained under *in vivo* experimental conditions, on the other hand, are influenced by numerous confounding effects of systemic hormonal and neurogenic origin. The isolated *ex vivo* rat lung model applied in the present study offers ideal conditions under which to investigate the effects of volatile anaesthetics on the airway tone under baseline conditions and in the presence of airway constriction at an organ level. Furthermore, excised lungs are an ideal model on which to investigate the direct effects of bronchoactive agonists in the absence of reflex mechanisms involving neural pathways and without the biasing effects of acutely released humoral mediators. This setting permits the maintenance of stable haemodynamic conditions, and also a separate assessment of the changes in the airway and parenchymal mechanics.

5.4. Protective potential of volatile anaesthetic agents against acetylcholine-induced bronchoconstriction

An important feature of the present study is the administration of the constrictor agent into the pulmonary circulation. No previous studies have characterized the changes in the airway and the lung parenchymal properties in small laboratory animals when the contractile agonist is administered via the pulmonary circulation. This route of the bronchoconstrictor agonist supplies the lung periphery, and it therefore offers a possibility to challenge the terminal airways and the alveolar contractile apparatus. The absence of systemic circulation in the isolated lung model applied in the present experimental setting avoids challenging the large conducting airways. This model resembles a human transplanted lung with no bronchial circulation and innervation exposed to a constrictor challenge via the circulation.

Many of the procedures used in anaesthetic practice may involve direct stimulation of the muscarinic receptors (via mechanical stimulation of the airways, i.e. tracheal intubation, bronchoscopy or bronchial suction), resulting in cholinergic-mediated bronchospasm. As ACh acts as a physiological ligand on the muscarinic receptors, adverse changes in lung function were induced by generating a spastic condition following stimulation of the muscarinic receptors.

As most previous studies applied global parameters to express the lung responses to volatile anaesthetic agents[101-105], the relative contributions of the airways and the lung parenchyma to the lung response to ACh-induced bronchospasm cannot be distinguished. In the present study, the low-frequency forced oscillatory technique and the model-based evaluation of the impedance data were applied to separate the airway and the lung parenchymal mechanical properties. This technique has been validated[77] and used successfully in small rodents[73,94] and in isolated perfused rat lungs[70,73], and has been shown to furnish selective parameters for the airway and parenchymal mechanics and to allow reliable quantification of the changes in these compartments. The airway and parenchmyal mechanical parameters under baseline conditions are in excellent agreement with those obtained previously[70,73].

In the present study, all of the currently used volatile agents exerted a marked protective effect against bronchoconstriction in the presence of an increased airway tone induced by the administration of ACh into the pulmonary circulation. The efficacy of this

bronchoprotective potential of the volatile agents was not affected by the magnitude of the constrictor stimuli. The ACh-induced increases in the lung tissue parameter related to parenchymal damping were not prevented by any of the volatile anaesthetics applied in the current investigations.

The observations of our study confirm the marked protective properties of desflurane against the airway constriction induced by cholinergic stimulation of the muscarinic receptors. Our finding that desflurane prevents ACh-induced bronchoconstriction may suggest that the humoral or neural pathways present *in vivo* are responsible for the lower protective potential of desflurane against airway constriction.

The results obtained in our study demonstrate that ACh induced marked increases in R_{aw} and G, but with no significant effects on H. This pattern of change in the lung mechanical parameters indicates that ACh induces heterogeneous airway constriction with marked ventilation heterogeneities, this phenomenon giving rise to changes in G rather than altered intrinsic parenchymal mechanics[77]. We observed the potential of these volatile anaesthetics to prevent increases in R_{aw}, while they were all ineffective in inhibiting the ACh-induced increases in G. The inability of the volatile agents to prevent the increases in G is in contrast with previous findings *in vivo*[94,103]. The fundamental difference in the site of action of the cholinergic stimulation may explain this controversy. While the muscarinic receptors are stimulated in the whole lung during intravenous challenges under *in vivo* conditions, ACh administered into the pulmonary circulation in isolated perfused lungs reaches primarily the receptors located in the lung periphery, leading to a heterogeneous constriction of more distal airways. Under these conditions, the volatile agents are able to prevent the overall airway constriction, while the stability of G indicates that they are not effective in reducing the ventilation heterogeneities.

6. Summary and Conclusions

The studies included in the present thesis focused on achieving a better understanding of the pulmonary effects of the different levels of intra-alveolar and systemic CO_2 and common volatile anaesthetic agents under various conditions that occur during routine anaesthetic practice. The mechanical properties of the lungs were partitioned into airway and

parenchymal components by a model-based evaluation of the low-frequency pulmonary input impedance spectra measured by a forced oscillation technique.

The separate assessment of the airway and pulmonary parenchymal responses revealed the following findings:

a) Alveolar hypercapnia with the maintenance of a physiological CO_2 level in the systemic circulation exerted no effect on the lung mechanics.
b) In contrast, we highlighted that systemic hypercapnia and acidosis mainly generated central airway constriction, mediated primarily by the vagus nerve.
c) Decrease of the CO_2 concentration in the intrapulmonary gas to below the physiological value had no detectable effect on the lung mechanics until a concentration of ~2% (15.2 mmHg) was reached, whereas severe bronchoconstriction with marked ventilation heterogeneities in the lung periphery developed sharply when the intra-alveolar CO_2 concentration was lowered to < 2% (15.2 mmHg). This biphasic feature of the dose-response curve is of importance as concerns decreases in the ventilation-perfusion mismatch via redirection of the airflow to the well-perfused lung areas from those lung regions where the pulmonary perfusion is severely compromised.
d) Constrictor provocation through the pulmonary circulation has a more peripheral effect than those observed previously following iv challenges performed *in vivo*. This finding can be explained by the absence of the bronchial circulation in our study, and may have implications in the clinical evaluation of the reactivity of transplanted lungs.
e) Desflurane and sevoflurane have the potential to decrease the basal airway smooth muscle tone, whereas isoflurane and halothane are ineffective in this regard on a denervated isolated perfused lung model.
f) All the currently used volatile agents exert a marked protective effect against ACh-induced bronchoconstriction, desflurane and sevoflurane having the most potent inhibitory effects. These findings provide evidence that desflurane exerts relaxation activity on the airway smooth muscle that is similar to or even stronger than the activities of other common volatile anaesthetics at an organ level under *ex vivo* conditions.

7. Acknowledgements

I would like to express my gratitude to both of my supervisors. I am grateful to my supervisor Ferenc Peták for his valuable guidance and for his support in my experimental work. His excellent advice, instructions and tutoring have been indispensable in my research activity. I am also very grateful to my supervisor Barna Babik, who introduced me to this research and clinical area. I also greatly appreciate his continuous guidance, his invaluable advice and wisdom, and his limitless trust.

I would like to express my appreciation to Professor Zoltán Hantos, who also introduced me to this research area. I am honoured to have received his selfless and invaluable generous help, which has had a fundamental influence on my research career, and also his essential philosophy, which represents perfectionism, and imaginative and precious reasoning.

My grateful thanks are due to Professor Walid Habre for his highly valuable help in my research and clinical career. I greatly appreciate his support, which has been so important during all these years.

I also wish to express my thanks to Professor Denis Morel and all the staff of the Anaesthesiological Investigation Unit at the University of Geneva, for their contribution, and especially to Fabienne, Manu and Sylvie. I extend my thanks to the staff of the Department of Anaesthesiology and Intensive Therapy of Cardiac Surgery and the staff of the Institute of Surgical Research, University of Szeged, for their excellent cooperation.

I shall always be grateful to my mother and my friends, Attila, Helga and Anna, for their continuous support in the most difficult days.

The work presented in this thesis was supported by a Swiss National Science Foundation Grant (No. 3200-064899.01 - Bern, Switzerland) and Hungarian Scientific Research Grants (OTKA K81179 and K67700 - Budapest, Hungary).

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