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

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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. 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. Experiments performed 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, and even the global matching of alveolar ventilation to perfusion can decrease[1]. The direct relaxation potential of CO_2 on the bronchial smooth muscle has been demonstrated against the bronchoconstriction induced by constrictor drugs[2]. Further, bronchoconstriction resulting from temporary regional pulmonary arterial occlusions is reversed by normalizing the partial tension of alveolar CO_2.

The systemic CO_2 level lowers the vagal withdrawal, and the consequent enhanced vagal tone may induce airway resistance through an indirect vagal nerve-mediated regulatory pathway[3]. 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[3].

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[1] Domino KB et al. *Respiration physiology.* 111:89-100, 1998
[2] Swenson ER et al. *Am J Respir Crit Care Med.* 149:1563-1569, 1994
[3] Waldron MA et al. *Respir Physiol* 72:271-282, 1988

1.2. Effects of volatile anaesthetic agents on lung mechanics

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 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)[4]. 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, 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[5]. 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[6]. 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. 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.

1.3. Characterization of respiratory mechanics

Low-frequency forced oscillation technique: 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 or between 0.25 and 32 Hz in anaesthetized and paralysed patients. 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. 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-

[4] von Ungern-Sternberg BS et al. *Anesthesiology*. 108:216-24, 2008
[5] Habre et al. *Anesthesiology*. 94:348-53, 2001
[6] Schütz et al. *Br J Anaesth*. 92:254-60, 2004

expiration. Several investigations have indicated that the airways can be described by a frequency-independent R_{aw} (airway resistance) and inertance (I_{aw}). 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[7], 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 $\eta = G/H$. 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.

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.

[7] Hantos et al. *J Appl Physiol*. 72:168-78, 1992

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

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.

3.1. Animal models

3.1.1. Open-chest dogs with a cardiopulmonary bypass: Adult mongrel dogs were anaesthetized, paralyzed, and mechanically ventilated. The frequency was set to maintain a normal arterial CO_2 level (40 mmHg) in the pre-bypass period. 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 with non-pulsatile blood flow at 100 ml/kg/min and use of a membrane oxygenator. 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, arterial blood gas samples were analysed.

3.1.2. Isolated perfused rat lung model: Thirty-five adult male Sprague-Dawley rats were anaesthetized, tracheotomised, and normoventilated mechanically. In one group of rats, the induction and maintenance of anaesthesia were achieved with Nembutal; these animals served as a control group without the application of volatile anaesthetics. Respiratory gases were monitored continuously, and the airway pressure (P_{aw}) was also measured continuously. A midline sternotomy was next performed; the chest was widely retracted, and a heart-lung block was prepared and it was placed in a thermostatically controlled and humidified Plexiglas chamber. 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.

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 forced oscillatory input impedance spectra of Z_L was recorded during short intervals of suspended mechanical ventilation. 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. 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. 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. 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. The wave-tube was equipped with side-arms and miniature identical transducers to measure the lateral pressures at the loudspeaker end (P_1) and the cannula end (P_2). The input impedance (Z_L) was computed from the P_1/P_2 spectra as the load impedance of the wave-tube by applying fast Fourier transformation:

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

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. 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. 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.

4. Results

4.1. Alterations in lung mechanics by alveolar hypocapnia and hypercapnia

The effects of Fi_{CO_2} on the airway and lung tissue mechanical parameters represent a tendency to a decrease in R_{aw} was 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.

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. 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

In the experiments performed on dogs *in vivo*, the airway and lung tissue mechanical parameters were determined before and after systemic acidosis, and the subsequent effects of atropine were observed. 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.

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

The percentage changes in the basic airway and lung tissue mechanical parameters in all groups of isolated perfused rat lungs were established. 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.

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

The airway and parenchymal mechanical parameters under the control conditions (C1 and C2) and following the administration of the two different concentrations of Ach were determined. 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*, the values of H remained at the baseline level throughout the study protocol.

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. 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.

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).

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. 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. 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. Study of the influence of severe hypocapnia (< 0.3% or < 2.3 mmHg CO_2), which is feasible only under *in vitro* conditions, 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, 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}).

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). Our data indicate no detectable bronchoconstriction under these conditions, 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. Such a regional bronchoconstriction that develops due to alveolar hypocapnia 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.

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.

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. 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. 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.

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

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 or isolated tracheal and/or bronchial rings. 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.

As most previous studies applied global parameters to express the lung responses to volatile anaesthetic agents, 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.

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. 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*. 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.

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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.

II. Lele EE, Hantos Z, Bitay M, Szívós B, Bogáts G, Peták F, Babik B. Bronchoconstriction during alveolar hypocapnia and systemic hypercapnia in dogs with a cardiopulmonary bypass. *Respiratory Physiology & Neurobiology.* 2011; 175: 140-145.

List of papers related to the subject of this thesis

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.

II. Habre W, Adamicza A, Lele E, Novák T, Sly PD, Petak F. The involvement of histaminic and muscarinic receptors in the bronchoconstriction induced by myorelaxant administration in sensitized rabbits. *Anesthesia & Analgesia.* 2008; 107: 1899-1906.

III. Peták F, Albu G, Lele E, Hantos Z, Morel DR, Fontao F, Habre W. Lung mechanical and vascular changes during positive- and negative-pressure lung inflations: importance of reference pressures in the pulmonary vasculature. *Journal of Applied Physiology.* 2009; 106: 935-42.