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RESPIRATORY EFFECTS OF GENERAL ANESTHESIA AND PULMONARY EMBOLISM IN RODENT MODELS

PhD Thesis

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List of scientific publications included in the present thesis:

I. Comparison of the respiratory effects of commonly utilized general anesthesia regimes in male Sprague-Dawley rats

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II. Changes in lung mechanics and ventilation-perfusion match: comparison of pulmonary air- and thromboembolism in rats

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Scientific publication related to the subject of the present thesis:

III. Lung and chest wall mechanical properties in metformin-treated and untreated models of type 2 diabetes

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1. Introduction

1.1. Anesthesia

Anesthesia is vital for a wide variety of medical procedures, providing pain relief and creating optimal conditions for surgical interventions and intensive care. It is also crucial in medical research, since procedures often require the use of general anesthesia. General anesthesia procedures are known to have significant impact on the respiratory system, potentially leading to alterations in respiratory mechanics and gas exchange. This can manifest in reduced functional residual capacity, hypoxia, hypercapnia, and increased intrapulmonary shunting. Such blood gas abnormalities can alter respiratory mechanics, although mechanical ventilation can often mitigate these effects and prevent most related complications. Anesthetic agents can have a variety of direct and indirect effects on the respiratory system. Moreover, the impact of some anesthetic agents on respiratory function and mechanics has not been fully characterized yet.

1.2. Respiratory monitoring: capnography

Capnography is an essential tool in patient monitoring, particularly in respiratory care. This technique offers a non-invasive, continuous analysis of exhaled carbon dioxide, providing both numerical data and graphical representations.

The capnogram is typically divided into three phases. Phase 1 marks the beginning of exhalation, where the exhaled gas mixture comes from the conducting airways. Phase 2 shows the transition zone, where the gas from both the alveoli and conducting airways mixes. Phase 3 reflects the CO_2 content of alveolar gas. At the end of phase 3, inhalation begins and the capnogram curve returns to the baseline.

1.3. Pulmonary embolism

Embolism is a life-threatening cardiovascular event, that occurs when an embolus gets lodged in the circulatory system and causes a circulation disorder. It ranks third in causes of cardiovascular death worldwide, following stroke and myocardial ischemia. Diagnosing pulmonary embolism is challenging for clinicians due to its highly variable clinical symptoms, with the most common being pleuritic chest pain, dyspnea at rest, tachypnea, tachycardia, and hypoxia. Laboratory tests (especially D-dimer), ultrasound, chest CT, and analysis of end-tidal CO_2 levels and the capnogram curve can assist in the diagnosis. Additionally, various clinical guidelines, such as the Wells score system are useful diagnostic tools.

Embolisms can be categorized based on their formation. Fat embolism, typically following fractures of long bones, involves fat droplets causing the blockage. Fluid embolism, a rare type,

mostly occurs during childbirth when amniotic fluid enters the bloodstream. Two more common types are pulmonary thromboembolism, where a blood clot lodges in the arteries of the pulmonary system, and air/gas embolism, where an air bubble blocks the vascular system.

Although both gas embolism and thromboembolism compromise pulmonary blood flow, they differ substantially, particularly in the regional distribution of pulmonary perfusion defects. Gas bubbles in venous blood undergo turbulent mixing and disruption in the right ventricle, leading primarily to gravity-dependent diffuse pulmonary hypoperfusion. In contrast, the spatial distribution of thromboembolism is more focal, primarily affecting distinct lung areas. These fundamental differences in the spatial distribution of lung perfusion defects may influence adverse changes in respiratory mechanics, gas exchange, and ventilation-perfusion matching.

Treatment for pulmonary embolism may vary according to the severity of the condition. The primary therapies include anticoagulation, thrombolysis, or thrombectomy, but close observation of the patient is recommended in all cases.

2. Aims

The general aim of this thesis was to better understand the effects of different anesthetics and pulmonary air and thromboembolism on the respiratory system. In the first study we aimed to assess the respiratory effects of agents frequently used for general anesthesia, with a specific focus on the following research topics:

I. To investigate the respiratory effects of five widely used anesthetics in a common rodent model at different positive end-expiratory pressure levels;
II. To measure the responsiveness of the respiratory system under general anesthesia using different anesthetic agents;
III. To compare the mechanical properties and bronchial hyperreactivity across the anesthetic regimens used.

In the second study, we aimed to compare changes in indices reflecting airway and respiratory tissue mechanics, gas exchange, and capnography parameters in cases of gas embolism and thromboembolism. The specific aims of this study were:

IV. To measure the changes in lung mechanics, gas exchange, and capnography indices during diffuse gas embolism and focal thromboembolism;
V. To compare the outcomes of these two different types of lung injury based on the observed parameters.

3. Materials and methods

3.1. Ethical approval

Both experimental protocols were approved by the National Food Chain Safety and Animal Health Directorate of Csongrád County, Hungary no. XXXII./2110/2019 on December 16, 2019 and no. XXXII./2096/2018 on September 24, 2018.

3.2. Measurement of end-expiratory lung volume (EELV)

We measured EELV using a custom-made whole body plethysmography box. For these evaluations, the animals were placed in a sealed plexiglas box and ventilated normally.

During measurements, the trachea and box were closed for 10–15 s at end-expiration and pressures inside the box and the trachea were measured during spontaneous breathing efforts of the animal against the closed trachea. Measurements were performed at different levels of positive end-expiratory pressure (PEEP) and EELV was calculated from simultaneously measured pressure signals by applying the Boyle–Mariotte law. To compensate for differences in body size between animals, EELV values were normalized to body mass.

3.3. Measurement of respiratory mechanics by forced oscillations

In both studies the mechanical parameters of the airway and respiratory tissues were determined by measuring the input impedance of the respiratory system (Zrs) using the wave-tube approach of forced oscillations. Briefly, the tracheal cannula was connected to a loudspeaker-in-box system during a short (8 s) end-expiratory apnea by turning a three-way tap. The loudspeaker delivered a small amplitude pseudorandom forcing pressure signal through a wave-tube. Lateral pressures on both ends of the wave-tube were measured using two identical differential pressure transducers, low-pass filtered at 25 Hz, and digitized using an analogue-digital converter of a data acquisition board at a sampling rate of 256 Hz. Pressure transfer functions were calculated with fast Fourier transformation from each recording. The Zrs was calculated as the load impedance of the wave-tube from the recordings. The input impedance of the endotracheal tube and the connections had previously been determined and subsequently subtracted from each Zrs spectrum.

A well-validated model was fitted to the Zrs spectra by minimizing the relative difference between the measured and modelled impedance data. The model included an airway resistance (Raw) and inertance (Iaw) in series with a constant-phase tissue compartment consisting of tissue damping (G) and elastance (H). G describes the dissipative properties (damping and resistance) while H describes the stiffness (elastance) of the respiratory tissues (lung and chest wall). Raw and Iaw mainly describe the flow resistance and inertance of the conducting airways.

3.4. Blood gas analyses

Samples of 0.15 ml arterial blood were collected for blood gas analyses, from which the arterial partial pressures of oxygen (PaO_2) and carbon dioxide ($PaCO_2$) were determined using a point-of-care blood analyzer system.

3.5. Recording and analyses of volumetric capnogram

The partial pressure of CO_2 in expired gas and ventilation airflow (V') were simultaneously recorded using a rodent sidestream volumetric capnograph at 256 Hz. Volumetric capnogram curves were generated for each expiratory cycle by combining the CO_2 and volume signals. Phase boundaries were determined, and shape factors were calculated based on established concepts. The phase 2 inflection point, identified as the maximum of the first derivative of the capnogram curve, determined the phase 2 slope (S2V), while phase 3 slope (S3V) was determined by a linear regression of the middle third of phase 3. Normalized phase 2 (Sn2V) and 3 (Sn3V) slopes were calculated by dividing S2V and S3V with end-tidal CO_2, allowing objective comparisons of capnogram shape changes due to variations of end-tidal CO_2, as seen in embolism models.

Volumetric capnography also enabled calculation of Fowler's anatomic dead space (VDF), and physiological dead space according to Bohr (VDB) and Enghoff (VDE); with the latter including intrapulmonary shunting.

3.6. Study protocols

3.6.1. Study protocol of anesthesia study

Following anesthesia and animal preparation, EELV was measured using whole-body plethysmograph with increasing levels of PEEP at 0, 3 and 6 cmH_2O. Respiratory mechanics were determined using forced oscillations while maintaining the same PEEP levels as with EELV. Arterial blood samples were collected and analyzed at each PEEP level. Finally, the responsiveness of the respiratory system to an exogeneous bronchoconstrictor stimulus was measured with administration of a continuous intravenous (iv.) infusion of methacholine (MCh) at increasing doses (0, 4, 8, 16 and 32 μg/kg/min) while maintaining a PEEP of 3 cmH_2O. All measurements were performed under anesthesia maintained in accordance with group allocation.

3.6.2. Group allocation according to anesthesia induction and maintenance

In the experiment where we compared the effects of different anesthetics on respiratory mechanics, we performed our measurements on forty male Sprague-Dawley rats. The animals were randomly allocated to one of five experimental groups (n=8). Groups were assigned according to the anesthetic agent used for induction: group PB were administered an

intraperitoneal (ip.) bolus of sodium pentobarbital, group KX were administered an ip. injection of ketamine and xylazine, group U were administered an ip. injection of urethane, and groups PF (propofol-fentanyl) and group S were administered inhalational sevoflurane. Animals were surgically prepared, and anesthesia was maintained according to group allocation. Anesthesia was maintained using repeated iv. boluses of sodium-pentobarbital in group PB, applying repeated ip. boluses of ketamine and xylazine in group KX, using a continuous iv. infusion of propofol and fentanyl in group PF or continuous inhalation of sevoflurane through a tracheostomy using a ventilator in group S. Additional maintenance of anesthesia following induction was not required in group U.

3.6.3. Study protocol of embolism study

Following anesthesia induction and surgical preparations, including the thoracotomy, the rats were ventilated with a PEEP of 5 cmH_2O throughout the study. Baseline data were collected by recording capnograph curves and Zrs data, along with blood gas analyses of arterial blood samples. Pulmonary air embolism was then induced by injecting 0.1 ml of air mixed with 0.9 ml of saline into the femoral vein while continuously monitoring the changes in systemic hemodynamics and the capnogram. When a peak decrease in $ETCO_2$ was observed, an additional data set was recorded. The effects of pulmonary air embolism on systemic hemodynamics and gas exchange parameters lasted approximately 2–4 minutes and then returned to the normal range after approximately 5 minutes, as verified by the continuous monitoring of relevant vital signs. After a 10–15-min stabilization period, the left pulmonary artery was clamped using a small-sized surgical clip, and a third data set, including capnography, forced oscillations, and blood gas analyses, was collected under this condition, i.e. during left pulmonary artery occlusion.

3.7. Statistical analyses

3.7.1. Statistical analysis of anesthesia study

Data are presented as mean ± SD for normally distributed variables. Normality was checked using the Shapiro-Wilk test. Two-way repeated-measures analyses of variance (ANOVA) with Holm-Šidák post hoc tests were performed to assess the effects of various anesthesia regimes. One-way ANOVA was applied to test the responsiveness of the respiratory system. Raw was the primary outcome variable for estimating the sample size.

3.7.2. Statistical analysis of embolism study

Data are presented as the median with interquartile ranges in each boxplot chart. The Shapiro-Wilk test was used to evaluate the data distributions for normality. For each parameter studied, one-way repeated-measures ANOVA with Holm–Šidák post hoc tests was applied.

Statistical analyses were performed with the SigmaPlot software. H was considered the primary outcome variable for estimating sample size.

4. Results

4.1. Results in anesthesia study

No significant differences in body masses were observed between groups ($p=0.92$). Significant increases in EELV were observed at higher PEEP levels in all groups ($p<0.001$ for all), with no differences observed between any of the experimental groups ($p>0.10$).

Raw significantly decreased with increasing PEEP ($p<0.001$ for all), with no differences observed between any of the experimental groups ($p=0.54$). Similar trends were observed in terms of the tissue mechanical parameters G and H, with marked effects of PEEP ($p<0.001$ for all) and no differences between experimental groups ($p=0.36$ for G and $p=0.33$ for H).

No significant differences in PaO_2 values were observed between groups ($p=0.27$); however, a negative PEEP-dependence was observed ($p<0.001$). Increased PEEP caused significant increases in $PaCO_2$ in all groups ($p<0.05$ in all), with no differences observed between any of the experimental groups ($p=0.52$). Increasing PEEP levels had no effect on arterial pH ($p=0.63$), with no differences in arterial pH values observed between experimental groups ($p=0.84$). Non-fasting serum glucose levels were not affected by PEEP and were within the normal range in groups PB and PF ($p>0.05$ in all groups). While PEEP had no significant effect on the serum glucose levels in group S ($p>0.50$), significantly higher serum glucose levels were observed in group S at a PEEP of 0 cmH_2O compared to groups PB and PF ($p=0.03$ and $p=0.02$, respectively). There was a tendency for this difference to be also present at a PEEP of 3 cmH_2O ($p=0.06$ for PB and $p=0.15$ for PF), while serum glucose levels in group S did not differ from those in groups PB or PF at a PEEP of 6 cmH_2O ($p=0.41$ and $p=0.61$, respectively). Of note, serum glucose levels remained within the normal range for most animals in group S. Conversely, markedly elevated serum glucose levels that exceeded the normal range were observed in groups KX and U, and these values were significantly higher than in all other groups ($p<0.001$). While PEEP had no effect on the above-normal glucose levels in group U ($P>0.05$), a significant increase in serum glucose levels with increasing PEEP was observed in group KX ($p<0.02$).

Heart rate was stable in all groups ($p=0.57$) regardless of the PEEP applied, with no significant differences between experimental groups ($p=0.18$). Mean arterial pressure decreased in response to elevated PEEP levels in all groups ($p<0.01$). Animals in groups S and U had significantly lower mean arterial pressure values compared to the other three groups ($p<0.01$).

During iv. MCh provocation, group KX exhibited increased reactivity in Raw compared to the other groups, with statistically significant increases observed at 8 and 16 μg/kg/min. Accordingly, the highest increases in Raw were in group KX compared to other groups ($p<0.001$). Due to severe bronchoconstriction (535%±223% increase compared to baseline), MCh provocation was aborted in all animals in group KX at 16 μg/kg/min instead of 32 μg/kg/min in other groups. In all other groups, significant elevations in Raw were observed during MCh infusion rates of 16 and 32 μg/kg/min compared to baseline.

Significant elevations of G were also observed in group KX at MCh infusions at 4 μg/kg/min and above compared to baseline ($p<0.001$). No differences were observed in G between the other experimental groups ($p>0.29$), with significantly higher G values observed at MCh infusions of 16 and 32 μg/kg/min ($p<0.001$). In terms of H, no significant differences were observed between experimental groups ($p=0.80$), with significant elevations observed starting at MCh infusion rates 4 μg/kg/min and above ($p<0.001$).

MCh administration had no effect on PaO_2 in most groups; however, lower PaO_2 values were observed in group S ($p<0.01$). Similar trends were observed with $PaCO_2$ values, with decreased $PaCO_2$ values observed in group U at the two highest doses of MCh ($p<0.04$). Significant difference in arterial pH were observed at the highest dose of MCh compared to baseline in all groups ($p<0.04$) with the exception of group U ($p=0.7$). Despite the differences in these trends, no between-group differences were observed at any of the MCh infusion rates used.

4.2. Results in embolism study

No significant changes in the mechanical parameters were observed after pulmonary air embolism. In contrast, significant increases in all parameters reflecting airway (Raw) and respiratory tissue mechanics (G and H) were obtained after clamping the left pulmonary artery compared to baseline values ($p<0.05$ for all). Significant differences in Raw and G were observed between the two forms of embolism ($p<0.05$ for all), while there was a trend toward a significant difference in H ($p=0.1$).

In accordance with our experimental approach to induce comparable gas exchange defects assessed in the expired CO_2 concentration, the significant decreases in $ETCO_2$ did not differ between the two embolism models ($p<0.001$ vs. baseline for both). Furthermore, increases in PaO_2-$ETCO_2$ gradient of comparable magnitude were observed in both embolism models ($P<0.05$ vs. baseline for both). A significant decrease in PaO_2 and a significant increase in $PaCO_2$ were observed following pulmonary air embolism ($p<0.001$ for both). However, these parameters were not significantly affected by left pulmonary artery occlusion, resulting in

significant differences in PaO_2 and $PaCO_2$ between the two forms of pulmonary embolism ($p<0.001$ for both). There was a strong tendency for an increase in the arterial lactate level, with increasing from the baseline value of 2.6±0.8 mmol/l to 3.6±1.3 and 4.3±1.8 mmol/l after pulmonary air embolism and left pulmonary artery occlusion, respectively ($p=0.056$).

No significant change in Sn2V was observed after either form of embolism. In contrast, significant increases in Sn3V were evidenced following left pulmonary artery occlusion compared to baseline and after pulmonary air embolism ($P<0.05$ for both).

There were no significant changes in VDF or VDB following either form of embolism. However, a significant increase in the VDE dead space fraction was observed following pulmonary air embolism and left pulmonary artery occlusion ($P=0.003$ and $P<0.05$, respectively).

Pulmonary air embolism and left pulmonary artery occlusion induced significant transient decreases in mean arterial pressure and heart rate ($p<0.001$, for all). Complete recoveries of these parameters were observed after pulmonary air embolism with no significant difference compared to the values obtained before the interventions.

5. Discussion

The results of the studies included in the present thesis demonstrated changes in the respiratory system in two different translational animal models.

The mechanical properties of the airways and the respiratory tissues are of critical importance in determining gas exchange. The respiratory mechanics is affected by a wide variety pulmonary disorders and diseases (e.g. COPD, asthma, pulmonary embolism) and medical interventions (e.g. various drugs, mechanical ventilation, general anesthesia). Although the pharmacological effects of the various anesthetic agents have been studied excessively, there is still a lack of knowledge of how they affect respiratory mechanics. Thus, we focused our first investigation on how sodium pentobarbital, ketamine-xylazine, propofol-fentanyl, sevoflurane, and urethane affect various properties of the respiratory system and we also assessed their modulation potential in response to bronchoprovocation.

Furthermore, pulmonary embolism is a common and potentially life-threatening condition. One of the quick and accurate diagnostic tools of embolism is the characteristic distortion of the capnogram curve. With regard to the type of embolism, different changes in the capnogram curve and respiratory mechanics can be observed when the pulmonary ischemia is subsequent to air bubble or a thrombus. Therefore, in our second study we aimed to compare these changes in translational models of air- and thromboembolism.

5.1. Anesthesia study

In this study we aimed to characterize the respiratory effects of iv. sodium-pentobarbital, iv. propofol-fentanyl, ip. ketamine-xylazine, inhaled sevoflurane and ip. urethane, which are all commonly used anesthesia regimes in rats. No significant differences in lung volume, baseline respiratory mechanics or blood gas parameters were observed between the anesthesia regimes at three different levels of PEEP. On the contrary, significant bronchial hyperreactivity was observed with the administration of increasing doses of iv. MCh in rats undergoing ketamine-xylazine anesthesia. Respiratory parameters in all groups were in good agreement with data acquired previously by us in healthy animals in the same mass range and rats undergoing pentobarbital anesthesia.

5.1.1. Effects of general anesthesia on baseline respiratory mechanics

The lack of differences in baseline respiratory mechanical parameters between experimental groups in this study may be attributable to a number of reasons. General anesthesia induces respiratory depression with practically all anesthetic agents to various extents, and such effects are particularly evident with the use of pentobarbital and propofol. However, all animals were mechanically ventilated in this study, which prevented lung volume loss and subsequently contributed to the similar EELV values observed between experimental groups. This feature of this study in addition to physiologically low basal airway smooth muscle tone can explain the lack of the previously described bronchodilator effects of sevoflurane and propofol. While the bronchodilator effect of sevoflurane has been consistently reported in subjects with increased bronchial tone (e.g. asthma, chronic obstructive pulmonary disease and following cardiopulmonary bypass), the bronchodilator effect of sevoflurane in normal subjects remains controversial. Similar to our findings, no bronchoactive properties of propofol or sevoflurane were demonstrated under baseline conditions in a previous study, with propofol found to have no bronchoprotective effect against bronchoconstrictive agonists.

5.1.2. Effects of general anesthesia on the responsiveness of the respiratory system

In contrast to our findings under baseline conditions, anesthesia regimes can significantly affect respiratory function during MCh-induced bronchoconstriction. Significant increases in Raw during ketamine-xylazine anesthesia demonstrated severe bronchial hyperreactivity compared to all other anesthetic regimes. The mechanism underlying this hyperreactivity remains unclear. Ketamine acts against cholinergic bronchoconstriction in isolated human and guinea pig bronchi, and sensitized rats and sheep *in vivo*. Previous studies on the bronchial effects of xylazine are limited; however, almost all describe a bronchodilatative effect of xylazine through activation of bronchial alpha-2 receptors. The discrepancy between the

previously described bronchial effects of these two anesthetics when used in isolation and our results with the combined administration of ketamine and xylazine indicates that the combination of the two drugs may result in increased airway reactivity. This increased Raw was also coupled with increased G and decreased arterial pH. While Raw is considered a measure of flow resistance in central conducting airways, G has more contributing factors. Elevation of G can most prominently indicate ventilation heterogeneity due to inhomogeneous small airway constriction and/or closure. As ketamine reportedly increases the collapsibility of the lung periphery, subsequent increases in ventilation heterogeneity may explain substantial increases in G. While the protective effects of volatile anesthetics (including sevoflurane) against cholinergic bronchoconstriction are well-established, we were unable to demonstrate a protective effect of sevoflurane on bronchoconstriction in this study. This finding may be attributable to the sustained bronchoconstriction and prolonged administration of sevoflurane in our study protocol. Indeed, sevoflurane has previously been shown to reverse cholinergic bronchoconstriction only transiently with a short duration of effect (approximately 5 minutes).

5.1.3. Effects of general anesthesia on blood sugar levels

Apart from the respiratory effects of the applied anesthetics, their applicability and other vital parameters also warrant discussion. Non-fasting serum glucose values were within the normal range in animals under sodium-pentobarbital, propofol-fentanyl or sevoflurane anesthesia. However, blood sugar values above the normal range were observed under urethane and ketamine-xylazine anesthesia. Urethane-induced hyperglycemia may be mediated by increased sympathetic activation via alpha-2 receptors. The progressive hyperglycemia observed in group KX has previously been observed in studies using ketamine or xylazine alone and in combination. This finding may also be attributable to an alpha-2 receptor-mediated response, resulting in inhibition of insulin secretion.

5.1.4. Effects of general anesthesia on systemic hemodynamics

Normal mean arterial pressure values and heart rate were observed in all groups, with the lowest pressure values (approximately 60 mmHg) observed in animals under S and U anesthesia. This finding is in concordance with the results of previous studies describing the hemodynamic effects of these anesthetic agents.

5.1.5. Effects of general anesthesia on other organ systems

Our findings on the effects of anesthetic agents on pulmonary system can also be considered in the context of their generalized potential to influence the function of various other organ systems. Tendency for neuroprotection was proposed for volatile agents, such as sevoflurane against neuronal injury in rats, barbiturates like pentobarbital, propofol and ketamine.

Conversely, the neurological effects of urethane are different compared to other anesthetics without a major effect on the peripheral nervous system and various subcortical areas, thereby allowing the induction of various peripheral reflexes or characterizing cerebral processes like natural sleep. This characteristic of urethane also results in a lesser amount of respiratory depression in spontaneously breathing animals without inducing apneic periods observed with other anesthetics. Related to the effects of short-term general anesthesia on the liver, various studies have described either dose-dependent or idiosyncratic hepatotoxicity after using sevoflurane or propofol, but not with most barbiturates used for sedation or anesthesia, or ketamine. These hepatotoxic effects need however, a longer timeframe to develop. It is important to note the carcinogenic effects described with urethane, resulting in the development of both benignant and malignant tumors in various organs of the animals, especially after repeated injections. Thus, it is not recommended to use urethane anesthesia in surviving animals.

5.1.6. Considerations with depth of anesthesia

Regarding the depth of anesthesia, no signs of inadequate anesthesia or inconsistency in the plane of anesthesia were observed in any of the experimental groups, as confirmed by the stability of heart rate and blood pressure in similar experimental conditions and also before and after the administration of boluses where applicable. The depth of anesthesia was also comparable between the different study groups, as the vital parameters of the animals were comparable with differences explainable by the known effects of the various regimes. Of note, the onset of anesthesia was extremely long in group U (approximately one hour), and while the dose was determined based on previous studies and published best practices, half of the animals (N=4) required an additional injection of approximately 50% of the dose administered before baseline measurements.

5.2. Embolism study

This study revealed fundamental differences between models of pulmonary embolism, although comparable decreases in the expired carbon dioxide concentration were induced by the gas- and thromboembolic models. Unilateral pulmonary arterial occlusion caused marked elevations in the bronchial tone and compromised lung tissue mechanics, whereas pulmonary air embolism did not affect lung mechanics. In contrast with these lung mechanical changes, pulmonary air embolism was the only insult that caused significant deteriorations in the arterial blood gas parameters, while partial pressures of oxygen and carbon dioxide in the arterial blood were unaffected by left pulmonary artery occlusion. No changes VDF or VDB reflecting ventilated but poorly perfused lung areas were observed in either embolism model. Conversely,

both pulmonary air embolism and unilateral pulmonary artery occlusion resulted in elevated alveolar dead space indices that indicate intrapulmonary shunting (Enghoff dead space).

5.2.1. Changes in airway mechanics following pulmonary embolism

The main finding of this study was that distinct lung mechanical responses developed in response to the different models of lung embolism, even in the presence of similarly deteriorated $ETCO_2$ levels. In contrast to the lack of changes in lung mechanics following pulmonary air embolism, marked bronchoconstriction developed in response to left pulmonary artery occlusion. This finding can be explained by the ability of the CO_2 content of the intrapulmonary gas to modulate the airway smooth muscle tone, resulting in severe bronchospasm below a threshold partial pressure of CO_2 of approximately 10 mmHg. Reaching this threshold CO_2 concentration in the ischemic left lung during left pulmonary artery occlusion explains the elevations in Raw. The magnitude of the overall increase in Raw was blunted by the intact right lung, which was probably somewhat overinflated. Conversely, the diffuse pulmonary hypoperfusion that developed after pulmonary air embolism was likely to cause a moderate and relatively evenly distributed hypocapnia in the bronchoalveolar system, in which the threshold concentration to trigger a bronchial smooth muscle contraction was not reached.

5.2.2. Changes in respiratory tissue mechanics following pulmonary embolism

These differences in the airway responses between pulmonary air embolism and left pulmonary artery occlusion were associated with distinct changes in the forced oscillatory parameters reflecting G and H. The excessive increase in G over those in H indicates the development of ventilation heterogeneities. As this pattern of change was observed only after left pulmonary artery occlusion, the impairment in respiratory tissue mechanics indicates that considerable ventilation inhomogeneity developed after left pulmonary artery occlusion due to uneven lung ventilation resulting from the additive effects of volume loss in the left lung and overdistension of the right lung. The presence of this phenomenon was confirmed by the elevations in mechanical and capnography parameters sensitive to ventilation heterogeneities (Sn3V).

5.2.3. Blood gas parameters following pulmonary embolism

Changes in blood gas parameters notably differed between the two embolism models, with significant deteriorations observed only after a diffuse pulmonary perfusion defect generated by pulmonary air embolism. In agreement with previous findings, acute pulmonary air embolism led to the development of hypoxia and hypercapnia. The adverse gas exchange defects were reflected in the alveolo-end-tidal CO_2 gradient and in the marked elevations in

VDE. Since VDE incorporates poorly ventilated but perfused alveoli with low V/Q, increased intrapulmonary shunting may be responsible for this finding as a consequence of a perfusion redistribution. Interestingly, blood gas parameters were unaffected by left pulmonary artery occlusion despite concomitant increases in capnography parameters reflecting intrapulmonary low V/Q areas and shunting ($PaCO_2$-$ETCO_2$ gradient and VDE) and in the airway and respiratory tissue mechanics. The absence of changes in PaO_2 following left pulmonary artery occlusion agrees with previous results demonstrating that oxygenation can remain normal following acute pulmonary thromboembolism. This finding is likely attributable to the unaffected right lung being able to maintain blood gas parameters within the normal range due to increased VT in response to hypocapnic bronchoconstriction in the ischemic left lung.

5.2.4. Anatomical and physiological dead spaces

No change in VDF was observed after inducing lung embolism using air bubbles or unilateral pulmonary vascular ischemia. As VDF reflects the amount of gas in the conducting airways, this finding demonstrates that there was no change in the anatomical dead space fraction following either intervention. While severe bronchoconstriction in the hypocapnic left lung may yield a unilateral reduction in apparent anatomic dead space, the redistribution of VT to the right lung may have overinflated this compartment, thereby counterbalancing the anatomic dead space.

The lack of change in VDB after either form of embolism is worth noting. Unilateral occlusion of the pulmonary artery is expected to result in nonperfused but well-ventilated lung regions. Furthermore, the development of a similar ventilation-perfusion mismatch is expected after introducing air into the pulmonary circulation. Accordingly, elevations in VDB can be anticipated in both models of embolism based on Riley's 3-compartment lung model, in which VDB reflects the amount of well-ventilated but poorly perfused alveolar compartments. This seemingly controversial result can be explained by the complex pathophysiological processes initiated by the massive ventilation-perfusion mismatch after embolism. Development of severe bronchoconstriction in the hypocapnic left lung following left pulmonary artery occlusion can reach a degree where airflow redistribution from the left into the right lung results in a constant VDB. The presence of this mechanical defect after left pulmonary artery occlusion was confirmed by our forced oscillatory data demonstrating marked elevations in Raw. The lack of change in VDB following pulmonary air embolism may be attributable to the relatively small amount of air reaching the pulmonary circulation (6%–8% of total intrapulmonary blood volume) that may have caused no major perfusion defects.

9. Summary and conclusions

This thesis presents a systematic comparison of the effects of commonly used anesthetic agents on the respiratory system at different PEEP levels and respiratory responsiveness to an exogenous constrictor stimulus.

Summarizing the effects of different anesthetic regimes on respiratory mechanics in rats, our findings led to the following conclusions:

I. No differences were observed in baseline respiratory mechanics and function between the anesthesia regimes at any PEEP levels.

II. Although baseline mechanical parameters were consistent, bronchial hyperreactivity developed under ketamine-xylazine anesthesia.

III. The baseline respiratory mechanics of ventilated rats are not influenced by the commonly used anesthesia regimes at generally administered doses, allowing for a fair comparison among experimental animals undergoing different anesthesia protocols. Furthermore, ketamine-xylazine anesthesia may not be suitable for studies where bronchial reactivity is a critical component (e.g., sensitization studies).

The outcomes of the second study provide unique data on the differences between diffuse lung injury induced by intravenous injection of air bubbles, modeling gas embolism, and focal thromboembolism caused by clamping the left pulmonary artery. Regarding the effects of pulmonary air and thromboembolism on respiratory mechanics and ventilation-perfusion matching in rats, our findings led to these conclusions:

IV. Deteriorations in lung mechanics, reflecting the development of heterogeneous bronchoconstriction, were observed after a focal ischemic insult to pulmonary perfusion. The severity of hypocapnia did not reach the threshold level to alter bronchial tone in any lung regions following a diffuse pulmonary perfusion defect subsequent to pulmonary air embolism.

V. Contrasting with the mechanical changes, arterial blood gas parameters deteriorated following a diffuse pulmonary perfusion defect due to pulmonary air embolism, while focal perfusion defects due to pulmonary vascular occlusion triggered a compensatory respiratory mechanical response. This response led to airflow redistribution to lung areas with maintained perfusion, thereby maintaining normal oxygenation and CO_2 elimination. The different impacts of air and thromboembolism on lung mechanics and gas exchange may have implications in tailoring respiratory support for patients.

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