Bronchoconstriction during alveolar hypocapnia and systemic hypercapnia in dogs with a cardiopulmonary bypass

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1. Introduction

Numerous factors contribute to the regulation of the bronchial tone via various excitatory and inhibitory mechanisms, including direct neural control and humoral/neural mediators. Carbon dioxide (CO₂) also plays a major role in this regulation: the relaxation of CO₂ on the bronchial smooth muscle has been demonstrated against the bronchoconstriction induced by constrictor drugs (Astin et al., 1973; Sterling et al., 1972). Further, the bronchoconstriction resulting from temporary regional pulmonary arterial occlusions is reversed by normalizing the alveolar CO₂ level (FACO₂) of 0.2–7% and during systemic hypercapnia before and after elimination of the vagal tone. Airway resistance (Rₐw), inertance (Iₐw), parenchymal damping (Gₚ) and elastance (H) were estimated from the Zₛ. The highest Rₐw observed at 0.2% FACO₂, which decreased markedly up to a FACO₂ of 2% (212 ± 24%), and remained unchanged under normo- and hypercapnia (FACO₂ 2–7%). These changes were associated with smaller decreases in Iₐw (−16.6 ± 3.7%), mild elevations in G (25.7 ± 4.7%), and no change in H. Significant increases in all mechanical parameters were observed following systemic hypercapnia; atropine counteracted the Rₐw rises. We conclude that severe alveolar hypocapnia may contribute to minimization of the ventilation-perfusion mismatch by constricting the airways in poorly perfused lung regions. The constrictor potential of systemic hypercapnia is mediated by vagal reflexes.

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increases the systemic CO₂ level which may lead to vagally mediated bronchoconstriction (Ingram, 1975; Nadel and Widdicombe, 1962). This phenomenon may have a synergistic constrictor effect on the affected airways, which becomes indistinguishable from the direct effects of altered intra-alveolar CO₂ 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 allow a detailed characterization of how the different CO₂ 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 CO₂ levels precisely in vivo, without involvement of the biasing effects of the an altered ventilation pattern or pulmonary ischemia, the advantages of this approach have not been utilized for a systematic exploration of the effects of an altered CO₂ level on the airway tone.

Hence, the aims of the present study were (i) to establish a dose–response curve relating to a wide range of alveolar CO₂ level without affecting the ventilation pattern, and (ii) to characterize the differences in the regulatory role of CO₂ on the airway tone when it is delivered to the resident gas in the airways or added directly to the blood. To achieve these aims, we subjected dogs to a CPB, which allows extensive manipulation of the alveolar and circulatory CO₂ levels independently in the airways and in the oxygenator. While manipulation of the alveolar CO₂ level has an impact on the entire lung, alterations in the systemic blood CO₂ level most probably affect primarily the proximal airways, since they are supported by the bronchial circulation with systemic origin. Thus, the lung mechanical changes were assessed by using the low-frequency forced oscillation technique, which permits separate the evaluation of the mechanical changes in the central and peripheral lung compartments.

2. Materials and methods

2.1. Animal and methods

After approval from the Institutional Animal Care and Use Committee of the University of Szeged, eight adult mongrel dogs (23.7 ± 5.0 kg) were anesthetized (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 vecuronium bromide (0.1 mg/kg). The dogs were then intubated with an 8–9-mm–internal diameter 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 cmH₂O were utilized for a systematic exploration of the effects of an altered CO₂ level on the airway tone.

The anesthesia was maintained by continuous iv infusion of propofol (50 μg/kg/min) and a muscle relaxant was administered as needed. After opening of the chest by midline sternotomy, anticoagulant (heparin, 3 mg/kg) 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% O₂ in air with controlled concentration of CO₂ added to this gas mixture from a cylinder attached to the low-pressure gas input of the respirator. The end-tidal partial pressure of CO₂ (PETCO₂) and FICO₂ were monitored (Datex, Oscar Helsinki, Finland). Arterial blood gas samples were analyzed (model 505, Acid Base Laboratory, Copenhagen, Denmark).

2.2. Measurement of airway and parenchymal mechanics

The measurement system for the collection of the forced oscillatory input impedance spectra of the pulmonary system (ZIC) was similar to that described in detail previously (Babik et al., 2003; Hantos et al., 1992, 1995; Kaczka et al., 2009). The set-up used for impedance measurements during short intervals of suspended mechanical ventilation is shown schematically in Fig. 1. 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 apneic period, small-amplitude (1.5 cmH₂O 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). To exclude endotracheal tube impedance from the measurements, tracheal pressure (Pₜ) was measured with an identical pressure transducer through a 1.5-mm-OD polyethylene-
lene 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 electric signal driving the loudspeaker and the measured signals of $P_{tr}$ and $\dot{V}$ were computed by fast Fourier transformation with 10-s time windows and 95% overlapping.

2.3. Separation of airway and parenchymal properties

To separate the airway and the lung tissue mechanics, a model containing a frequency-independent airway resistance ($R_{aw}$) and inertance ($I_{aw}$) in series with a constant-phase tissue model (Hantos et al., 1992), including parenchymal damping ($G$) and elastance ($H$), was fitted to the $Z_L$ spectra:

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

where $j$ is the imaginary unit, $\omega$ 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 ($\eta$) was calculated as $\eta = G/H$ (Fredberg and Stamenovic, 1989). The model was fitted to each average impedance spectrum by minimizing the differences between the measured and the modeled impedance data. The optimization procedure was used with a relative (weighted) fitting criterion: the differences between the measured and modeled impedance values were normalized by the impedance magnitude at each frequency point (Hantos et al., 1990).

2.4. Experimental protocol

After surgical preparation, alveolar hypocapnia was induced by applying a total bypass with cessation of the pulmonary blood flow. This maneuver allowed the decrease of $\text{PET}_{\text{CO}_2}$ 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 $F_{ICO_2}$, accomplished by altering the CO$_2$ flow from the cylinder attached to the low-pressure input of the respirator. After a 2–3-

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**Fig. 2.** Group means ± SE ($n=8$) for the airway and lung parenchymal mechanical parameters at different levels of $FICO_2$. $R_{aw}$, airway resistance; $I_{aw}$, airway inertance; $G$, lung parenchymal damping; $H$, lung parenchymal elastance; $FICO_2$, fraction of inspired carbon dioxide. *p<0.05 vs. the values obtained under normocapnia ($FICO_2 = 4\%$, 30.4 mmHg).
Scatters in parameters were expressed in SE values. Repeated measures one-way analysis of variance (ANOVA) with FlCO₂ as variable was applied to compare mechanical parameters. Pairwise comparisons were performed by using Student–Newman–Keuls multiple comparison procedures. The effects of systemic acidosis and the administration of atropine were assessed by using paired t-tests on the mechanical parameters. Statistical significance was accepted at the p < 0.05 level.

3. Results

For a precise statistical evaluation of the changes in the respiratory mechanics in response to the alterations of FlCO₂, the values of the model parameters were obtained at discrete FlCO₂ 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 FlCO₂ vs. Raw, Iaw, G, H, and η data in each individual animal. Fig. 2 depicts the group means of these values for the airway and lung parenchymal mechanical parameters. This analysis revealed a markedly elevated Raw (243.2 ± 334.7%, p < 0.05 vs. normocapnia defined as 5% or 38 mmHg) at FlCO₂ levels lower than 1% (7.6 mmHg), associated with a smaller decrease in Iaw (−17.2 ± 25.0%, p < 0.05) at lower FlCO₂ levels. In regards to the lung parenchymal parameters, G and η were moderately elevated (38.4 ± 63%, 22.5 ± 27%, respectively; p < 0.05 for both) at low FlCO₂, whereas no significant changes occurred in H (15.2 ± 21.5%; NS) throughout the study protocol. There were no detectable alterations in Raw, Iaw, G, H and η in the presence of alveolar hypercapnia.

The airway and lung tissue mechanical parameters determined before and after systemic acidosis, and the subsequent effects of atropine, are demonstrated in Fig. 3. Raw and Iaw 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 Raw statistically significantly, while atropine had no effects on the elevated Iaw.

The 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. Discussion

In the present study, the alterations in the airway and lung parenchymal mechanical properties were investigated when different levels of CO₂ were maintained in the alveoli and in the systemic circulation. The application of extracorporeal circulation in the experimental design allowed the manipulation of intra-alveolar CO₂ 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 the Raw to CO₂ revealed that, instead of a gradual increase in the bronchial tone, there was a sharp elevation in Raw at very low alveolar CO₂ levels (≤1% or ≤7.6 mmHg). In contrast with the neutral effects of alveolar hypocapnia on the lung mechanics, hypercapnia induced into 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.
The present study was designed to characterize the effects of CO₂ through wide-ranging chances of its concentration in the alveolar gas or in the blood in the systemic circulation, while the intact neurohormonal control was maintained. Utilization of a CPB was ideal for these purposes, since this approach not only allows the investigation of severe alveolar hypopcapnia, but also permits independent manipulation of the alveolar and systemic CO₂ levels. 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 CPB), for otherwise the elevated systemic CO₂ would consequently appear in the alveoli. Vice versa, the elevated PₐCO₂ during CPB has no direct systemic effects, as the interrupted pulmonary circulation cannot transmit the CO₂ induced into the systemic circulation. We elevated PETCO₂ levels in increasing 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 CO₂ level. Although the randomized order of the CO₂ levels would have decreased the potential temporal effects in the experiments, the increasing order of PETCO₂ was not likely to affect our findings significantly. While the bronchoprotecting potential of propofol might have blunted the constrictor effects of CO₂ (Brown and Wagner, 1999), our dose was much lower than that proved to be effective against methacholine-induced bronchoconstriction.

While the airway parameters Rₐw and Iₐw determined in the present study 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 (Hantos et al., 1990, 1992). 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 (Petak et al., 2004). However, this bias was independent of the alveolar or systemic CO₂ levels; and thus, the main findings of the present study are not affected by this phenomenon.

4.1. Effects of alveolar hypocapnia and hypercapnia

A number of previous in vivo or ex vivo studies have reported the constrictor response of the lungs to alveolar hypopcapnia. The bronchoconstrictive effect of a moderately low airway CO₂ 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 (Coleridge et al., 1978; D’Angelo et al., 2001; Newhouse et al., 1964; Nielsen and Pedersen, 1976; O’Cain et al., 1979; Severinghaus et al., 1961; Smith et al., 1979; Sterling, 1968; Tsi et al., 1970). Study of the influence of severe hypocapnia (<0.3% or <2.3 mmHg CO₂), which is feasible only under in vitro conditions (Duane et al., 1979; Ingram, 1975; Lindeman et al., 1998), further confirmed the development of a severe airway narrowing while extremely low alveolar CO₂ levels were maintained. Similarly to those earlier findings, in the present study we observed significant increases in Rₐw in response to decreases of the level of alveolar CO₂. These changes were associated with small elevations in G and H, and mild decreases in Iₐw. Since this pattern of change in the lung mechanical parameters was manifested during airway constriction with marked ventilation heterogeneities (Lutchen et al., 1996; Petak et al., 1997), we may conclude that alveolar hypopcapnia exerts constrictions on both the central (leading to marked elevations in Rₐw) and the peripheral airways (leading to ventilation heterogeneities reflected by apparent increases in H and decreases in Iₐw).

The results relating to the effects of alveolar hypercapnia on the lung mechanics are more conflicting. Alveolar hypercapnia has been reported to elevate (Jammes et al., 1985; Nadel and Widdicombe, 1962; Parker et al., 1963; Waldron and Fisher, 1988), decrease (Astin et al., 1973; Ingram, 1975; Sterling et al., 1972), or cause no changes in (Green and Widdicombe, 1966; Rodarte and Hyatt, 1973) the total lung resistance (RL). The results of the present study, obtained under well-controlled conditions, corroborate the lattermost of the 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 (Rₐnt) during wide-ranging alterations in the level of intra-alveolar CO₂ (D’Angelo et al., 2001). Our results indicating an increase in Rₐw correspond well with those obtained previously in the Rₐnt in the range where the intra-alveolar and intra-airway CO₂ overlap. Nevertheless, in consequence of the presence of a pulmonary circulation in the previous experiments, the minimum alveolar CO₂ 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 Rₐw to the altered intra-alveolar CO₂, the sharp increase in this parameter when PₐCO₂ (decreased to 1% (7.6 mmHg), remained undetectable in that previous study. This highly non-linear feature of the dose–response curve may be of importance in the adaptation mechanisms of the lungs to altered conditions via regulation of the ventilation distribution. Hyperventilation initiated regularly by the central nervous system to compensate hypoxemia (e.g. during exercise) may reduce the intra-alveolar CO₂, but this decrease cannot reach a concentration of <2% (15.2 mmHg) (D’Angelo et al., 2001). Our data indicate no detectable bronchoconstriction under these conditions (Fig. 2), which is a sensible physiological response as the lung function remains normal to maintain optimum gas exchange. Intra-alveolar CO₂ 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. Our data suggest the development of a severe airway smooth muscle contraction in these affected lung regions, which may subsequently contribute to redirection of the airflow to the lung areas where the pulmonary perfusion is intact; consequently, this regulatory mechanism reduces the ventilation–perfusion mismatch. 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 hypopcapnia-induced bronchoconstriction is substantiated by the proportionally greater increases in G than in H leading to elevations in H, a hallmark feature of the presence of heterogeneous peripheral airway constriction with the development of ventilation heterogeneities (Lutchen et al., 1996).

4.2. Systemic hypercapnia and acidosis

In the present study, systemic acidosis via systemic hypercapnia was produced by supplying CO₂ into the extracorporeal circulation (into the oxygenator). An elevated level of systemic CO₂ may exert its pulmonary effects via direct and indirect pathways. As concerns the direct effects of excess systemic CO₂, it most probably reaches the cells of the tracheobronchial tree in the terminal bronchioles, via the bronchial circulation, and then gains access direct 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 CO₂-mediated bronchodilatation (Duckles et al., 1974; Nadel and Widdicombe, 1962). This discrepancy suggests that, in the present study, the direct bronchodilatation activity of CO₂ 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