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Elevated CO_2 modulates airway contractility

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Carbon dioxide (CO_2), a primary product of oxidative metabolism, can be sensed by eukaryotic cells eliciting unique responses via specific signalling pathways. Severe lung diseases such as chronic obstructive pulmonary disease are associated with hypoventilation that can lead to the elevation of CO_2 levels in lung tissues and the bloodstream (hypercapnia). However, the pathophysiological effects of hypercapnia on the lungs and specific lung cells are incompletely understood. We have recently reported using combined unbiased molecular approaches with studies in mice and cell culture systems on the mechanisms by which hypercapnia alters airway smooth muscle contractility. In this review, we provide a pathophysiological and mechanistic perspective on the effects of hypercapnia on the lung airways and discuss the recent understanding of high CO_2 modulation of the airway contractility.

1. Introduction

Cells and tissues sense and respond to changes in the concentration of gaseous molecules through specific signalling pathways. Oxygen- and nitric oxide-activated cellular signalling pathways have been extensively studied [1–3], but much less is known about the mechanisms by which non-excitable cells sense and respond to changes in carbon dioxide (CO_2) concentrations [3–5]. CO_2 is a primary product of oxidative metabolism and its physiological levels in mammals are significantly higher than atmospheric levels (approx. 5% versus approx. 0.04%, https://scripps.ucsd.edu/programs/keelingcurve/) [4,6], suggesting that CO_2 is inextricably linked to physiological conditions. In humans, the elevation of CO_2 levels in tissues and the bloodstream (hypercapnia) is a consequence of inadequate alveolar gas exchange in patients with lung diseases such as the acute respiratory distress syndrome (ARDS) [7–9], chronic obstructive pulmonary disease (COPD) [10–12] and others [13–15]. In clinical situations, hypercapnia has been initially proposed to be benign or even protective in the lung since hypercapnia and its associated acidosis have been shown to attenuate systemic cytokine response in mechanically ventilated patients with acute lung injury and ARDS [7,8,16]. However, it is becoming increasingly evident that elevated CO_2 conditions have deleterious pathophysiological effects on various organs, including the lung [9,17–19], skeletal muscles [20–22] as well as innate immunity system [18,23–27]. In the lung, recent studies suggest that high concentrations of CO_2 activate specific gene expression [19,28,29] and signal transduction pathways with adverse consequences on alveolar epithelial function (alveolar fluid clearance) [17,30–35] and epithelial cell repair [36–39].

Hypercapnia is also reported to modulate the tone of lung airways which is a dynamic equilibrium between various excitatory and inhibitory mechanisms. The effects of hypercapnia on the airways and airway smooth muscle are controversial, as there are reports attesting to it causing increased airway contractility [19,40–49] or airway relaxation [50–61]. Here, we review recent advances in our understanding of how elevated CO_2 conditions modulate the airway tone, focusing on the effects of hypercapnia and respiratory acidosis.

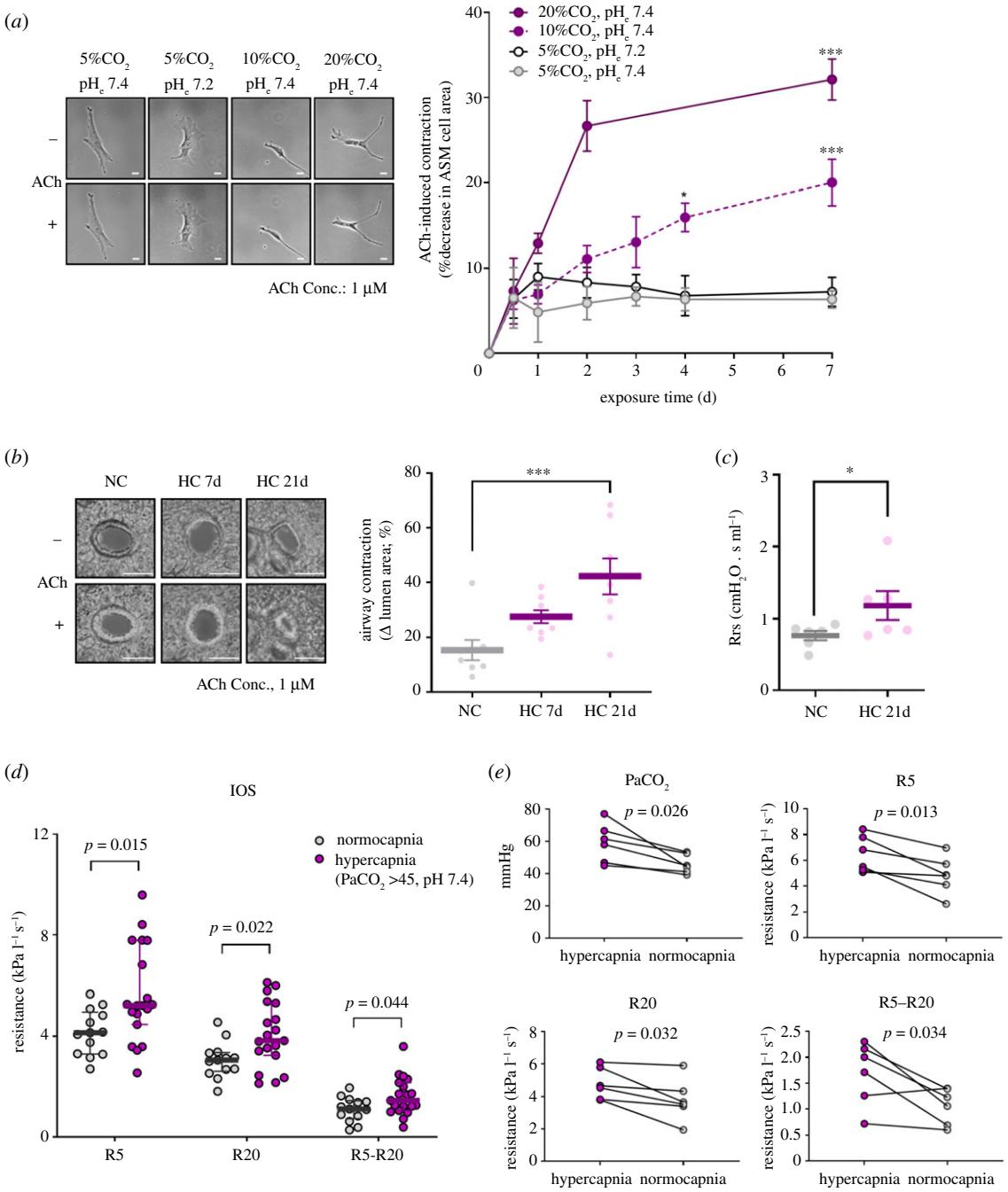

Figure 1. Normoxic hypercapnia increases airway smooth muscle contractility. (*a*) Acetylcholine (ACh)-induced cell contraction in mouse airway smooth muscle cells exposed to different conditions. Left, representative images from 7-day exposure conditions (scale bar, 50 µm). Right, time-course quantification of ACh-induced cell contraction. (*b,c*) C57BL/6 J wild-type mice were exposed to 21% O_2 and 10% CO_2 (HC) or maintained in room air (NC) for up to 21 days. Representative images (top; scale bar, 100 µm) and quantification (bottom) of ACh-induced airway contraction in precision-cut lung slices (*b*). Total resistance of the respiratory system (Rrs) at baseline on a FlexiVent instrument (*c*). (*d*) Comparison of respiratory resistance measured by impulse oscillometry between normocapnic and hypercapnic patients with chronic stable COPD. Values of R5, R20 and R5–R20 indicate total, proximal and peripheral respiratory resistance, respectively. (*e*) Changes of respiratory resistance in hypercapnic patients. All data are expressed as means ± s.e.m. (*a–c*) or median with interquartile range (*d,e*). *$p < 0.05$, ***$p < 0.001$. Reproduced from [19]. Copyright © 2018 American Association for the Advancement of Science.

2. Hypercapnia-induced bronchoconstriction

Evidence suggesting that changes in the level of CO_2 in the blood influence the airway tone was first reported by Einthoven in 1892 [40]. He described that inhalation of high concentrations of carbonic acid (CO_2-rich mixtures) caused bronchoconstriction in dogs, which was confirmed in various models of normoxic hypercapnia-exposed dogs [41–45] and cats [46,47]. Airway tone is regulated by interaction of the sympathetic and parasympathetic nerves [46,62] and the stimulation of vagal efferent nerves can increase the tone, resulting in bronchoconstriction [46,62–64]. As the hypercapnia-induced bronchoconstriction was abolished by blocking the vagus nerve, it was understood to be dependent on the integrity of vagal conduction [40–44,46,47]. In healthy humans, there have been few reports describing that the inhalation of high CO_2 concentrations decreases specific airway or pulmonary conductance which is the mathematical inverse of airway resistance [48,49]. The increases in airway resistance during high CO_2 exposure were interpreted as extrathoracic airway narrowing [48] such as larynx narrowing [49], because the hypercapnic effect was not blocked by atropine or β_1/β_2

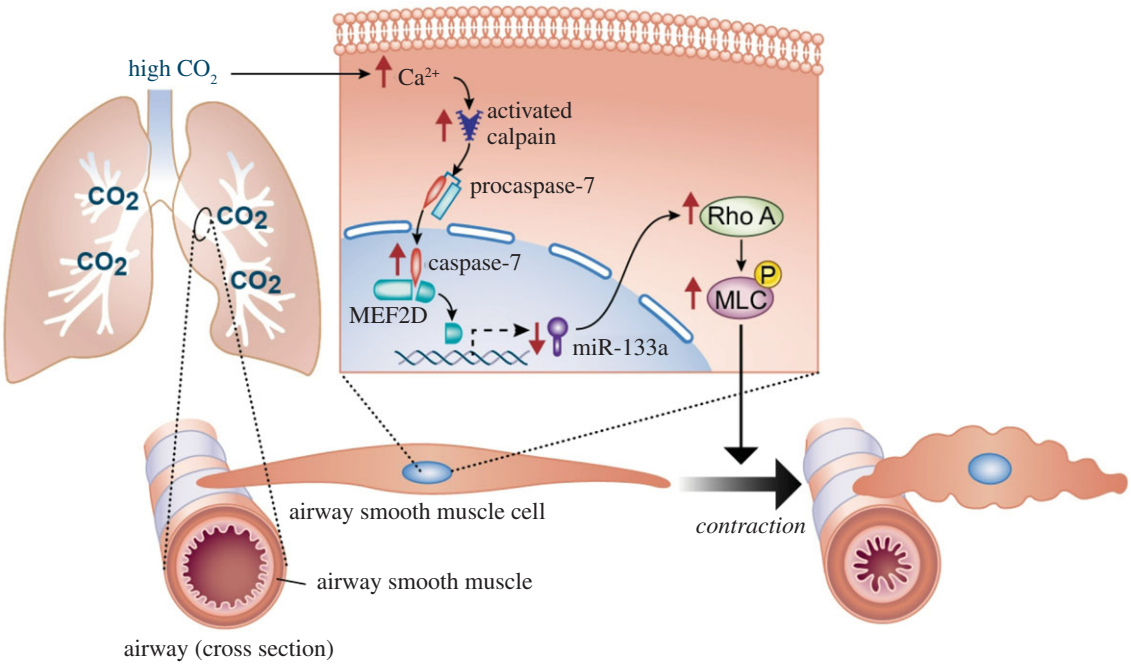

Figure 2 . Schematic of calcium–calpain signalling in airway smooth muscle cell contraction during hypercapnia. Hypercapnia promotes airway smooth muscle contractility through an increase in intracellular calcium (Ca^{2+}) and consequent activation of calpain which cleaves caspase-7. Cleaved caspase-7, in turn, cleaves the transcription factor myocyte enhancer factor 2D (MEF2D) that reduces miR-133a expression, thereby increasing Ras homologue family member A (RhoA) abundance and MLC phosphorylation. Reproduced from [19]. Copyright © 2018 American Association for the Advancement of Science.

adrenergic receptor agonists. However, the direct studies of laryngeal resistance during high CO_2 exposure indicated no change in anaesthetized animals [65] and healthy human subjects [66]. Furthermore, several reports of bronchoconstriction in the hypercapnia-exposed animals [42,44,46] showed that the blockage of the vagus nerve did not entirely abolish the bronchoconstrictor response to the high CO_2 exposure. These reports suggest that other mechanisms can contribute to the airway response to hypercapnia. Recently, we have reported that CO_2 operates as a signalling molecule that increases contraction of mouse and human airway smooth muscle cells [19]. We found that high concentrations of CO_2, independently of hypoxia and extracellular pH, increased acetylcholine (ACh)-induced cell contraction, which is both time- and dose-dependent in cultured cells (figure 1*a*). In a murine model, the exposure to normoxic hypercapnia, particularly chronic hypercapnia, increased ACh-induced airway contraction in precision lung cut slices (figure 1*b*) as well as airway resistance (figure 1*c*). Furthermore, we found that, in a small cohort of patients with chronic COPD, patients with hypercapnia had higher airway resistance (figure 1*d*), which improved after correction of hypercapnia (figure 1*e*). Our study also provided novel insights into the molecular mechanisms by which hypercapnia promotes airway smooth muscle cell contractility via calcium–calpain signalling. The signalling was mediated by caspase-7, which by cleaving the transcription factor myocyte-specific enhancer factor 2D (MEF2D), leads to downregulation of the microRNA-133a (miR-133a) and consequent upregulation of Ras homologue family member (Rho) A and myosin light-chain (MLC) phosphorylation (figure 2). Our data suggest that the elevation of CO_2 levels activates specific signal transduction pathways in airway smooth muscle cells, which results in deleterious changes in the airway tone, leading to bronchoconstriction. Taken together, these reports suggest that hypercapnia can contribute to airway constriction by activating vagus nerve and high CO_2-responsive signal transduction pathways. In lung disease conditions, hypercapnia may worsen airway constriction and limit ventilation to poorly functioning lung units setting up a feedback loop that could culminate in respiratory failure.

3. Respiratory acidosis-related bronchodilation

There have been studies reporting that hypercapnia shows airway relaxation [50–61]. We have reported that airway smooth muscle relaxation occurs during acute hypercapnia-associated acidosis, but it was transient and modest [19]. We reason that hypercapnia may acutely contribute to bronchodilatation when the tone of airways is previously increased by various constrictor stimuli such as drugs [52–54], hypoventilation [55,67–69] or when the reduction of ventilation in one lung following the occlusion of its pulmonary artery leads to bronchoconstriction in response to local airway ischaemia [70] and hypocapnia [50–52,71]. The inhalation of high CO_2 concentrations reduces constriction of airways as well as the tension developed by isolated bronchial rings caused by drugs such as 5-hydroxytryptamine [52–54]. It also reverses the airway constriction associated with pulmonary artery occlusion in ventilated animal models [50,52]. In humans, the administration of high CO_2 can relax the constriction of airways in the patient with unilateral pulmonary artery occlusion [51] and young asthmatic adults with hyperventilation (hypocapnia) [55] or exercise-induced bronchoconstriction [55,56]. These *in vivo* and *in vitro* effects of hypercapnia were not stimulated by the nerve reflexes and were understood to be a result of changes in extracellular/intracellular pH level, possibly elevated CO_2-related acidosis (respiratory acidosis) in airway smooth muscle cells. Many of the cellular responses to CO_2 elevation are thought to be a consequence of acidosis because of the rapid conversion of CO_2 in solution into H_2CO_3 and subsequently HCO_3^- and H^+ [5,72]. Several

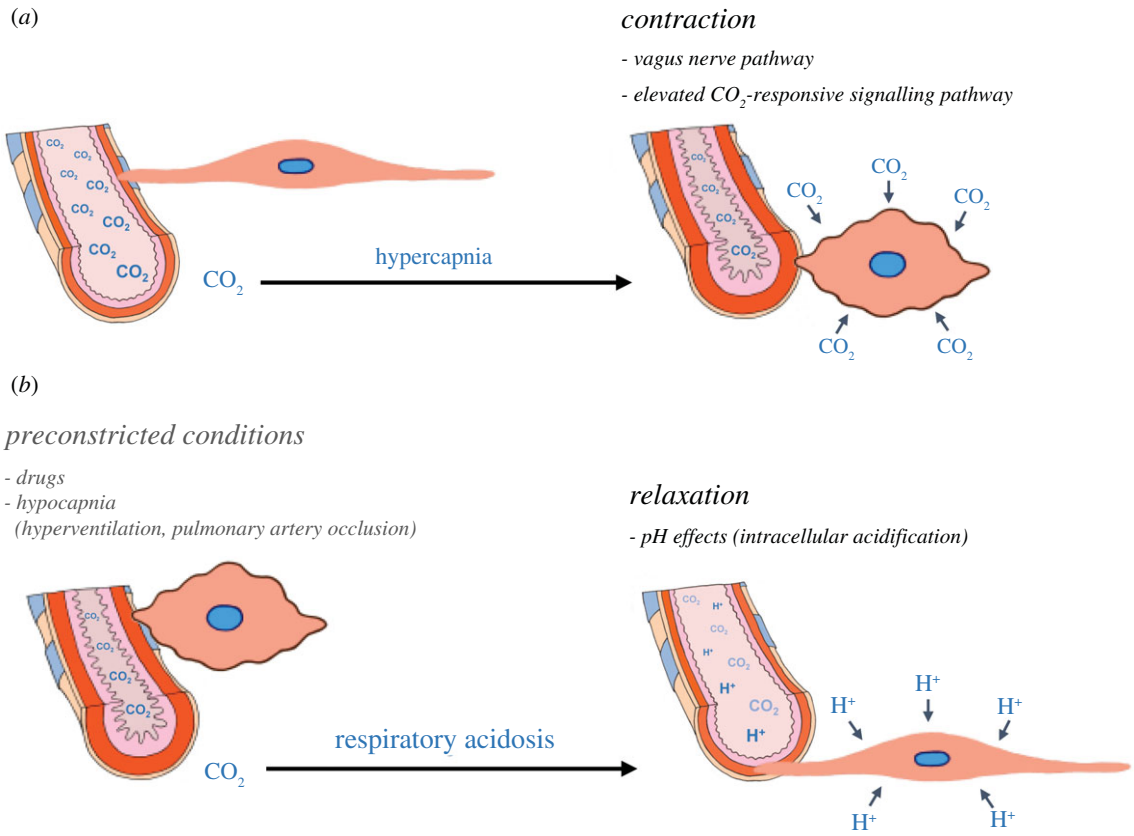

Figure 3. A proposed model for the modulation of CO_2 in airway tone. Lung airway cells sense and respond to changes in CO_2 levels, which modulates the tone of airways, airway contraction or relaxation, via specific mechanisms of the vagus reflexes, molecular CO_2 and pH effects. (*a*) Hypercapnia. Acute and chronic hypercapnia promote airway contractility via either vagus reflexes or molecular CO_2 effects. (*b*) Respiratory acidosis. Elevated CO_2 conditions particularly showing acute respiratory acidosis can have a potent relaxing effect on contracted airways via pH effects.

in vitro reports show that respiratory or normocapnic (metabolic) acidosis produced a reversible reduction in active tension of bronchial rings [53,54,57,58]. Extracellular pH can alter airway smooth muscle tone by changing the levels of pH and intracellular calcium (Ca^{2+}) [58,59,73]. Intracellular acidification has been reported to decrease intracellular Ca^{2+} levels through voltage-dependent Ca^{2+} channels in the potassium-induced contractile model, thereby inhibiting airway smooth muscle cell contraction [60]. On the other hand, an *in vitro* study reported that high concentrations of CO_2, independently of extracellular pH, enhanced airway smooth muscle relaxation via the epithelium-dependent mechanism induced by substance P in the model of methacholine-precontracted bronchial smooth muscle [61]. Collectively, elevated CO_2 conditions, specifically showing acute respiratory acidosis, appear to have a potent relaxing effect on contracted airways.

4. Effect of hypocapnia on airway contractility

Low levels of CO_2 (hypocapnia) have been also reported to increase airway constriction in humans with pulmonary artery occlusion [51,71], hyperventilation [67,68] and exercise-induced asthma attacks [55,56] and other models *in vivo* [50,52,70,74] and *in vitro* [59,61,75,76]. The bronchoconstrictor effect of hypocapnia is largely attributed to local mechanisms on the bronchial smooth muscle since it was not abolished by vagotomy or atropine in intact animals [50,70] and asthmatic patients [55,56]. Several reports suggest that the hypocapnic response involves additional contribution of cholinergic reflexes in the airways [67,68]. The cellular mechanisms involved in local airway response to hypocapnia are likely dependent on intracellular alkalosis elicited by hypocapnia on airway smooth muscle cells. *In vitro* studies suggest that intracellular alkalosis can increase airway smooth muscle contractility [77] by increasing intracellular Ca^{2+} levels through voltage-dependent calcium channels in airway smooth muscle [60,73,76].

5. Conclusion

A proposed model for the effects of CO_2 levels on the airway tone, airway smooth muscle contractility or relaxation, is presented in figure 3. Lung airway cells appear to sense and respond to changes in CO_2 levels via specific mechanisms of the vagus reflexes, molecular CO_2 and pH effects. Thus, the effect of elevated CO_2 levels to lung diseases is somewhat controversial. Hypercapnia is associated with worse outcomes in patients with obstructive lung diseases such as asthma [13], obesity hypoventilation syndrome [14] and COPD [10–12]. Furthermore, the recently reported strategy of mechanical ventilation aimed at reducing the partial pressure of CO_2 in arterial blood can provide beneficial effects including improvement of airway resistance, health-related quality of life and mortality for patients with COPD and hypercapnia [11,12,19]. Understanding the elevated CO_2 effects on airway contractility is of significant clinical interest for those patients and could help with the design of innovative therapeutic approaches.

Data accessibility. This article has no additional data.

Authors' contributions. M.S.: conceptualization and writing—original draft preparation; M.S. and J.I.S.: writing—review and editing . All authors have read and agreed to the submitted version of the manuscript.

Competing interests. We declare we have no competing interests.

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