Hypercapnia: clinical relevance and mechanisms of action

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Purpose of review
Multiple clinical and laboratory studies have been conducted to illustrate the effects of hypercapnia in a range of injuries, and to understand the mechanisms underlying these effects. The aim of this review is to highlight and interpret information obtained from these recent reports and discuss how they may inform the clinical context.

Recent findings
In the last decade, several important articles have addressed key elements of how carbon dioxide interacts in critical illness states. Among them the most important insights relate to how hypercapnia affects critical illness and include the effects and mechanisms of carbon dioxide in pulmonary hypertension, infection, inflammation, diaphragm dysfunction, and cerebral ischemia. In addition, we discuss molecular insights that apply to multiple aspects of critical illness.

Summary
Experiments involving hypercapnia have covered a wide range of illness models with varying degrees of success. It is becoming evident that deliberate hypercapnia in the clinical setting should seldom be used, except wherever necessitated to avoid ventilator-associated lung injury. A more complete understanding of the molecular mechanisms must be established.

Keywords
carbon dioxide, hypercapnia, hypertension, ischemia, pneumonia

INTRODUCTION

The use of lower tidal volumes as a method of protective ventilation in patients with acute respiratory distress syndrome (ARDS) has been documented to show a significant reduction in mortality rates [1]. This protective ventilation leads to an increase in arterial carbon dioxide (hypercapnia), and the associated drop in pH resulting is termed hypercapnic acidosis (HCA). From studies spanning the last 30 years, HCA has been associated with improvement in the outcome of patients with acute lung injury/ARDS [2–6] and also with favorable effects in acute myocardial ischemia and brain injury [7] as well as gut mucosal injury due to sepsis [8]. However, in various in-vivo, ex-vivo, and in-vitro models of acute lung injury, there has also been some evidence for harmful effects of HCA, even when they seem to be outweighed by the beneficial effects [9]. Here, we highlight and interpret information obtained from more recent experimental series and, taken with what we already know, discuss their impact in leading toward the routine use of hypercapnia.

VASCULAR EFFECTS

Recent studies have focused on the pulmonary and systemic vasculature. Hypercapnia has previously been shown to reverse hypoxia-induced pulmonary hypertension in adult and neonatal rats [10,11], and a number of recent publications have recapitulated these important effects with the aim of deciphering underlying mechanisms.

In an infant rat model of right ventricular dysfunction induced by inhaled nitric oxide and hypoxia, hypercapnia was shown to normalize ventricular function by modulating interleukin-1 [12].

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KEY POINTS

- Neutrophil phagocytosis and oxidative reactions in pulmonary sepsis are impaired by hypercapnia.
- Hypercapnia protects against central nervous system ischemia by mediating levels of proapoptotic Bax and antiapoptotic B-cell CLL/lymphoma-2.
- Hypercapnia modulates cytoskeletal remodeling via cAMP induction of α-adductin activation.
- cAMP pooling has been suggested in cells that are sensitive to increased bicarbonate levels; this may have impact on buffering HCA.
- An important sheddase, ADAM-17 is impaired by hypercapnia demonstrating a new pathway by which carbon dioxide is anti-inflammatory in stretch-induced lung injury.

Here, hypercapnia inhibited interleukin-1 secretion that, in turn, reduced nitric oxide synthase 2 upregulation and therefore lessened the generation of reactive nitrogen species that contribute to hyperoxia-induced pulmonary vascular remodeling.

Another study using the same model of lung injury investigated the effects of two different levels of inhaled carbon dioxide (7 and 10%) on established pulmonary hypertension [13]. Addition of inhaled carbon dioxide to chronic hypoxia attenuated pulmonary hypertension; this was manifested as improvements in hemodynamic and structural markers of pulmonary hypertension compared to exposure to hypoxia alone. In this case, it was discovered that inhaled carbon dioxide inhibited Rho-associated protein kinase; thus, Rho-associated protein kinase-mediated vasoconstriction was diminished and pulmonary hypertension ameliorated.

Bleomycin is not only an important chemotherapeutic but also important as a contributor to lung injury in some critically ill patients. It is also used to generate lung inflammation and fibrosis for experimental models of critical illness. In experimental bleomycin-induced lung injury, inhaled carbon dioxide was shown to ameliorate deterioration of lung function, in addition to attenuating macrophage influx and the development of pulmonary hypertension [14]. In pulmonary hypertension induced by chronic hypoxia, hypercapnia attenuated pulmonary hypertension but preserved endothelial integrity [15]. Importantly, attempts to buffer HCA increased inducible nitric oxide synthase activity, and this in turn increased endothelial permeability.

A clinical study by Perry *et al.* [16] recruited 15 healthy volunteers to examine the effects of hypercapnia in steady state, nonpharmacological increases in mean arterial pressure (MAP). Hypercapnia (5% carbon dioxide in air) was shown to impair the control of blood flow velocity during steady state increases in MAP, whereas in normocapnic conditions, increases in MAP lead to a decrease in middle cerebral artery blood velocity, and hypercapnia prevented such autoregulation. Such maintenance of middle cerebral artery blood velocity suggests that hypercapnia impairs the regulatory mechanism that protects against induced hypertension.

PNEUMONIA

Previous studies have examined the effects of hypercapnia in bacterial pneumonia, with apparently conflicting results. Hypercapnia was shown to be protective in early *Escherichia coli* infection [17,18]; in contrast, another study reported that prolonged hypercapnia worsened outcome in longer-term pneumonia [19]. The overall synthesis from this work is that hypercapnia impairs neutrophil phagocytosis; while in the short term, this results in less tissue injury, in the longer term, such impairment of phagocytosis causes increased bacterial load and a greater burden of disease.

Nichol *et al.* [20] have reported an antioxidant action of hypercapnia. In a rat model of endotoxin-induced lung injury, with and without a nonspecific NOS inhibitor, hypercapnia was shown to decrease pulmonary oxidative reactions during established inflammation. In a study of the effects of therapeutic hypercapnia in endotoxin-induced lung injury, proinflammatory responses were enhanced in the lungs; however, the opposite was shown in the spleen where an anti-inflammatory cytokine milieu was observed [21]. More recently, the adverse effects of hypercapnia have been demonstrated in a mouse model of pneumonia induced by *Pseudomonas aeruginosa* [22]; here, high levels of inhaled carbon dioxide led to increased mortality. Bacterial load was increased in the lungs and neutrophil phagocytosis was decreased, and cytokine levels were reduced in early but not prolonged pneumonia.

NONINFECTIVE ACUTE LUNG INJURY

The potential for beneficial effects of hypercapnia on ventilator-induced lung injury (VILI) are promising. Peltekova *et al.* [23] – in a mouse model of VILI – demonstrated that hypercapnia attenuates the inflammatory response; this occurred in a dose (and time)-dependent manner, without adverse effects in control animals. They suggested that the positive effect of hypercapnia might be a result of the suppressed production of the COX-2 protein.

Using an isolated lung model of severe VILI, Kapetanakis *et al.* [24] have compared the effects of respiratory vs. metabolic acidosis. They reported that both respiratory acidosis and metabolic acidosis (induced by HCl) were equally effective in reducing lung edema.

An important study of the effects of hypercapnia in VILI – and possible mechanisms – was conducted by Contreras *et al.* [25]. Here, the effects of hypercapnia on moderate and severe VILI were shown to be anti-inflammatory, and were overall beneficial. In-vitro analysis demonstrated that hypercapnia inhibited the NF-κB pathway, thereby reducing levels of interleukin-8 and NF-κB-driven luciferase production.

Although not conventionally considered to be VILI, mechanical ventilation can induce significant dysfunction in the diaphragm. An experimental study in rats suggests that hypercapnia may protect against this problem [26]. Here, diaphragm myofiber myosin concentration was significantly increased compared with controls; in addition, tumor necrosis factor-α, interleukin-1β, and keratinocyte-derived chemokines were all significantly decreased in diaphragm homogenates of hypercapnia animals.

Fuchs *et al.* [27] performed an interesting study comparing progressively lower tidal volumes while permitting the resultant hypercapnia. Compared with 8–10 ml/kg, reductions in tidal volume below 4 ml/kg did not provide additional protection; reassuringly, the severe hypercapnia (i.e., 160 mmHg), which arose at extremely low tidal volume (i.e., 2 ml/kg), did not reverse the protection. The study utilized a surfactant-depletion rabbit model of ARDS.

Finally, carbon dioxide preexposure blocks degranulation of mast cells and significantly reduces histamine release implicated in allergic rhinitis in response to stimulation by compound 48/80 [28]. The mechanism of action behind this antiallergy effect was demonstrated to be in correlation with a decrease in intracellular calcium levels. This may point toward a possible mechanism by which carbon dioxide exerts some of its effects.

ISCHEMIA

Severe, sustained hypercapnia in newborn rats (10% inspired carbon dioxide) appears to impair brain growth by increasing nitrative stress, which, in turn, leads to microvascular degeneration [29]. This negative effect was not seen in animals treated with reactive nitrogen species inhibitors showing that high levels of carbon dioxide causes nitrative stress in neonatal brain development.

The effects of therapeutic hypercapnia were investigated in a rodent model, where impaired spatial memory and sensorimotor function was induced by central nervous system ischemia [30]. Hypercapnia improved both conditions via antiapoptotic mechanisms. Levels of proapoptotic Bax (and antiapoptotic B-cell CLL/lymphoma-2) proteins were decreased (and increased, respectively) in brain tissues of animals exposed to hypercapnia.

Hypercapnia improved lung injury indices such as perfusion pressure elevation, lung wet weight, broncheoalveolar lavage protein concentration, and lactate dehydrogenase, in the setting of ischemia reperfusion [6]. Filtration coefficient, an index of barrier integrity, was also reduced in the setting of hypercapnia. This study also documented the correlation between hypercapnia-associated improvements and NF-κB inhibition, via a decrease in ischemia-reperfusion-induced inhibitor of nuclear factor kappa-B kinase subunit alpha/beta (IKK-α/β) phosphorylation that, in turn, prevented degradation of IκBα and NF-κB activation and translocation.

MOLECULAR MECHANISMS

Previous studies have pointed out that hypercapnia may impair alveolar fluid clearance by decreasing the levels of Na,K-ATPase on plasma membranes [31,32]. Further investigations by this group have shown that hypercapnia induces endocytosis of the Na,K-ATPase transporter [33] and this occurs via an extracellular-signal-regulated kinase-regulated pathway [34] (see Fig. 1 [34–37,38▪▪,39]). Most recently, it has been shown that this is dependent on cyclic AMP (cAMP) production; cAMP activated PKA-Iα, which, in turn, led to phosphorylation of α-adductin, a known regulator of Na,K-ATPase endocytosis [37].

It has been suggested that pools of cAMP exist in pulmonary microvessels, and such pools are sensitive to bicarbonate [38▪▪]. Indeed, addition of bicarbonate decreased transendothelial resistance and increased filtration coefficient in isolated perfused lungs. This may ultimately be an important issue to consider when attempting to buffer the acidosis resulting from increased hypercapnia.

Hypercapnia has been shown to inhibit NF-κB-dependent cytokines and, perhaps consequentially, decrease macrophage phagocytosis [40]. However, such inhibition of cytokine production does not occur via blockade of either NF-κB activation or its translocation, and appears to occur via other transcription factors [40].

In a study by Cummins *et al.* [35], mouse embryonic fibroblast cells were exposed to 0.03% or 10% carbon dioxide, with or without pH-buffering. The group also conducted graded pH experiments to compare effects of hypercapnic and metabolic acidosis on NF-κB activity. Here, nuclear translocation of IKK-α was independent of O_2 concentration, but was

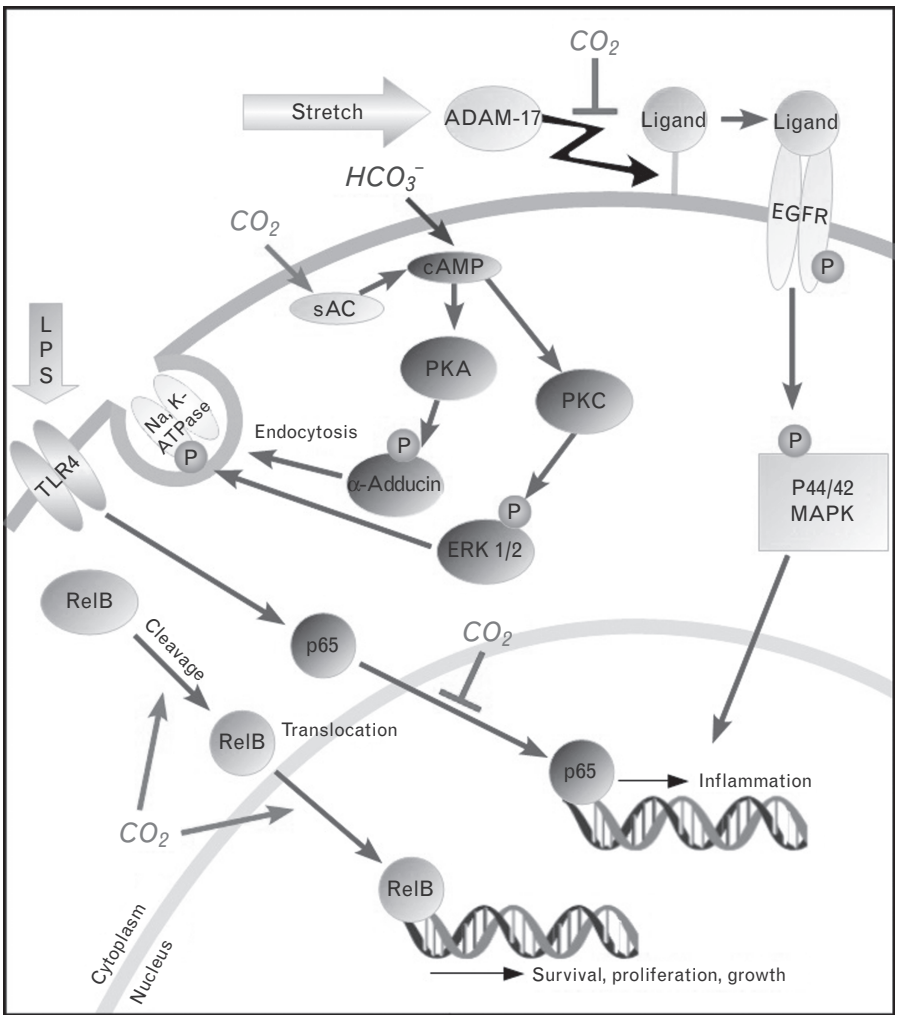

FIGURE 1. Schematic representation of the recently established intracellular molecular responses to elevated carbon dioxide. Carbon dioxide has been shown to confer positive anti-inflammatory effects by increasing the translocation of RelB and impairing the translocation of p65 [35,36]. Carbon dioxide has also been demonstrated to increase the endocytosis of the Na,K-ATPase transporter [34,37,38▪▪] leading to reduced edema clearance from injured lungs. P44/42 activation by stretch-induced injury was shown to be decreased after carbon dioxide exposure via inhibition of the sheddase ADAM-17 reducing stretch-induced inflammation [39].

dependent on carbon dioxide concentration, and the carbon dioxide effect was rapidly reversible. In the same experimental series, hypercapnia was also shown to inhibit p65 translocation in mouse embryonic fibroblast cells in response to lipopolysaccharide injury. In a further study by the same group [36], RelB – a protein of the noncanonical NF-κB pathway – was shown to undergo cleavage and subsequent translocation to the nucleus under conditions of increased carbon dioxide; these effects were independent of pH changes induced by buffering cell culture media.

A recent article by Otulakowski *et al.* [39] has pointed toward a mechanism whereby hypercapnia inhibits p44/42 mitogen activated protein kinase (MAPK) activation. Activation of p44/42 MAPK correlates with the degree of stretch-induced lung injury [41] and is mediated via epidermal growth factor receptor activity [42]. This, in turn, is dependent on the binding of endogenous ligands whose shedding is induced by a sheddase – ADAM-17 [43]. Hypercapnia was shown to prevent ligand shedding and thus prevent downstream activation of epidermal growth factor receptor and p44/42 MAPK in rodent alveolar epithelial cells [40] (see Fig. 1). Stretch-induced shedding of tumour necrosis factor receptor (another ADAM-17 substrate) was also shown to be reduced in an isolated perfused mouse lung model of injury.

The cellular mechanisms of action of carbon dioxide in cerebral microvascular endothelial cells and human fetal astrocytes have shed light on

interactions with nitrogen-derived free radical mechanisms [44]. The production of nitric oxide by these cell types is increased in hypercapnia (and decreased in hypocapnia) during stable neutral pH levels.

GASTROINTESTINAL IMPACT

Using a ventilated canine model of gastric hemorrhage, Schwartges *et al.* [45] aimed to determine the effects of hypercapnia, induced by reduced tidal volumes, in a canine model of hemorrhage. Hypooxygenation of the splanchnic region is a risk factor of hemorrhage and endangers mucosal barrier function. Here, prophylactic and therapeutic hypercapnia appeared to preserve splanchnic mucosal oxygenation. However, intraoperative hypercapnia in patients undergoing elective colon resection was shown to have little or no ability to prevent surgical site infection [46].

CONCLUSION

In conclusion, this article reviews key research developments in the study of hypercapnia in critical illness and describes many effects in experimental models of human disease, along with exploring key novel molecular mechanisms of effect. As our ability to better characterize critical illness states improves, in terms of organ and molecular-specific mechanisms, we will be better able to apply the insights learned over the last 3–5 years.

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Conflicts of interest

There are no conflicts of interest.

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