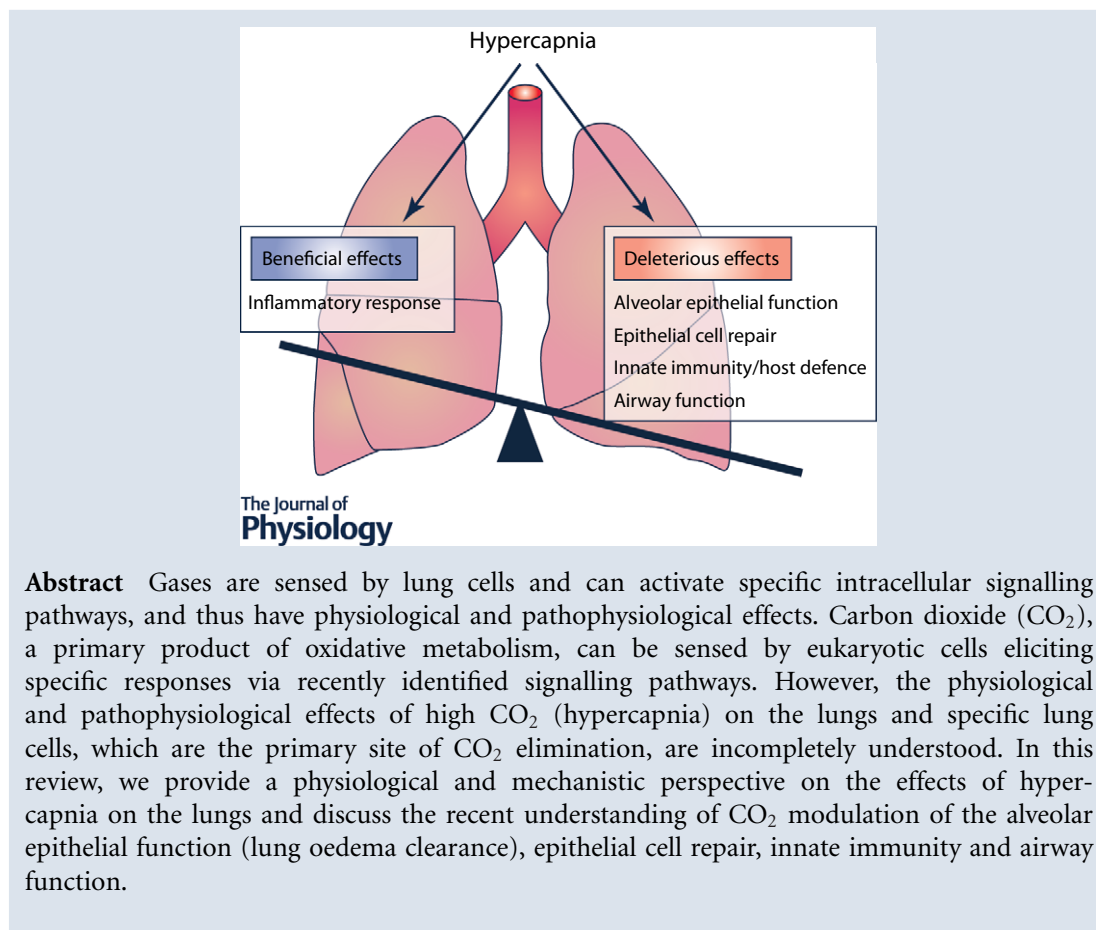


## SYMPOSIUM REVIEW

## Effects of hypercapnia on the lung

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**Jacob I. Sznajder** (left) is the Ernest S. Bazley Professor of Medicine and Cell and Molecular Biology at Northwestern University and has been conducting research related to lung injury and alveolar epithelial function. His laboratory is using integrative approaches to study molecular mechanisms regulating lung injury in infectious models (influenza) and non-infectious models. He is also studying the effects of high CO<sub>2</sub> levels on the lungs and muscle biology. **Emilia Lecuona** (centre) obtained her PhD in Spain (University of La Laguna) and completed her postdoctoral training at Northwestern University. She is a Research Associate Professor at Northwestern University and is interested in the resolution of lung injury as well as the pathophysiology of hypercapnia on the lungs. **Masahiko Shigemura** (right) completed his PhD in Japan (Hokkaido University) and is now a research fellow in Dr Sznajder's laboratory. He is currently conducting research on the effects of hypercapnia on airways and lung pathophysiology.



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**Abstract figure legend** Hypercapnia, especially hypercapnic acidosis, has been historically associated with improvement in the outcome of patients with acute lung injury. Its protective effects are thought to be mediated through inhibition of the NF- $\kappa$ B inflammatory pathway. In contrast, recent studies suggest that molecular CO<sub>2</sub> can act as a signaling molecule and that hypercapnia can have deleterious effects in the lung via impairment of alveolar epithelial function, epithelial cell repair, innate immunity/host defence and airway function.

**Abbreviations** ALI, acute lung injury; AMPK, AMP kinase; AQP, aquaporin; ARDS, acute respiratory distress syndrome; cAMP, 3',5'-cyclic adenosine monophosphate; CAMKK- $\beta$ , Ca<sup>2+</sup>/calmodulin-dependent protein kinase kinase- $\beta$ ; COPD, chronic obstructive pulmonary disease; ERK, extracellular signal-regulated kinase; IL-6, interleukin-6; IDH2, isocitrate dehydrogenase-2; JNK, c-Jun-N-terminal kinase; PAL, prolonged air leak; PKA-I $\alpha$ , protein kinase A-I $\alpha$ ; PKC- $\zeta$ , protein kinase C- $\zeta$ ; sAC, soluble adenylyl cyclase; TNF, tumour necrosis factor.

## Introduction

Carbon dioxide (CO<sub>2</sub>) is a primary product of oxidative metabolism. The physiological levels of CO<sub>2</sub> in exhaled breath of mammals are significantly higher than the room air (~5% vs. ~0.04%) (Monastersky, 2013; Cummins *et al.* 2014) and inextricably linked to physiological conditions. In humans, elevated CO<sub>2</sub> (hypercapnia) can occur as a consequence of lung diseases when inadequate gas exchange takes place (Vadasz *et al.* 2012b). Despite the fact that the lung is the primary site of CO<sub>2</sub> elimination, the effects of hypercapnia have been argued and contradictory data have been reported. Here, we review recent advances in our understanding of the effects of hypercapnia on the lung.

## CO<sub>2</sub> transport and sensing in the lung

CO<sub>2</sub> is a small non-polar molecule thought to traverse biological cell membranes via passive diffusion, depending upon the transmembrane concentration gradient of CO<sub>2</sub> and the lipid/water partition behaviour of the gas (Missner & Pohl, 2009). However, this view has been challenged with the discovery of the effect of cholesterol on CO<sub>2</sub> permeability and of protein channels used by CO<sub>2</sub> to cross membranes, aquaporins (AQPs) (Verkman, 2007; Musa-Aziz *et al.* 2009) and rhesus proteins (Endeward *et al.* 2008). Functionally, high permeability for CO<sub>2</sub> seems to be exhibited by AQP1, AQP4-M23, AQP5 and AQP6 (Musa-Aziz *et al.* 2009). Several AQPs are expressed in the lung: AQP1 in microvascular endothelia, AQP3 and AQP4 in airway epithelia, and AQP5 in type I alveolar epithelial cells and a subset of airway epithelial cells (Verkman, 2007). Once inside the cell, CO<sub>2</sub> very rapidly equilibrates with its hydrated form, H<sub>2</sub>CO<sub>3</sub>, which in turn rapidly dissociates into H<sup>+</sup> and HCO<sub>3</sub><sup>-</sup> catalysed by carbonic anhydrases (Casey *et al.* 2010). Cellular enzymes and chemical reactions are sensitive to pH, and cells actively transport H<sup>+</sup> and HCO<sub>3</sub><sup>-</sup> across their cell membrane

to maintain intracellular pH (Casey *et al.* 2010). Cells appear to sense CO<sub>2</sub> via different mechanisms: soluble adenylyl cyclase senses CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup>, generating the second messenger 3',5'-cyclic adenosine monophosphate (cAMP), which is a key signalling molecule affecting a range of processes (Kamenetsky *et al.* 2006; Lecuona *et al.* 2013). Transmembrane adenylyl cyclases have also been described to play a role in CO<sub>2</sub> sensing in the carotid body (Holmes *et al.* 2015). Connexin 26 hemichannels, causally linked to respiratory chemosensitivity, respond to an increase in CO<sub>2</sub> and are an important conduit for the CO<sub>2</sub>-dependent ATP release (Meigh *et al.* 2013). Receptor protein tyrosine phosphatase- $\gamma$ , which has an extracellular ligand binding domain 40% identical to the catalytic domain of carbonic anhydrases, is an extracellular CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup> sensor critical for pH homeostasis (Zhou *et al.* 2016). The role of transmembrane adenylyl cyclases, connexin 26 and receptor protein tyrosine phosphatase- $\gamma$  in CO<sub>2</sub> sensing in the lung has not yet been established.

## Beneficial effect of hypercapnia/hypercapnic acidosis

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 (ARDS Team Network, 2000). This protective ventilation leads to hypercapnia and the associated drop in pH resulting in hypercapnic acidosis. From studies spanning the last 30 years, hypercapnia, especially hypercapnic acidosis, has been associated with improvement in the outcome of patients with acute lung injury (ALI)/ARDS and the concepts of 'permissive' and even 'therapeutic' hypercapnia have been proposed in treating these patients (Hickling *et al.* 1994; Contreras *et al.* 2015). The protective effects of hypercapnic acidosis in preclinical models are mediated through effects on the host immune system, with key effects mediated through inhibition of the NF- $\kappa$ B pathway, a pivotal transcriptional activator

in inflammation, injury and repair (Contreras *et al.* 2015). On the other hand, hypercapnia-mediated NF- $\kappa$ B inhibition may also explain several deleterious effects, including delayed epithelial wound healing and decreased bacterial killing (Wang *et al.* 2010).

### Deleterious effects of hypercapnia/hypercapnic acidosis

There have been also studies on the harmful effects of hypercapnia as described below.

**Alveolar epithelial function.** One of the most extensively investigated effects of hypercapnia focused on the high CO<sub>2</sub> effects on alveolar epithelial function and particularly on the clearance of lung oedema. Disruption of the alveolo-capillary barrier, which is a hallmark of ARDS, results in accumulation of alveolar oedema, which results in impaired gas exchange. The Na<sup>+</sup>,K<sup>+</sup>-ATPase plays a key role in the active transport of Na<sup>+</sup> and K<sup>+</sup> across membranes, thus, maintaining cellular ion homeostasis and favouring water reabsorption by generating an ion gradient (Sznajder *et al.* 2002). Reduction of lung oedema clearance is associated with the endocytosis of the Na<sup>+</sup>,K<sup>+</sup>-ATPase from the plasma membrane of alveolar epithelial cells, which leads to decreased Na<sup>+</sup>,K<sup>+</sup>-ATPase activity (Lecuona *et al.* 2007). Exposure of lungs, in an isolated rodent lung model *ex vivo*, to hypercapnia leads to impaired alveolar fluid reabsorption, independently of extra- and intracellular acidosis (Briva *et al.* 2007; Vadasz *et al.* 2008, 2012a). The mechanism of impaired alveolar fluid in hypercapnia involves activation of protein kinase C (PKC)- $\zeta$ , which directly phosphorylates the Na<sup>+</sup>,K<sup>+</sup>-ATPase  $\alpha$ 1-subunit at the Ser-18 residue, leading to endocytosis of the Na<sup>+</sup>,K<sup>+</sup>-ATPase (Briva *et al.* 2007; Vadasz *et al.* 2008). The activation of PKC- $\zeta$  is regulated by AMP kinase (AMPK) via Ca<sup>2+</sup>/calmodulin-dependent protein kinase kinase- $\beta$  (CAMKK- $\beta$ ) and extracellular signal-regulated kinase (ERK) (Vadasz *et al.* 2008; Welch *et al.* 2010). The endocytosis of the Na<sup>+</sup>,K<sup>+</sup>-ATPase by hypercapnia is also regulated by c-Jun-N-terminal kinase (JNK) via an AMPK-PKC- $\zeta$  signalling (Vadasz *et al.* 2012a). However, JNK does not phosphorylate the Na<sup>+</sup>,K<sup>+</sup>-ATPase, but promotes the phosphorylation of LMO7b, which regulates the actin cytoskeleton in epithelial cells, followed by its colocalization and interaction with the Na<sup>+</sup>,K<sup>+</sup>-ATPase and several components of the clathrin-dependent endocytic machinery (Dada *et al.* 2015). The protein kinase A (PKA)-I $\alpha$  has also been reported to play a role in the Na<sup>+</sup>,K<sup>+</sup>-ATPase endocytosis during hypercapnia. Namely, a novel pathway was proposed whereby hypercapnia via a CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup>-sensitive soluble adenylyl cyclase (sAC) increases the production of cAMP, activates

PKA-I $\alpha$  and in turn, the phosphorylation of the actin cytoskeleton component  $\alpha$ -adducin, culminating in the Na<sup>+</sup>,K<sup>+</sup>-ATPase endocytosis from the cell plasma membrane (Lecuona *et al.* 2013). Taken together, these reports suggest that hypercapnia has deleterious effects on the alveolar epithelial function by impairing the resolution of lung oedema via a pH-independent mechanism that involves the endocytosis of the Na<sup>+</sup>,K<sup>+</sup>-ATPase (Fig. 1).

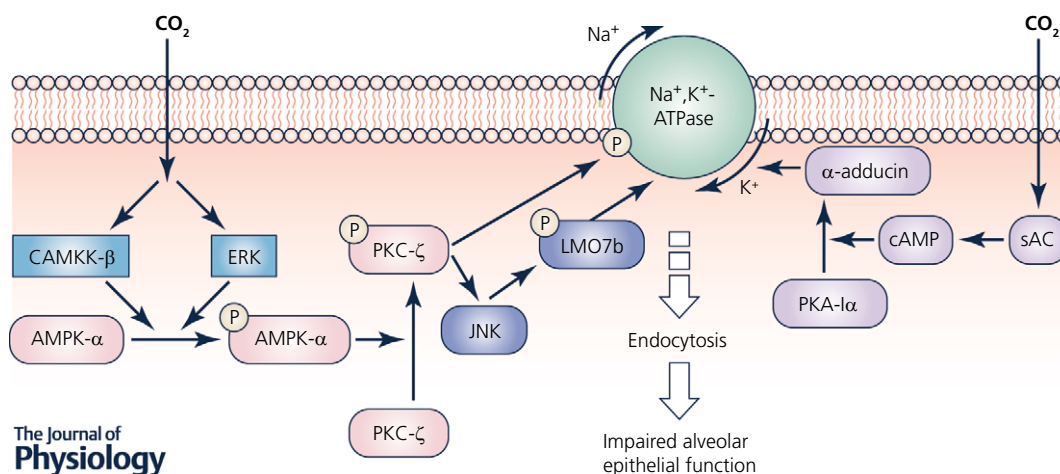
**Alveolar epithelial repair.** Alveolar epithelial repair is critical for patients to recover from lung injury with the repair process involving cell proliferation and migration (Berthiaume *et al.* 1999). Hypercapnia, independently of extracellular acidosis, has been shown to impair proliferation of alveolar epithelial cells (Vohwinkel *et al.* 2011). The decreased cell proliferation was due to hypercapnia-mediated mitochondrial dysfunction, resulting from hypercapnia-induced miR-183, which down-regulates the tricarboxylic acid (TCA) cycle enzyme isocitrate dehydrogenase-2 (IDH2) (Fig. 2). In a different model, hypercapnic acidosis was shown to impair plasma membrane wound resealing in ventilator-injured lungs (Doerr *et al.* 2005). In line with this observation, hypercapnic acidosis has been shown to decrease alveolar epithelial wound repair via reduced NF- $\kappa$ B activation (O'Toole *et al.* 2009) (Fig. 2). A recent report demonstrated that miR-183 inhibits NF- $\kappa$ B by directly targeting its 3'-untranslated region (Sha *et al.* 2014). In addition, miR-183 is known to negatively regulate cell migration in cancer cells (Lowery *et al.* 2010; Zhu *et al.* 2012). These observations raise the possibility that miR-183 may play an important role in alveolar epithelial cell migration as well as proliferation in hypercapnic conditions. Recently, a clinical study reported an association between hypercapnia and prolonged air leaks (PALs) in patients after thoracic surgery. PAL is an important cause of morbidity and mortality after lung resection (Okereke *et al.* 2005). Intrapleural hypercapnia was associated with delayed resolution of PAL in patients after lobectomy, and reducing pleural CO<sub>2</sub> levels was associated with faster resolution of air leaks (Bharat *et al.* 2016). Collectively, hypercapnia appears to impair alveolar epithelial cell proliferation and migration, which is deleterious to alveolar epithelial repair.

**Innate immunity and host defence.** Although previous studies have reported that patients with lung injury may have benefited from permissive hypercapnia (Hickling *et al.* 1994; Amato *et al.* 1998; Contreras *et al.* 2015), it has been associated with increased mortality in hospitalized patients with community-acquired pneumonia (Laserna *et al.* 2012) and in patients with cystic fibrosis awaiting lung transplantation (Belkin *et al.* 2006). These studies raised the question whether hypercapnia may be associated with a dysregulated host immune response to fight infection

in patients with severe lung disease. The effects of hypercapnia on host immune response have been explored in *in vitro* and *in vivo* studies. Hypercapnia selectively inhibits the expressions of interleukin-6 (IL-6) and tumour necrosis factor (TNF), the innate immune effectors that play a role in host defence, and it has been reported to decrease phagocytosis in human and mouse macrophage cell lines as well as alveolar macrophages isolated from both species (Wang *et al.* 2010) (Fig. 3A). The inhibition of phagocytosis occurred independently of hypoxia, heat shock-responsive pathways or NO signalling. Furthermore, hypercapnia also inhibited autophagy and bacterial killing in human macrophages by increasing the expression of Bcl-2 and Bcl-xL, which bind Beclin 1 and prevent autophagy initiation (Casalino-Matsuda *et al.* 2015) (Fig. 3B). Recent studies reported that hypercapnia inhibits activation of the canonical NF- $\kappa$ B pathway that drives the expression of many host defence genes while promoting activation of the non-canonical NF- $\kappa$ B component RelB, whose function is largely anti-inflammatory and immunosuppressive (Cummins *et al.* 2010; Oliver *et al.* 2012) (Fig. 3C). In these *in vitro* studies, suppression of cytokine gene expression, phagocytosis, autophagy and NF- $\kappa$ B signalling by hypercapnia was independent of pH. In contrast, there are reports suggesting that hypercapnia might regulate the immune response by decreasing extracellular and/or

intracellular pH. Acidosis is known to impair the function of immune cells (Lardner, 2001), including alveolar macrophages (Lang *et al.* 2005). Thus, it appears that hypercapnia may modulate innate immunity and host defence via pH-independent or -dependent mechanisms. More recently, it has been reported that normoxic hypercapnia impairs antimicrobial host defence in a model of murine pneumonia caused by *Pseudomonas aeruginosa*, an important cause of pulmonary infection in patients who may have hypercapnia, such as those with advanced chronic obstructive pulmonary disease (COPD) and cystic fibrosis (Gates *et al.* 2013). Mice exposed to hypercapnia had higher mortality and increased burden of *Pseudomonas aeruginosa* in the lungs and other organs. The lung levels of IL-6 and TNF were decreased during the early phase of infection, and inhibited the phagocytosis of bacteria and generation of reactive oxygen species by lung neutrophils (Gates *et al.* 2013).

**Airway function.** Several studies suggest that hypercapnia is a marker of poor prognosis in patients with obstructive lung disease such as COPD (Köhnlein *et al.* 2014) and obesity hypoventilation syndrome (Piper, 2016). In addition, there is increasing evidence that the strategy of mechanical ventilation aimed at reducing the partial pressure of CO<sub>2</sub> in arterial blood ( $P_{aCO_2}$ ) can have beneficial effects including improvement of forced



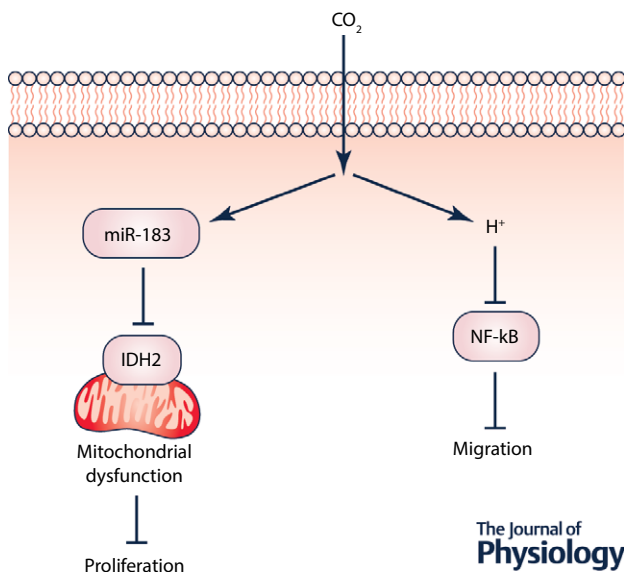
**Figure 1. Hypercapnia impairs alveolar fluid reabsorption**

Reduction of lung edema clearance is associated with the endocytosis of the Na<sup>+</sup>,K<sup>+</sup>-ATPase from the plasma membrane of alveolar epithelial cells, which leads to decreased Na<sup>+</sup>,K<sup>+</sup>-ATPase activity. During hypercapnia protein kinase C (PKC)- $\zeta$  directly phosphorylates the Na<sup>+</sup>,K<sup>+</sup>-ATPase  $\alpha$ 1-subunit at Ser 18 residue, leading to endocytosis of the Na<sup>+</sup>,K<sup>+</sup>-ATPase. The activation of PKC- $\zeta$  is regulated by AMP kinase (AMPK) via Ca<sup>2+</sup>/calmodulin-dependent protein kinase kinase- $\beta$  (CAMKK- $\beta$ ) and extracellular signal-regulated kinase (ERK). The endocytosis of the Na<sup>+</sup>,K<sup>+</sup>-ATPase by hypercapnia is also regulated by c-Jun-N-Terminal Kinase (JNK) via an AMPK-PKC- $\zeta$  signaling. JNK promotes the phosphorylation of LMO7b, which regulates the actin cytoskeleton in epithelial cells, followed by its colocalization and interaction with the Na<sup>+</sup>,K<sup>+</sup>-ATPase and several components of the clathrin-dependent endocytic machinery. The protein kinase A (PKA)-I $\alpha$  also plays a role in the Na<sup>+</sup>,K<sup>+</sup>-ATPase endocytosis during hypercapnia. Namely, hypercapnia via a CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup>-sensitive soluble adenylyl cyclase (sAC) increases the production of cAMP, activates PKA-I $\alpha$  and in turn, the phosphorylation of the actin cytoskeleton component  $\alpha$ -adducin, culminating in the Na<sup>+</sup>,K<sup>+</sup>-ATPase endocytosis from the cell plasma membrane.



expiratory volume, health related quality of life and mortality in hypercapnic patients with COPD (Windisch *et al.* 2008; Köhnlein *et al.* 2014). Changes in  $\text{CO}_2$  are known to modulate airway smooth muscle tone. However, reported effects of  $P_{\text{aCO}_2}$  changes in respiratory mechanics of spontaneously breathing, unanaesthetized healthy human subjects are controversial. With inhalation of  $\text{CO}_2$  mixtures, pulmonary resistance has been shown to increase, decrease, or remain unchanged (Sterling, 1969; Rodarte & Hyatt, 1973; Badr *et al.* 1991). Part of this variability probably reflects the multiple sites of action of  $\text{CO}_2$ . An *in vitro* study showed that hypercapnic

acidosis produced a reversible reduction in active tension of bronchial rings while normocapnic acidosis was without any effect (Stephens *et al.* 1968). This is probably related to a difference in intracellular pH under the different conditions studied. Intracellular pH directly modulates the entry of  $\text{Ca}^{2+}$  into airway smooth muscle cells through voltage dependent  $\text{Ca}^{2+}$  channels (Yamakage *et al.* 1995), suggesting that modulation of  $\text{Ca}^{2+}$  influx into airway smooth muscle cells by intracellular pH contributes to the regulation of airway tone by  $\text{CO}_2$ . On the other hand, hypercapnia, independently of acidosis, also leads to a rapid and transient increase in intracellular  $\text{Ca}^{2+}$  (Vadasz *et al.* 2008).

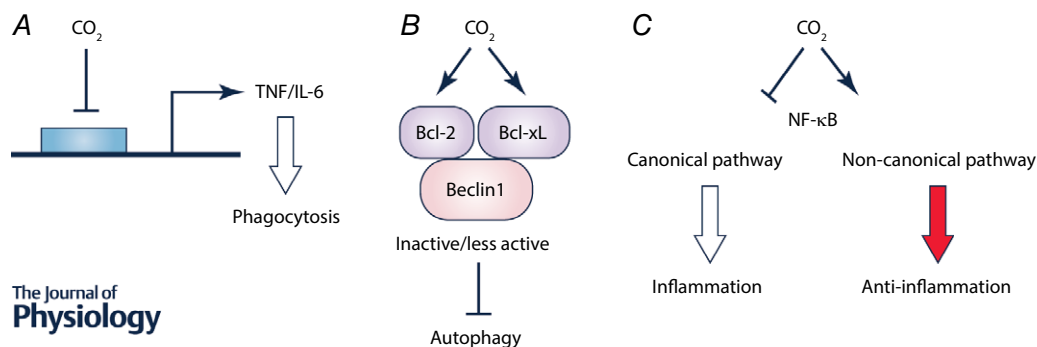


**Figure 2. Hypercapnia inhibits alveolar epithelial repair**

Hypercapnia inhibits proliferation of alveolar epithelial cells due to mitochondrial dysfunction resulting from hypercapnia-induced miR-183 which down-regulates the TCA cycle enzyme isocitrate dehydrogenase-2 (IDH2). Hypercapnic acidosis impairs alveolar epithelial cell migration by the NF- $\kappa$ B-dependent mechanism.

## Conclusion

Historically, it has been proposed that hypercapnia, and especially hypercapnic acidosis, may have beneficial effects in mechanically ventilated patients and patients with ALI. The term 'permissive hypercapnia' has been proposed and is being used in treating patients (Hickling *et al.* 1994; Amato *et al.* 1998; Contreras *et al.* 2015). Many of the cellular responses to hypercapnia were thought to be a consequence of acidosis because of the rapid conversion of  $\text{CO}_2$  in solution into  $\text{H}_2\text{CO}_3$  and subsequently  $\text{HCO}_3^-$  and  $\text{H}^+$ . Recent studies suggest that molecular  $\text{CO}_2$  can act as a signalling molecule and that hypercapnia can have deleterious effects in the lung (Briva *et al.* 2007; Vadasz *et al.* 2008; Vohwinkel *et al.* 2011; Gates *et al.* 2013; Casalino-Matsuda *et al.* 2015; Dada *et al.* 2015) and patient survival (Köhnlein *et al.* 2014; Bharat *et al.* 2016). The impairment of these lung physiological functions by hypercapnia probably underlies the negative impacts of hypercapnia in patients with severe acute or chronic lung diseases. Thus, further preclinical and clinical studies are needed to define which of these (or other) effects of hypercapnia are beneficial or deleterious in patients with lung diseases.



**Figure 3. Hypercapnia suppresses innate immunity and host defence**

A, hypercapnia selectively inhibits mRNA and protein expressions of IL-6 and TNF and decreases phagocytosis in macrophages. B, hypercapnia inhibits autophagy in macrophages by increasing expressions of Bcl-2 and Bcl-xL which bind Beclin 1. C, hypercapnia inhibits activation of the canonical NF- $\kappa$ B pathway that drives expression of inflammatory cytokine genes while promoting activation of the non-canonical NF- $\kappa$ B pathway.

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## Additional information

### Competing interests

None declared.

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