In Search of a Sensor: How Does CO_2 Regulate Alveolar Ion Transport?

Levels of carbon dioxide (CO_2), which is an end product of cellular energy metabolism, are well controlled by respiration. Therefore, abnormal alveolar ventilation strongly affects blood pH, in that hyperventilation and hypoventilation cause respiratory alkalosis and acidosis, respectively. Hypercapnia often occurs in patients on mechanical ventilation with elevated fractional oxygen content in the inspired air because of ventilation with low tidal volume designed to minimize ventilator-induced lung damage (1, 2), such as in acute respiratory distress syndrome. But hypercapnia severely impairs alveolar fluid clearance (1), causing or exaggerating alveolar edema in the already injured lung and resulting in tissue hypoxia, which also impairs alveolar fluid clearance and thus aggravates edema formation.

Alveolar fluid clearance is driven by vectorial Na transport mediated by apical epithelial Na channels (ENaC) and basolateral Na/K-ATPase (NKA). Both fluid reabsorption and Na transport are inhibited by hypercapnia, as shown in a series of publications by Sznajder and colleagues using various models that have unraveled bit by bit the signaling cascade (3–9). These publications show that hypercapnia, by transiently increasing the cytosolic Ca^{2+} concentration ($Ca^{2+}{}_i$), initiates a cascade involving calcium–calmodulin–dependent kinases, mitogen-activated protein (MAP)-kinases, adenosine monophosphate (AMP)-activated kinase, protein kinase C (PKC)ζ, and c-Jun-terminal kinases (JNK) to cause internalization of the NKA and ENaC subunits and retention of the β1-NKA subunit in the endoplasmic reticulum (ER). It remains to be shown whether internalization of α/β-NKA and of α/β ENaC are caused by the same or by independent mechanisms.

In this issue of the *Journal*, Kryvenko and colleagues (pp. 615–629) (10) report that acute hypercapnia-exposed lung slices and A549 cells similarly attenuate the maturation of the β-NKA, a modulator of activity and supporter of membrane sorting of NKA (11). In both systems, hypercapnia caused retention of β-NKA in the ER by increasing the release of Ca^{+2} from the ER via 1,4,5-trisphosphate (IP3) receptors. This activates IRE1-α to cause degradation of β-NKA. Genetic ablation or inhibition of IRE1-α prevented the hypercapnia effect, and induction of ER stress confirmed the role of endoplasmic-reticulum-associated degradation (ERAD) in degrading β-NKA during hypercapnia.

Although this extraordinary line of experiments clarifies many downstream signaling events leading to the internalization of Na^+ transporters, the view on the CO_2 sensor and the initial signaling machinery remains blurred. Surprisingly, the most obvious mechanism, acidification, a potent regulator of both ENaC and NKA, seems not to play a significant role because CO_2 effects persist after clamping the extracellular pH and after inhibition (although incomplete) of carbonic anhydrases (CA) (3, 4). This ancient and ubiquitously expressed enzyme catalyzes with high speed the interconversion between CO_2, H^+, and bicarbonate and significantly contributes to lung CO_2 elimination (12). The elevation of bicarbonate in hypercapnia certainly affects the intracellular chloride concentration, which in turn might affect transepithelial transport of ions by altering the electrochemical gradients and might thus act independent of complex Ca^{2+} signaling. If it is not the CA mediating the CO_2 effects in alveolar epithelium in culture, in the *in vivo* situation, it might well play a role because of high activity of CA in erythrocytes (13).

At first view, one mechanism common to the internalization of α1- and β1-NKA subunits seems to be an increase in the intracellular Ca^{2+} concentration. However, the results are not consistent. BAPTA-AM, which is often used to scavenge intracellular Ca^{2+} and to prevent its signaling-induced concentration changes, fully prevented the increase in phospho-AMPK by hypercapnia (5). In the present paper, BAPTA-AM at normal CO_2 decreased β1-NKA in the ER and increased p-IRE1 levels, similar to hypercapnia. However, in presence of BAPTA-AM, hypercapnia still exerted its effects (*see* Figure 5 in Reference 10). Also, eliminating extracellular Ca^{2+} did not prevent hypercapnia-induced effects (*see* Figure E4 in Reference 10). Thapsigargin, which transiently increases $Ca^{2+}{}_i$ and prevents filling of intracellular stores by blocking SERCA, increased p-IRE1 and decreased β1-NKA in the ER (*see* Figure E1 in Reference 10) consistent with $Ca^{2+}{}_i$ being the relevant signal. The lack of effect of hypercapnia in the presence of BAPTA-AM plus thapsigargin was interpreted as indicative that a decrease in ER Ca^{2+} levels might be the relevant signal. These inconsistencies await clarification.

How should we proceed with shedding light on the signaling in hypercapnia? First, the CO_2 sensor and its immediate downstream signals need to be identified. Is there a target protein controlling Na^{2+} transport, whose function is altered directly by CO_2 binding (14) similar to changes in oxygen affinity of hemoglobin (15)? There is literature indicating CO_2-induced ATP-release. Extracellular ATP might cause an increase in Ca_i via purinergic receptors and Ca-induced Ca^{2+} release from intracellular stores modulates Ca-induced effects on ion transport in epithelial cells (16). CO_2-induced release of ATP via connexin hemichannels was found in medulla oblongata and Hela cells independent of acidification (17), which might be caused by formation of carbamate (18).

Might comparison with below-ground–dwelling animals and hibernators provide insight into adjustments to hypercapnia? In fact, these species experience not only hypercapnia but also hypoxia, a situation very similar to patients with respiratory distress syndrome (ARDS) and acute lung injury (ALI). This points to another open and clinically very relevant question: What happens with ion transport in

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hypercapnia combined with hypoxia, which also inhibits alveolar reabsorption (19) by inhibiting ENaC and NKA (20)?

Is there a treatment to prevent the adverse effects of reduced alveolar fluid clearance in hypercapnia? Extracorporeal membrane oxygenation, which is used when CO_2 reaches dramatically high levels in ventilated patients, maintains both oxygen supply and removal of CO_2 and thus resolves the inhibition of reabsorption caused by hypercapnia. Considerably less invasive is the stimulation of Na^+ reabsorption with β2-adrenergic agents, which not only prevented but even reversed hypercapnic (5) and hypoxic inhibition (21) of alveolar reabsorption, but there might be contraindications for their use. Directly targeting the CO_2 sensor or early CO_2-specific signals might have the fewest unwanted effects. Intervention at levels of MAP kinases, AMPK, or at further distal sites might have too many side effects because of their involvement in multiple signaling events. ■

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