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Review

# The respiratory chemoreception conundrum: Light at the end of the tunnel?

*Patrice G. Guyenet, Stephen B.G. Abbott, Ruth L. Stornetta**

*Department of Pharmacology, University of Virginia, 1300 Jefferson Park Ave, Charlottesville, VA 22908, USA*

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ABSTRACT

Arterial $PCO_2$ is tightly regulated via changes in breathing. A rise in $PCO_2$ activates the carotid bodies and exerts additional effects on neurons located within the CNS, causing an increase in lung ventilation. Central respiratory chemoreception refers to the component of this homeostatic reflex that is triggered by activation of receptors located within the brain (central chemoreceptors). Throughout the body, $CO_2$ generally operates via the proxy of pH. Since countless proteins, ion channels and neurons display some degree of pH-sensitivity, the notion that central respiratory chemoreception could rely on a few specialized neurons seems a priori counter-intuitive. Yet, two types of neurons currently stand out as critically important for breathing regulation by $CO_2$: the retrotrapezoid nucleus (RTN) and the raphe. RTN neurons are glutamatergic, strongly activated by hypercapnia in vivo and by $CO_2$ or protons in slices. These neurons target selectively the pontomedullary regions implicated in generating the respiratory rhythm and pattern. Their response to $CO_2$ seems to involve both cell-autonomous and paracrine effects of $CO_2$, the latter presumably mediated by the surrounding glia. The specific connections that these excitatory neurons establish with the rest of the breathing network are likely to be the main explanation of their importance to respiratory chemoreception. Serotonergic neurons have a powerful stimulatory effect on breathing, they facilitate the chemoreflexes and a subset of them likely function as $CO_2$ sensors.

Opto- and pharmacogenetic methods have played an important role in assessing the contribution of RTN and serotonergic neurons as well as glial cells to respiration. These particular experiments are emphasized here for thematic reasons although the current perception of the importance of the RTN and serotonergic cells to respiratory chemoreception also relies on many other types of evidence. A small portion of this evidence is presented as background.

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## Contents



*Corresponding author. Fax: +1 804 982 3878, 1 434 982 3878.
E-mail addresses: pgg@virginia.edu (P.G. Guyenet), sba6t@virginia.edu (S.B.G. Abbott), rs3j@virginia.edu, stornetta@virginia.edu (R.L. Stornetta).

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## 1. The conundrum of central respiratory chemoreception

A conundrum is "a confusing and difficult problem or question". Central respiratory chemoreception is the extremely sensitive mechanism by which very small changes in brain $PCO_2$ produce very large changes in breathing. The conundrum is that the mammalian brain has a plethora of neurons or channels that individually respond to $CO_2$/pH and, in most cases, it has been very difficult to establish which of these widespread effects of pH are germane to central respiratory chemoreception. The interpretative problems experienced by the field of central chemoreception are in many ways similar to those encountered by investigators who are trying to sort out which of the myriads of glucose- or temperature-sensitive CNS neurons regulate glycemia or body temperature.

The amplitude and frequency of respiratory movements were already measured over a century ago (Haldane and Priestley, 1905). By using a simple procedure for measuring $PCO_2$ in expired alveolar air, Haldane and Priestley established two fundamental aspects of breathing regulation, namely the powerful stimulatory effect of $CO_2$ on lung ventilation and the fact that arterial $PCO_2$ is very tightly regulated regardless of physical activity or altitude. The notion that $PCO_2$ is "the main factor that normally determines lung ventilation" originates from these studies (Haldane and Priestley, 1905). In reality breathing adjusts to any change in behavior and, unless airways are obstructed, these adjustments do not operate in reaction to changes in $PCO_2$ but primarily in a feed-forward mode via multiple CNS inputs to the brainstem respiratory centers. These inputs are related to emotions, exercise, metabolic status, body temperature and the state of vigilance. Although behavior- or emotion-related changes in breathing are not driven by variations in $PCO_2$, $PCO_2$ stability is essential to maintain tissue pH constant and this homeostatic regulation requires a powerful feedback control that operates regardless of the magnitude of lung ventilation. This feedback is driven by $CO_2$ sensors located in the carotid bodies (peripheral chemoreceptors) and within the CNS, the central respiratory chemoreceptors (Smith et al., 2010). The location and cellular nature of the central respiratory chemoreceptors (neurons, glia, vascular cells, pluricellular functional units) is still not definitively established (Nattie and Li, 2009; Gourine et al., 2010; Guyenet et al., 2010). $CO_2$ is thought to work via protons (the "reaction theory" (Loeschcke, 1982)), a plausible theory that has been difficult to put to the test given the plethora of pH-modulated proteins, particularly ion channels, that could serve as "proton receptors". Molecular $CO_2$ may also exert direct effects on glial cells or neurons, perhaps by changing the properties of connexin hemi-channels (Huckstepp et al., 2010). The identification of central respiratory chemoreceptors has also been hampered by the fact that many neurons are activated or inhibited by acidification in vitro or in culture, regardless of whether their anatomical connections in the intact brain would enable them to exert any influence on the activity of the breathing network (Su et al., 2007). These relatively widespread and sometimes sizeable effects of pH on neurons in vitro (Wang et al., 2001) are not always seen in vivo (Mulkey et al., 2004) and have been variously interpreted.

This review focuses on the contribution to central respiratory chemoreception of the retrotrapezoid nucleus (Sections 2–7), the medullary raphe (Section 9, for further details on the latter topic see: (Hodges and Richerson, 2010b)) and the glia (Section 8). These cells have been selected because they have been subjected to the most exhaustive recent investigations and optogenetics has helped to understand their role in respiration.

This article is not intended as a comprehensive review on chemoreception. The following reviews are recommended for additional information on this topic (Nattie and Li, 2008; Corcoran et al., 2009; Duffin, 2010; Buckler, 2010; Forster and Smith, 2010; Hodges and Richerson, 2010a; Nattie, 2011). Although these authors also highlight the contribution of RTN neurons and the raphe to central chemoreception, their view is that central chemosensitivity is caused by a widespread sensitivity of the lower brainstem respiratory centers to pH/$P_{CO2}$ combined with a potentially equally widespread pH/$P_{CO2}$ sensitivity of the many neurons that regulate the activity of this network. These interpretations rely on microdialysis experiments in which focal acidification of various regions of the brain is shown to increase respiration to some degree and on the result of brain lesions or pharmacological interventions.

## 2. Identification and early evidence for the role of the RTN in respiration

The neurons that generate the respiratory rhythm and the pattern of activity of the various breathing muscles (respiratory pattern generator, RPG) are located within the ventrolateral medulla oblongata and dorsolateral pons (Smith et al., 2009). Experiments conducted in anesthetized cats in the early 1960s suggested that the central respiratory chemoreceptors might be confined to the ventral surface of the medulla oblongata, one of the key regions being located just caudal to the trapezoid bodies (Mitchell et al., 1963). While searching for inputs to the RPG with conventional retrograde