Central chemoreception is a complex system function that involves multiple brain stem sites

Eugene Nattie and Aihua Li
Department of Physiology, Dartmouth Medical School, Lebanon, New Hampshire

Central chemoreception refers to detection of CO₂/pH within the brain and the subsequent reflex effects on breathing. It involves multiple sites within the hindbrain (9, 12, 19) as focal acidification in vivo uniquely at these sites stimulates breathing, indicating detection and chemoreflex initiation. This Viewpoint is not all inclusive but focuses on recent reports of substantial decreases in the CO₂ response arising from disruption in each of three central chemoreceptor sites (1, 2, 6) as well as in a broad distribution of neurokinin-1 receptor immunoreactive (NK1R-ir) neurons and processes (21). These latter results are significant as two of the chemoreceptor sites of present interest express NK1Rs and the other contains substance P, the natural ligand for the NK1R. At issue is how the central chemoreceptor system is organized if disruption in many locations causes substantial loss of chemosensitivity in vivo. The emphasis is on data obtained in conscious animals as anesthesia substantially reduces the CO₂ response (1).

Glutamatergic neurons in the retrotrapezoid nucleus (RTN) that express the transcription factor Phox2b have been proposed as putative central chemoreceptors (11, 28) and suggested to be of predominant importance (11). The RTN in the rodent lies between the ventral medullary surface and facial nucleus and extends a few hundred micrometers caudal to it (5, 11, 28). It appears to overlap with or lie adjacent to the parafacial respiratory group (pFRG) thought to be involved in rhythm generation in neonatal rats (24). The exact relationship between the RTN and the pFRG is unclear. Very small lesions produced by moderately sized lesions. The overall importance of the RTN in the conscious adult animal remains uncertain. The medullary raphe 5-HT cells are certainly of import in the development of breathing control in early life as their loss produced by a mutation of the transcription factor pet-1 is associated with unstable breathing and death of some pups in some conditions (8).

The Phox2b cells in the RTN are certainly of import in early life as their loss by genetic manipulation in mice is associated with absent chemoreception, unstable breathing, and death within hours of birth (7), although there may be additional functional abnormalities, e.g., in the carotid body. That patients with Central Congenital Hypoventilation Syndrome (CCHS) have Phox2b abnormalities and markedly diminished CO₂ responses suggests an important role for Phox2b RTN cells, although again these patients can have multiple abnormalities of the autonomic nervous system including brain noradrenergic neurons (27).

Serotonergic (5-HT) neurons of the medullary raphe have been proposed as putative central chemoreceptors due to their chemosensitivity in vitro and to their proximate location to cerebral blood vessels (26). If all 5-HT neurons are absent from early prenatal life, as in the floxed Lmx1b/Pet-1 Cre mouse, the CO₂ response is reduced by >50% (13). This could reflect loss of 5-HT effects on 1) neuronal development, 2) chemosensitivity, and/or 3) excitatory modulation of chemoreceptor and respiratory neurons (see below). Adult rat lesion data also implicate 5-HT neurons in chemoreception. A 28% loss of 5-HT neurons distributed over the medullary raphe reduced the CO₂ response by up to 18% (23). More focal and effective killing, via injection of the cell specific toxin anti-SERT-saporin, of 5-HT neurons in raphe magnus (50% loss) reduced the CO₂ response by up to 62%, a very substantial effect (6). Removal of the 5-HT transport protein (5-HTT) by genetic knockout in mice also results in a substantially reduced response to CO₂ that is more pronounced in males (~68%) than females (~22%), a result attributed to a medullary 5-HT system that is “downregulated” by the constant excess of extracellular 5-HT (15). Acute inhibition of raphe magnus 5-HT neurons by microdialysis of the 5-HT₁A receptor agonist 8-OH-DPAT decreased the CO₂ response by up to 30% (29) and focal acidic stimulation of raphe magnus increased ventilation (12, see 9, 19). More caudally, inhibition of medullary raphe 5-HT neurons had no effect on the CO₂ response (16) and enhanced the inhibition of the CO₂ response at the RTN produced by simultaneous application of muscimol (16). The rostral medullary raphe (raphe magnus and pallidus) may be a site of chemosensitivity that directly affects respiratory neurons while the caudal medullary raphe (raphe obscurus) acts as a modulator of other chemoreceptor sites, e.g., the RTN. Phox2b positive RTN neurons that are putative chemoreceptors possess 5-HT receptors and can be excited by local 5-HT application (18). The overall importance of 5-HT neurons in the medullary raphe in the conscious adult animal in chemoreception remains uncertain. The medullary raphe 5-HT cells are certainly of import in the development of breathing control in early life as their loss produced by a mutation of the transcription factor pet-1 is associated with unstable breathing and death of some pups in some conditions (8).

Noradrenergic neurons of the locus ceruleus have been proposed as putative central chemoreceptors due to their che-
mosensitivity in vitro (25). They are also located close to blood vessels (10). Widespread lesions of catecholamine neurons via injection into the cisterna magna of the cell specific toxin anti-dopamine-β-hydroxylase-saporin with a 73–84% loss at A5, A6, and A7 reduced the CO₂ response by 28% (14), a moderate effect. But specific and substantial lesions of locus ceruleus noradrenergic neurons bilaterally (>50% loss of NA neurons) in rats reduced the ventilatory response to CO₂ by 64% (2). Here, as in raphe magnus (6), a large specific lesion at a specific location has a profound effect on the CO₂ response. One caveat is that these lesions were produced by injection of 6-hydroxy-dopamine, which may also affect other neurons. The overall importance of the NA neurons for chemoreception in the conscious adult animal remains uncertain but the NA cells of the locus ceruleus are certainly of import in early life as their loss produced by a Phox2a mutation is associated with unstable breathing and death just after birth (30).

NK1R-ir in the medulla is present as a broad network of processes and cells (see 21, 22) including noradrenergic cells in locus ceruleus (4) and Phox2b glutamatergic cells of the RTN (28) but not 5-HT cells of the medullary raphe (23). Focal lesions of NK1R-ir neurons and processes in RTN (22) and along the medullary raphe (23) decreased the CO₂ response by 21 to 30%. Larger lesions of NK1R-ir cells and processes throughout the ventral medulla (−79% in the RTN, −65% in the A5 region, −38% in the medullary raphe and −49% in the pre-Bötzinger complex/rostral ventral respiratory group) reduced the CO₂ response by 65% and resulted in hypoventilation during air breathing (21). NK1Rs and SP are importantly involved in central chemoreception. Medullary substance P is predominantly localized in 5-HT neurons (17), which could be the source of activation of brain stem NK1Rs involved in chemoreception. It also provides a drive to breathe.

In summary:

1) That disruption of locus ceruleus NA neurons, raphe magnus 5-HT neurons, and NK1R-ir cells and processes can each dramatically reduce the CO₂ response argues for a complex system organization and against a predominant single site for central chemoreception that acts alone, although a recent review argues otherwise (11). Central chemoreception involves monoamine neurons that can detect CO₂/pH and affect respiratory neurons and other chemoreceptor sites. It is of interest that the activity of these monoamine neurons is arousal state dependent and their activation by CO₂ likely contributes to arousal as well as ventilation.

2) The medullary raphe appears to be heterogeneous in function related to chemoreception and the 5-HT neurons in the medullary raphe that contain SP may be of particular importance.

3) Many types of brain stem neurons are excited by CO₂ in reduced preparations (26). The role of each in vivo is challenging to demonstrate. Some may influence respiratory output directly; others may do so indirectly.

4) There are arguments for a predominant role of the RTN in chemoreception (see 9, 11) but its role in the intact conscious animal remains uncertain. This statement is also true for the other putative central chemoreceptor sites, but the data outlined above show severe deficits in chemoreception from specific, focal lesions in raphe magnus, locus ceruleus, and in NK1R-expressing neurons and processes in the ventral medulla, suggesting to us a complex organization.

of neurons and sites that participate in central chemoreception.

We hypothesize that the many sites involved in central chemoreception provide stability in a “closed loop” control system. Excitation at a single site is modulated by subsequent hypocapnic inhibition at other sites. Excitation at many sites produces an enhanced response. Multiple sites allow correction of regional imbalances of VA, cerebral blood flow, and metabolism. They also provide redundancy and malleability with the importance of each site likely differing with development, arousal state, stimulus intensity, pathophysiology, and sex.

GRANTS
The authors’ work is supported by National Heart, Lung, and Blood Institute Grant R37-HL-28066 and by National Institute of Child Health and Human Development Grant PO1-HD-036379.

REFERENCES
17. Mulkey DK, Rosin DL, West G, Takakura AC, Moreira TS, Bayliss DA, Guyenet PG. Serotonergic neurons activate chemosensitive retro-


