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Review Article

The Retrotrapezoid Nucleus and Central Chemoreception

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Abstract

The functional role of retrotrapezoid nucleus (RTN) neurons as the central chemoreceptors and the potential implications of Phox2b expressed in these neurons will be discussed. RTN resides at the ventral medullary surface. RTN lesions reduce central respiratory chemoreception (CRC). RTN neurons are glutamatergic propriobulbar interneurons that selectively innervate the ventral respiratory column and other medullary regions essential to breathing. Their response to CO_2 is presumably intrinsic. RTN neurons uniformly express Phox2b, a transcription factor whose mutation in man causes a loss of CRC and central sleep apnea. RTN neurons are activated by stimulation of the carotid bodies, restrained by inhibitory inputs from the central respiratory pattern generator and from lung afferents and their response to CO_2 is sensitized by serotonin and by peptides released by serotonin neurons. The properties of RTN neurons are consistent with those expected from specialized central respiratory chemoreceptors. These data also suggest that respiratory reflexes operate in part by regulating the activity of central chemoreceptors. RTN neurons and the neurons that relay carotid body inputs to the respiratory centers express Phox2b. This peculiarity probably accounts for the loss of CRC associated with Phox2b mutations in man (central congenital hypoventilation syndrome). [*Tzu Chi Med J* 2008;20(4):239–242]

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1. Introduction

Central respiratory chemoreception (CRC) is the mechanism by which brain pCO_2 regulates breathing. This mechanism is especially critical to sustain respiration during sleep. Brain pCO_2 is almost certainly detected via changes in pH, but the molecular, cellular and integrative basis of CRC is still being defined [1]. Airway motor neurons and many of the neurons that make up the central respiratory pattern generating network (CPG) respond to acidification in various brainstem preparations *in vitro* [2,3]. Yet, in such preparations, even severe acidification only causes a weak activation of the oscillatory frequency of the respiratory network and typically decreases rather than increases respiratory motor neuronal output. These results are in

striking contrast to the exquisite sensitivity of the respiratory outflow to pCO_2 *in vivo*. One possible reason for this discrepancy is that CRC requires the participation of specialized pH-responsive neurons (central chemoreceptor neurons) and that the integrity of these cells or their connections to the CPG is disrupted *in vitro*. The existence of a genetic disease in humans in which CRC is selectively attenuated causing severe sleep apnea [4] strongly supports the existence of specialized central chemoreceptors.

The first experimental evidence suggesting the existence of specialized central chemoreceptors came from manipulations of the ventral medullary surface (VMS) (acidification, lesions) performed in the early 1960s [5]. The VMS chemoreceptors have not been definitively identified and the literature suggests that other chemoreceptors may reside elsewhere in the ventrolateral medulla and in the raphe [6].

This brief review summarizes our recent work on a group of VMS neurons whose properties are consistent with a central respiratory chemoreceptor role. These neurons are located in the retrotrapezoid nucleus (RTN), a name originally coined by Smith et al [7] to describe a cluster of cells that innervate more caudal regions of the ventral respiratory column. The RTN resides approximately at the level of area M, a region of the ventral medullary surface already known for its high pH sensitivity [5,8].

2. Properties of RTN neurons *in vivo*

2.1. *Sensitivity to hypercapnia and evidence of intrinsic sensitivity to CO_2 in vivo*

A unique combination of characteristics distinguishes RTN neurons from most if not all other respiratory neurons examined to date. Under anesthesia, RTN neurons are silent at low levels of end-expiratory pCO_2 (threshold around arterial pH of 7.5), increasingly active as pCO_2 is raised, reaching about 8–14 Hz at 10% end-expiratory pCO_2, and their CO_2 threshold is always lower than that of the respiratory network [9–11]. Also, especially in vagotomized animals, RTN neurons typically do not display an ON-OFF discharge pattern like classic CPG neurons, even at very high levels of pCO_2 [10]. Third and perhaps most critical, the CO_2 sensitivity of RTN neurons *in vivo* is resistant to blockade of ionotropic glutamatergic receptors and to the administration of high doses of morphine, whereas such treatments silence most CPG neurons and/or render them insensitive to CO_2 [9]. These observations indicate that the CO_2 sensitivity of RTN neurons is not driven by the activity of the CPG and is unlikely to be caused by synaptic inputs from pH-sensitive neurons located elsewhere in the brain.

2.2. *Feed-forward regulation of RTN neurons by the carotid bodies*

RTN neurons are strongly activated by carotid chemoreceptor stimulation [11]. The pathway from the carotid bodies to RTN may involve only two neurons, namely the primary afferents innervating the carotid bodies and second-order glutamatergic neurons located in the commissural portion of the solitary tract nucleus. In essence, RTN neurons have the properties of a chemosensory integrating center: their activity reflects both brain pH and the composition of the arterial blood (pCO_2 and pO_2).

2.3. *Feedback regulation of RTN neurons by pulmonary afferents*

A large proportion of RTN neurons are inhibited by lung inflation [12]. This inhibition is caused by activation of slowly-adapting pulmonary mechanoreceptors (SARs). The pathway from SARs to RTN may also be di-synaptic, consisting of the primary afferents and GABAergic pump cells (the second-order neurons) located in the interstitial portion of the solitary tract nucleus. This finding indicates that the excitatory drive from central chemoreceptors (RTN) to the CPG can be downregulated by inputs from lung mechanoreceptors. This process may be viewed as a negative feedback that decreases the influence of central chemoreceptors under conditions when breathing is already highly active due to other causes than a high pCO_2.

2.4. *Feedback regulation of RTN neurons by the CPG*

In vagotomized rats, there is clear evidence that RTN neurons receive CPG-related inputs. These inputs seem predominantly inhibitory and are most likely responsible for the saturation of the discharge of RTN neurons and of the phrenic nerve that is observed at high levels of CO_2 [10]. The feedback from the CPG, like that from lung mechanoreceptors, may serve to downregulate the CO_2-dependent excitatory drive from RTN when the CPG is being vigorously activated by other inputs (hypothetically by pain, emotions and exercise).

2.5. *Transmitters, structure and anatomical projections of RTN neurons*

RTN neurons express the vesicular glutamate transporter type-2, suggesting that they release glutamate and are therefore excitatory [9]. This fact is consistent with prior evidence that the RTN region is a source of excitatory drive to the CPG [11,13]. RTN neurons have

two dendritic domains, one of which resides within the marginal layer of the ventral medulla (9,10). RTN neurons should therefore be exceptionally responsive to local application of drugs or acid at the VMS, consistent with the possibility that these neurons could be the VMS chemoreceptors identified earlier as area M (5,14).

Also consistent with a central chemoreceptor role, RTN neurons do not innervate the spinal cord but they do innervate the regions of the brainstem that contain the CPG, i.e., the ventral respiratory column, dorsolateral pons and selected lateral regions of the solitary tract nucleus (9,15,16).

2.6. *Phox2b, RTN and the central congenital hypoventilation syndrome*

Mutations of the homeobox gene *PHOX2B* are responsible for most cases of central congenital hypoventilation syndrome (CCHS) (4,17). The cardinal symptoms of CCHS are a very severe central sleep apnea and a profound reduction of peripheral and central chemoreflexes (17–19). A remarkable feature of the disease is that breathing is usually fairly normal during waking and responds normally to exercise and emotions (20,21). These characteristics suggest that the CPG of CCHS patients must be largely intact, hence the greatly reduced respiratory activity during sleep may be due to the selective loss of neurons implicated in CRC.

Consistent with this notion, Phox2b is expressed by an unbroken chain of neurons involved in the integration of peripheral and central chemoreception (22). This circuit includes the carotid bodies, carotid body sensory afferents, and the NTS neurons that are activated by carotid body afferents and innervate the ventrolateral medulla and the RTN neurons themselves (11,22–24). The prevalence of Phox2b in this circuit and its scarcity in regions involved in respiratory rhythm and pattern generation (ventral respiratory column caudal to RTN, cardiorespiratory regions of the dorsal pons) explains why *PHOX2B* mutations selectively impair the chemical drive to breathe and hence cause sleep apnea without major breathing disruption while awake.

3. Properties of RTN neurons *in vitro* and mechanisms of pH sensitivity

In slices (postnatal age P4 to P12), the RTN region contains neurons that respond vigorously to acidification by increasing their discharge rate (9). These neurons are located at the ventral surface of the medulla and they have the characteristic dendritic structure of the CO_2-sensitive neurons that have been recorded *in vivo* (10). The response of RTN neurons to CO_2 *in vitro* is mediated by changes in pH (9). It is temperature-sensitive (Q_{10}~2.5) and robust (2.2 Hz per 0.1 pH unit at 37°C) (10). In slices, RTN neurons discharge tonically, regardless of the recording temperature (10). Their pH sensitivity is independent of glutamatergic, GABAergic, glycinergic and purinergic transmission. The pH sensitivity of RTN neurons is therefore largely intrinsic (9,25), an interpretation that is consistent with the presence of a TTX-resistant pH-modulated resting potassium conductance in these cells (9). This current has the properties of a leak current but the responsible channel, still unidentified, is not TASK (Twik-related acid-sensitive potassium channel). The pH-sensitive neurons that we study *in vitro* are almost certainly the same as those that we record *in vivo* because they have the same location and structure and they contain Phox2b mRNA as revealed by single cell polymerase chain reaction.

4. Conclusions

RTN neurons have numerous structural and physiological characteristics that are consistent with a central chemoreceptor role. RTN neurons are located at the VMS, their sensitivity to pH is high and independent of the activity of the rest of the respiratory network. RTN neurons release an excitatory transmitter (glutamate), their CO_2 threshold is below that at which the CPG is activated, and they have selective projections to the brainstem regions that contain the CPG. Consistent with the notion that their pH sensitivity is intrinsic, RTN neurons express a pH-sensitive background potassium conductance. Finally, RTN neurons in rodents also express a developmental gene (*Phox2b*) which, when mutated, is associated with a dramatic and selective loss of central respiratory chemosensitivity in man (4).

This checklist is compelling, but it does not constitute proof that the Phox2b-expressing neurons of RTN are critical to CRC nor that these neurons are the only central respiratory chemoreceptors. Such proof would require demonstrating that their selective destruction eliminates or, at least, attenuates the response of the respiratory network to hypercapnia. This goal has not been achieved yet but lesions of the region where these chemosensitive cells are located have produced results that are consistent with this notion (26).

Finally, if one accepts the interpretation that RTN neurons are central chemoreceptors, this type of cell should not be viewed as solely regulated by pH. Clearly, at any given brain pH, the firing rate of RTN neurons can also be greatly modified by lung inflation (via mechanoreceptors), blood gases (via the carotid bodies), the degree of activation of the CPG and, most likely, by a host of inputs still to be discovered.

References

1. Nattie E, Li A. Central chemoreception 2005: a brief review. *Auton Neurosci* 2006;126–127:332–8.
2. Kawai A, Ballantyne D, Muckenhoff K, Scheid P. Chemosensitive medullary neurones in the brainstem—spinal cord preparation of the neonatal rat. *J Physiol* 1996;492: 277–92.
3. Bayliss DA, Talley EM, Sirois JE, Lei Q. TASK-1 is a highly modulated pH-sensitive 'leak' K^+channel expressed in brainstem respiratory neurons. *Respir Physiol* 2001;129: 159–74.
4. Amiel J, Laudier B, Attie-Bitach T, et al. Polyalanine expansion and frameshift mutations of the paired-like homeobox gene *PHOX2B* in congenital central hypoventilation syndrome. *Nat Genet* 2003;33:459–61.
5. Loeschcke HH. Central chemosensitivity and the reaction theory. *J Physiol* 1982;332:1–24.
6. Feldman JL, Mitchell GS, Nattie EE. Breathing: rhythmicity, plasticity, chemosensitivity. *Annu Rev Neurosci* 2003;26: 239–66.
7. Smith JC, Morrison DE, Ellenberger HH, Otto MR, Feldman JL. Brainstem projections to the major respiratory neuron populations in the medulla of the cat. *J Comp Neurol* 1989;281:69–96.
8. Okada Y, Chen Z, Jiang W, Kuwana S, Eldridge FL. Anatomical arrangement of hypercapnia-activated cells in the superficial ventral medulla of rats. *J Appl Physiol* 2002;93:427–39.
9. Mulkey DK, Stornetta RL, Weston MC, et al. Respiratory control by ventral surface chemoreceptor neurons in rats. *Nat Neurosci* 2004;7:1360–9.
10. Guyenet PG, Mulkey DK, Stornetta RL, Bayliss DA. Regulation of ventral surface chemoreceptors by the central respiratory pattern generator. *J Neurosci* 2005;25:8938–47.
11. Takakura AC, Moreira TS, Colombari E, West GH, Stornetta RL, Guyenet PG. Peripheral chemoreceptor inputs to retrotrapezoid nucleus (RTN) CO_2-sensitive neurons in rats. *J Physiol* 2006;572:503–23.
12. Moreira TS, Takakura AC, Colombari E, West GH, Guyenet PG. Inhibitory input from slowly adapting lung stretch receptors to retrotrapezoid nucleus chemoreceptors. *J Physiol* 2007;580:285–300.
13. Nattie EE. The retrotrapezoid nucleus and the 'drive' to breathe. *J Physiol* 2006;572:311.
14. Millhorn DE, Eldridge FL. Role of ventrolateral medulla in regulation of respiratory and cardiovascular systems. *J Appl Physiol* 1986;61:1249–63.
15. Cream C, Li A, Nattie E. The retrotrapezoid nucleus (RTN): local cytoarchitecture and afferent connections. *Respir Physiol Neurobiol* 2002;130:121–37.
16. Rosin DL, Chang DA, Guyenet PG. Afferent and efferent connections of the rat retrotrapezoid nucleus. *J Comp Neurol* 2006;499:64–89.
17. Weese-Mayer DE, Berry-Kravis EM, Marazita ML. In pursuit (and discovery) of a genetic basis for congenital central hypoventilation syndrome. *Respir Physiol Neurobiol* 2005; 149:73–82.
18. Spengler CM, Gozal D, Shea SA. Chemoreceptive mechanisms elucidated by studies of congenital central hypoventilation syndrome. *Respir Physiol* 2001;129:247–55.
19. Gaultier C, Amiel J, Dauger S, et al. Genetics and early disturbances of breathing control. *Pediatr Res* 2004;55: 729–33.
20. Paton JY, Swaminathan S, Sargent CW, Hawksworth A, Keens TG. Ventilatory response to exercise in children with congenital central hypoventilation syndrome. *Am Rev Respir Dis* 1993;147:1185–91.
21. Shea SA, Andres LP, Shannon DC, Banzett RB. Ventilatory responses to exercise in humans lacking ventilatory chemosensitivity. *J Physiol* 1993;468:623–40.
22. Stornetta RL, Moreira TS, Takakura AC, et al. Expression of Phox2b by brainstem neurons involved in chemosensory integration in the adult rat. *J Neurosci* 2006;26: 10305–14.
23. Dauger S, Pattyn A, Lofaso F, et al. Phox2b controls the development of peripheral chemoreceptors and afferent visceral pathways. *Development* 2003;130:6635–42.
24. Guyenet PG, Stornetta RL, Bayliss DA, Mulkey DK. Retrotrapezoid nucleus: a litmus test for the identification of central chemoreceptors. *Exp Physiol* 2005;90:247–53.
25. Mulkey DK, Mistry AM, Guyenet PG, Bayliss DA. Purinergic P2 receptors modulate excitability but do not mediate pH sensitivity of RTN respiratory chemoreceptors. *J Neurosci* 2006;26:7230–3.
26. Nattie EE, Li A. Substance P-saporin lesion of neurons with NK1 receptors in one chemoreceptor site in rats decreases ventilation and chemosensitivity. *J Physiol* 2002;544: 603–16.