CENTRAL CHEMORECEPTION is the mechanism by which the brain regulates breathing in response to changes in tissue pH, wherein CO$_2$/H$^+$ chemoreceptors sense pH changes to regulate the depth and frequency of breathing. Several brain regions are thought to participate in chemoreception including the nucleus tractus solitarius, locus coeruleus, medullary raphe, prebolzing-inger complex, fastigial nucleus of the cerebellum, hypothalamus, and retrotrapezoid nucleus (RTN) (14, 22). Although the relative contributions of these putative chemoreceptor regions remains controversial (24, 42), there is compelling evidence that the RTN is an important site of chemoreception (1, 6, 26, 38, 43). In this review, we will summarize the properties of chemosensitive RTN neurons; further details on this topic can be found in several recent reviews (23, 24, 41). Surprisingly, pH-sensitive RTN glial cells, first described by Fukuda et al. (16) more than 30 years ago, have received relatively little attention (9–11, 46, 53) despite evidence from other brain regions that glial cells can function as important modulators of both neural network activity and vascular tone (25). Therefore, we will also describe general characteristics of glial cells and propose an expanded model of chemoreception that includes a role for RTN glia as a source of excitatory drive to pH-sensitive neurons as well as a regulator of local vascular tone.

THE RTN IS AN IMPORTANT SITE OF CENTRAL CHEMORECEPTION

The early work of Mitchell and colleagues more than 40 years ago established that acidification of the ventral medullary surface (area M) stimulates breathing (34); therefore, they concluded that pH-sensitive neurons in this region, later defined as the RTN (57), are able to provide the chemical drive to breathe. This possibility has been strengthened by a series of in vivo experiments by Nattie and colleagues who showed that chemical activation of the RTN led to increased respiratory drive and the ventilatory response to CO$_2$ (6, 30, 43), whereas chemical inhibition of the RTN had the opposite effect (40, 44). These studies also found that the contribution of the RTN to respiratory drive is more pronounced in anesthetized animals compared with conscious animals. These observations together with those described below led to the working hypothesis that the RTN provides a tonic excitatory drive to the central pattern generator: during sleep or anesthesia, input from the RTN provides a tonic excitatory drive to breathe; however, during wakefulness, the respiratory central pattern generator as well as upstream chemoreceptor regions (e.g., RTN) receive excitatory input from many sources (e.g., hypothalamus, med-
ullary raphe), and so input from any one source, including the RTN, becomes less essential to respiratory drive (22, 23).

More recently, together with Patrice Guyenet and Douglas Bayliss, we were able to determine the cellular identity of RTN chemoreceptors (38). Some of the properties of RTN chemoreceptors are summarized in Fig. 1. In anesthetized rats ventilated to maintain an end-expiratory CO2 level approximating the physiological range, CO2/H^+ -sensitive RTN neurons exhibit a low tonic level of activity and are robustly activated by hypercapnia (Fig. 1, A1 and A2); the CO2/H^+ sensitivity of these neurons remains intact when inputs from peripheral chemoreceptor are removed or when respiratory neuronal activity is blocked with morphine (38), indicating that the CO2/H^+ sensitivity of these cells is centrally mediated and not dependent on feedback from respiratory centers. As traditionally expected for central respiratory chemoreceptors (34, 56), CO2/H^+ -sensitive RTN neurons have extensive dendrites within the marginal layer of the ventral medullary surface (Fig. 1A3), are glutamatergic, as evidenced by expression of the vesicular glutamate transporter VGLUT2 (Fig. 1A5) and project directly to key pontomedullary respiratory centers, including the pre-Bötzinger complex (38). In addition, CO2/H^+ -sensitive RTN neurons express the transcription factor Phox2b (Fig. 1A5) (39, 59). Mutations in the gene that encodes PHOX2B have been shown to cause severe respiratory deficits that characterize a condition known as congenital central hypoventilation syndrome (CCHS) (7), the principal symptom of which is hypoventilation during sleep and reduced (or absent) chemical drive to breathe. In addition, a transgenic animal model of CCHS developed by creating a knock-in of the most frequent CCHS-causing mutation (Phox2b^{27Ala+}) show reduced ventilatory response to CO2 at birth, apparently due to a

![Diagram of RTN chemoreceptors](image-url)

**Fig. 1.** Defining characteristics of RTN chemoreceptors in vivo and in vitro. **A1:** the typical firing rate response of a CO2/H^+ -sensitive neuron in vivo to changes in end expiratory CO2; increasing Exp CO2 increased neuronal activity in a reversible and repeatable manner. **A2:** average firing rate of CO2/H^+ -sensitive (n = 26) and CO2/H^+ -insensitive (n = 39) neurons in vivo under control (4% CO2) and hypercapnic (10% CO2) conditions. After recording cells were labeled with biotinamide for later conformation of location, morphology, and neurochemical phenotype. **A3:** plots the location of 17 CO2/H^+ -sensitive neurons (black dots) recorded in vivo. Bregma level = -1.4 mm (Amb, nucleus ambiguous; FN, facial nucleus; ML, marginal layer; Py, pyramidal tracts). **A4:** a biotinamide (Alexa Fluor 488 fluorescence)-labeled CO2/H^+ -sensitive RTN neuron recorded in vivo. **A5:** a biotinamide with Cy-3 (red), and bottom shows that the same cell is immunoreactive for Phox2b (Alexa 488, green), biotinamide with Cy-3 (red); colocalization is shown in yellow.

**B1:** trace of firing rate and bath pH show characteristic responses of a pH-sensitive RTN neuron to pH values ranging from 6.9 to 7.5; these cells are spontaneously active at control pH 7.3, nearly silent at pH 7.5, and robustly active at pH 6.9. **B2:** average firing rate of pH-sensitive (n = 40) and pH-insensitive (n = 47) neurons recorded in vitro at varying pH. *P < 0.01 for effect of pH. **B3:** structure of three biocytin-filled pH-sensitive neurons that are reminiscent of CO2/H^+ -sensitive RTN neurons recorded in vivo. **B4:** composite map shows the location of 11 pH-sensitive neurons (black dots) recorded in vitro (Amb, nucleus ambiguous; FN, facial nucleus; ML, marginal layer; Py, pyramidal tracts). **B5:** agarose gel of single cell RT-PCR for Phox2b and GAPDH; the chemosensitive RTN neuron expresses Phox2b, but the Purkinje cell does not.
massive reduction in the number of RTN neurons since other respiratory regions appeared normal in these animals (7). Furthermore, conditional Phox2b mutations that selectively disrupt RTN development also reduced CO₂ sensitivity at approximately embryonic day 15 measured in vitro using the brain stem-spinal cord preparation (8). The presence of Phox2b in RTN neurons further supports the possibility that they function as important chemoreceptors. Perhaps the most convincing evidence that Phox2b-expressing RTN neurons contribute to respiratory drive was obtained in the elegant work of Abbot et al. (1), who used a lentivirus to target expression of the light-activated cationic channel channelrhodopsin-2 in Phox2b-expressing cells. Photo-stimulation of even a small percentage of Phox2b-expressing RTN neurons caused a marked increase in phrenic nerve activity (1).

In brain stem slices, we identified a population of RTN neurons in a similar location and of a similar morphology and neurochemical phenotype as CO₂/H⁺-sensitive RTN neurons identified in vivo (38, 39). The cells were characterized as being pH-sensitive by making cell-attached current clamp recordings of membrane potential during exposure to acidic (pH 6.9) and alkaline (pH 7.5) perfusate (Fig. 1, B1 and B2). The structure of these cells is strikingly similar to that of pH-sensitive RTN neurons recorded in vivo (38); specifically, the locations of their soma and long secondary dendrites, which project along the marginal layer of the surface of the ventrolateral medulla, were nearly identical between preparations (Figs. 2, B3 and B4). In addition, we used single-cell RT-PCR to demonstrate that pH-sensitive RTN neurons recorded in vitro represent the cellular correlate of the glutamatergic and Phox2b-expressing chemoreceptors characterized in vivo (Fig. 1B5; Ref. 39).

The mechanism by which RTN neurons sense pH remains unresolved. These cells appear to be intrinsically pH-sensitive because their firing-rate response to pH changes persisted after blocking ionotropic glutamate receptors with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM) and antagonist 2-amino-5-phosphovalerate (APV, 20 μM) and also when P2-receptors were blocked with pyridoxal-phosphate-6-azophenyl-2,4'-disulfonate (PPADS; 100 μM), an ATP-receptor antagonist (36, 39). In addition, voltage-clamp experiments (in the presence of 0.1 μM tetrodotoxin to block action potentials) showed that RTN chemoreceptors express a pH-sensitive voltage-independent K⁺ conductance that likely confers pH sensitivity to these cells (39). The properties of this pH-sensitive current suggest involvement of the TASK family of background K⁺ channels (i.e., TASK-1 and TASK-3). However, the pH-sensitive current in RTN neurons is not sensitive to halothane (39), a volatile anesthetic known to activate TASK channels. In addition, central chemoreception and pH sensitivity of RTN neurons was retained in TASK-1, TASK-3, and double TASK-1/3 knockout animals (39), indicating that these channels do not confer pH sensitivity RTN neurons or are required for central chemoreception. Similar results were obtained by Trapp et al. (60), using independently generated TASK-1 and TASK-3 knockout animals. The properties of the pH-sensitive K⁺ current expressed by RTN neurons (i.e., high pH sensitivity in the physiological range and a lack of halothane sensitivity) are not consistent with the known properties of other background K⁺ channels. Note that TWIK-1 channels are inhibited by H⁺, but their response to halothane has not been described.

A number of alternative pH-sensitive K⁺ channels have been proposed to contribute to respiratory chemoreception (for review, see Ref. 51). For example, several pH-sensitive inward rectifying K⁺ channels are expressed in brain stem neurons. However, we find no evidence for inward rectification in I-V relationships recorded from pH-sensitive RTN neurons (38, 39). Large conductance Ca²⁺-dependent K⁺ channels are also pH-sensitive and thought to contribute to chemoreception, but we found that inhibition of these channels had no effect on the pH sensitivity of RTN neurons (Mulkey DK, Bayliss DA, unpublished observations). Several members of the voltage-dependent K⁺ (Kᵥ) family of channels are expressed in brain stem neurons. For example, the RTN also contains pH-sensitive glial cells (12, 16, 53) that may exhibit a constitutively active “window” current that could contribute to the background pH-sensitive K⁺ current in RTN neurons.

In addition to responding to pH changes, the activity of RTN neurons can also be modulated by various neurotransmitters. For example, the RTN received extensive innervation from medullary serotonergic neurons, and it was shown both in vivo and in vitro that exposure to serotonin increased baseline activity of RTN chemoreceptors and caused an upward parallel shift in their CO₂/H⁺ sensitivity (37). These results suggest that serotonergic raphe neurons contribute to chemoreception at least in part by modulating excitability of RTN neurons. In a similar fashion, RTN glia may also contribute to chemoreception by modulating activity of pH-sensitive RTN neurons. For example, the RTN also contains pH-sensitive glial cells (12, 16, 53). Since tetrodotoxin will not inhibit glial transmitter release, it remains possible that local paracrine mechanisms, including excitatory input from pH-sensitive glial cells, could contribute to pH sensitivity of RTN neurons and respiratory drive. Based on this possibility, we have expanded our working model of RTN chemoreception to include possible contributions from pH-sensitive glial cells (Fig. 2).
GENERAL CHARACTERISTICS OF GLIAL CELLS

The term “glia” was coined in 1856 by Rudolf Virchow because these cells appeared to “glue” the nervous system together and currently refers to all nonaction potential forming cells of the nervous system. During the decades following their discovery, glial cells have been classified based on their location, morphology, and reactivity to different staining methods. Recently, more functional roles for glial cells have been established including myelination, immune response, ion and transmitter homeostasis, and active participation in synaptic plasticity and neuronal excitability. Although many subpopulations of glial cells have been described, they all can be classified into one of four categories: microglia, oligodendrocytes, polydendrocytes, and astrocytes. Each of these subtypes differs in proposed function, immunoreactivity, and electrophysiology.

Microglia are immunocompetent cells of the central nervous system that can be identified immunohistochemically by expression of the cluster differentiation marker CD11b (a β-integrin marker of microglia). In their “resting” state microglia can interact with all elements of the CNS and can respond to a variety of physiological signals, including pH (13). However, microglia have a resting membrane potential (approximately −40 mV) that is considerably more depolarized than the resting membrane potential of pH-sensitive glia (approximately −70 mV) (16, 53), suggesting that pH-sensitive RTN glia are probably not microglia. Other characteristics of resting microglia include high membrane resistance and the presence of inward rectifying K+ (Kir) channels, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors, and glutamate transporters (3, 55). Once activated by injury or infection, microglia increase expression of outward rectifying K+ channels (3).

Oligodendrocytes and polydendrocytes belong to the same lineage. Oligodendrocytes are myelin-producing cells usually identified by antibody labeling for myelin basic protein (17). Oligodendrocytes are also electrically distinct in that they have low membrane resistance, exhibit mostly voltage-independent K+ currents, and express glutamate transporters but not AMPA receptors (17). Polydendrocytes are glia progenitors present in all brain regions throughout life (45); they are identified immunohistochemically by expression of NG2 (45), an integral membrane chondroitin sulfate proteoglycan. The electrical profile of polydendrocytes includes high membrane resistance, outward rectifying K+ currents, distinctive inward Na+ currents, and presence of AMPA receptors but not glutamate transporters (31, 64).

Astrocytes are the largest class of glial cells and can be further classified based on location, morphology, and association with vasculature, e.g., protoplasmic astrocytes are located in the gray matter and fibrous astrocytes are located in white matter. Both cell types have numerous fine processes and form close associations with neurons and blood vessels. Astrocytes are commonly differentiated from other glial cell types based on expression of glial fibrillary acidic protein (GFAP) or S100β (a Ca2+ binding protein expressed by astrocytes) (15). However, both GFAP and S100β activity are found in glial progenitor cells (45), and protoplasmic astrocytes can be GFAP-negative (45). Recent transcriptome analysis has revealed aldehyde dehydrogenase 1L1 to be a more specific marker of protoplasmic astrocytes (4). In addition, electrophysiological studies have identified two subtypes of astrocytes in the hippocampus (33, 64) and brain stem (20): one type has passive electrical properties, low membrane resistance, and expresses glutamate transporters (e.g., passive astrocytes); the second type has voltage-dependent channels, comparatively higher membrane resistance, and preferentially expresses AMPA receptors over glutamate transporters (e.g., variable rectifying astrocytes). The distribution of these astrocyte subtypes vary across development (64) and is thought to reflect distinct cell types with unique functions, but this has yet to be demonstrated.

POSSIBLE ROLES OF GLIA IN CENTRAL CHEMORECEPTION

Extracellular pH Regulation

Originally, it was proposed that glial cells in the RTN contribute to the mechanism of chemoreception by potentiating CO2-induced extracellular acidosis (11). It is well known that astrocytes and oligodendrocytes help regulate extracellular K+ by taking up extracellular K+ ions during times of increased neuronal activity (47). This process effectively buffers changes in extracellular K+; however, it can also increase Na+/HCO3− co-transport into glia (5), which would enhance the CO2-induced fall in extracellular pH and potentiate activation of RTN chemoreceptors (Fig. 2, arrow 4). This possibility is supported by a series of in vivo experiments in which glial cells were artificially depolarized by microinjection of fluorocitrate, a glia-specific metabolic toxin, into the RTN. The fluorocitrate-mediated glia depolarization, presumably by depletion of ATP and consequent loss of cytoplasmic K+ (28), caused extracellular acidification followed by increased respiratory frequency and ventilatory sensitivity to CO2 (11, 26). It is important to note that these studies used subtoxic doses of fluorocitrate; therefore, the glia had responded to fluorocitrate-mediated depolarization by either exacerbating extracellular acidification or releasing excitatory transmitters and consequently increasing the activity of pH-sensitive neurons.

Purinergic Signaling and Central Chemoreception

There is increasing evidence that ATP, possibly released by astrocytes (58), is an important mediator of central chemoreception (18, 19). For example, in vivo studies showed that exposure to high CO2 (i.e., hypercapnia) caused discrete release of ATP within the RTN (19). In addition, application of ATP into the RTN stimulated respiratory output, whereas application of the ATP receptor antagonist (PPADS) to the same region lowered CO2 respiratory responsiveness (19).

It was proposed that purinergic P2X-receptors mediate the effects of ATP on ventral surface neurons (18); however, mice lacking P2X2-receptors exhibit normal CO2 sensitivity (54), and this theory still lacks evidence at the cellular level. It was shown in the brain stem-spinal cord preparation that exposure to ATP stimulated respiratory output but that CO2-mediated respiratory drive was unaffected by block of P2 receptors (32), suggesting that purinergic signaling can modulate respiratory activity without directly affecting chemosensitivity. At the level of the RTN, we found that purinergic signaling can have opposing effects on excitability of pH-sensitive RTN neurons in the brain slice preparation. For example, activation of P2X receptors, presumably on interneurons, leads to GABA_A- or glycine-mediated inhibition of pH-sensitive neurons; con-
versely, activation of P2Y receptors directly activates pH-sensitive RTN neurons even when glutamatergic transmission was blocked by CNQX (10 μM) and APV (20 μM) (36). As mentioned above, the pH sensitivity of RTN neurons in vitro was retained when P2 receptors were blocked with PPADS (36, 38). These results suggest that CO2/H+ was retained when P2 receptors were blocked with PPADS mentioned above, the pH sensitivity of RTN neurons in vitro was blocked by CNQX (10 M) and APV (20 M) (36). As mentioned above, the pH sensitivity of RTN neurons in vitro was retained when P2 receptors were blocked with PPADS (36, 38). These results suggest that CO2/H+ was retained when P2 receptors were blocked with PPADS.

The source of ATP release in the RTN during hypercapnia is poorly understood. In other brain regions, it is well established that activated astrocytes can release excitatory transmitters (e.g., ATP, glutamate) (25). Furthermore, observations first made three decades ago showed that a subset of nonspiking RTN cells are CO2/H+-sensitive (16), suggesting that these pH-sensitive glia could be the source of ATP released during hypercapnia (Fig. 2, arrows 1–3). However, the identity of these pH-sensitive glial cells, the mechanism by which they sense changes in pH, and their potential contribution to respiratory drive remain unknown.

Vascular Control and Central Chemoreception

There is a close relationship between the mechanism of chemoreception and blood flow. The same stimulus that drives breathing (e.g., CO2/H+) also acts as a potent vasodilator to coordinate blood flow with the metabolic needs of tissues (2). The effects of CO2/H+ on blood flow buffers tissue pH and stabilizes chemoreceptor activity during fluctuations in arterial CO2. For example, CO2/H+-mediated vasodilation increases blood flow and accelerates removal of metabolically produced CO2, thereby increasing tissue pH and limiting chemoreceptor activation for a given increase in arterial CO2 (2). The opposite has been shown to occur with a drop in arterial CO2. An additional factor to consider is that changes in blood flow affect tissue O2 levels and so may affect the formation of reactive oxygen species (ROS). This is relevant because ROS can exert direct effects on cerebral vasculature (i.e., vasodilation; Ref. 48), and ROS have been shown to activate putative chemoreceptors in certain brain stem regions (35). These effects of vascular CO2/H+ reactivity on respiration have been demonstrated in several in vivo preparations, including in humans, and it is thought that disruption of this mechanism contributes to breathing instability observed in patients with central sleep apnea (2, 61).

The cellular and molecular mechanisms underlying CO2/H+-mediated cerebral vasodilation are not fully known. It is generally thought that the vascular response to CO2/H+ is an intrinsic property of blood vessels (2). However, astrocytes are also known to regulate blood flow in response to neural activity (25), and evidence suggests that astrocytes in the glia limitans contribute to CO2/H+-mediated vasodilation (62). Astrocytes can release a number of vasodilatory signals, including ATP (21), which can initiate arteriole dilation by activation of P2Y receptors on smooth muscle or endothelial cells (27). Alternatively, ATP can be readily hydrolyzed by ectonucleotidases to adenosine, which has been shown to mediate vasodilation by activation of P1 receptors (63). This latter possibility may contribute to CO2-mediated cerebral vasodilation because inhibition of the A2A subtype of P1 receptors has been shown to attenuate the effects of CO2 on blood flow (50). Therefore, it is possible that CO2/H+-evoked ATP release by pH-sensitive RTN glial cells initiates vasodilation, via P1-receptors, to buffer tissue pH and effectively “fine tune” chemoreceptor activity.

An interesting alternative possibility is that chemosensitive regions regulate blood flow and tissue pH differently from nonchemosensitive regions. It has been shown in brain slices from rat pups <11 days postnatal that putative chemosensitive neurons in the nucleus tractus solitarius and RTN regulate pH, differently from neurons in nonchemosensitive regions; the Na+/H+ exchanger is more sensitive to inhibition by extracellular acidification in neurons from chemosensitive areas than in neurons from nonchemosensitive areas (46, 52). These differences in pH regulation are thought to reflect the unique function of chemoreceptors as pH sensors (52). By analogy, perhaps hypercapnia causes constriction of vasculature in chemosensitive regions rather than vasodilation that is typically observed in other brain regions (2). This unique vascular response could be mediated by the discrete release of ATP in the RTN. In this case, ATP may mediate capillary vasoconstriction by activation of P2Y receptors on pericytes (contractile cells that regulate blood flow through capillaries) (49). The effects of purinergic signaling on vascular tone in the RTN have not been determined, and so they are depicted in Fig. 2 as a single arrow (arrow 3) representing vasoconstriction or vasodilation. In addition, recent evidence indicates that Phox2b-expressing neurons directly innervate vascular pericytes (29), implying that chemosensitive neurons may directly regulate capillary diameter (Fig. 2, arrow 4). To understand how vascular control contributes to chemoreception, it will be important for future experiments to determine the effects of CO2/H+ on vascular tone in chemosensitive and nonchemosensitive regions.

CONCLUSIONS

Current evidence supports the possibility that the RTN is an important site of central chemoreception. Despite the apparent intrinsic pH sensitivity of RTN neurons, evidence also suggests that purinergic signaling also contributes to RTN chemoreception (19). Although the source of CO2-evoked ATP release in the RTN has not been determined, these findings raise the interesting possibility that pH-sensitive neurons may contribute to chemoreception by providing a purinergic drive to pH-sensitive neurons. Purinergic signaling can stimulate activity of pH-sensitive neurons by activation of P2Y receptors or decrease activity of pH-sensitive neurons by activation of P2X receptors on inhibitory interneurons. It is also conceivable that glial cells contribute to the mechanism of chemoreception indirectly by regulating vascular tone. For these reasons, understanding the physiological significance of RTN glial cells will be an important topic in future studies of central chemosensitivity.

GRANTS

This work was supported by a large faculty grant from the University of Connecticut Research Foundation.

DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES

2. Ainslie PN, Duffin J. Integration of cerebrovascular CO2 reactivity and chemoreflex control of breathing: mechanisms of regulation, measure-
NEW INSIGHTS INTO THE MECHANISM OF RTN CHEMORECEPTION


