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Review

Julius H. Comroe, Jr., Distinguished Lecture: Central chemoreception: then…and now

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Nattie E. Julius H. Comroe, Jr., Distinguished Lecture: Central chemoreception: then…and now. *J Appl Physiol* 110: 1–8, 2011. First published November 11, 2010; doi:10.1152/japplphysiol.01061.2010.—The 2010 Julius H. Comroe, Jr., Lecture of the American Physiological Society focuses on evolving ideas in chemoreception for CO_2/pH in terms of what is "sensed," where it is sensed, and how the sensed information is used physiologically. Chemoreception is viewed as involving neurons (and glia) at many sites within the hindbrain, including, but not limited to, the retrotrapezoid nucleus, the medullary raphe, the locus ceruleus, the nucleus tractus solitarius, the lateral hypothalamus (orexin neurons), and the caudal ventrolateral medulla. Central chemoreception also has an important nonadditive interaction with afferent information arising at the carotid body. While ventilation has been viewed as the primary output variable, it appears that airway resistance, arousal, and blood pressure can also be significantly affected. Emphasis is placed on the importance of data derived from studies performed in the absence of anesthesia.

carbon dioxide; control of breathing; pH

IT IS AN HONOR to give the Comroe Lecture. When I began my career in physiology, Dr. Comroe was the leader of the Cardiovascular Research Institute in San Francisco, where he provided encouragement and support for Robert Mitchell in his pioneering studies of central chemoreception, the subject of this lecture. In fact, Dr. Comroe himself was a central chemoreceptor man. "Comroe…microinjected solutions containing CO_2 near the dorsal surface of the medulla, which increased ventilation…his general conclusion…was that the CO_2 of blood perfusing the medulla is the major determinant of normal ventilation…the peripheral chemoreceptors protected…against hypoxia" (53).

CO_2 vs. O_2 in the Regulation of Resting Breathing

Ventilation is sensitive to small increases in arterial P_{CO_2} but relatively insensitive to decreasing arterial P_{O_2} until it reaches ~70 Torr (97). Alveolar ventilation would have to decrease considerably to produce this arterial P_{O_2} level, indicating that O_2 sensing is certainly not the means by which a fine-tuned regulation of ventilation is achieved. Thus chemoreception for CO_2/H^+ acts as a primary, sensitive detector of the adequacy of alveolar ventilation.

What Is the Stimulus? pH? CO_2?

Recent data suggest that CO_2 per se can be a chemoreceptor stimulus acting via stimulation of glial ATP release at connexin hemichannels within the ventrolateral medulla (49). This mechanism at these sites was estimated to account for ~20% of the total CO_2 response in anesthetized rats (49). The relative contribution and importance of this glial mechanism in the unanesthetized state are unknown. This review focuses on pH-dependent mechanisms, acknowledging that, in many cases, both CO_2 and pH change.

Where Are Such CO_2/H^+ Chemoreceptors Located and When (in Evolution) Did They Arise?

The invertebrate pulmonate snail *Helix aspersa* has centrally located chemoreceptors (17). Increased CO_2 opens the pneumostome, an opercular structure that allows greater diffusional CO_2 loss, and the CO_2 is detected by single neurons at one central site within the subesophageal ganglion. The vertebrate frog *Rana catesbeiana* has central chemoreceptors located on the ventrolateral medulla at a rostral and a caudal site. When the frog brain stem is studied in situ, focal application of CO_2 at either site increases fictive respiratory output. Furthermore, the stimulation of this output by system-wide application of CO_2 is abolished by lesions at either site (92). These two examples illustrate the broad phylogenetic nature of central chemoreception and suggest an early evolutionary presence (for further discussion see Ref. 66).

In the 1950s, Leusen (57) perfused the brains of anesthetized mammals with acidic fluids and observed a stimulation of breathing, indicating the presence of central chemoreception. A few years later, focal application of acidic fluids localized this response to sites on the rostral and caudal ventral medullary surface in anesthetized cats (63, 67, 84). The history of this work has been reviewed by Severinghaus (85).

Anesthesia

All the experiments that identified putative locations for central chemoreception were performed under deep anesthesia. However, the ventilatory response to changes in pH of cerebral

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fluids produced in conscious goats is exquisitely sensitive; e.g., a decrease of 0.05 pH unit can double alveolar ventilation (79), and inhalation of 5.6% CO_2 increases alveolar ventilation by 300%, a response that is decreased to 40% by light anesthesia (10 mg/kg sodium thiopental iv) (79). We have shown similar blunting of the CO_2 response in rats studied in the conscious state and then quickly again after induction of anesthesia (1). Anesthesia severely depresses the sensitivity to chemoreceptor stimulation!

The Carotid Body Is an Important CO_2 Chemoreceptor

Denervation studies in anesthetized and conscious animals attributed ~40% of the steady-state ventilatory CO_2 response to the carotid body and ~60% to central chemoreceptors (21, 23) and indicated that the response of the carotid body is more rapid than the response of the central chemoreceptors. However, carotid denervation can induce adaptive responses over days to weeks (23).

Recent work in unanesthetized dogs with one carotid body denervated and the other perfused allows separate control of CO_2/H^+ at the peripheral and central chemoreceptors (5, 86). Stimulation of the carotid bodies increased the ventilatory response to central CO_2 by 223% of normal, while carotid body inhibition resulted in a central response that was only 19% of normal. The response to central chemoreceptor stimulation depends on the amount of carotid body excitation! These remarkable data led to a hypothesis of "interdependence" of the carotid body and the central chemoreceptors, i.e., an important synergism between them. The high sensitivity of the chemoreceptor system in conscious animals is best served by this interdependence between peripheral and central chemoreception.

Central Chemoreception

Is central chemoreception uniquely localized to regions accessible to surface areas on the ventrolateral medulla (37)? Data showing other potential central chemoreceptor sites and arguments for and against the unique ventrolateral medulla hypothesis have been reviewed (37, 70, 71). Many sites and neurons have been shown to respond to CO_2/H^+ in vitro (see below). What was missing was a way to demonstrate in vivo that there are multiple locations within the neuraxis that, when stimulated by focal acidification, can increase ventilation.

Multiple Sites

Lee Coates, while a fellow in my lab, asked whether the stimulatory effects on ventilation of systemic carbonic anhydrase inhibition with acetazolamide, as used to prevent high-altitude sickness, could be attributed to a central chemoreceptor effect. He found that inhibition of carbonic anhydrase focally at the ventral surface of the medulla increased ventilatory output and decreased focal pH (7). We then produced focal stimulation via very small injections of acetazolamide at various locations; i.e., we probed in vivo for putative central chemoreceptor sites. Coates et al. (6) showed that a 1-nl injection of acetazolamide (50 μM) produced a focal acidosis that increased ventilation at many sites in anesthetized cats and rats, including the locus ceruleus (LC), caudal nucleus tractus solitarius (NTS), medullary raphe, rostral aspect of the ventral respiratory group, and a region near the ventral medullary surface now known as the retrotrapezoid nucleus (RTN). These findings coincided with work of others that began to broaden the scope of inquiry into central chemoreception and resulted in a renaissance of interest in the last 15–20 yr in terms of *1*) chemosensitive cells and molecular sensing mechanisms (11, 19, 32–35, 37, 49, 81–83, 98) and *2*) integrative physiology (8, 22, 23, 27, 37, 39, 40, 47, 49, 52, 55, 58, 69–71, 88). The degree of focal acidification at the center of the acetazolamide injections was relatively severe: the change in pH was similar to that associated with a 36-Torr increase in arterial P_{CO_2}. However, the application of CO_2 directly is problematic, especially in the conscious animal, as cerebral blood flow is so effective in clearing any exogenously applied CO_2. Aihua Li, in my lab, developed an approach using reverse microdialysis of an artificial cerebrospinal fluid that is equilibrated with high CO_2, which maintains a constant source of CO_2. With dialysate equilibrated with 25% CO_2, measured pH at ~100–200 μm from the dialysis probe tip decreased by an amount similar to that observed with a 6.6-Torr increase in systemic (arterial) P_{CO_2}, a stimulus intensity much lower than that produced by the acetazolamide injections and much lower than that observed in the same rats given 7% CO_2 in the inspired gas, an ~15-Torr increase (60).

We and others have applied this approach at many sites and observed *1*) an ~24% increase in ventilation in wakefulness, but not in sleep, at the RTN region (61), *2*) an ~20% increase in ventilation in sleep, but not in wakefulness, in the rostral medullary raphe (raphe magnus) (75), *3*) an ~20–30% increase in ventilation in sleep and wakefulness in the caudal NTS (74), *4*) an ~17% increase in ventilation at the caudal ventral medullary surface area (9), and *5*) a significant increase in ventilation in wakefulness at the perifornical region of the hypothalamus, the location of orexin neurons (Li, Li, and Nattie, unpublished observations). In addition, focal acidification of the pre-Bötzinger region increased ventilation in conscious goats (54). In the caudal medullary raphe (raphe obscurus), focal acidification had little effect on ventilation in rats (12, 62). In conscious goats, Hodges et al. (42) observed an increase in ventilation findings somewhat different from ours, perhaps explicable by their use of a greater stimulus intensity. In summary, a mild focal acidosis at many brain stem sites can stimulate ventilation in conscious animals, with some sites being responsive in a state-dependent manner (Fig. 1).

Is There Interdependence Among Central Chemoreceptor Sites?

Using dialysis probes implanted in the RTN and the caudal aspect of the medullary raphe in the rat, Dias et al. (12) showed that focal acidification of the caudal raphe alone had little effect on ventilation but simultaneous acidification at the raphe and the RTN region induced a much greater response (51% increase) than focal RTN acidification alone (24% increase) (12). Thus the caudal medullary raphe in the rat can detect CO_2/H^+, but the ventilatory response requires an interaction with the RTN. We also examined the effect of isolated and simultaneous inhibition of *1*) the RTN with muscimol and *2*) the caudal medullary raphe with the serotonin ($5\text{-}HT_{1A}$) autoreceptor agonist 8-hydroxy-2-(di-*N*-propylamino)tetralin (8-OH-DPAT). Inhibition of the RTN alone reduced the ventilatory response to systemic hypercapnia (7% CO_2) by 24%,

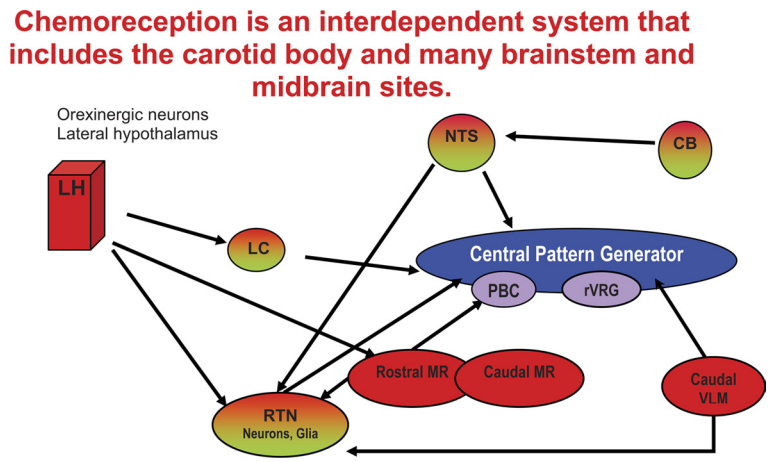

Fig. 1. A simplified, schematic view of chemoreceptor sites that, when stimulated, can increase ventilation in wakefulness. Red areas represent chemosensitive sites identified via focal acidification in vivo or via effects of focal, specific neuronal inhibition. Red-green areas identify chemosensitive sites that also express a Phox2b background. Solid lines identify connections known to be present in wakefulness. In non-rapid eye movement sleep, orexinergic neurons of the lateral hypothalamus (Li, Li and Nattie; unpublished data) and retrotrapezoid nucleus (RTN) neurons may participate less (61), while serotonergic neurons may participate more (75). LH, lateral hypothalamus; LC, locus ceruleus; NTS, nucleus tractus solitarius; CB, carotid body; PBC, pre-Bötzinger complex; rVRG, rostral ventral respiratory group; MR, medullary raphe; VLM, ventrolateral medulla.

while inhibition of the caudal medullary raphe alone reduced the CO_2 response by 2.5% (62). In dramatic contrast, simultaneous inhibition of both sites reduced the CO_2 response by a substantial 51% (62). These two central chemoreceptor sites, the RTN and the caudal medullary raphe, appear to function in an interdependent manner.

The RTN as a Putative Central Chemoreceptor Site

The RTN is a small group of dispersed neurons that are located along the base of the medulla just below the rostral ventral surface area (87), initially described by Mitchell and colleagues (67) as a site of central chemoreception. These neurons were originally discovered in cats by retrograde tracing experiments (87). At the time of these experiments, we had found that a very small injection of the excitotoxin kainic acid into the RTN region directly from the ventral medullary surface in anesthetized cats induced a brief stimulation of phrenic nerve activity followed by decreased activity and then apnea, along with a severely reduced CO_2 response (77). As noted above, focal acidification of the RTN region stimulates ventilation in wakefulness (61). A series of studies in anesthetized rats and brain stem slices described the chemical phenotype of neurons within the RTN that are likely detectors for CO_2/H^+; they express the vesicular glutamate transporter VGLUT2, as well as the "master gene" of the autonomic nervous system, Phox2b (2, 34, 35, 38). In conscious rats, inhibition of the RTN region by muscimol (72) or excitotoxin lesion (1) reduces the CO_2 response, but this effect is much less drastic than that observed under anesthesia (1). More specific lesions of neurons that express the neurokinin-1 receptor reduced the number of RTN Phox2b-expressing neurons and the CO_2 response (73). Under anesthesia, these lesions shifted the apneic threshold to the right, indicating a possible loss of a tonic source of excitation to respiration, but the response sensitivity to CO_2 once threshold was reached was not decreased (91). With specific RTN Phox2b neuron lesions induced by injection of a lentivirus construct containing the *Drosophila* receptor allatostatin and a PRSx8 promoter specific for Phox2b, a similar pattern of results was observed. After administration of allatostatin to activate the receptor and inhibit the neurons, under anesthesia the apneic threshold was shifted to the right, but the CO_2 response slope was unaffected (64). In contrast, in the conscious state, the CO_2 response slope was substantially reduced by ~60% without effect on baseline breathing, suggesting an important role for the RTN Phox2b neurons in chemoreception, insofar as the percentage of transfected RTN Phox2b-immunoreactive neurons was only 50–64%. It is puzzling why nonspecific inhibition or lesion severely reduces the CO_2 response under anesthesia while specific inhibition or lesions of RTN Phox2b neurons alone under anesthesia do not reduce the CO_2 response but, instead, shift the apneic threshold and subsequent CO_2 response to the right. In marked contrast, anesthesia with isoflurane, a known respiratory depressant, stimulates RTN neurons, while phrenic nerve activity remains unchanged or is transiently stimulated and then inhibited (56). That overall chemosensitivity is severely reduced in isoflurane anesthesia compared with the conscious state while it appears to be enhanced within the RTN suggests the presence of other chemoreceptor sites that are depressed by isoflurane.

RTN Glia

Recently, attention has been directed to the role of glial cells in central chemoreception (18–20, 30–33, 46, 68). While glial cells may participate at each central chemoreceptor site, they have been mostly studied in or near the RTN region. In vivo, focal disruption of glial function in the RTN region acidifies extracellular fluid pH and stimulates ventilation in anesthetized and conscious rats (18, 20, 46). Thus one theory of glial participation in chemoreception focuses on their function in the regulation of extracellular fluid pH. An alternative theory is that glial cells are a source of CO_2-stimulated release of ATP, which then activates nearby neurons that express appropriate receptors (30, 32, 33). In this view, the chemoresponsivity of putative chemosensitive neurons either depends on, or is augmented by, glial ATP release.

Is the RTN Preeminent?

There has been an ongoing focus on describing a single dominant or preeminent site for central chemoreception (37). It is difficult to understand this focus. Even at the time of the initial

studies that "localized" central chemoreception to areas on the ventral medullary surface, there were two areas, a rostral area and a caudal area. There is little doubt that the RTN participates importantly in central chemoreception, especially in wakefulness (61). The relative roles of Phox2b-expressing neurons, other neurons within the RTN, and glial cells remain to be determined, especially in unanesthetized, adult animals studied with physiological stimulus intensities in different states of arousal. Of note, the RTN is known to receive direct input (90) from the NTS and, thus, the carotid body, so the interdependence between the carotid body and central chemoreception may involve the RTN.

One argument for a dominant role of the RTN in central chemoreception involves an analogy between transgenic mice with altered Phox2b gene expression and the human central congenital hypoventilation syndrome (CCHS) (2, 10, 15, 29, 78, 80). CCHS occurs in young children who exhibit hypoventilation at rest and a reduced CO_2 response in sleep (80). Multiple other autonomic dysfunctions are also present in varying degrees. These children have a defect, most commonly a polyalanine expansion, that involves the Phox2b gene (2). Furthermore, a transgenic mouse with this defect, with an abnormal breathing rhythm and a reduced CO_2 response, dies shortly after birth (2). Thus there is the temptation to attribute CCHS to altered RTN function, as shown in these mice, and to infer that the RTN is the preeminent central chemoreceptor site. However, this comparison between mice that die at birth with very focally altered brain stem Phox2b expression and CCHS children who *1*) do not die at birth, despite quite abnormal CO_2 responses in sleep, and *2*) express multiple other physiological and anatomic abnormalities may be a bit tenuous. Phox2b is a transcription factor that determines differentiation of neurons involved in pathways important in autonomic and respiratory control, including the carotid body and the NTS (10). The polyalanine expansion Phox2b transgenic mouse appears to exhibit a limited, although severe, defect, which may also involve the parafacial respiratory group neurons (78) and account for the severe respiratory rhythm disturbances, as well as their early mortality. Furthermore, these mice die well before the age at which serotonergic neurons become chemosensitive (83). Also, the CCHS-related effects on the CO_2 response are most dramatic in sleep, when RTN neurons, at least in the adult rat, may not be as involved in chemoreception (61).

Data from a recent study with a TASK2 gene-null mouse (28), uniquely expressed within the RTN, indicates that the function of the RTN may be more complex than imagined. Were this potassium channel to be of importance in RTN chemosensing function, its absence would be expected to decrease the CO_2 response, which it did, but only when the mice were exposed to 6% CO_2. When exposed to 2% CO_2, a more physiological stimulus intensity, these mice had an enhanced CO_2 response. This channel may be implicated in RTN chemosensing, but in a complicated manner. As in all studies in knockout mice, here adaptation to the absence of TASK2 could be involved in determining the phenotype.

The Medullary Raphe as a Putative Central Chemoreceptor Site

The medullary raphe serotonergic neurons are more widely dispersed in the brain stem than are Phox2b-expressing cells, and they are grouped in multiple clusters, largely within the midline of the medulla and pons (50). They are sensitive to CO_2/H^+ when studied in vitro in slice preparations and in culture, with their response becoming apparent at approximately postnatal *day 12*; that is, they do not appear to be responsive at younger ages (82, 83). Consistent with this is the observation in vivo that specific inhibition of medullary raphe serotonergic neurons with 8-OH-DPAT (a 5-HT_{1A} receptor agonist) in awake newborn piglets decreased the CO_2 response in an age-dependent manner (65). Some raphe neurons, presumed to be serotonergic on the basis of their firing properties, increase their firing with systemic CO_2 stimulation in conscious cats (94). Cell-specific lesions of medullary raphe serotonergic neurons (28% loss) reduce the CO_2 response in rats by 15–18% in sleep and wakefulness (76), and acute specific inhibition of rostral medullary raphe 5-HT neurons by dialysis of 8-OH-DPAT decreases the CO_2 response in conscious rats by ~20% (93).

Mice with a complete absence of brain stem serotonergic neurons, the conditional Lmx1b-null mice, exhibit transient, severe apneas at birth (43–45) and a reduced CO_2 response as adults. While there is some early-life mortality, most of these mice survive and function well unless stressed by cool temperatures or exogenously administered CO_2. Replacement of serotonin [5-hydroxytryptamine (5-HT)] by injection into the cerebral ventricles in adult mice corrects the reduced CO_2 response, suggesting that serotonergic neurons that detect changes in CO_2/H^+ may, in part, affect other chemoreceptor sites to add to the overall response. Further support for this concept comes from studies in conscious rats discussed above with separate dialysis probes implanted in the RTN and in the caudal aspect of the medullary raphe (12, 62).

The Locus Ceruleus as a Putative Central Chemoreceptor Site

As for the RTN and the medullary raphe, data obtained in vitro and in vivo support a role for noradrenergic (NA) neurons of the LC in central chemoreception. Studies of these neurons in brain stem slices and in culture have demonstrated responses to CO_2/H^+ (16, 26, 27, 59, 81). Similar to the medullary raphe serotonergic neurons, but unlike the RTN, the brain stem NA neurons are dispersed in clusters throughout the brain stem. The A1/C1, A2/C2, A5, and A6 (LC) groups have been linked to the control of breathing (41, 96), but only the LC neurons have been studied in any detail in respect to chemoreception (51). Of interest is the observation that these neurons express Phox2b (10), as do the RTN neurons, but not the medullary raphe serotonergic cells. In conscious rats, large, (−80%) specific lesions of NA neurons of the LC by injection of 6-hydroxydopamine decreased the CO_2 response by 64%, a substantial effect (4). There are no data on whether LC neurons interact with other central chemoreceptor sites, as shown for the caudal medullary raphe and the RTN.

Orexin Neurons as a Putative Central Chemoreceptor Site

The orexin neurons form a small cluster located in the lateral hypothalamus that projects widely into forebrain and hindbrain and participates in a variety of physiological functions, including sleep-wake regulation and feeding. They were shown in culture to be CO_2-sensitive (98). There are substantiating in vivo experiments in conscious rodents. The preproorexin knockout mouse has a substantially reduced CO_2 response

(−30 and −50% at 5% and 10% CO_2, respectively), focal inhibition of the orexin-1 receptor at the RTN reduces the CO_2 response by 30% in wakefulness (14), focal inhibition of the orexin-1 receptor at the medullary raphe reduces the CO_2 response by 16% in wakefulness (13), focal acidification of the lateral hypothalamus/perifornical region stimulates ventilation by 15% in wakefulness (N. Li, A. Li, and E. Nattie, unpublished observations), and systemic administration of a dual orexin receptor antagonist previously shown to enhance sleep reduces the CO_2 response in wakefulness, but only during the dark, active period of the rat diurnal cycle (58). Orexin neurons do participate importantly in chemoreception, perhaps by augmenting responses during wakefulness at lower hindbrain chemoreceptor sites, such as the RTN (24) and medullary raphe. Orexin neurons may be a source of the sleep-wake difference in CO_2 sensitivity.

Chemoreception Is an Interdependent System That Includes the Carotid Body and Many Brain Stem and Midbrain Sites

Figure 1 summarizes a current view of chemoreception for CO_2/H^+ that emphasizes a system of multiple sites, many of which express Phox2b. In fact, development of the carotid body, cranial ganglia, and NTS requires Phox2b. In Phox2b-null animals, the carotid body degenerates, and the geniculate, petrosal, and nodose ganglia and the NTS do not form (10). Thus the development of many chemoreceptive structures in the "periphery" and "centrally" depend on the transcription factor Phox2b, and they require each other for optimum function (5, 86). However, importantly, included are orexin neurons of the lateral hypothalamus/perifornical region and serotonergic neurons of the medullary raphe, which do not express Phox2b. Thus the transcription factor Phox2b is involved in neuronal fate determination in some, but not all, putative chemoreceptor sites. Why are there so many sites? Sleep, wakefulness, anesthesia, arousal, stimulus intensity, and circadian time are variables that can affect central chemoreception. To really understand central chemoreception, investigators need to keep these variables and this scheme, or one like it, in mind. Furthermore, studies that utilize systemic CO_2 tests employ relatively high inspired levels. One approach that allows systemic CO_2 tests but examines lower stimulus intensities is a ramp CO_2 test (48, 99) in which the inspired CO_2 increases steadily over a period of seconds within a whole body plethysmograph and the threshold at which ventilation increases is observed. Once threshold is determined for a given animal in a particular arousal state, the steady-state response to that stimulus intensity can be determined as well.

The functions of central chemoreception are as follows: *1*) chemical feedback via CO_2 for the regulation of alveolar ventilation, *2*) acid-base homeostasis via pH sensing, and *3*) a tonic drive for ventilation. In addition, chemoreceptors likely serve an important role in *1*) control of airway caliber, *2*) upper airway resistance regulation, *3*) determination of sympathetic tone and blood pressure, *4*) arousal from sleep, and *5*) the "defense" response.

Control of Airway Caliber

Haxhiu et al. (39, 40) proposed a model with brain stem airway vagal preganglionic neurons (AVPNs) at the core to explain the relationship between central chemoreception and airway caliber. These cholinergic neurons innervate small airways, and their stimulation induces bronchoconstriction. The AVPNs receive inhibitory inputs from *1*) 5-HT neurons from the raphe, *2*) NA neurons of the LC, and *3*) histaminergic neurons of the tuberomammillary nucleus. In turn, these three sources receive inhibitory inputs from the ventrolateral preoptic region and excitatory inputs from orexinergic neurons of the lateral hypothalamus/perifornical regions. In this model, an increase in CO_2 will inhibit AVPNs and bronchodilate. Of potential translational interest is the circadian variation of airway resistance, with the greatest resistance occurring in humans at ~4 AM, when the CO_2 response is also low, as are orexin levels (89). This is the time of greatest airway resistance in asthmatic patients and is associated with morbidity and mortality. While chemoreception does not cause asthma, circadian variations in airway tone related to function of neurons that are putative chemoreceptors likely play an important role.

Upper Airway Resistance Regulation

Increased CO_2 decreases upper airway resistance and increases genioglossus activity (48, 95). The sources of this CO_2 effect remain uncertain, as studies in awake rats did not clearly verify results obtained under anesthesia that implicated raphe 5-HT inputs to the hypoglossal nucleus (47). Nevertheless, it is intriguing that central chemoreception via detection of CO_2/H^+ can modulate upper and lower airway caliber in physiological conditions.

Determination of Sympathetic Tone and Blood Pressure

This topic has been reviewed recently (36). Briefly, increased CO_2 stimulates breathing and sympathetic activity via a mechanism within the ventrolateral medulla. In addition, there is a tonic nonrespirophasic sympathetic nerve activity also determined by CO_2 via an unknown site.

Arousal

Some central chemoreceptor neurons, e.g., orexin, 5-HT, and NA neurons, are more active in wakefulness than in sleep. Increased CO_2 does cause arousal (3), although this effect seems to require a surprisingly high level of CO_2. Inspired CO_2 levels <5% do not affect arousal, even though ventilation is substantially increased (48). In addition, focal acidification of the caudal NTS by dialysis with 25% CO_2 (inducing a focal tissue pH change similar to that associated with a ~6- to 7-Torr increase in arterial P_{CO_2}) does not arouse the rat, but dialysis with 50% CO_2 does (74). Part of this CO_2-induced arousal likely involves raphe 5-HT neurons, as the amount of time required to arouse (without 5-HT neurons) to a 7% CO_2 challenge is twice as long for Lmx1b mice as for wild-type mice (G. F. Buchanan and G. B. Richerson, unpublished observations). Surprisingly, breathing low CO_2 levels (2%) improves sleep (25). Thus, while one might suspect that CO_2-induced arousal from sleep would have a low threshold as a protection against apnea or hypoventilation, the limited data to date suggest the opposite.

"Defense Response"

The preproorexin knockout mouse has a significantly reduced defense response (100). The apparent requirement for

orexin in this response to stress raises important questions. *1*) Is the CO_2-activated involvement of orexin neurons and other putative central chemoreceptors neurons actually a stress response due to the level of stimulation usually applied? Our recent results with focal stimulation of the lateral hypothalamic region via dialysis with CO_2 argue against this, as the estimated increase in P_{CO_2} is small. *2*) Do the orexin neurons, via their effects on other central chemoreceptor groups, govern the "normal CO_2 response," at least during wakefulness? Recent observations from our lab support this view. Using a systemically administered antagonist of both orexin receptors, we found inhibition of the CO_2 response, but only in wakefulness in the dark, active period of the circadian cycle (58). This effect occurs at the RTN (14), the medullary raphe (13), and possibly other chemoreceptor sites.

Summary

An increase in arterial P_{CO_2} marks a failure in the regulation of alveolar ventilation relative to metabolism. The chemoreceptor system, central and peripheral, via interdependence, displays a high sensitivity to small increases in arterial P_{CO_2}. It is a very sensitive control system in the conscious, awake animal! There are numerous physiological responses to chemoreceptor stimulation, e.g., ventilation, blood pressure, airway caliber, and arousal, all of which serve to normalize the relationship between alveolar ventilation and metabolism and promote blood gas and pH homeostasis. Chemoreception reflects a system of responsive sites that interact in an overlapping manner (Fig. 1). Disruption of many individual sites can curtail, but not abolish, sensitivity.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author.

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