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Role of chemoreceptors in mediating dyspnea

Gordon F. Buchanan^{a,c,*} and George B. Richerson^{a,b,c}

^aDepartment of Neurology, Yale University, School of Medicine, New Haven, CT 06520, United States

^bDepartment of Cellular and Molecular Physiology, Yale University, School of Medicine, New Haven, CT 06520, United States

^cVeteran's Affairs Medical Center, West Haven, CT 06516, United States

Abstract

Dyspnea, or the uncomfortable awareness of respiratory distress, is a common symptom experienced by most people at some point during their lifetime. It is commonly encountered in individuals with pulmonary disease, such as chronic obstructive pulmonary disease (COPD), but can also be seen in healthy individuals after strenuous exercise, at altitude or in response to psychological stress. Dyspnea is a multifactorial sensation involving the brainstem, cortex, and limbic system, as well as mechanoreceptors, irritant receptors and chemoreceptors. Chemoreceptors appear to contribute to the sensation of dyspnea in two ways. They stimulate the respiratory control system in response to hypoxia and/or hypercapnia, and the resultant increase respiratory motor output can be consciously perceived as unpleasant. They also can induce the sensation of dyspnea through an as yet undetermined mechanism—potentially via direct ascending connections to the limbic system and cortex. The goal of this article is to briefly review how changes in blood gases reach conscious awareness and how chemoreceptors are involved in dyspnea.

Keywords

Hypercapnia; Hypoxia; Serotonin; 5-HT; Raphe; Dyspnea; Chemoreceptors; Breathing

1. Introduction

Dyspnea is the uncomfortable awareness of breathing, the sensation of breathlessness or the experience of air hunger (American Thoracic Society, 1999). It is an important clinical symptom used to assess functional status of chronically ill patients with cardiorespiratory or neuromuscular disease, and has thus been the subject of extensive recent reviews (Manning and Mahler, 2001; McConnell and Romer, 2004; O'Donnell et al., 2007). Healthy subjects can experience dyspnea during strenuous exercise, at high altitude, after breath-holding, or during stressful situations that cause anxiety or panic. Dyspnea occurs more frequently in the aged, the obese and the deconditioned. Certain medications can induce dyspnea as a side

*Corresponding author at: Department of Neurology, Yale University, School of Medicine, 333 Cedar Street, LCI-706, New Haven, CT 06520, United States. Tel.: +1 203 785 6054; fax: +1 203 737 1425, gordon.buchanan@yale.edu (G.F. Buchanan).

effect, and it is often the reason cited for cessation of a new medication or for medication non-compliance. It has been a difficult symptom to study, because it is a subjective and uniquely human experience so animal models have limited use in understanding its mechanisms, and it relies on often vague descriptions by patients (American Thoracic Society, 1999).

Dyspnea is a normal phenomenon that is protective against abnormalities in gas exchange. It can be caused by any number of derangements of normal cardiorespiratory function including primary pulmonary, cardiac or neuromuscular diseases. Pulmonary diseases include COPD, asthma, emphysema, interstitial lung disease, pulmonary edema, pulmonary embolism and pulmonary infections. Cardiac diseases include congestive heart failure and acute myocardial infarction (American Thoracic Society, 1999). Neurological diseases commonly leading to dyspnea include, but are not limited to, amyotrophic lateral sclerosis, myasthenia gravis, Guillain-Barré syndrome, multiple sclerosis and Parkinson's disease.

Awareness of respiratory sensation can occur in normal situations or during dyspnea. During dyspnea there is a heightened level of awareness of respiratory sensation and a strong emotional component. The neural basis of dyspnea is therefore likely to involve activation of both the cortex and the limbic system. As will be discussed below there is emerging evidence for cortical and limbic activation associated with dyspnea.

Dyspnea can be induced by an increase in CO_2 or decrease in O_2 . There are several possibilities for how this occurs including direct effects from chemoreceptor activation or indirect effects either through interactions with other respiratory afferents or through activation of corollary discharges. There is evidence in the literature for each of these possibilities; however the specific mechanisms of the chemoreceptor contribution have not yet been elucidated. In order to understand how changes in blood gases cause dyspnea we need to understand how changes in O_2 and CO_2 are detected, how they influence breathing, and how these changes are transmitted to the forebrain.

Our goal with this review will be fourfold. First we will describe peripheral and central chemoreception and their contribution to the control of breathing. Second, we will describe the evidence that there can be conscious awareness of chemoreceptor input and that this input can induce the sensation of dyspnea. Third, we will speculate as to the mechanisms by which changes in blood gases reach consciousness and how they induce the sensation of dyspnea. Finally, we will discuss chemoreceptor contributions to dyspnea in certain human disease states.

2. Role of chemoreceptors in breathing

Chemoreceptors are instrumental in the regulation of breathing. Blood concentrations of O_2 and CO_2 as well as serum pH need to be maintained within a narrow range to ensure normal function of the body's tissues. Changes in the partial pressure of O_2 (P_{O_2}) are sensed primarily by peripheral O_2 chemoreceptors. Similarly, small changes in the partial pressure of CO_2 (P_{CO_2}) are sensed primarily by central CO_2 chemoreceptors. Activation of either chemoreceptor type leads to an increase in ventilation in an attempt to correct the chemical aberration.

2.1. Oxygen chemoreception

The majority of O₂ chemoreception occurs peripherally by the type I glomus cells of the carotid and aortic bodies (Kumar, 2007). Ultimately, activation of the afferent nerve depends on K⁺ channel inhibition, glomus cell depolarization, influx of Ca²⁺ via voltage-gated Ca²⁺ channel activation, and secretion of neurotransmitters. There are several mechanisms by which hypoxia might inhibit K⁺ channels including elevation in intracellular cyclic adenosine monophosphate (cAMP) through an undefined pathway, inhibition of NADPH-oxidase in mitochondria, or dissociation of O₂ from a heme-containing protein near the K⁺ channel (Richerson and Boron, 2005). The carotid bodies send afferent information regarding P_{O₂} to the nucleus tractus solitarius (NTS) via the carotid sinus nerve branch of the glossopharyngeal nerve (Kubin et al., 2006). In single-fiber recordings from carotid sinus nerve fibers there is tonic discharge in hyperoxia. The discharge rate increases as P_{O₂} is decreased below the normal level of 100v mm Hg (Buckler and Vaughan-Jones, 1994). Though the majority of O₂ chemoreception occurs peripherally, there is also evidence from animal models of a central component of O₂ chemoreception. Implicated regions include the pre Böttinger complex (preBötC) (Ramirez et al., 1998; Solomon et al., 2000), the ventrolateral medulla (Mazza et al., 2000) and hypothalamus (Dillon and Waldrop, 1992). Carotid body resected human patients do not have a hypoxic ventilatory response (Honda, 1992), suggesting that in humans there is little O₂ chemoreception occurring centrally.

2.2. Carbon dioxide chemoreception

Whereas the majority of O₂ chemoreception occurs peripherally, CO₂ chemoreception primarily occurs centrally. In unanesthetized intact dogs, there is some peripheral CO₂ chemoreception through carotid body activation constituting approximately one-third of the overall CO₂ response (Smith et al., 2006). In humans, there is also peripheral CO₂ chemoreception. The sensitivity of these chemoreceptors to CO₂ is increased by hypoxia (Mohan and Duffin, 1997). Neurons in a number of brainstem nuclei have properties consistent with being central CO₂ chemoreceptors, though the relative importance of the specific sites and neuronal cell types responsible for central respiratory chemoreception have been the subject of debate (Richerson et al., 2005; Guyenet et al., 2005; Nattie and Li, 2008).

Candidate chemoreceptive nuclei include the medullary raphé (Richerson, 1995, 2004), NTS (Dean et al., 1990), locus coeruleus (LC; Pineda and Aghajanian, 1997; Oyamada et al., 1998; Dean et al., 2001), retrotrapezoid nucleus (RTN; Nattie et al., 1993; Li and Nattie, 1997; Mulkey et al., 2004), hypothalamus (Dillon and Waldrop, 1992; Williams et al., 2007) and cerebellar fastigial nucleus (Martino et al., 2007). As we will discuss, putative chemoreceptors in the raphé, LC and NTS are particularly attractive as potential mediators of the sensation of dyspnea.

2.2.1. Serotonin neurons as CO₂ chemoreceptors—When studied in slices and in culture many raphé 5-HT neurons are intrinsically chemosensitive (Richerson, 1995; Wang et al., 1998, 2001; Bradley et al., 2002). This is true of 5-HT neurons in the medulla, as well as the midbrain (Fig. 1A and B) (Severson et al., 2003). Though hypercapnia is the respiratory stimulus ultimately leading to chemoreceptor activation, central chemoreceptors

use the change in brain tissue pH resulting from the change in P_{CO_2} as their stimulus. Consistent with this, 5-HT neurons appear to respond to changes in intracellular pH, and not CO_2 directly (Wang et al., 2002; Bouyer et al., 2004). Many CO_2 -sensitive 5-HT neurons are intimately associated with large, penetrating arteries (Fig. 1C) (Bradley et al., 2002; Severson et al., 2003), where tissue pH would be closely related to arterial P_{CO_2} , thus allowing them to more faithfully detect changes in lung ventilation than if they were near capillaries.

In awake behaving cats, a subset of 5-HT neurons increases their firing rate in response to inhaled CO_2 (Fig. 1D) (Veasey et al., 1995). Similarly, hypercapnia *in vivo* increases c-fos staining in neurons of the medullary raphe (Larnicol et al., 1994; Pete et al., 2002), including those that are serotonergic (Haxhiu et al., 2001; Johnson et al., 2005). Consistent with these findings, hypercapnia causes an increase in extracellular 5-HT in the hypoglossal nucleus of mice (Kanamaru and Homma, 2007). Additionally, focal acidification of the medullary raphe with either microinjection of acetazolamide (Bernard et al., 1996) or reverse microdialysis of a CO_2 -enriched solution (Nattie and Li, 2001; Hodges et al., 2004) induces an increase in breathing.

Lesion studies also implicate 5-HT neurons as central CO_2 chemoreceptors. For example, acute lesions or silencing of 5-HT neurons with 5,7-dihydroxytryptamine (5,7-DHT; Mueller et al., 1984), lidocaine, ibotenic acid, muscimol (Dreshaj et al., 1998; Messier et al., 2002; Hodges et al., 2004), 8-OH-DPAT (Messier et al., 2004), or focal saporin conjugated to an antibody for the 5-HT transporter (Nattie et al., 2004) have each been found to depress the hypercapnic ventilatory response. Mice with genetic deletion of the transcription factor *Lmx1b* selectively in 5-HT neurons (*Lmx1b^{fl/fp}* conditional knockout mice) have near complete loss of 5-HT neurons (Zhao et al., 2006). These mice have an ~50% reduction in the hypercapnic ventilatory response compared to WT after inhibiting peripheral CO_2 chemoreception with hyperoxia (Hodges et al., 2008).

These and other data reviewed in detail previously (Richerson, 2004) provide strong evidence that 5-HT neurons sense changes in P_{CO_2} /pH *in vivo*, which then causes release of 5-HT, substance P and thyrotropin releasing hormone (TRH) onto neurons within the respiratory network. These neurotransmitters are well known to increase excitability of rhythm-generating, premotor and motor neurons, thereby increasing ventilation and restoring homeostatic levels of P_{CO_2} (Richerson, 2004). Furthermore, 5-HT neurons have recently been shown to play an additional role in chemoreception, by enhancing the response of the rest of the respiratory network to hypercapnia (Hodges et al., 2008).

The hypothesis that 5-HT neurons are central CO_2 chemoreceptors has recently been challenged (Guyenet et al., 2005, 2008; Richerson et al., 2005; Richerson, 2005), in part based on data that suggests that TASK channels are responsible for chemosensitivity of 5-HT neurons, and that mice with genetic deletion of TASK channels have a normal ventilatory response to CO_2 (Mulkey et al., 2007b; Guyenet et al., 2008). However, the *in vitro* recordings reported by Mulkey et al. (2007b) were from immature (7–12 day old) 5-HT neurons, and their responses were induced by a pathologically large decrease in pH (to 6.9). We have previously shown that 5-HT neurons in rats do not develop a significant response

to physiologically relevant changes in pH until they are at least 12 days old (Wang and Richerson, 1999), and have recently replicated these findings in mouse neurons (Wu et al., 2008). In contrast, adult rat and mouse 5-HT neurons have a much larger response, averaging 300% of control in response to a decrease in pH from 7.4 to 7.2 (Wang et al., 1998; Wang et al., 2001; Wang et al., 2002; Wu et al., 2008). These large responses occur in both culture and brain slices (Fig. 1) (Richerson, 1995; Wang et al., 1998; Bradley et al., 2002; Severson et al., 2003). Thus, TASK channels may mediate chemosensitivity in immature 5-HT neurons to pathological changes in pH, but other mechanisms may become involved when they mature.

2.2.2. Other candidate central CO₂ chemoreceptor sites—Hypercapnia stimulates LC neurons *in vivo* and *in vitro* (Fig. 2A) (Haxhiu et al., 1996; Pineda and Aghajanian, 1997; Oyamada et al., 1998; Filosa et al., 2002; Johnson et al., 2008). Likewise, neurons within the NTS are also stimulated by acidosis (Fig. 2B) (Miles, 1983; Dean et al., 1990), as are neurons in the RTN (Nattie et al., 1993; Mulkey et al., 2004). Neurons in each of these regions, except the RTN (Guyenet et al., 2008) have been shown to retain their chemosensitivity after chemical synaptic blockade or physical isolation. RTN neurons are strongly stimulated by 5-HT, SP and TRH (Mulkey et al., 2007a), and as recently discussed it is possible that some of their pH sensitivity is due to synaptic input from 5-HT or other neurons (Hodges and Richerson, 2008; Guyenet, 2008).

Neurons in the caudal hypothalamus of rabbits (Cross and Silver, 1963), cats (Dillon and Waldrop, 1993) and rats (Dillon and Waldrop, 1992) are stimulated by hypercapnia. There are also chemosensitive neurons in the lateral hypothalamus that contain orexin (Fig. 2C) (Williams et al., 2007). Mice with genetic deletion of hypothalamic orexin neurons have an attenuated hypercapnic ventilatory response, and this can be partially restored by exogenous orexin (Nakamura et al., 2007). Treatment with an orexin receptor antagonist attenuates the hypercapnic ventilatory response in WT mice (Kuwaki et al., 2008).

It has recently been proposed that central chemoreception is a widely distributed function of neurons in many brainstem nuclei (Nattie and Li, 2008). This possibility is supported by studies showing that focal acidosis in many of the nuclei discussed above causes an increase in ventilation *in vivo* (Feldman et al., 2003). What remains unclear is whether all of these sites are equally important under normal physiological conditions, or if some of them only play an important role under specific conditions, such as during development, under anesthesia, during sleep or in various pathological states.

3. Conscious awareness of blood gases and the role of chemoreceptors in dyspnea

It is well known that intense dyspnea can be induced by breathing a gas mixture with high CO₂ or low O₂ (American Thoracic Society, 1999). Hypercapnia-induced dyspnea is subjectively more intense than dyspnea induced by voluntary hyperventilation or exercise (Chonan et al., 1990). Dyspnea induced by hypercapnia could theoretically result either directly from activation of chemoreceptors, or indirectly by the increase in respiratory afferent feedback from the resulting increase in respiratory motor output. It is thought that

the majority of the dyspnea that occurs is due to the latter mechanism. For example, two studies examining tubocurarine-paralyzed healthy subjects found that increasing P_{CO_2} did not induce the sensation of dyspnea (Campbell et al., 1967, 1969). In another study of healthy, passively ventilated subjects, dyspnea was sensed in response to increased P_{CO_2} only after respiratory efferent activation (Castele et al., 1985) implying that it is the respiratory afferent activation that causes the dyspnea and not the elevated P_{CO_2} per se. However, there is also evidence from several other studies suggesting that hypercapnia can induce dyspnea independent of changes in respiratory output. Quadriplegic patients paralyzed by polio (Opie et al., 1959; Patterson et al., 1962) or high cervical cord lesions (Banzett et al., 1989) experience dyspnea when P_{CO_2} is increased. A similar phenomenon was also seen in healthy, mechanically ventilated human subjects paralyzed with vecuronium and exposed to progressive elevations in inspired CO_2 (Fig. 3) (Banzett et al., 1990).

There is other evidence indicating that changes in CO_2 can induce dyspnea independent of changes in respiratory effort (Manning and Schwartzstein, 1995). For example, individuals breathing a hypercapnic gas mixture experience more dyspnea compared to eucapnic individuals with the same level of ventilation and presumably equivalent motor output (Adams et al., 1985; Chonan et al., 1987). The intensity of dyspnea is also heightened when ventilation is voluntarily decreased below the level dictated by chemical drive despite the lesser respiratory motor output (Schwartzstein et al., 1989). Interestingly, when ventilation was increased above the level dictated by chemical drive, 50% of subjects reported increased intensity of dyspnea while the other 50% did not experience dyspnea (Schwartzstein et al., 1989). That dyspnea occurs when there is a mismatch between ventilation and the demand set by the chemical drive exemplifies the importance of maintaining chemical homeostasis. If ventilation is maintained at a constant level, subjects still experience an increased intensity of dyspnea when P_{CO_2} is increased; however their subjective estimation of respiratory effort is actually decreased (Demediuk et al., 1992). Thus, although respiratory afferent feedback may play a large role under some conditions, it appears that there is also a direct and independent effect of hypercapnia itself.

Like hypercapnia, hypoxia can also cause intense dyspnea, especially during exercise, as anyone who has exerted themselves at altitude can attest. As with hypercapnia, it is possible that hypoxia induces dyspnea through the increase in feedback from respiratory afferents. It is also possible that hypoxia acts directly via chemoreceptors to induce the sensation of dyspnea. Indeed, one study demonstrated that relief of exercise-induced dyspnea is achieved following oxygen supplementation out of proportion to the reduction in ventilation (Lane et al., 1987). Another study demonstrated that supplemental oxygen can also improve the sensation of exercise-induced dyspnea in healthy human subjects in the absence of an appreciable change in ventilation (Chronos et al., 1988). These studies suggest that hypoxia can induce dyspnea independent of any increase in respiratory afferent activity. The relative importance of afferent feedback versus direct chemoreceptor input is unclear, but hypoxia is thought to be less dyspnea-inducing than hypercapnia (Manning and Schwartzstein, 1995).

4. Mechanisms leading to dyspnea in response to blood gas changes

In order to understand the mechanisms of dyspnea induced by hypercapnia we must define how chemoreceptor activation can directly or indirectly activate the forebrain.

4.1. Forebrain regions activated by CO₂ and dyspnea

There are several lines of evidence suggesting that elevating P_{CO₂} activates forebrain regions and that these same regions are activated in association with dyspnea induced by other factors. Data from respiratory-related evoked potential (RREP) studies in humans show that brief periods of breathlessness induced by upper airway occlusion activate the somatosensory cortex (Davenport et al., 1986). Functional imaging studies with positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) reveal that hypercapnia causes activation of multiple limbic regions of the brain, including the cingulate cortex, hippocampus, insula, amygdala and hypothalamus, but not forebrain regions that are activated by volitional breathing, such as the primary motor cortex, premotor area and supplementary motor area (Fig. 4A and B) (Liotti et al., 2001; Brannan et al., 2001; von Leupoldt and Dahme, 2005). Rarely, dyspnea is induced by seizures originating in the medial temporal lobe (Cohen-Gadol et al., 2004). Interestingly, over one hundred years ago it was observed that uncus stimulation in epilepsy could result in apneusis (Jackson, 1899). Functional imaging with PET and fMRI demonstrate that each of these same regions is also activated by dyspnea induced by other means (e.g. lung volume restriction), as are the operculum and amygdala (Fig. 4C and D) (Evans et al., 2002; von Leupoldt and Dahme, 2005). These results are consistent with the possibility that dyspnea induced by hypercapnia is due to activation of the limbic system by direct or indirect projections from chemoreceptors to limbic regions.

4.2. Afferents potentially involved in conscious respiratory sensation

The fact that CO₂ and dyspnea can activate cortical and limbic structures is not surprising. There are several possibilities for how hypercapnia can be consciously sensed, including: (1) direct projections from chemoreceptors to the forebrain; (2) input from peripheral respiratory afferents to the forebrain, and; (3) corollary discharges from respiratory neurons to the forebrain (Fig. 5).

4.2.1. Chemoreceptor afferents—It is reasonable to conclude that any of the chemoreceptor candidates discussed above could influence the limbic system. Neuroanatomical studies reveal that many central CO₂ chemoreceptor candidate nuclei including the NTS, raphe, LC and hypothalamus have direct connections to limbic structures. Such direct connections have not been identified for the RTN.

Immunohistochemical analyses show that catecholaminergic and serotonergic terminals localize to limbic regions. Various 5-HT and adrenergic receptor subtypes are also found in these regions. Anterograde labeling studies have also elucidated projections from medullary raphe 5-HT neurons to higher structures including cortex, thalamus and limbic structures such as the hippocampus and amygdala (Kosofsky and Molliver, 1987). Within the thalamus, 5-HT terminals are most abundant in those regions subserving limbic function,

such as the anterior dorsal nucleus (Jacobs and Azmitia, 1992). Immunohistochemical methods reveal abundant diffuse 5-HT neuronal terminals throughout the brain including amygdala, hippocampus, hypothalamus, basal ganglia, substantia nigra and NTS (Steinbusch, 1981). Immunohistochemical methods also reveal that various 5-HT receptor subtypes are distributed throughout the forebrain including limbic structures (for review see Jacobs and Azmitia, 1992).

The 5-HT system plays a key role in control of mood and emotional processing. For instance, depletion of the 5-HT precursor tryptophan, which decreases available 5-HT, produces an emotional state consistent with depression in healthy subjects. Administration of selective serotonin reuptake inhibitors (SSRIs) improves the mood of depressed patients and also reduces anxiety and panic thought to be due to limbic activation (Harmer, 2008; Hensler, 2006).

Anterograde labeling studies also reveal projections from the LC to bilateral telencephalic and diencephalic structures including cortex, cingulate, amygdala, thalamus, hypothalamus and hippocampus (Jones and Moore, 1977; Jones et al., 1977). Blockade of norepinephrine reuptake also improves the mood of depressed patients (Rueter et al., 1998). Through interactions with the 5-HT system norepinephrine may also be involved with anxiety and panic (Coplan and Lydiard, 1998). Interestingly, lesions of the LC induce prolonged limbic seizures in a rodent model of epilepsy (Giorgi et al., 2006).

There are also abundant forebrain projections from the NTS, including from many neurons that receive respiratory afferents. Anterograde tracing studies using wheat germ agglutinin conjugated horseradish peroxidase identified efferent projections from the NTS to the central nucleus of the amygdala, bed nucleus of the stria terminalis, paraventricular, dorsomedial and arcuate nuclei of the hypothalamus, ventral preoptic area and thalamus (Ricardo and Koh, 1978). Retrograde tracing studies also identified connections to the central nucleus of the amygdala (Reyes and Van Bockstaele, 2006). Forebrain projections from the NTS are primarily catecholaminergic (Riche et al., 1990), expressing tyrosine hydroxylase (TH) (Reyes and Van Bockstaele, 2006), but also contain some neuropeptides including neuropeptide Y, somatostatin and substance P (Riche et al., 1990).

Orexinergic neurons from the lateral hypothalamus are connected to many higher structures. Orexin immunoreactive nerve terminals are seen throughout the hypothalamus, thalamus, brainstem, cerebral cortex and spinal cord (Nambu et al., 1999). Projections to the brainstem include those to the median raphe and RTN (Kuwaki et al., 2008). There are additionally connections from prefrontal area (PFA), a known orexinergic region, to amygdala evidenced by retrograde labeling following injection of cholera toxin B subunit into the PFA (Sakurai et al., 2005). A recent fMRI study in patients with narcolepsy and cataplexy and a dysfunctional orexin system demonstrates that viewing emotionally-charged photographs induces enhanced activation of limbic structures when compared to control subjects viewing the same photographs (Schwartz et al., 2008).

The presence of diffuse projections from many of the candidate chemoreceptive nuclei to important forebrain and limbic structures involved in conscious awareness and processing of

emotional content, and the influence of neurotransmitters released by some of the putative chemoreceptor neurons on limbic function, supports the notion that direct projections from chemoreceptors could subserve the sensation of dyspnea in response to blood gas challenges.

Consistent with the above mentioned connections from chemoreceptive nuclei to forebrain regions, hypercapnia is a potent arousal stimulus. In adult humans hypercapnia induces arousal from both NREM and REM sleep in a state-dependent manner, with significantly less hypercapnia required to induce arousal out of REM compared to NREM (Berthon-Jones and Sullivan, 1984). In dogs, hyperoxic hypercapnia induces arousals from both NREM and REM but does so more quickly and at a lower CO₂ concentration in NREM than in REM (Phillipson et al., 1977). In lambs, repeated 60-s challenges with 8% CO₂ greatly increased the arousal probability compared with a eucapnic environment (Johnston et al., 2007). It has previously been proposed that chemosensitive 5-HT neurons could contribute to this arousal response to hypercapnia (Washburn et al., 2002; Severson et al., 2003). Consistent with this possibility, preliminary data from *Lmx1b^{fl/p}* conditional knockout mice (see above) indicate that these mice do not demonstrate robust electroencephalographic or behavioral evidence of a change in arousal state in response to hypercapnia (Buchanan et al., 2007). It is possible that 5-HT neurons may induce arousal in response to low levels of CO₂, whereas anxiety and dyspnea could be induced by the same projections at higher CO₂ levels.

4.2.2. Feedback from respiratory afferents—Changes in blood gases might also be detected consciously by indirect activation from non-chemoreceptor sources. For example, the relative magnitude of respiratory effort appears to be monitored by the cortex, and when this becomes a large percentage of the maximum capable effort it is perceived as unpleasant (Manning and Schwartzstein, 1995). Embedded within the intercostal muscles and joints of the chest wall are muscle spindle fibers that sense expansion and contraction of the thorax and relay this information to higher centers. Afferent signals from muscle spindles are involved with reflex control of respiratory activity. Similarly the diaphragm contains Golgi tendon organs, which sense muscle tension and have a primarily inhibitory impact on respiratory activity. These muscle mechanoreceptors allow detection of changes in volume (length) and pressure (tension) status. The balance between volume and pressure is conveyed to higher centers as “length–tension appropriateness” (Campbell et al., 1961). Any load on this system conveys an “inappropriate” signal upward and leads to adjustments in medullary respiratory motor activity. It has recently been proposed that a neuromechanical uncoupling mediates the sensation of dyspnea in COPD. In this model, there is an increase in the ratio of percent maximal esophageal pressure (effort) to tidal volume response (displacement), or effort–displacement ratio (O'Donnell et al., 2006). As discussed above, one mechanism of dyspnea that has been proposed is a mismatch between motor output from the central respiratory network and afferent inputs from peripheral receptors involved with respiratory sensation (Schwartzstein et al., 1989). Changes in blood gases could increase this mismatch via indirect effects from chemoreceptors.

In addition to chest wall mechanoreceptors, there may also be activation of vagal afferents from lung and airway receptors. Expansion of the lungs stimulates pulmonary stretch receptors that send afferent signals to respiratory sensory pathways. Similarly there are

irritant receptors within bronchial walls that are stimulated by changes in smooth muscle tone, tactile stimulation of bronchial mucosa, and rapid airflow through the bronchi. All of these afferents relay through the NTS to modulate respiratory activity (Kubin et al., 2006), and from there could project to forebrain regions. Dyspnea may be caused in part by the increase in this ascending activity when respiratory motor output is increased by hypercapnia.

4.2.3. Corollary discharges—Changes in respiratory output are also transmitted through corollary discharges to higher centers. These corollary discharges are widely believed to contribute to the perception of dyspnea (American Thoracic Society, 1999). Corollary discharges project from respiratory control nuclei in the lower brainstem to the sensory cortex during automatic breathing and from motor cortex to sensory cortex during voluntary breathing (Killian et al., 1984). They occur simultaneously with the primary respiratory discharges and convey information regarding the breathing effort to the sensory cortex to keep the cortex “aware” of the level of respiratory activity. Corollary discharges are known to activate the cortex (Shea et al., 1993), midbrain (Chen et al., 1991) and thalamus (Chen et al., 1992) in response to hypercapnia. Mesencephalic and thalamic activation by corollary discharges occurs independent of afferent input suggesting that these are internally generated signals related to respiratory motor output (Chen et al., 1992). Little is known about the cellular and molecular mechanisms underlying these corollary discharges. Certainly most of the chemoreceptive nuclei are well positioned to influence these corollary discharges in part by causing an increase in the level of respiratory activity.

4.2.4. Interactions between chemoreceptors and other ascending pathways—Peripheral afferent information can modify the intensity of dyspnea induced by hypercapnia (Manning and Schwartzstein, 1995). For example, when normal humans are allowed to take larger breaths they are better able to tolerate hypercapnia (Mithoefer et al., 1953; Remmers et al., 1968). When the airway is anesthetized there is an increased hypercapnic ventilatory response possibly owing to removal of inhibitory signals from airway stretch receptors (Winning et al., 1985). At a given end-tidal CO₂ humans experience greater dyspnea when there is lower ventilation (Schwartzstein et al., 1989) or when lung volumes are restricted either voluntarily or involuntarily (Chonan et al., 1987). These data all suggest interplay between respiratory afferents and chemoreceptors.

The NTS may be a key site for relaying chemoreceptor information to the forebrain to mediate the sensation of dyspnea. First, this is a key site for relaying all respiratory afferent signals to the forebrain (Kubin et al., 2006). In addition, NTS neurons are putative central chemoreceptors themselves. Thus, the NTS may mediate effects of blood gases on the sensation of dyspnea in two ways: via direct activation of the NTS through its chemoreceptive properties, and through activation of NTS neurons by input from respiratory afferents and/or other chemoreceptors, including peripheral chemoreceptors and chemosensitive 5-HT neurons in the nearby medullary raphé nuclei.

5-HT terminals synapse on and modulate firing of NTS neurons. These NTS neurons contain 5-HT_{1A}, 5-HT_{1B} (Manaker and Verderame, 1990), 5-HT_{2A}, 5-HT_{2B}, 5-HT₃ (Fonseca et al., 2001) and 5-HT_{4A} receptors. 5-HT_{4A} receptors typically suppress C-fiber

input to the NTS neurons (Edwards and Paton, 2000). NTS neurons are also modulated by substance P, which is colocalized with 5-HT (Mutoh et al., 2000). This implies a potential joint effect between 5-HT neurons in the raphé and catecholaminergic neurons in the NTS in mediating chemoreceptor-mediated dyspnea. Given the evidence that 5-HT neurons are central CO₂ chemoreceptors; the interconnections with the NTS which, as mentioned above, is a prime relay nucleus for respiratory afferent information; interconnections between 5-HT neurons and other respiratory nuclei including preBötC, hypoglossal nucleus and nucleus ambiguus (Hodges and Richerson, 2008; for review see Richerson, 2004); and extensive connections from the raphé to the thalamus, cortex and limbic structures, the medullary raphé is also an attractive potential site for hypercapnia interacting with respiratory afferents that contribute to the sensation of dyspnea.

5. Chemoreceptors and dyspnea in human disease

Chemoreceptors likely contribute to the sensation of dyspnea in normal, healthy individuals in some circumstances, but there are certain disease entities in which activation of chemoreceptors may be of particular relevance.

5.1. COPD

Aside from signaling blood gas abnormalities related to respiratory distress to help bring the distress to conscious awareness, chemoreceptor abnormalities may also be a more direct part of the pathophysiology of COPD. In COPD a point can be reached where elevations in P_{CO₂} become so severe that they no longer activate chemoreceptors to effect respiratory changes to correct the abnormalities. In essence this creates a mismatch between chemical drive and ventilation. These patients develop acidosis-associated mental status changes and lose the protective sensation of dyspnea. When they reach this point they require ventilator support to correct the abnormality. Identification of the defect responsible for allowing such profound elevations in P_{CO₂} may lead to better monitoring and earlier intervention to reduce COPD-related morbidity and mortality.

5.2. Obstructive sleep apnea (OSA)

Dyspnea, especially nocturnal dyspnea, is a prominent feature in OSA. OSA affects as many as 4% of the middle-aged population, males more often than females. Both anatomic and neurophysiologic factors contribute to airway obstruction in OSA, but abnormally low upper airway tone during sleep is an important factor. Decreased upper airway tone increases airway compliance and increases resistance at a given negative airway pressure. 5-HT input to motor neurons innervating upper airway dilator muscles decreases during sleep (Veasey, 2003), and this may enhance airway obstruction. Given that 5-HT and LC neurons are putative central chemoreceptors (Richerson, 2004), 5-HT and norepinephrine increase tone of upper airway muscles, and 5-HT and LC neurons are part of the ascending arousal system (Saper et al., 2005), defective chemoreception owing to abnormalities in 5-HT and LC neurons could contribute to OSA. Data from fMRI studies suggest that there is impaired activation of hippocampus, cingulate, insula and cerebellum in OSA patients during brief periods of breathlessness induced by Valsalva maneuvers suggesting a neural defect in limbic processing of respiratory sensations (von Leupoldt and Dahme, 2005).

5.3. Congenital central hypoventilation syndrome (CCHS)

Further evidence of impaired processing of respiratory sensations in human diseases comes from the study of patients with CCHS. These patients have impaired ventilatory responses to hypercapnia and hypoxia, loss of breathing drive during sleep and impaired perception of dyspnea (Kumar et al., 2005). Furthermore, fMRI studies reveal that forebrain regions activated in normal subjects in association with dyspnea do not activate well in CCHS patients (Kumar et al., 2005). The impairment in dyspnea is likely to be due in part to a lack of chemoreceptor afferent input in these patients. There is evidence that neurons of the RTN may be impaired in mice with the most common mutation of *Phox2b* that causes CCHS (Dubreuil et al., 2008). There is also evidence for abnormal peripheral chemoreceptors (Macey et al., 2004), and there may be defects in neurons of the NTS as well as 5-HT neurons, both of which depend on *Phox2b* for normal development (Dauger et al., 2003; Pattyn et al., 2003). Thus, defects in many of the different putative chemoreceptor neurons could contribute to the impairment of dyspnea in CCHS.

5.4. Panic disorder

Panic disorder is thought to result from a catastrophic misinterpretation of physiologic sensations including, but not limited to, dizziness, palpitations and breathlessness (Clark, 1986). One theory of the pathophysiology of panic disorder is that the misinterpretation of sensation is due to a hypersensitive “suffocation alarm” (Klein, 1993) whereby a small increase in blood P_{CO_2} leads to physiological and behavioral changes of panic, foremost among these being dyspnea (Goetz et al., 2001). Consistent with this, patients with panic disorder can be induced to have an attack by breathing air enriched with CO_2 (Van den Hout and Griez, 1984). Thus, fundamentally chemoreceptors must be involved, either as the site of pathology or as the trigger of paroxysmal changes that occur at a site downstream of chemoreceptor projections. It was recently found that the threshold and sensitivity of the hypercapnic ventilatory response was not different in panic disorder patients and healthy subjects (Katzman et al., 2002). This was interpreted as being in conflict with the false suffocation alarm theory of Klein. However, these data do not rule out dysfunction in central chemoreceptor neurons or pathways in panic disorder. One explanation for these results is that the chemoreceptors that trigger the response could have a normal response to CO_2 when tested in patients between panic attacks, but high CO_2 triggers a paroxysmal increase in firing of chemoreceptors that causes a panic attack. Similar to patients with epilepsy that have normal interictal cortical function, or “classic” migraine patients with normal visual cortex function between migraine attacks with visual aura, panic disorder patients may sometimes have paroxysmal dysfunction of chemoreceptor neurons. There are also other explanations consistent with both the basic theory as proposed by Klein and the observation of a normal baseline hypercapnic ventilatory response, including a paroxysmal response to elevated CO_2 induced downstream of chemoreceptors, and increased sensitivity of a subset of CO_2 chemoreceptors that selectively project to elements of the limbic system.

There is a large body of data suggesting that 5-HT may be instrumental in the manifestation of panic disorder (Coplan et al., 1992). For example, selective serotonin reuptake inhibitors (SSRIs) are first line treatment for panic disorder (Coplan et al., 1997). A potential explanation for these associations is that changes in CO_2 induce abnormal excitability of 5-

HT neurons, which in turn activate thalamocortical mechanisms, the limbic system and respiratory centers to produce heightened arousal, increased respiratory rate and dyspnea.

5.5. Sudden infant death syndrome (SIDS)

SIDS remains the leading cause of post-neonatal death in the U.S. occurring in 0.6 of every 1000 live births, or 6 infants per day in the U.S. alone. Certain consistent risk factors have been identified including maternal smoking, prone sleeping position, sleeping on soft surfaces, low birth weight and male sex (Task Force on Sudden Infant Death Syndrome, 2005). SIDS victims have been found to have abnormalities in the brainstem 5-HT system (Panigraphy et al., 2000) including an increase in the number of 5-HT neurons in the medulla. It is not clear if there is an increase or decrease in function of the 5-HT system, but there is a reduction in the number of multipolar 5-HT neurons consistent with a delay in their maturation (Paterson et al., 2006).

Dysfunction of the 5-HT system may represent the underlying vulnerability in SIDS (Richerson, 1997), whereby rebreathing or hypoventilation during a critical developmental period results in hypercapnia, hypoxia and failure to arouse. If the 5-HT system was functioning properly in these children the elevation of P_{CO_2} would trigger chemosensitive 5-HT neurons to induce arousal either directly via thalamocortical activation (Washburn et al., 2002; Severson et al., 2003; Buchanan et al., 2008) or indirectly by inducing dyspnea and activating corollary discharges. Therefore, while dyspnea per se is not a feature of SIDS, lack of dyspnea, as mentioned above for CCHS, may be important in the pathophysiology of this disease.

5.6. Parkinson's disease

In a manner similar to other diseases mentioned above, there is an impaired perception of dyspnea in patients with Parkinson's disease exposed to hypoxia (Onodera et al., 2000). At the same time, 40% of patients with Parkinsonism complain of dyspnea (Witjas et al., 2002) due in some cases to irregular and incoordinated breathing. It has recently been found that defects in the brainstem of patients with Parkinson's disease are much more widespread than just in the substantia nigra (Braak et al., 2003; Braak and Del, 2008). Thus, the impaired perception of dyspnea could be due to defects in 5-HT neurons that occur in these patients (Halliday et al., 1990; Braak et al., 2004; Benarroch et al., 2007) or other brainstem neurons involved in respiratory control, respiratory afferent processing or chemoreception. This could also include carotid body glomus cells, which like substantia nigra neurons contain dopamine (Iturriaga and Alcayaga, 2004).

6. Summary

Dyspnea is a multifactorial sensation involving interplay of sensory afferent information onto the central respiratory control nuclei and connections to and from higher cortical and limbic centers (Fig. 5). Chemoreceptors certainly play a role in the sensation of dyspnea, likely via a combination of (1) increasing respiratory output and subsequent respiratory afferent activation, (2) activation of corollary discharges and (3) direct projections from chemoreceptors to forebrain structures—especially parts of the limbic system. Central

chemoreceptive regions such as the medullary raphe, NTS and LC may all play an important role in this since each of them has direct projections to the limbic system. Dyspnea is an important clinical symptom seen in a wide variety of cardiopulmonary and neuromuscular disease. It can be protective in that it allows conscious recognition of blood gas derangements. Further understanding the role of chemoreception in the mechanisms of dyspnea may lead to improved management of patients and reduced morbidity and mortality.

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Fig. 1.

5-HT neurons are central CO₂ chemoreceptors. Patch clamp recordings from a rat medullary raphé neuron in cell culture (A) and a dorsal raphé neuron in a rat brain slice (B). (A) Adapted with permission from Wang et al. (2002), (B) adapted with permission from Severson et al. (2003). (C) Confocal imaging after immunohistochemistry for tryptophan hydroxylase showing juxtaposition of medullary 5-HT neurons (yellow and green) to the basilar artery (BA) and its branches (red). Scale bar, 200 μm. Adapted with permission from Bradley et al. (2002). (D) The firing rate of 5-HT neurons increases in awake behaving cats *in vivo* in response to elevated CO₂. Adapted with permission from Veasey et al. (1995).

Fig. 2. LC, NTS and hypothalamic neurons are sensitive to CO₂. Unit recordings from LC (A), NTS (B) and hypothalamus (C). (A and B) Depict single-unit extracellular recordings from rat brain slices integrated over time. (A) Adapted with permission from Pineda and Aghajanian (1997); (B) adapted with permission from Dean et al. (1990). (C) Depicts a whole-cell current-clamp recording from a lateral hypothalamus orexin neuron. Adapted with permission from Williams et al. (2007). Copyright 2007 National Academy of Sciences, U.S.A. In all traces baseline CO₂ was 5% and increased to 10% as indicated.

Fig. 3.

Hypercapnia induces dyspnea independent of respiratory afferent input. Record from a single subject paralyzed with vecuronium at the beginning of the tracing. During the experiment inhaled CO₂ concentration was altered and the subject communicated their level of respiratory discomfort by moving a finger that was left unparalyzed. The subject was suctioned during the gap in the tracing and vecuronium was re-administered at the end of the gap. SEV, severe; MOD, moderate; SLT, slight. Adapted with permission from Banzett et al. (1990).

Fig. 4.

Limbic regions are activated by either hypercapnia or dyspnea. (A) Coronal section from a PET scan showing activation of the amygdala during hypercapnia. Adapted with permission from Brannan et al. (2001). Copyright 2001 National Academy of Sciences, U.S.A. (B) Sagittal section from a PET scan showing anterior cingulate activation during hypercapnia. Adapted with permission from Liotti et al. (2001). Copyright 2001 National Academy of Sciences, U.S.A. (C) Axial section from an fMRI scan showing amygdala activation during dyspnea induced by tidal volume restriction. (D) Sagittal section from an fMRI scan showing anterior cingulate activation during dyspnea induced by tidal volume restriction. (C and D) modified with permission from Evans et al. (2002). AC, anterior cingulate; Am, amygdala; Fo, orbitofrontal cortex.

Fig. 5.

Pathways involved in chemoreceptor-mediated dyspnea. Respiratory nuclei generate respiratory motor output. Respiratory motor output leads to changes in feedback from respiratory afferents and peripheral chemoreceptors. Afferent signals from these receptors are relayed through the NTS to respiratory nuclei, limbic structures and sensory cortex. Respiratory motor output also alters the CO₂ level which affects central chemoreceptive nuclei which in turn project to respiratory nuclei, limbic structures and sensory cortex. As respiratory nuclei send signals to alter motor output they also send “corollary discharges”

(shown as dashed lines) to limbic structures and sensory cortex. The motor cortex has the ability to directly control the activity of respiratory neurons, while also sending corollary discharges to the limbic system. CR, chemoreceptor; LC, locus coeruleus; NTS, nucleus tractus solitarius.

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