SLEEP NETWORKS AND THE ANATOMIC AND PHYSIOLOGIC CONNECTIONS WITH RESPIRATORY CONTROL

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1. ABSTRACT

A central neuronal network regulates airway functions from the nares to the bronchioles and is an integral component of a regulatory system for brain control of breathing and airway patency during wakefulness and sleep. This network, components of which include sleep generating sites and monoaminergic neurons in particular, is characterized by reciprocal interconnections, parallel organization, and state-dependent activity patterns, which can be influenced by both genes and environment. Sleep generating neurons are interconnected with the monoaminergic containing cells to the extent that sleep-related changes in upper and lower airway patency could be due to inhibitory influences of sleep-activated neurons on serotonergic and noradrenergic producing cells. Neurochemical studies and physiologic experiments show that serotonergic and noradrenergic producing cells can make parallel pathways, directly innervating the hypoglossal motor cells regulating upper airway dilating muscles, and vagal preganglionic neurons providing cholinergic outflow to the airways. Activation of serotonergic and noradrenergic cell groups preferentially increases activity of the genioglossus muscle, but diminishes cholinergic outflow to the airways. Hence, inhibition of monoaminergic neurons during sleep may lead to a decrease in upper airway dilating forces and an elevation of cholinergic outflow to the airways. Qualitatively different responses of hypoglossal and airway-related vagal preganglionic neurons (AVPNs) occur in response to endogenously released serotonin or norepinephrine and could be related to its simultaneous action on different serotonin or norepinephrine receptor subtypes. Dysfunction of monaminergic cell groups during sleep may predispose to upper airway occlusion as well as bronchoconstriction. Pharmacological corrections of alterations of these transmitter specific converging systems might be an avenue for treatment of sleep related airway disorders such as sleep apnea and worsening of asthma.

2. INTRODUCTION

The airways, extending from the nares to the alveoli, function to conduct airflow from the environment to the gas-exchanging units of the lung for the purpose of eliminating carbon dioxide and acquiring oxygen. This is an active process, controlled by neural mechanisms that regulate respiratory-related drive to different controlled elements of the upper airway (nasal and pharyngeal passages), chest wall (intercostals and abdominal muscles and the diaphragm); and airway smooth muscle, submucosal glands, and lung vasculature. Chemo- and mechano-sensors provide feedback to neural networks of the controller and also play an important role in regulation of breathing rate (frequency) and depth (tidal volume). In this review, we discuss these systems within the broad concept of how sleep networks interact with respiratory control and regulate upper airway geometry, the conductivity of the tracheobronchial system, and the implications of these neural control systems on human health and disease.

Sleep in healthy people is generally considered to be protective to cardiopulmonary functions; however, there are a number of sleep-related disorders of respiratory control. The most common is obstructive sleep apnea (OSA), a condition found in perhaps 2-4% of the population and responsible for significant morbidity from

sleepiness to cardiovascular co-morbidity (1). Patients with neuromuscular disease (failure of the controlled system) or healthy people at altitude (low oxygen environment) are prone to alterations in gas exchange during sleep, further emphasizing the interdependence of sleep and breathing. Current treatments for sleep-disordered breathing are mechanical or surgical in nature, and there is a need to develop pharmacologic options. Another respiratory disease with a prominent sleep component is bronchial asthma, in which the symptoms may be first manifested during sleep.

One area of focus will be how upper airway patency is influenced by sleep. Sensory inputs, activity of sensory neurons, as well as tone of upper airway dilating muscles such as the genioglossus and posterior cricoarytenoid muscles are reduced during both non-rapid eye movement (NREM) and rapid eye movement (REM) sleep (2-6). These changes are associated with variable and often significant narrowing of the upper airway passage. In all conditions where OSA occurs, the onset of the event is associated with a fall in upper airway dilating muscle activity. Sleep deprivation, moderate weight gain, sedatives, enlarged tonsils or nasal congestion influence the presence, number, and consequences of events (1). The consequences are hypoxemia, cardiac arrhythmia, autonomic stress, and sleep state discontinuity (7-15). It appears that the same cell groups that innervate the hypoglossal motoneurons also project to vagal motoneurons, which in turn provide cholinergic outflow to the tracheobronchial tree. One area of focus will be on the controller aspects of the monoaminergic neurotransmitter system.

Another feature will be a review of autonomic control of the lower airways. In humans, the caliber of the tracheobronchial system also undergoes cyclic oscillations, with decreases at night and increases during the day. These fluctuations are greatly amplified during sleep in disease states such as bronchial asthma. Up to 90% of asthmatic patients experience nocturnal symptoms severe enough to awaken them at least periodically. The mechanisms of worsening of asthma during sleep are complex. Some factors proposed to account for nocturnal increase in airway resistance include circadian variations in blood-hormone concentrations, nighttime exposure to antigen, and airway inflammation (16-18). Although, these may play a role in nocturnal worsening of asthma, in most cases they do not completely explain airway narrowing and the dynamic features of airflow obstruction. Thus, there is a controller effect consistent with literature that suggests that specific neuronal networks determine cholinergic outflow to the airways and a subset of these neurons also are integrated in circuits that regulate sleep and circadian rhythm.

Throughout the discussion, the focus will be on parallel neural control of upper airway dilating muscles and airway smooth muscle tone as a model system for displaying the manner in which sleep and breathing interact at different levels of central control and promote repetitive apneas. Finally, we will briefly review clinical and animal model evidence for a genetic component to sleep disordered breathing.

3. OVERVIEW OF THE RESPIRATORY CONTROLLER

Understanding the neural basis of the control of breathing begins with an appreciation of the discharge of putative pacemaker neuronal groups located in the medulla oblongata (19, 20). This network of neurons is embedded in a system of adjacent medullary neurons, and is interconnected with pontine neurons, and regions like the nucleus tractus solitarius (NTS) that receive neural impulses resulting from lung inflation, lung deflation, blood pressure, and other afferent systems. Our current conceptual model is based on two possible models of respiratory rhythm generation: 1) an autorhythmic population of "pacemaker" neurons embedded in a pattern formation network, and 2) a network-based route that has inspiration being terminated by an inhibitory process and expiration continuing until inhibitory influences wane, leading to the onset of the next inspiration. In the first model, respiratory rate is determined by the excitability of the autorhythmic pacemaker neurons, while in the second model, the rate of inspiration is determined by the intensity of medullary discharge; duration and depth are determined by the timing and intensity of inhibitory influences. The models may be complimentary, changing from a pacemaker based model in the neonatal period to a network/inhibition dependent model as the animal develops.

Intensity of discharge in medullary neurons is largely modulated by chemoreceptors, specialized cells and organs sensing oxygen and carbon dioxide. The carotid bodies are located bilaterally at the bifurcations of the common carotid arteries; the aortic bodies are situated anterior and posterior to the arch of the aorta and left main pulmonary artery. The primary event is a low PaO_2 that will stimulate these peripheral chemoreceptors, although hypercapnia, acidemia, and possibly hyperthermia may influence the gain of the response to hypoxemia (21). Within the CNS, an increase in $PaCO_2$ will stimulate cells on or with dendritic processes close to the ventral medullary surface (VMS) (22, 23), primarily by lowering the pH of medullary extracellular fluid (22) However, there is widespread distribution of cells in the brainstem and suprapontine regions with chemosensory properties similar to those on the VMS such as monoaminergic neurons (25). All these cell groups are involved in behavioral state control and can influence cardiopulmonary behavior during sleep.

Specialized sensory cells for mechanical deformation (mechanoreceptors) and for temperature are located in the upper airway mucosa; other receptors are located in the structures of the chest wall and lung (26). Afferent nerve signals from these receptors are directed to the medulla (NTS), where information is integrated with chemoreceptor information to influence medullary timing and volume of ventilation. From the NTS there are connections to the midbrain and cortex, partly via brainstem monoaminergic neurons. Arousals from sleep induced by hypercapnia probably utilize this pathway. This receptor system acts in concert with chemoreceptors to control the timing of expiration (and therefore functional residual
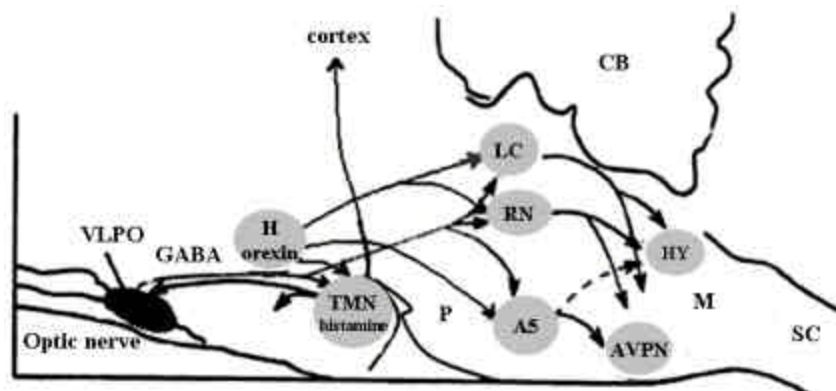
Figure 1. Schematic presentation of structures involved in regulation of behavioral control of upper airway patency and lower airway conductivity. Only GABAergic hypothalamic neurons involved in the generation of sleep and their projections to monoaminergic cell groups are presented. It is assumed that VLPO neurons, which have been shown to be involved in generation of NREM and recently REM sleep, project to histaminergic neurons in the TMN as well as brainstem noradrenergic cell groups such as the LC and A5, and serotonergic neurons of raphe nuclei. As schematically presented, noradrenergic and serotonergic cell groups project to hypoglossal motoneurons and vagal preganglionic cells providing cholinergic outflow to the airways. In addition, projections from hypothalamic orexin containing neurons to brainstem monoaminergic cells are also shown. A5: A5 adrenergic cell group, AVPN: airway vagal preganglionic neuron, CB: cerebellum, H: hypothalamus, HY: hypoglossal nucleus, LC: locus coeruleus, M: medulla, P: pons, RN: raphe nucleus, SC: spinal cord, TMN: tuberomammillary nucleus, VLPO: ventrolateral preoptic area.

capacity), ventilation, as well as upper airway patency and cholinergic outflow to the airways (4, 27, 28). There are "set-points" for regulation of respiratory drive that are best understood in the context of homeostatic control of acid-base balance (pH) and oxygen delivery. One example of a set point is the "apneic threshold" which is defined as that level of arterial (or central) carbon dioxide below which there is no detectable neural inspiration. This "set point" is higher in sleep (3, 28). Certainly, brain centers other than the medulla and pons contribute to breathing rate and depth and can override to a certain extent brainstem mechanisms for breathing. During sleep, "higher" centers have less influence and may be actively inhibiting respiratory activity, unless engaged in an arousal response from quiet sleep or by REM sleep. This concept is discussed below in greater detail.

Integration and coordination of neuromuscular activity is needed during inspiration. In constrast, exhalation occurs often as a result of the passive recoil of the lungs and chest wall; however, the second phase (pause) in expiration and the start of a new inspiration are active events. Hence, in a healthy person, breathing is a sequence of inhalations and exhalations that serve to maintain alveolar ventilation at a level that is appropriate for meeting metabolic demands during wakefulness and sleep, as well as during exercise and other activities of daily living.

4. SLEEP GENERATION AND RESPIRATORY CONTROL

Sleep is one brain state with ultradian and circadian expression. It is actively generated and induced by specific networks that alter the firing patterns of different cell populations along the neuraxis, including

activity of monoaminergic neurons (29). There is little evidence that any single cell group independently causes all of the traits comprising the sleep state (29). The neuroanatomical connections among sleep networks as shown in Figure 1 introduces some relationships relevant to respiratory control.

Slow wave sleep (SWS) is initiated both in brain regions within and outside the brainstem; however, a prominent site for generation of SWS is the ventrolateral preoptic area (VLPO) and the adjacent basal forebrain (30). A lower brainstem region that is thought to participate in generation of SWS is located within the dorsal aspect of the caudal brainstem, and includes the rostral part of the nucleus tractus solitarius and the nucleus reticularis ventralis (31). Sleep-activated cells also innervate cell bodies and dendrites of the ipsilateral tuberomammilary histaminergic neurons of the posterolateral hypothalamus in a region that participates in arousals from sleep (32, 33). Stimulation of each sleep-inducing site promotes sleep, lesions of each suppress it, and single units in each area show SWS-related activity. Previously, it was shown that damage to a cluster of sleep-active neurons (cFos-positive cells during sleep) in the VLPO decreases SWS or non-rapid eye movement (NREM) sleep in rats, whereas injury to sleep-active cells extending dorsally and medially from the VLPO cluster (the extended VLPO) diminishes REM sleep. Projections from the VLPO to other sites in the brain including the locus coeruleus and the raphe nuclei of the brainstem (31-33), indicating additional ways to link these neuronal pools to arousal and vigilance systems.

Events of REM sleep are controlled by structures of the brainstem (31,34-42, 44-46). Microinjection of cholinergic agonists or acetylcholine esterase inhibitors such as physostigmine into the medial pontine reticular formation
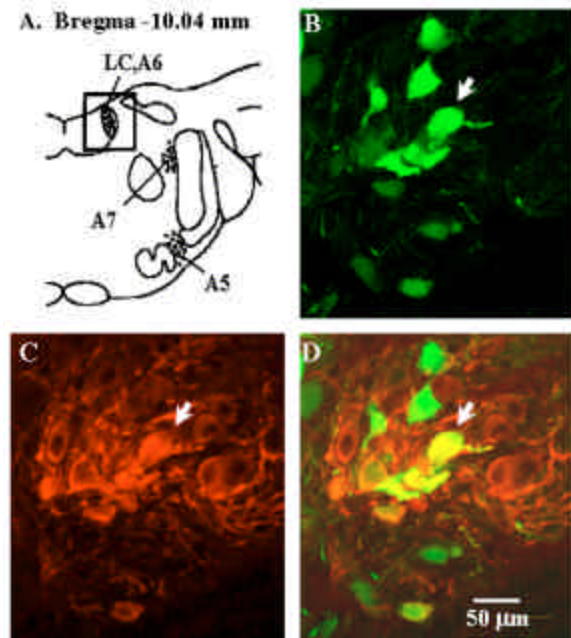
Figure 2. Projections of locus coeruleus (LC) noradrenergic cells to hypoglossal motoneurons. A: Schematic of a coronal section showing location of pontine noradrenergic cell groups. B: LC neurons labeled with transsynaptic pseudorabies virus that expresses green fluorescent protein (PRV-GFP) following injection of PRV-GFP into the genioglossus muscle and a survival time of 5 days. C: Tyrosine hydroxylase-containing cells (TH, red) were identified by immunohistochemisty in the same section of the LC. D: Overlay of confocal microscopy images shown in B and C; yellow color indicates neurons that are colabeled for TH and PRV-GFP (yellow) as indicated by the arrows.
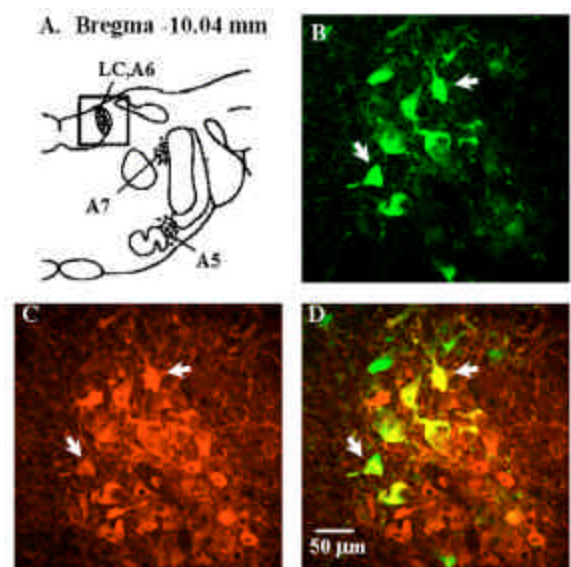
Figure 3. Projections of LC noradrenergic cells to airway-related vagal preganglionic neurons (AVPNs). A: Schematic of a coronal section showing location of pontine noradrenergic cell groups. B: LC neurons were retrogradely labeled neurons with PRV-GFP following injection of PRV-GFP into the upper lobe of the right lung and a survival time of 5 days. C: TH- containing cells (red) were identified in the same section of the LC. D: The majority of LC neurons projecting to AVPNs innervating the lung are noradrenergic, as indicated by the arrows.

(mPRF) causes a REM sleep like state (34-40), which can be enhanced by carbachol microinjections into the amygdala (41). Neurons within the mPRF express muscarinic cholinergic receptors, and are innervated by choline acetyl transferase expressing cells of the laterodorsal and pedunculopontine tegmental nuclei (LDT/PPT; (34, 42-44). Activation of LDT/PPT cholinergic and mPRF cholinoceptive cells stimulates neurons in the nucleus gigantocellularis which are located in the region that corresponds to the bulbospinal inhibitory zone (BIZ) of Magoun and Rhines (45), and lie in the ventral medulla just rostral to the inferior olive. These cells project to serotonergic as well as noradrenergic neurons, and mediate REM sleep related skeletal muscle atonia. In the rat, as in the cat, REM sleep can be induced by carbachol infusion into the pontine reticular formation, specifically into the posterior oral pontine reticular nucleus (PnO), as recently reported (46). During REM sleep, the activity of upper airway dilating muscles decreases (3, 43), but cholinergic outflow to the airways is increased (18). These influences are in part due to inhibition of monoaminergic neurons that project to motoneurons of the cranial nerves.

5. MONOAMINERGIC NEURONS AND STATE DEPENDENT CONTROL OF THE AIRWAY GEOMETRY

During the last 30 years, diencephalic and pontomesencephalic networks were well defined, as well as the reciprocal relationships in sleep generating sites in regard to norepinephrine-, serotonin-, and histamine-containing neurons(48-50,52). Furthermore, using recent neurochemical tools, significant progress has been made in determining the precise connectivity of the monoaminergic neurons with the sleep generating cells, and monoaminergic cell groups with the motoneurons of the cranial nerves innervating upper airway-dilating muscles and smooth muscle of the tracheobronchial system.

5.1. Brainstem norepinephrine-containing neurons and behavioral state control of airway geometry
Since the pioneering work of vonEuler (53) demonstrating the presence of catecholamines in nerve terminals, numerous studies have shown the role of catecholamines in regulation of autonomic functions (25, 54, 55). The availability of antisera against specific enzymes involved in catecholamine synthesis and against specific catecholamines themselves, allowed for a more precise determination of catecholamine cell groups. In the mammalian central nervous system, most of the noradrenergic neurons are located within several nuclei in the brainstem, and they promote different physiological and behavioral modulatory influences by their widespread targets. These effects are related primarily to the central regulation of autonomic functions and motor activity, and with sleep-wake-arousal cycles. The noradrenergic inputs to the motoneurons innervating-upper airway dilating muscles and smooth muscle of the tracheobronchial system arise mainly from the locus coeruleus (LC) and the A5 cell group (25,147). Figures 2 and 3 show that noradrenergic neurons of the locus coeruleus innervate both the
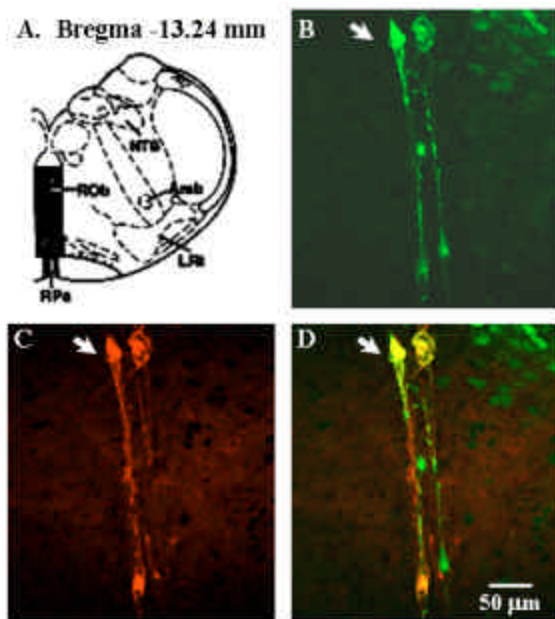
Figure 4. Projections of raphe cells to hypoglossal motoneruons. A: Schematic of a coronal section showing location of raphe nuclei. B: Raphe obscurus neurons were retrogradely labeled with PRV-GFP following injection of PRV-GFP into the genioglossus muscle and a survival time of 5 days. C: Serotonin-containing cells (5-HT, red) were identified in the same section of the raphe obscurus. D: Overlay of confocal microscropic images shown in B and C indicates neurons that are colabeled with 5-HT and PRV-GFP (yellow) as indicated by the arrows.

hypoglossal and airway-related vagal preganglionic neurons, i.e. parallel control.
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Noradrenergic projections often do not have the same degree of synaptic specialization as glutamatergic or GABAergic fibers, and appear to release their content, norepinephrine, in a less punctate and a more diffuse manner (25,110). Following vesicular release, norepinephrine is actively cleared from the extracellular spaces by presynaptic transporters. The response of the cranial motoneurons to released norepinephrine depends on the expression of the adrenergic receptor subtype. The adrenergic receptor family comprises a number of subfamilies. The alpha-1, alpha-3, and beta-adrenergic receptor families and each sub-family of these families contain a minimum of three distinct subtypes. Subtypes are coded by separate genes and display a distinct tissue distribution, drug specificity, and regulatory property (56-60).

Many of the modulatory effects of norepinephrine are attributable to alpha-2 adrenergic receptors (alpha-2AR; 80). The expression of the alpha-2AR subtypes has been very well documented in the brain with the use of in situ hybridization, autoradiography, and immunolocalization techniques (56-59). The alpha-2AR is particularly abundant in the ventrolateral medulla, where neurons involved in cardiorespiratory control are located

(57). As opposed to the alpha-1 and beta-adrenergic receptors, activation of the alpha-2AR can inhibit neuronal activity. It is proposed that norepinephrine-containing neurons are the potential source of the "noradrenergic" inhibitory drive to the medullary respiratory network, acting via alpha-2AR (25). Prejunctional alpha-2ARs are located on many peripheral and central nerve terminals, where their activation inhibits neurotransmitter release (60). Furthermore, there is a subdivision of the alpha-2AR into four subtypes, based primarily on radioligand binding characteristics in tissue homogenates (60), so that functional differences could be anticipated to occur. To date, all alpha-2AR subtypes are coupled to the same signaling systems, which include inhibition of adenyl cyclase, activation of receptor operated K^+ channels, and inhibition of voltage-gated Ca^{2+} channels (60). Our recent studies indicate that AVPNs innervating the tracheobronchial tree express the alpha-2_A subtype of the adrenergic receptor (alpha-2_AAR), which has a relatively high affinity for yohimbine and rauwolscine. In addition, physiological studies showed that activation of neurons within the LC or A5 cell group caused inhibition of cholinergic outflow to the airways (110). Hence, it is possible for specificity in control of vagal outflow in relation to vigilance or sleep-wake behavior.

Noradrenergic neurons project to cranial motoneurons innervating upper airway dilating muscles such as the genioglossus and posterior cricoarytenoid. It has been shown that norepinephrine is excitatory to orofacial (61-63), and hypoglossal motoneurons (64). It appears that only the mRNA for alpha-1B receptors is expressed by the majority of XII motoneurons, and the alpha-1A or alpha-1D receptors whose mRNAs are expressed in a fraction of XII motoneurons may mediate postsynaptic excitation in a distinct subpopulation of motoneurons, e.g. those targeting selected muscles innervated by the XII nerve (65).

The activity of norepinephrine-containing cells is highest during waking and lowest during REM sleep (50). Since norepinephrine has predominantly excitatory effects on hypoglossal motoneurons, the loss of this adrenergic excitation may also contribute to sleep-related decrements in motor tone of skeletal muscles in slow-wave sleep and motor atonia of REM sleep. If disfacilitatory effects occur in orofacial motoneurons, sleep-related decrements may occur in upper airway patency that, in turn, may influence expression of snoring and obstructive apnea (65). In contrast, loss of noradrenergic control of AVPNs may increase cholinergic outflow to the airways, precipitate nocturnal worsening of airway functions, and produce nocturnal asthma.

5.2. Serotonergic neurons
There is increasing evidence suggesting that midline neurons in the rostral ventral medulla play a role in the ventilatory response to hypercapnia (24,25,66,67,72). A subpopulation of these cells (25%) expresses serotonergic traits (51) and is involved in diverse physiological functions. A regulatory role is witnessed by findings that neurons of this system are localized in the medial aspect of the brainstem, the most primitive portion
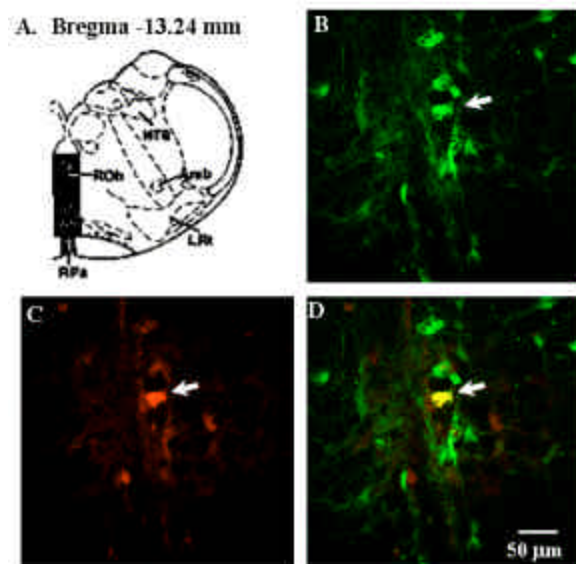
Figure 5. Projections of raphe cells to airway-related vagal preganglionic neurons (AVPNs). A: Schematic of a coronal section showing location of raphe nuclei. B: Raphe obscurus neurons were retrogradely labeled with PRV-GFP following injection of PRV-GFP into the upper lobe of the right lung and a survival time of 5 days. C: 5-HT cells were identified in the same section of the raphe obscurus. D: Overlay of confocal microscropic images shown in B and C indicates neurons that are colabeled with 5-HT and PRV-GFP (yellow) as represented by arrows. For further data on methods and correlation to other observations see Haxhiu et al(147).

of the CNS; these cells develop early in ontogeny in humans and are highly conserved. Furthermore, their axonal projections and terminal arborizations invade almost the entire neuraxis, from the most caudal segments of the spinal cord to the frontal cortex (52).

Projections emanating from serotonin-containing neurons of the caudal raphe nuclei extend to the NTS, ventrolateral medulla, and the spinal cord (68-70). Furthermore, serotonin-immunoreactive boutons synapse with phrenic motoneurons, respiratory- related neurons of the ventral and dorsal respiratory groups (71), and motoneurons of cranial nerves, including AVPNs (Figure 4 and Figure 5) and these neurons also express GABA$_A$ receptors (Figure 6). Focal acidification of raphe nuclei by microinjection of acetalozamide in anesthetized, vagotomized animals increases the amplitude of the integrated phrenic moving average (72). Extensive chemical lesioning of midline neurons in anesthetized and decerebrate piglets reduces the response of phrenic and hypoglossal nerves to progressive hypercapnia with hypoglossal nerve activity being substantially more affected than phrenic nerve discharge (24). For example, in ferrets, chemical stimulation of the raphe pallidus produces an increase in respiratory output, preferentially to the genioglossus muscle (Figure 7 a and b). In addition, activation of these neurons induces the release of serotonin in the rostral nucleus ambigualis region (Figure 8) and

inhibits cholinergic outflow to the airways as shown in Figure 9.

It is conceivable that a reduction in serotonergic activity could lead to an imbalance between the activity of upper airway (dilating) and chest wall (pumping) muscles, leading to upper airway obstruction, and withdrawal of inhibitory inputs to cholinergic neurons, causing bronchoconstriction. Conceivably, the effects of stimulation of midline neurons on respiratory drive could be partly mediated via release of other transmitters. In support of this concept, hypercapnic loading will activate substance P-containing neurons within raphe nuclei (73). It is likely that more neural circuits are involved and a challenge will be to define those factors that determine the relative hierarchy of effects and how such activation is managed or withdrawn.

Entering NREM, neuronal activity slows to approximately 50% of the quiet waking level and loses its regularity. Finally, during REM sleep, most serotonin neurons become nearly quiescent (49, 74). Furthermore, serotonin levels in the hypoglossal nucleus region and hypoglossal nerve activity are reduced during carbachol-induced REM sleep (75). Stimulation of serotonin-containing neurons is associated with inhibition of AVPNs, but with excitation of hypoglossal motoneurons. Following stimulation of midline neurons, there occurs release of serotonin (using in situ voltametry measurements) and a decrease in airway smooth muscle tone. These actions are diminished by blockade of serotonergic receptors (27). Furthermore, the data suggest that state-related changes in serotonergic inputs may explain oscillations in the patency of upper airways and in airway smooth muscle tone across the wake-sleep-arousal cycle. During sleep, diminished activity of serotonin neurons may lead to a decrease in airway patency. Entering NREM or REM sleep, respiratory drive to upper airway dilating muscles is preferentially decreased (4,65,76). In humans, this pathway may lead to sleep obstructive apnea, hypopnea, and autonomic stress (3, 77). In addition, during sleep, an increase in cholinergic outflow to the tracheobronchial system is observed, which is greatly amplified in disease states such as bronchial asthma (17, 18). Taken together, these findings indicate that sleep-producing neurons may utilize specific neuronal networks that inhibit the activity of serotonergic neurons. This in turn will influence respiratory drive to the diaphragm and genioglossal muscles but in the opposite direction also influence AVPNs and cholinergic outflow to the lower airways (Figure 10).

Serotonin exerts modulatory effects in many brain nuclei involved in regulation of motor and sensory responses as well as on behavior. Actions of serotonin are due to the existence of at least 15 different 5-hydroxytryptamine (5-HT) receptor subtypes. For example, activation of 5-HT2A receptors induces cell depolarization, while activation of 5-HT1A has an opposite effect (78). Hypoglossal and phrenic motoneurons and motor cells innervating pharyngeal and laryngeal muscles express 5-HT2A receptors that provide tonic excitatory inputs in the awake state (79). During sleep, particularly entering REM
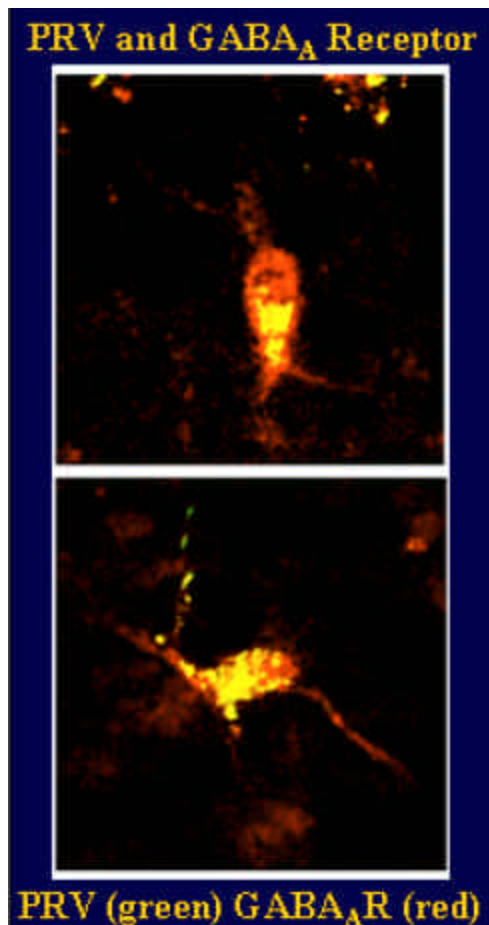
Figure 6. Confocal microscopic images showing GABA$_A$ receptors visualized with rhodamine (red) on transneurally labeled inspiratory bulbospinal neurons in the raphe obscurus. PRV infected neurons are visualized with fluorescein (green). In these double-labeled cells the yellow signal is the result of superimposed red on green signals. For further information see Haxhiu et al (25).

sleep, the activity of serotonergic receptors reaches its nadir (65). Microinjection of 5-HT into the hypoglossal nucleus can significantly attenuate the REM sleep-like suppression of XII nerve activity, in part, by substituting for decreased endogenous 5-HT in the XII nucleus (75).

The 5-HT1A receptors are expressed throughout the CNS (78), including AVPNs (27). In most of these nuclei, the receptor functions postsynaptically, responding to serotonin release from raphe nuclei projections. The most obvious effect of 5-HT, which is mediated by 5-HT1A receptors, is membrane hyperpolarization(78). This membrane-delimited signaling mechanism involves a G protein of the Gi/Go family, opening of inwardly rectifying potassium channels, and inhibition of calcium currents (78). Alterations in serotonergic pathways may cause failure of homeostatic responses to life-threatening challenges (e.g. asphyxia, hypercapnia) during sleep (67). In children or adults, altered serotonergic pathways may contribute to OSA occurrence or severity or to the appearance of asthma during sleep.

5.3. Histaminergic neurons

Histaminergic neurons in the adult vertebrate brain are confined to the posterior hypothalamic area. Scattered groups of these neurons are referred to as the tuberomammillary nucleus. These cells give rise to widespread projections extending via the basal forebrain to the cerebral cortex, as well as to the thalamus and pontomesencephalic tegmentum. The morphological features of these neurons suggest that the histaminergic system acts as a regulatory network for whole-brain activity. Indeed, this amine regulates hormonal functions, sleep, food intake, thermoregulation and locomotor behavior (80). The histaminergic system is also involved in the control of arousal. In addition to their ascending cortical axons, these neurons send numerous descending inputs to the mesopontine tegmentum, which plays a key role in cortical activation (32), and to NTS subnuclei (80), which are known to play an important role in cardiovascular control.

Histaminergic neurons may partly mediate changes in behavioral state in response to changes in CO_2 and H+ within the brain, and alter the gain of physiological effects on the respiratory network. We demonstrated c-Fos expression in histamine-containing neurons, a finding that suggests that hypercapnic loading activates a subset of histamine-containing cells (25). The functional significance of chemosensory traits in histaminergic neurons is not known. However, it is expected that activation of histamine-containing cells by an increase in CO_2 and/or H+ may affect central respiratory drive, via activation of NTS neurons which are heavily innervated by histaminergic fibers (80). Furthermore, a pharmacological study of respiratory rhythm in isolated brainstem-spinal cord preparations of newborn rats showed that histamine increases the frequency of spontaneous periodic depolarization (81), acting via H-1 receptors. There is pharmacological evidence suggesting that most of the centrally acting drugs, i.e. benzodiazapines, barbiturates, and ethanol, share an antihistaminergic effect (82, 83). These substances decrease central histaminergic transmission acting pre-(decrease of the turnover rate of histamine) or postsynaptically (H1 -receptor blockade) that could contribute to sedation and sleep related disturbances such as the occurrence of complete obstruction in those at risk. Sleep related disturbances following administration of sedatives could be a consequence of preferential inhibition of hypoglossal nerve activity and upper airway dilating muscles (25).

Histaminergic neurons of the tuberomamillary nucleus are involved in behavioral state regulation. These cells express the highest discharge rate during waking and are virtually silent during NREM and REM sleep (32, 84). Activation of histamine-containing neurons inhibits basal forebrain-preoptic cells involved in the generation of NREM sleep. Conversely, blockade of histamine synthesis in this region promotes sleep, and decreases wakefulness. Hence, it is expected that alterations in central histaminergic control may contribute to an arousal deficit upon exposure to hypercapnia.
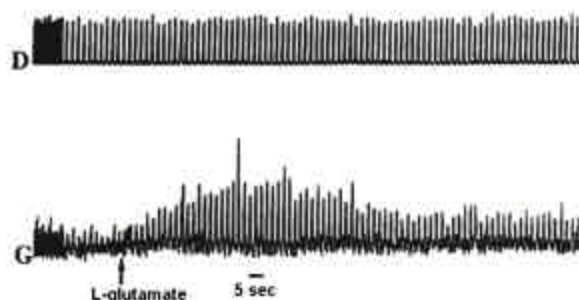
Figure 7a. The effect of stimulation of the midline neurons on genioglossus and diaphragm actitivity in the ferret breathing oxygen at an end-tidal CO2 level above the apneic point. Microinjection of L-glutamine (1nmol, 100nl) caused an increase in electromuographic activity in the genioglossal muscle without a change in diaphragmatic activity.
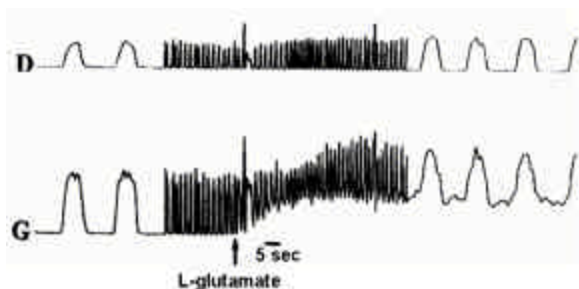
Figure 7b. Example of the effect of stimulation of midline neurons on genioglossus and diaphram activity in a ferret breathing oxygen at an end-tidal CO2 above apneic point. Microinjection of L-glutamate (4 nmol, 100 nl) significantly increased tonic activity of genioglossus muscle, which was followed by a slight change in amplitude of the diaphragm and a transient increase in breathing frequency.

The link between sleep-related respiratory disorders in infants and maternal smoking during pregnancy (85) could be partly due to the interference of nicotine with the development of the histaminergic system. Nicotine inhibits histamine- N-methyltransferase (86), leading to altered histaminergic transmission, arousal deficit, and possibly to events that are presumed to lead to sudden infant death syndrome (87). The morphological features of these neurons suggest that the histaminergic system acts as a regulatory network for whole-brain activity.

5.4. Orexin/hypocretin system

Hypocretin-1 (hcrt-1) and hypocretin-2 (hcrt-2) are also called orexin-A and orexin-B. Mainly neurons of the lateral hypothalamus produce these peptides (88) that are processed from a common precursor, prepro-hypocretin or prepro-orexin, encoded by a gene localized to human chromosome 17q2. Hypocretin containing neurons affect autonomic, neuroendocrine and sleep-wakefulness neuroregulatory systems (89-92). It should be noted that hcrt can be found in the circulation and hcrt receptors are present outside of the central nervous system in the gastrointestinal tract, pancreas, and adrenal gland (93), and thus are in position to regulate feeding behavior and metabolism.

Recent findings indicated that hcrt neuronal systems are associated with the maintenance of wakefulness (for review see (93)). Perfusion of hcrt-1 by microdialysis in both the basal forebrain and brain stem increases wakefulness in freely behaving adult rats (94). Fos expression in hcrt-containing neurons correlates positively with the amount of wakefulness and negatively with the amounts of NREM and REM sleep (91). The majority of hcrt cells express c-fos during active waking, whereas only a small number of these cells express c-fos during quiet waking. In rat brain slices containing centromedial (CM) and rhomboid nuclei in the thalamus, hcrt depolarizes and excites all neurons tested through a direct postsynaptic action. The hcrts stimulate target cells via hypocretin G protein-coupled receptors, hcrt receptor 1 (hcrt-R1; Orexin-R1) and hcrt-receptor 2 (hcrt-R2; Orexin R2) (95, 96). One proposal is that the hcrt neuronal types promote wakefulness by excitation of cholinergic neurons in the basal forebrain, which release acetylcholine and thereby contribute to the cortical activation of wakefulness; however, the causality of these associations remains to be determined as wakefulness is often accompanied by behavioral activation. Suppression of REM occurs through an inhibition of the cholinergic neurons in the lateral dorsaltegmental and pedunculopontine nuclei (94).

In the central nervous system, hcrt-containing cells directly activate noradrenergic neurons in the locus coerulus , serotonergic (5HT) cells of the DRN, and histaminergic neurons in the tuberomamillary nucleus (92,93,99,100,103). Hcrt excites noradrenergic and serotonergic neurons in the LC and DRN respectively, via hcrt-R1 (99, 100), activating both potassium-dependent (101), as well as sodium-dependent (102) mechanisms. In histaminergic neurons of the tuberomammillary nucleus, the hcrt excitatory response is mediated via the Na^+-Ca^+ exchanger (103).

The hcrt peptides may exert influences upon upper airway dilating muscles and ventilation via neuroendocrine cells within the paraventricular nucleus (PVN), and/or direct projections to hypoglossal (XII) (104) and phrenic motoneurons (105). We have recently performed a series of neuroanatomical and physiological experiments investigating hypothalamic pathways underlying cardiorespiratory control, upper airway patency, and lower airway conductance. These results provide a neuroanatomic and physiologic framework explaining the humoral, autonomic and behavioral interactions through the common source and parallel pathways, and indicate that PVN neurons are an important component of the central neurocircuitry regulating respiration and autonomic functions (106-110) including sympathetic nerve outflow (111).

Peripheral or humoral factors of the orexin network may also alter central respiratory control; but, here again the evidence is more associative than causative. In

obese and morbidly obese individuals, plasma hcrt- 1 levels
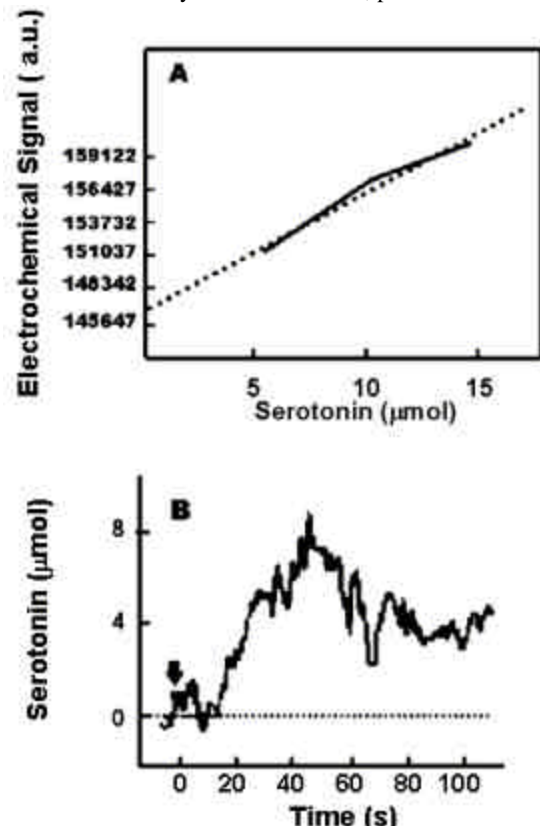
Figure 8. An example of the effect of raphe neuron stimulation (L-glutamate, 4 nmol/80 nl) on serotonin release in the rostral ventrolateral medulla in an anesthetized cat. A) Calibration curve for serotonin. B) Release of serotonin following stimulation of raphe nuclei. Serotonin release was associated with a decrease in airway smooth muscle tone (not shown). For further information see references (25, 27).
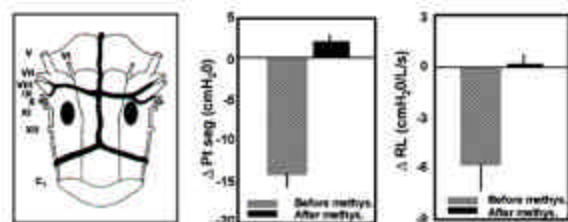
Figure 9. The average decreases in tracheal tone and lung resistance induced by stimulation of raphe neurons before and after blockade of 5-HT receptors within the ventrolateral medulla oblongata.

are significantly lower and leptin levels are significantly higher when compared to normal individuals (112). Hypocretin-containing cells express leptin receptors (113), and leptin attenuates respiratory complications and respiratory depression associated with the obese phenotype (114, 115). It is therefore possible that leptin modulates body weight and breathing partly via its action on hcrt-containing cells, hence, alterations in number and/or function of the hcrt producing cells could lead to

respiratory changes, particularly during sleep. Furthermore, this raises interesting issues in regard to the uncommon syndromes involving changes in body weight and respiratory control during sleep. The Prader-Willi syndrome is characterized by developmental delay, obesity, and behavioral problems. Children with this disorder frequently suffer from excessive daytime sleepiness and have a primary abnormality of circadian rhythm and expression of REM sleep. In addition, such individuals have abnormal ventilatory responses to hypoxia and hypercapnia, and are at risk for a variety of abnormalities of breathing during sleep, including obstructive sleep apnea and sleep-related alveolar hypoventilation (116). Future studies in this field should consider the role of these interactions in modulating sleep-wakefulness states, upper airway patency and lower airway conductivity during sleep.

6. THE PRODUCTION OF REPETITIVE APNEAS DURING SLEEP

The occurrence of an apnea during sleep appears to set in motion several consequences that conspire to promote further breathing instability (77). The inertia of the ventilatory control system prevents resumption of rhythmic breathing after apnea until arterial carbon dioxide levels increase by 4 to 6 mmHg above an apneic threshold (117, 118). In addition, both central and obstructive apneas are associated with narrowing or occlusion of the pharyngeal airway (7). This narrowing of the upper airway may explain the overlap between central and obstructive apnea (i.e., mixed apnea). Resumption of ventilation requires opening of a narrowed or occluded airway passage overcoming tissue adhesion forces, and craniofacial gravitational forces (119). This non-linear function of the controlled element may prolong an apnea and permit chemoreceptor drive to increase further than what might be expected from merely a controller viewpoint. Finally, an increased respiratory drive will result in ventilatory overshoot, subsequent hypocapnia, and further apnea/hypopnea. The transient arousal from sleep results in the cycle being re-initiated (1).

A short-term potentiation of ventilation (STP), or ventilatory after-discharge, occurring after hypoxia promotes ventilatory stability, and protects against dysrhythmic breathing or multiperiodic breathing , as represented by repetitive apnea and Cheyne-Stokes respiration (CSR)(120). Absence of STP would promote periodic behavior, a proposal supported by studies showing its absence in patients with OSA hypopnea syndrome (121) or congestive heart failure patients with episodic central apneas (122). Central and obstructive apneas may occur in the same patient over a night indicating that an unstable breathing pattern is a fundamental event in sleep-disordered breathing.

There is a need for a higher level of analysis of neural control of respiration that becomes apparent once one considers the clinical conditions of obstructive sleep apnea syndrome, Cheyne-Stokes respiration, and to a less extent the appearance of bronchoconstriction. Clinical illness usually becomes apparent through assessments of people with complaints and/or signs associated with
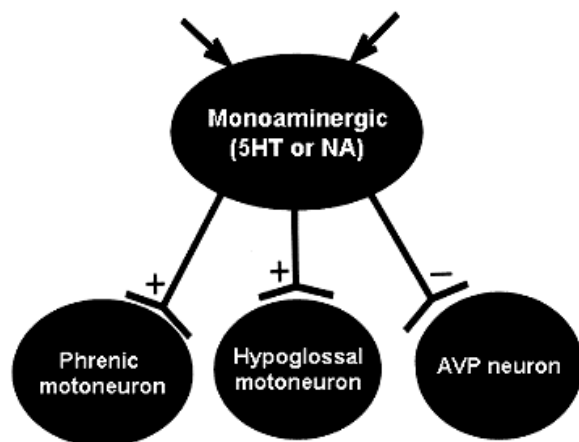
Figure 10. Summary diagram of the differential effects of monoaminergic stimulation (noradrenergic and serotonergic) on phrenic, hypoglossus, and AVPNs.

repetitive events during sleep. Thus, there is a need to link the neurophysiology data discussed above with the appearance of multiple apneas during sleep. In this regard, it may be the neural networks of sleep and breathing that interact alone or more likely in combination to produce repetition of apneas (3, 12, 18, 123).

Sleep is known to promote respiratory instability through "loop gain" (120). Mathematical models and studies on humans have focused on statistical correlations between the incidence of periodic breathing and hypoxic sensitivities (122, 124). Post-hypoxic instability occurs more frequently in individuals during sleep, especially in those individuals with a higher peripheral chemosensitivity (77). There are alternative explanations for repetitive apneas during sleep. The oscillations in individuals with repetitive apneas appear to be similar to those observed in normal individuals, but some excitatory factor may contribute to greater swings in ventilation. Recurrent apneas would then result from an intrinsic property in the feedback control of breathing in regard to either stability or instability in ventilation over time (125). The respiratory control system may be intrinsically designed in such a way as to produce a spontaneous oscillatory phenomenon that in turn would promote central apneas (126). Theoretically at least, if in response to the cyclic changes in drive, the mechanical outputs of chest-wall muscles and upper airway muscles are not identical either in phase or in amplitude, there could be an occurrence of obstructive events in the context of central apneas.

Alterations in the monoaminergic system in particular may play a role in the creation of breathing stability or instability (76). A decrease in tone would augment reflex bronchoconstriction induced by transient hypoxemia or hypercapnia. Enhanced monoaminergic tone may prevent ventilatory undershoot upon recovery from an apnea. There is some evidence that enhancing monoaminergic function will improve some aspects of sleep-disordered breathing in the bulldog model of snoring and sleep apnea (14). Hence, direct and indirect modifiers of the monaminergic pathways are a focus of interest for

those that want to develop pharmacologic treatments of snoring, sleep apnea, and nocturnal asthma.

7. GENETIC CONSIDERATIONS IN BREATHING DURING SLEEP

Animal studies indicate that genetic background directly influences ventilatory behavior (respiratory frequency, tidal volume, and minute ventilation). There is nearly absent respiratory depression in response to brief hyperoxia in nitric oxide synthase (NOS)-3 mutant mice (127) and an altered breathing pattern in knock out NOS-1 mice (128, 129). Other studies of knock-out mouse models report effects on hypoxic response with endothelin converting enzyme-1(ECE-1), endothelin-1, dopamine, neutral endopepidase (NEP), and hypoxic inducible factor (HIF); on the other hand, knock out models for other supposedly critical factors for hypoxic or hypercapnic responses show little or no effect on ventilation, e.g. NADPH nor Endothelin-3 (130). More complex models with a single spontaneously mutant gene include the ob/ob mutant B6 mouse in which leptin is absent (115). In this model the basic structure of resting breathing is unaffected, but the functions of carbon dioxide responses are modified by leptin, even in the absence of obesity. Thus, a candidate gene approach provides evidence that individual genes can affect tidal volume and breathing rate.

There are a number of case reports or collections of patients that exhibit both sleep disordered breathing and well recognized disorders that are based on familial transmission (131-134). Congential hypoventilation syndrome is one in which there is obvious familial transmission (135). Another interesting condition, Prader-Willi syndrome (discussed briefly above in the context of the orexin system) demonstrates the interplay of neurotransmitters and the resulting associated changes in sleep apnea, orexin regulation, and obesity (116, 136-139). These syndromes are rather uncommon.

There is evidence that genes influence the adult expression of sleep symptoms and sleep apnea in the absence of a recognizable genetic disease. Symptoms of sleep apnea (snoring and sleepiness) are two to six times more frequently observed in family members of affected patients than in a control population. Sleep apnea is present in higher numbers in first-degree relatives of patients than in age-, sex-, and socioeconomic-matched control families (140, 141). These family studies also suggest that the frequency of sleep apnea is underestimated in the community and that the symptomatic sequelae of multiple apneas are quite variable.

From human studies of families with one (or more) people with sleep apnea syndrome, there is statistical evidence for a transmission of a limited number of "genes" that would explain some 27% of the expression of apnea-hypopnea index (AHI) (142). In Caucasians, analyses suggest a recessive Mendelian inheritance accounting for >20% of the variance in AHI values, with an additional 8-9% of the variation due to other non-specified familial effects. Transmission patterns in an African-American

sample are also consistent with Mendelian inheritance, accounting for 25% of the total variation with an additional 8% due to other familial effects. Adjustment for obesity in Caucasians reduced the major gene effect; however, in African Americans, there remained a moderately strong effect (19%). These results suggest that an underlying genetic basis for sleep apnea exists independent of the contribution of obesity to the disease in African-Americans.

Experience in searching for gene regions associated with human sleep-disordered breathing is limited. In sleep apnea, there is observed an increased prevalence (2-fold) in a polymorphism for apolipoprotein E, which is also associated with cardiovascular disease and Alzheimer's disease (143). This has not been confirmed in other studies (144, 145), perhaps because of age or other demographic differences. The first familial linkage study (146) utilized a 9-cM genome scan and multipoint variance-component linkage analysis in an attempt to link apnea-hypopnea index (AHI) and body mass index (BMI). Candidate regions on chromosomes 1p (LOD score 1.39), 2p (LOD score 1.64), 12p (LOD score 1.43), and 19p (LOD score 1.40) gave the most evidence for linkage to AHI. BMI was also linked to multiple regions, and the evidence for linkage to AHI was effectively removed after adjustment for BMI, with the exception of regions on chromosomes 2p (adjusted LOD score 1.33) and 19p (adjusted LOD score 1.45). This report offers the first direct evidence for shared and unshared genetic factors underlying susceptibility to OSA and obesity and suggests that the interrelationship of OSA and obesity may result from a common pathway involving one or more genes. It should be noted that this linkage study was performed in Caucasians.

8. FUTURE DIRECTIONS

There is a renewed focus on the central neural mechanisms that produce apneic activity during sleep and contribute to state-related ventilatory and airway instability either directly at the level of the brainstem, or indirectly in regard to appearance and the repetitive nature of apneas or bronchoconstriction during sleep. Better definition of the sleep-related neurochemical features of breathing and of the control of airway caliber might provide insight into novel treatments. The availability of functional genomics will permit a dissection of respiratory control mechanisms from the direction of altering cell function producing anatomic and functional alterations in breathing. How such models will illuminate the the physiology of sleep and breathing is not entirely clear, as many genes operate to produce the interactions outlined above. Certainly, the development of pharmacologic interventions would create a paradigm shift in the management of sleep apnea and nocturnal asthma. One obvious target for sleep disordered breathing is respiratory drive; however, the way that the respiratory controller interacts with the systems for sleep will require the neuroexcitatory effects that probably maintain ventilation at a higher level during wakefulness to be dissociated from a normal sleep-wake cycle.

9. ACKNOWLEDGMENTS

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