

Respiratory motor activity: influence of neuromodulators and implications for sleep disordered breathing¹

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Abstract: Sleep, especially rapid-eye-movement sleep, causes fundamental modifications of respiratory muscle activity and control mechanisms, modifications that can predispose individuals to sleep-related breathing disorders. One of the most common of these disorders is obstructive sleep apnea (OSA) that affects approximately 4% of adults. OSA is caused by repeated episodes of pharyngeal airway obstruction that can occur hundreds of times per night, leading to recurrent asphyxia, arousals from sleep, daytime sleepiness, and adverse cardiovascular and cerebrovascular consequences. OSA is caused by the effects of sleep on pharyngeal muscle tone in individuals with already narrow upper airways. Moreover, since OSA occurs only in sleep, this disorder by definition is a state-dependent process ultimately caused by the influence of sleep neural mechanisms on the activity of pharyngeal motoneurons. This review synthesizes recent findings relating to control of pharyngeal muscle activity across sleep-wake states, with special emphasis on the influence of neuromodulators acting at the hypoglossal motor nucleus that innervates the genioglossus muscle of the tongue. The results of such basic physiological studies may be relevant to identifying and developing new pharmacological strategies to augment pharyngeal muscle activity in sleep, especially rapid-eye-movement sleep, as potential treatments for OSA.

Key words: genioglossus, neurotransmitters, obstructive apnea, sleep.

Résumé : Le sommeil, notamment le sommeil paradoxal (SP), provoque des modifications fondamentales de l'activité et des mécanismes de régulation des muscles respiratoires, modifications qui peuvent prédisposer les individus à des troubles respiratoires liés au sommeil. L'un des troubles les plus courants est l'apnée obstructive (AOS) qui touche approximativement 4 % des adultes. L'AOS est causée par des épisodes répétées d'obstruction des voies aériennes supérieures, qui peuvent se répéter des centaines de fois par nuit et entraîner une asphyxie récurrente, des épisodes de réveil nocturne, une somnolence diurne ainsi que des effets cardiovasculaires et cérébrovasculaires indésirables. L'AOS est causée par les effets du sommeil sur le tonus du muscle pharyngé chez les individus qui ont des voies aériennes supérieures étroites. De plus, comme l'AOS se produit uniquement durant le sommeil, ce trouble est, par définition, un processus dépendant de l'état, causé par l'influence des mécanismes neuronaux sur l'activité des motoneurones des voies respiratoires supérieures. La présente synthèse résume les récentes découvertes sur la régulation de l'activité du muscle pharyngé au cours des états de sommeil-éveil, et porte une attention particulière à l'influence des neuromodulateurs agissant au niveau du nerf grand hypoglosse qui innerve le muscle génio-glosse de la langue. Les résultats de ces études physiologiques fondamentales pourraient servir à l'identification et à l'élaboration de nouvelles stratégies pharmacologiques pour augmenter l'activité du muscle pharyngé durant le sommeil, en particulier le sommeil SP, comme traitements potentiels de l'AOS.

Mots clés : muscle génio-glosse, neurotransmetteurs, apnée obstructive, sommeil.

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Upper airway muscles and their respiratory function

In contrast to the trachea and bronchi, the upper airway is the only region of the respiratory tract not surrounded by rigid cartilaginous support. Instead, the upper airway is sur-

rounded by a complex anatomical arrangement of skeletal muscles and soft tissues that support essential nonrespiratory functions such as vocalization, suckling, and swallowing, i.e., behaviors that require dynamic changes in airway size to move air, liquids, and solids. Nevertheless, this property of a collapsible tube compromises the essential respiratory

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function of the upper airway, i.e., the airway must remain open during breathing, in all postures, to allow adequate pulmonary ventilation and gas exchange.

Sleep, especially rapid-eye-movement (REM) sleep, however, causes fundamental modifications of pharyngeal muscle tone and reflex responses that in normal individuals lead to airway narrowing and increased resistance to breathing. This increased upper airway resistance contributes significantly to the hypoventilation and increased arterial PCO_2 of 3–5 mmHg normally observed in sleeping humans, and elimination of this increased upper airway resistance reverses the major component of the sleep-induced hypoventilation (Henke et al. 1992). In individuals with an already anatomically narrow upper airway, these effects of sleep on pharyngeal muscle tone can predispose to inspiratory flow limitation (hypopneas) and obstructive sleep apnea (OSA) (Remmers et al. 1978). OSA affects ~4% of adults (Young et al. 1993) and is associated with increased risk for development of adverse cardiovascular events such as angina, myocardial infarction, stroke, and daytime hypertension and has adverse effects on sleep-regulation producing excessive daytime sleepiness, impaired work performance, and increased risk of having a vehicular accident (Bassiri and Guilleminault 2000). Overall, OSA is a significant public health problem with adverse clinical, social, and economic consequences (Phillipson 1993).

Sleep mechanisms are critical to OSA

Suppression of pharyngeal muscle activity in sleep is critical to OSA by producing a narrower airspace that is more vulnerable to collapse on inspiration (Horner 1996; Smith and Schwartz 2002). Anatomical factors that produce an already narrowed upper airspace (e.g., pharyngeal fat deposition, hypertrophied adenoids and tonsils, retrognathia, micrognathia, macroglossia) predispose to OSA by reducing the critical pressure needed for suction collapse (Horner 1996). Likewise, changes in respiratory control system stability and decreased lung volume in sleep also play a role in OSA (Younes et al. 2001; Heinzer et al. 2005). Despite these predisposing factors, however, it is important to note that the airway remains open in wakefulness and closes only in sleep. Therefore, even in individuals with a narrow upper airway, OSA is ultimately caused by the impact of brain sleep mechanisms on the processes controlling motor outflow to the pharyngeal muscles whose tone is necessary and sufficient to keep the airway open in wakefulness (Mezzanotte et al. 1992). Accordingly, determining the brain mechanisms mediating the effects of sleep on pharyngeal muscle tone not only has important basic relevance to respiratory neurobiology, it is also important to understand the pathogenesis of a common and serious breathing disorder at the central neuronal level. Ultimately, this knowledge may lead to novel strategies to prevent the critical sleep-related decrements in pharyngeal muscle tone as a potential treatment for OSA.

This review will synthesize recent findings relating to the control of pharyngeal muscle activity and the influence of neuromodulators associated with alterations in sleep-wake states. For more in-depth background information the

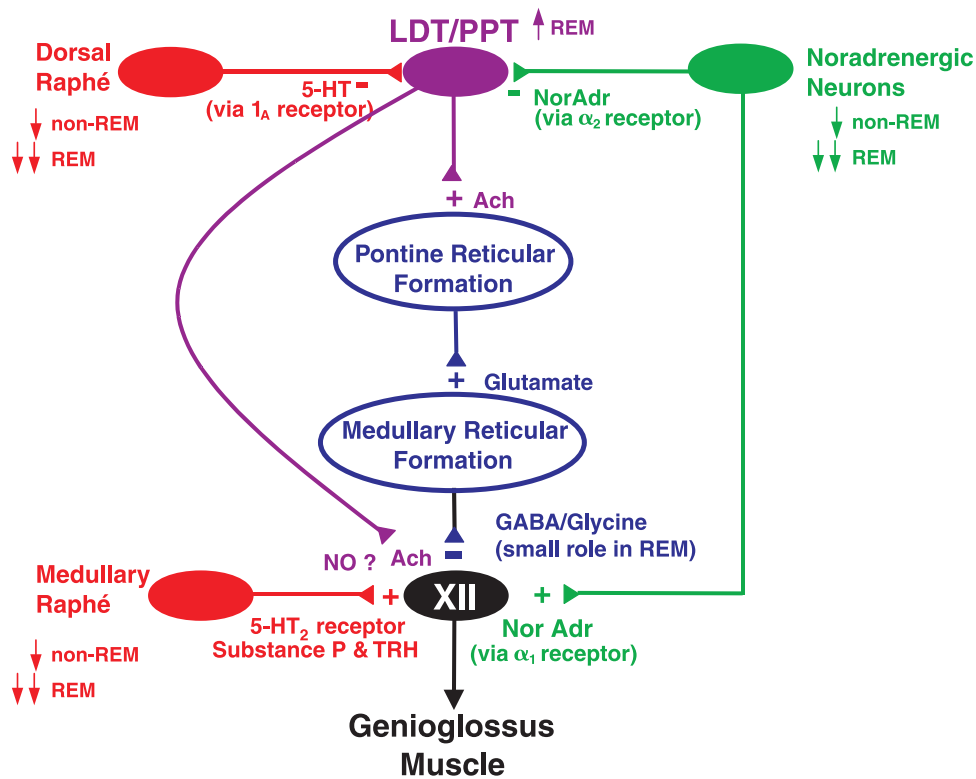
reader is referred to other reviews (Horner 1996; Kubin and Davies 2002). Wherever appropriate, instances of lack of information and indications for future research are also highlighted.

Upper airway muscles: respiratory and tonic activation

This review will focus specifically (but not exclusively) on the neural mechanisms modulating motor output to the genioglossus (GG) muscle of the tongue in wakefulness and natural sleep. The GG is the main focus because it is central to OSA in humans, with airway obstructions occurring behind the tongue both at the level of the soft palate and below (Horner et al. 1989). In awake animals and humans, there is respiratory modulation of GG activity superimposed on a background of tonic activation (Sauerland and Harper 1976; Mezzanotte et al. 1992; Horner et al. 2002). This background tonic activity is important in setting baseline airway size and stiffness, with the respiratory activation counteracting the subatmospheric collapsing pressures generated during inspiration (Horner 1996). In nonREM sleep, there is suppression of GG activity, mainly in the tonic component, but in REM sleep, there are periods of major suppression of pharyngeal muscle activity resulting in episodes of atonia that renders the upper airspace particularly vulnerable to collapse (Horner 1996). These periods of major GG motor suppression in REM sleep are interspersed with occasional flurries of brief muscle twitches. Such brief twitches also occur in postural muscles and are responsible for the transient body twitches commonly observed during REM sleep. For the pharyngeal muscles, such brief motor activations in REM sleep are thought to be responsible for the sporadic restorations of airflow observed in OSAs in humans (Younes 2003).

Some pharyngeal muscles exhibit more respiratory-related activity than others, with some being more tonically active, e.g., the GG compared with the tensor palatini (Worsnop et al. 1998). The tensor palatini displays mostly tonic activity and is thought to enhance stiffness in the rostral upper airway. Decreases in palatal muscle activity in sleep are associated with increased resistance in the airspace behind the soft palate (Tangel et al. 1992), a consistent site of occlusion in OSA patients (Horner et al. 1989). Overall, understanding the mechanisms underlying pharyngeal muscle activation in wakefulness and sleep is relevant to understanding the factors responsible for the maintenance of an open upper airway during breathing and preventing OSA. The mechanisms underlying the tonic drives to pharyngeal motoneurons may also be particularly relevant. For example, unlike phrenic motoneurons innervating the diaphragm, hypoglossal motoneurons innervating the GG muscle of the tongue are not inhibited throughout expiration (Withington-Wray et al. 1988; Woch and Kubin 1995; Peever et al. 2001). Accordingly, even though pharyngeal muscles conform to the classic concept of being “respiratory muscles” and show respiratory-related activity, the activity in expiration is simply the manifestation of prevailing tonic inputs of nonrespiratory origin that are revealed

Fig. 1. Schema of the neuronal circuitry currently thought to be involved in the pontine regulation of rapid-eye-movement (REM) sleep and the modulation of muscle activity. Decreased discharge in dorsal raphe and noradrenergic neurons preceding and during REM sleep progressively disinhibits pontine cholinergic neurons of the laterodorsal and pedunculopontine tegmental nuclei (LDT/PPT) via withdrawal of serotonin (5-HT)- and noradrenaline (NorAdr)-mediated inhibitory inputs. Activation of these LDT/PPT neurons then leads to increased acetylcholine (ACh) release into the pontine reticular formation producing activation of neuronal systems mediating ascending and descending signs of REM sleep (e.g., cortical desynchronization and motor atonia, respectively). Exogenous application of a cholinergic agonist (e.g., carbachol) by microinjection into the pontine reticular formation is used to mimic this process and trigger REM-like neural events in reduced preparations (e.g., anesthetized or decerebrate animals). Motor atonia in postural muscles in REM sleep is produced by postsynaptic inhibition of motor neurons by GABA (γ -amino-butyric acid) and glycine. Neurons of the medullary reticular formation are thought to drive this inhibition, themselves being driven by neurons in the pontine reticular formation. There is evidence that hypoglossal (XII) motoneurons are minimally influenced by such postsynaptic inhibitory mechanisms in REM sleep (Morrison et al. 2003a, 2003b). Hypoglossal motoneurons also receive excitatory noradrenergic and serotonergic inputs, with the former contributing significantly to alterations in GG activity across sleep–wake states in freely behaving rodents. The self inhibition of raphe neurons by axon collaterals is not shown for clarity. There are excitatory nicotinic and inhibitory muscarinic receptor influences of Ach at hypoglossal motoneurons but their role across sleep–wake states have not yet been determined. Likewise, the influences of nitric oxide (NO) in suppressing hypoglossal motor responses to locally applied serotonin and α_1 receptor agonists also remain to be determined. See text for further details. +, excitation; −, inhibition; ?, speculation based on literature and preliminary data but physiological role in-vivo has not been determined.



when inspiratory activation is withdrawn. Therefore, understanding the basis for these tonic inputs and their sleep state dependence is particularly relevant to understanding the predisposition of the upper airway to narrowing and closure in sleep.

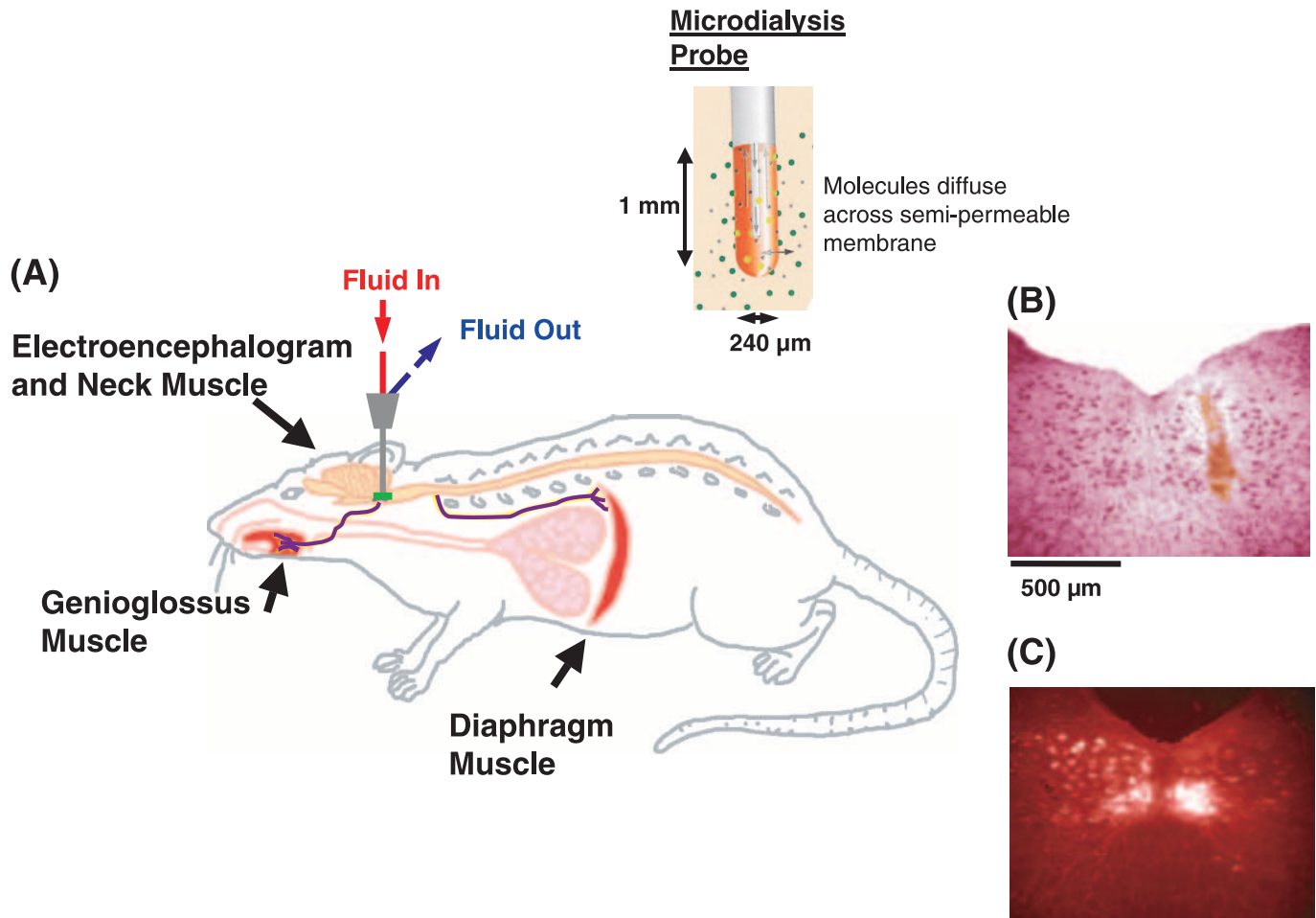
Key mechanisms modulating GG motor activity in sleep

Figure 1 shows the interactions between some of the neuronal groups principally involved in sleep regulation and their potential involvement in modulating motor outflow to the GG muscle of the tongue. We developed an animal model for manipulation of neurotransmission at the hypoglossal motor nucleus in the caudal medulla of freely behaving rats (Jelev et al. 2001) to determine for the first time

those mechanisms modulating motor outflow to GG muscle across natural sleep–wake states (Fig. 2). Accordingly, the data obtained from this model are important to advancing and translating the fundamental concepts in respiratory neurobiology derived from basic in vitro studies (Bellingham and Funk 2000; Rekling et al. 2000) or heavily reduced decerebrate or anesthetized animals (Kubin 2001) to the intact organism in vivo.

Sleep–wake states exert profound, and clinically important, influences on GG muscle activity, and these can be summarized into 3 major observations whose underlying mechanisms need to be determined. Firstly, there is the concept of the “wakefulness stimulus” to the respiratory musculature, i.e., a neural drive to respiratory motoneurons that activates respiratory muscle in wakefulness but the influence of which is withdrawn in sleep (Phillipson and Bowes 1986;

Fig. 2. (A) Freely behaving animal preparation for in-vivo microdialysis of the caudal medulla to modulate neurotransmission at the hypoglossal motor nucleus across natural sleep–wake states. Chronically implanted electrodes are used to record the electroencephalogram and neck electromyogram for determination of sleep–wake states, and genioglossus and diaphragm electrodes for respiratory muscle recordings. The tip of the microdialysis probe (shown enlarged at the top of the figure) allows for diffusion of neuro-chemicals across a semipermeable membrane to modulate the hypoglossal motor nucleus, the source of motor outflow to the genioglossus muscle of the tongue. (B) A histological section illustrating an example of a lesion site made by the microdialysis probe in the hypoglossal motor nucleus is also shown. The microdialysis probe is located within the cluster of motoneurons that innervate the genioglossus muscle, as shown by (C) retrograde transport of fluorescent probe injected at the site of the recording electrodes.



Orem 1994). This notion has been a significant and enduring concept in respiratory medicine, not least because it is the root mechanism to model the effects of sleep on breathing and understand the pathogenesis of sleep-related breathing disorders. However, a neurotransmitter substrate-modulating respiratory-muscle activation across sleep–wake states had not been identified. Secondly, REM sleep is associated with periods of major suppression of GG activity that renders the upper airspace particularly vulnerable to collapse (Horner 1996). However, as reviewed below, the mechanisms mediating this profound GG motor suppression are not through the same classic inhibitory neurotransmitters that operate in the spinal cord, with other mechanisms more importantly involved (Morrison et al. 2003a, 2003b). Thirdly, once GG activity is suppressed in REM sleep, it is difficult to re-activate the muscle, even in the presence of high respiratory drive (Horner et al. 2002) or local application of neurotransmitters (Jelev et al. 2001; Chan et al. 2006). These latter observations have particular relevance to those clinical studies aiming to increase pharyngeal muscle activity by pharmaco-

logical methods as a treatment for OSA, a topic also discussed in more detail below.

Activation of GG muscle in wakefulness and reduced activity in sleep

The activity of brainstem serotonin (5-hydroxytryptamine, 5-HT) and noradrenergic neurons (Fig. 1) is highest in wakefulness, reduced in nonREM sleep and minimal in REM sleep (Horner 1996; Kubin and Davies 2002). Hypoglossal motor neurons receive excitatory 5-HT and noradrenergic inputs, with the former from medullary raphe neurons also containing coreleased thyrotropin-releasing hormone (TRH) and Substance P, which are also excitatory (Rekling et al. 2000). Based on this circuitry, withdrawal of these excitatory inputs in sleep, especially REM sleep, may contribute to decreased GG activity.

From studies in reduced preparations, withdrawal of 5-HT was long hypothesized to be the main mechanism underlying decreased GG activity in sleep, especially REM sleep (Kubin et al. 1992, 1998). We showed for the first time in

vivo, however, that despite robust GG activation with delivery of 5-HT to the hypoglossal motor nucleus in conscious rats (Jelev et al. 2001), endogenous 5-HT plays a minimal role in the normal modulation of GG activity (Sood et al. 2005). Inhibition of serotonergic medullary raphe neurons, the source of 5-HT inputs to the hypoglossal motor nucleus, confirmed this result (Sood et al. 2006). We also showed that the potential role of 5-HT in modulating GG activity was over-emphasized from previous studies in reduced preparations (Kubin et al. 1992, 1994; Woch et al. 1996; Dreshaj et al. 1998; Fenik and Veasey 2003; Fenik et al. 2005), including our own experiments that used the same methodology as our subsequent studies in conscious animals (Sood et al. 2003). This over-estimation of the normal physiological role of 5-HT in pharyngeal motor control was likely because of the use of vagotomy in those previous studies using reduced preparations, a procedure that augments the role of 5-HT at the hypoglossal motor nucleus (Sood et al. 2005, 2006). Indeed, most medullary raphe neurons that project to motoneurons are inhibited by vagal afferents (Blair and Evans 1991; Evans and Blair 1993). Overall, these results emphasize the importance of studies in intact preparations to determine mechanisms of respiratory motor control as procedures such as de-afferentation can alter the neurochemical environment and normal physiological processes. The lack of a significant role of endogenous 5-HT in pharyngeal motor control in this intact animal model may also help explain the lack of clinically significant effects following manipulation of endogenous 5-HT to increase pharyngeal muscle activity in sleep and prevent OSA in humans (Hanzel et al. 1991; Berry et al. 1999; Kraiczki et al. 1999).

In contrast to 5-HT, however, we identified a functional endogenous noradrenergic drive to respiratory motoneurons that contributes to both the respiratory-related and tonic components of GG muscle activation in wakefulness and the residual respiratory-related GG activity that persists in nonREM sleep (Chan et al. 2006). However, the noradrenergic contribution to GG motor tone was minimal in REM sleep, so explaining, at least in part, the decreased GG activity encountered in this state (Chan et al. 2006). This result is significant because since the first clinical descriptions of the occurrence and mechanism of OSA (Gastaut et al. 1966, 1969), this was the first identification of a neural drive contributing to the sleep state-dependent activity of a respiratory muscle that is central to this disorder. This result is also of broader significance in identifying a mechanistic substrate underlying respiratory muscle activation in wakefulness whose influence is withdrawn in sleep, i.e., the “wakefulness stimulus” to the respiratory musculature (Phillipson and Bowes 1986; Orem 1994).

Nevertheless, the source of the noradrenergic input to hypoglossal motoneurons responsible for the sleep state-dependent modulation of GG activity has not yet been determined. Noradrenergic neurons of the locus coeruleus and its closely adjacent ventral extension (sub-coeruleus) show robust decrements in activity from wakefulness to nonREM and REM sleep (Aston-Jones and Bloom 1981; Reiner 1986). The main noradrenergic innervation of the hypoglossal motor nucleus arises from subcoeruleus (Aldes 1990; Aldes et al. 1992; Aston-Jones et al. 1995). Although A7 neurons also have significant projections to the hypoglossal

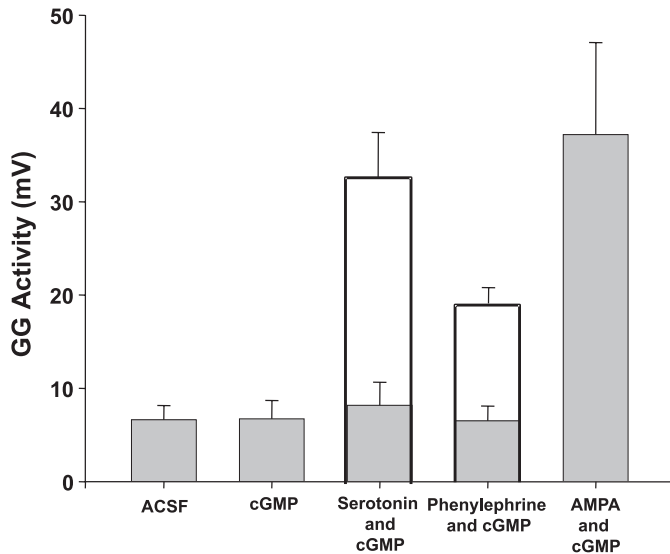
motor nucleus (Aldes et al. 1992), the sleep-state-dependent activity of those neurons is not well studied. In addition, although A5 neurons show decreased activity in a pharmacological model of REM sleep in anesthetized animals, a sample of those neurons showed that they did not project to hypoglossal motoneurons (Fenik et al. 2002). Accordingly, subcoeruleus and perhaps A7 neurons are the most likely source of the endogenous excitatory noradrenergic drive modulating GG activity across sleep-wake states, but this remains to be tested. Given the widespread projections of brainstem catecholaminergic neurons, they are also positioned to provide an endogenous input to other respiratory and nonrespiratory neurons and motoneurons, and so influence diaphragm activity and ventilation across sleep-wake states (Rekling et al. 2000; Fenik et al. 2002; Li and Nattie 2006).

Pharyngeal motor suppression in REM sleep

Decreased dorsal raphe and locus coeruleus activity preceding and during REM sleep also progressively withdraws inhibition of cholinergic neurons of the laterodorsal and pedunculopontine tegmental nuclei (LDT/PPT) via reduced 5-HT and noradrenaline (Fig. 1). Activation of LDT/PPT neurons then increases acetylcholine release into the pontine reticular formation to trigger REM sleep (Horner 1996; Kubin and Davies 2002). Exogenous application of a cholinergic agonist (e.g., carbachol) by microinjection into this region in anesthetized or decerebrate animals is used to mimic this process and trigger REM-like neural events, i.e., the carbachol model of REM sleep (Kubin and Davies 2002). However, carbachol does not produce the whole range of electrocortical and respiratory events that characterize natural REM sleep and the general applicability of this model is debated (Orem 1994; Horner 1996; Kubin et al. 1998).

Studies in the carbachol model of REM sleep provide evidence that spinal motoneurons are inhibited by glycine and γ -amino-butyric acid-A ($GABA_A$) receptor mechanisms (Morales et al. 1987; Chase and Morales 2000) but few studies have been performed in natural sleep, likely because of technical difficulties accessing spinal motoneurons (Soja et al. 1987b; Chase and Morales 2000). However, whether such inhibitory glycinergic and $GABA_A$ -ergic mechanisms contribute to the major suppression of hypoglossal motor outflow to GG muscle was controversial with conflicting data from the carbachol model of REM sleep (Kubin et al. 1993; Yamuy et al. 1999; Fung et al. 2000) and no studies in natural sleep. We first characterized glycine and $GABA_A$ receptor effects at the hypoglossal motor nucleus in vivo (Morrison et al. 2002; Liu et al. 2003) and then showed that antagonism of these receptors (either alone or in combination) increased respiratory-related GG activity across all sleep-wake states (Morrison et al. 2003a, 2003b). This non-specific increase in respiratory-related GG activity may have been caused by blockade of end-inspiratory inhibition (Withington-Wray et al. 1988; Woch and Kubin 1995; Peever et al. 2001) mediated by glycine and $GABA_A$ receptor mechanisms (Shao and Feldman 1997; Singer et al. 1998; O'Brien and Berger 1999; Donato and Nistri 2000). Importantly, there was no evidence for a major recruitment of glycine and $GABA_A$ receptor-mediated inhibition that was specific to REM sleep (Morrison et al. 2003a, 2003b). Re-

Fig. 3. Schema to show that application of a cell permeable cyclic guanosine-3'-5'-monophosphate (cGMP) analogue has no independent effect on genioglossus motor activity when applied to the hypoglossal motor nucleus. However, the cGMP analogue abolished responses to serotonin (5-HT) and the α_1 receptor agonist phenylephrine (grey bars), which otherwise produce robust excitatory effects (open bars). Iontropic responses to application of (S)-2-amino-3-(3-hydroxy-5-phenyl-4-isoxazolyl) propionic acid (AMPA) (a non-NMDA receptor agonist) were preserved.



cent data modulating trigeminal motoneurons and recording masseter muscle activity supports this result (Brooks et al. 2006), again highlighting the importance of studies in conscious animals in natural REM sleep compared with the carbachol model in reduced preparations (Soja et al. 1987a; Pedroarena et al. 1994).

Overall, these results showed that inhibitory glycine and GABA_A receptor mechanisms contribute minimally to the major suppression of GG activity that occurs in REM sleep and that other mechanisms are more importantly involved (Morrison et al. 2003a, 2003b). There is an interesting case report describing an attempt to counteract putative glycinergic inhibition of pharyngeal motoneurons with systemically applied strychnine in a patient with OSA (Remmers et al. 1980). In that study, strychnine caused an increase in tensor palatini muscle activity, however, changes in GG activity were less obvious and nonREM and REM sleep were not distinguished, i.e., again agreeing with data from the animal studies on the lack of effect specific to REM sleep (Morrison et al. 2003a, 2003b; Brooks et al. 2006).

Reduced excitation of hypoglossal motoneurons via α_1 receptor mechanisms contributes to decreased GG activity in natural REM sleep (Chan et al. 2006) and in the carbachol model of REM sleep (Lai et al. 2001; Fenik et al. 2005). However, loss of excitation is not the sole explanation for the periods of major GG suppression in REM sleep as this also occurs despite continuous delivery to the hypoglossal motor nucleus of the α_1 receptor agonist phenylephrine (Chan et al. 2006), 5-HT (Jeleu et al. 2001), or combined 5-HT and phenylephrine (Chan et al. 2006), all of which produce otherwise robust excitatory responses.

Cholinergic PPT neurons are retrogradely labeled follow-

ing injection of fluorescent tracers into the trigeminal, facial, and hypoglossal motor nuclei (Woolf and Butcher 1989). Cholinergic inputs to the hypoglossal motor nuclei also originate from the reticular formation (Connaughton et al. 1986). Numerous cholinergic axons make synaptic contact with hypoglossal motoneurons, whereas other cholinergic endings are opposed to noncholinergic terminals (Connaughton et al. 1986; Davidoff and Irintchev 1986; Hellstrom et al. 2003). The former may provide the anatomical substrate for post-synaptic modulation of hypoglossal motoneurons (Zaninetti et al. 1999; Bellingham and Funk 2000; Lape and Nistri 2000; Chamberlin et al. 2002; Robinson et al. 2002), whereas the latter may provide the basis for modulation of presynaptic inputs (Bellingham and Berger 1996), specifically the excitatory glutamatergic inputs associated with respiratory drive (Greer et al. 1991; Funk et al. 1993; Steenland et al. 2006). We characterized cholinergic influences at the hypoglossal motor nucleus in vivo and showed in anesthetized rats that cholinergic stimulation led to net decreases in respiratory-related GG activity, with muscarinic receptor-mediated suppression predominating over nicotinic excitation (Liu et al. 2005).

Although REM sleep is a cholinergic event and a subset of PPT neurons show increases in activity specific to REM sleep (el Mansari et al. 1989; Thakkar et al. 1998), it remains to be determined if modulation of central cholinergic activity importantly contributes to changes in GG activity across sleep-wake states. For example, REM sleep-active cholinergic neurons may mediate a component of GG motor suppression via inhibitory muscarinic receptor mechanisms (Bellingham and Berger 1996; Liu et al. 2005). Likewise, it is also conceivable that decreased cholinergic neuronal activity from wakefulness to nonREM sleep (el Mansari et al. 1989; Thakkar et al. 1998) could also contribute to decreased hypoglossal motor activity via reduced nicotinic excitation (Zaninetti et al. 1999; Chamberlin et al. 2002; Robinson et al. 2002; Liu et al. 2005). Overall, characterization of cholinergic influences on motor output to pharyngeal muscles is clinically relevant because modulation of central cholinergic activity has been attempted as a pharmacological treatment for OSA (Gothe et al. 1985; Hedner et al. 2003), despite no knowledge of the sites and mode of action of the cholinergic agents being tested.

Second messengers and modulation of hypoglossal motor responses to excitatory inputs

All previous studies in animals and humans aiming to increase pharyngeal muscle activity in sleep have targeted receptors on pharyngeal motoneurons via manipulation of extracellular neurotransmitters. However, sophisticated in vitro experiments show that respiratory motoneuron excitability is dynamically modulated by a variety of intracellular signaling molecules that are amenable to manipulation (Richter et al. 1997; Feldman et al. 2005) and it has been suggested that modulation of intracellular targets downstream from surface receptors may offer new and alternative approaches to develop pharmacological strategies for OSA (Greer 2005). We examined the effects on respiratory muscle activity of manipulating intracellular signaling molecules at respiratory motoneurons in freely behaving animals in vivo and we also determined the effects of prevailing

sleep–wake states on those responses (Aoki et al. 2006). In that study, we showed that modulation of the cyclic adenosine-3'-5'-monophosphate (cAMP)–protein kinase A (PKA) pathway at the hypoglossal motor nucleus led to increases in GG activity in wakefulness and nonREM sleep but not REM sleep (Aoki et al. 2006). This result further emphasizes the consistent finding that REM sleep recruits powerful mechanisms that can overcome excitatory stimuli and powerfully suppress GG activity (Jelev et al. 2001; Horner et al. 2002; Chan et al. 2006) but also emphasized that responses observed *in vitro* are not intractable but are importantly modified by interactions with other state-dependent systems that are normally engaged in the intact organism *in vivo*.

In that study, we also demonstrated a new mechanism in respiratory motor control by which cyclic guanosine-3'-5'-monophosphate (cGMP) at the hypoglossal motor nucleus abolished the normally potent excitatory responses to locally applied 5-HT and phenylephrine, whereas the ionotropic responses to the non-N-methyl-D-aspartate (non-NMDA) glutamate receptor agonist (S)-2-amino-3-(3-hydroxy-5-phenyl-4-isoxazolyl) propionic acid (AMPA) were preserved (Fig. 3). Given that this effect of cGMP mimics the effects of natural REM sleep on responses to 5-HT and phenylephrine (Jelev et al. 2001; Chan et al. 2006), the data suggested that recruitment of the nitric oxide (NO)–cGMP pathways in REM sleep may contribute to the major suppression of GG muscle activity and reduced responses to excitatory monoaminergic inputs. Increased cGMP in REM sleep would also explain why muscle twitches are preserved in REM sleep because these excitatory events are produced by nonNMDA receptor mechanisms (Soja et al. 1995), and responses to non-NMDA receptor stimulation with AMPA were unaffected by cGMP (Fig. 3) (Aoki et al. 2006).

Both soluble guanylate cyclases and protein kinase G (PKG) are expressed at the hypoglossal motor nucleus (de Vente et al. 2001; Lin and Talman 2005) and cGMP decreases hypoglossal motoneuron excitability *in vitro* (Neverova et al. 2002). There are also nitrenergic projections from the reticular formation to hypoglossal and trigeminal motoneurons (Pose et al. 2005). Cholinergic LDT/PPT neurons also express nitric oxide synthase (Vincent and Kimura 1992; Gautier-Sauvigne et al. 2005) with these neurons involved in arousal and REM sleep generation (el Mansari et al. 1989; Thakkar et al. 1998) and influences at the hypoglossal motor nucleus (Bellingham and Berger 1996; Liu et al. 2005). However, it is not known whether the major suppression of GG muscle responses to 5-HT and phenylephrine at the hypoglossal motor nucleus in natural REM sleep (Jelev et al. 2001; Chan et al. 2006) is due to recruitment of NO and cGMP via soluble guanylate cyclase, and this needs to be determined. Characterizing the mechanisms underlying the lack of excitatory responses to applied monoamines (e.g., 5-HT) are also clinically relevant because although pharmacological strategies aiming to increase 5-HT at pharyngeal motoneurons have some beneficial effects in OSA patients, any benefit is largely confined to nonREM sleep with minimal effects observed in REM sleep (Hanzel et al. 1991; Berry et al. 1999; Kraiczi et al. 1999; Veasey et al. 1999; Sunderram et al. 2000).

Summary

There have been several previous attempts in humans to increase upper airway muscle tone and alleviate OSAs by neurochemical approaches, with a resurgence of interest in these approaches as knowledge of the neural systems affecting pharyngeal motor control increases. To date, however, these clinical studies have met with only limited success, in large part because the basic mechanisms underlying suppression of upper airway muscle activity in natural sleep and the neurotransmitters and receptor subtypes involved have not yet been determined. Once these neural systems and receptors have been identified, however, and their relative importance determined, it is expected that more rational and systematic approaches can be devised for the systemic administration of drugs to centrally modulate motor output to the pharyngeal muscles. Indeed, as in other disciplines, an effective route for overcoming the many obstacles in this field will likely be forthcoming only after the basic physiological experiments guide the clinical and therapeutic approaches to target specific receptors and problems of targeted delivery have been addressed.

It is with respect to determining both physiological mechanisms of respiratory motor control in sleep and identifying strategies to reactivate GG muscle that studies with intact rodent models and modulation of central respiratory motoneurons are important. The presence in rats of tonic and respiratory-related GG activity in wakefulness, reduced tonic GG activity in sleep, and abolition of any residual respiratory-related GG activity in REM sleep (Horner et al. 2002; Chan et al. 2006) is, overall, similar to humans (Sauerland and Harper 1976; Mezzanotte et al. 1992; Horner 1996). However, it remains to be determined whether the central neuromodulators found important in controlling GG activity across sleep–wake states in rats are equally involved in humans. Although there is no currently available way to specifically address central neural mechanisms of pharyngeal motor control in humans, evidence for a minimal effect of 5-HT in the control of GG activity in rats (Sood et al. 2005, 2006) may also apply to humans and help explain the overall lack of clinical benefit of 5-HT strategies for OSA (Horner 2001). Of perhaps greatest value to future studies in humans, however, is the ability to target pharyngeal motoneurons directly to address mechanisms responsible for decreased activity in sleep, especially REM sleep, and devise strategies to reactivate these motoneurons. Pharmacological strategies for sleep-disordered breathing is of significant current interest in pulmonary sleep medicine (Hudgel and Thanakitcharu 1998; Horner 2001; Veasey 2001, 2003; Smith and Quinnell 2004; Greer 2005). The field is now in a position to develop such approaches as the relevant neurotransmitters and receptors are identified by basic experiments and then test the effects on pharyngeal muscle activity in sleep at their desired site of action using such experimental models.

From a clinical perspective, the importance of understanding basic neural mechanisms of pharyngeal motor control, especially the differences in neurobiology between nonREM and REM sleep, is especially important both in the adequate interpretation of clinical data and planning future therapeutic interventions. For example, if progressive disfacilitation or

inhibition significantly contributes to the further GG suppression from non-REM to REM sleep then a suitable combination of neuropharmacological agents may be more beneficial to maintaining pharyngeal muscle tone in REM sleep than modulating a single neurotransmitter that may only be effective in nonREM sleep. The implications of this consideration are that any potential therapy may have to be tailored to the individual patient based on whether their sleep-disordered breathing predominates in nonREM and (or) REM sleep. Accordingly all studies investigating potential treatments for sleep-disordered breathing should rigorously control for such variables that influence OSA, such as sleep stage and even the body position in which the apneas occur.

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