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Control of Upper Airway Motoneurons During REM Sleep

Leszek Kubin, Richard O. Davies, and Allan I. Pack

The loss of tone in upper airway muscles contributes to disorders of breathing during sleep. In an animal model of rapid eye movement sleep atonia, decrements in the activity of upper airway motoneurons are caused by withdrawal of excitation mediated by serotonin and other transmitters, rather than by state-dependent inhibition.

Understanding the control of the upper airway muscles of the larynx and pharynx presents a long-standing and interesting challenge to physiologists. These muscles are multifunctional, playing roles in respiration, vocalization, ingestive behaviors, and protective reflexes such as coughing and sneezing. Consequently, their behaviors provide a unique window on central neural mechanisms controlling these functions. Recently, special attention has focused on the role of these muscles in the pathogenesis of sleep-disordered breathing, especially obstructive sleep apnea.

Sleep exerts profound effects on the control of all skeletal muscles and central neuronal activity; in particular, the changes occurring during rapid eye movement (REM) sleep are highly unique. At the cortical and subcortical level, REM sleep has as its hallmarks a desynchronization of the cortical electroencephalogram, similar to that in wakefulness, and ponto-geniculo-occipital waves (characteristic electrical potentials generated in the midbrain and pons). Two REM sleep-specific phenomena occur at the motoneuronal level: a strong suppression of postural muscle tone (atonia) and intermittently generated flurries of activity (phasic events), manifested by rapid eye movements, muscle twitches, and increments or decrements in the activity of those muscles not rendered completely atonic. Thus, during REM sleep, excitatory and inhibitory phasic events are superimposed on a tonic cortical activation and motor inhibition.

The REM sleep-specific phenomena are expressed not only in postural but also in respiratory muscles. Respiratory rate and tidal volume are very variable from breath to breath, whereas upper airway muscle tone is suppressed in parallel to the atonia of postural muscles. This loss of tone is greater than that seen during other phases of sleep and more profound than the depression of respiratory pump muscle (such as the diaphragm and intercostal muscles) activity.

Obviously, the respiratory pump muscles cannot all become atonic, or ventilation would cease. However, complete atonia may occur in some upper airway muscles, one function of which is to secure upper airway patency. Because the hypotonia of these muscles occurs simultaneously with the atonia of postural muscles, it is logical to hypothesize that the REM sleep suppression of these two motor outputs has a common origin. The neural mechanisms of atonia in postural muscles have been the subject of intense research since the discovery of REM sleep over 40 years ago. Intracellular studies in chronically instrumented behaving cats by Chase and collaborators (reviewed in Ref. 3) demonstrated that lumbar postural motoneurons are actively inhibited during REM sleep. The inhibition is manifested by motoneuronal membrane hyperpolarization, a decrease in membrane resistance, and the appearance of large “state-specific” inhibitory postsynaptic potentials. This inhibition is due to an increased motoneuronal membrane permeability to chloride ions, which suggests that one or both of the two common inhibitory neurotransmitters, glycine and γ-aminobutyric acid (GABA), are released onto the motoneuronal membrane during REM sleep. Indeed, the inhibition of lumbar motoneurons can be abolished by the extracellular application of strychnine, an antagonist of glycnergic neurotransmission (3). This result, derived from the study of lumbar postural motoneurons, is commonly extrapolated to all motoneuronal populations whose activity is suppressed during REM sleep. However, recent data do not support the contention that glycnergic inhibition plays a major role in the atonia of hypoglossal (XII) motoneurons (see below) (6). XII

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motoneurons are a frequently studied pool of upper airway motoneurons whose motor nucleus is located in the medulla rather than the spinal cord. They have an inspiratory-related firing pattern and innervate the genioglossus muscle of the tongue, a major pharyngeal dilator.

Features of the carbachol model of REM sleep atonia

Studies on the neural mechanisms underlying REM sleep phenomena have concentrated on the brain stem because decerebrate animals (a reduced preparation, with thalamus and cortex removed and only the brain stem and spinal cord left intact, and lacking all conscious perception of pain) show recurring episodes of the major hallmarks of REM sleep, such as postural muscle atonia and rapid eye movements. Also, neurons located in the dorsolateral pons contain and release from their terminals the neurotransmitter acetylcholine (ACh) (cholinergic neurons) and increase their firing in association with REM sleep. One of the projection targets of these cholinergic cells is another region of the pons, the dorsal pontine tegmentum (also called the subceruleus region because it is located just ventral to the anatomically distinct locus ceruleus). The extracellular level of ACh rises at this site during REM sleep. Finally, when a powerful cholinergic receptor agonist, such as carbachol, is injected into the subceruleus area in chronically instrumented, intact animals (cats or rats), it evokes a state similar to REM sleep, including a profound postural atonia. The effects of carbachol can be reversed by microinjections of atropine, an antagonist of muscarinic cholinergic receptors, into the same pontine site. Such microinjections give researchers full control over the timing of the REM sleep-like atonia and provide a tool to study the accompanying changes in respiratory motor output.

Importantly, microinjections of carbachol into the same specific region of the pons also evoke the major signs of REM sleep in decerebrate animals (12) (Fig. 1A). This reduced preparation provides a model in which one can employ a wide range of neurophysiological and pharmacological techniques, many of which are difficult to use in chronic animals. In these animals, the effects of carbachol can be reversed by microinjections of atropine, an antagonist of muscarinic cholinergic receptors, into the same pontine site. Such microinjections give researchers full control over the timing of the REM sleep-like atonia and provide a tool to study the accompanying changes in respiratory motor output.

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associated with the atonia produced by carbachol and not confounded by secondary phenomena resulting from changes in muscle tone or pulmonary gas exchange.

Injections of carbachol in decerebrate cats produce at least four major signs that are also characteristic of natural REM sleep: atonia of postural muscles, eye movements, reduction in upper airway motor tone, and reduction in the activity of serotonin (5-HT)-containing neurons (Fig. 1, A and B, and Fig. 2, B and C). Important-ly, simultaneous recordings from multiple distinct respiratory motor nerves show that the carbachol-induced depression of respiratory motor output has a stereotyped pattern: phrenic nerve (innervating the diaphragm) activity is least depressed; inspiratory intercostal and inspiratory laryngeal activity are more suppressed; and expiratory pharyngeal (vagal), XII, and expiratory intercostal nerve activities are the most suppressed (Fig. 1, B and C) (5). Although in individual studies in behaving cats usually not more than three respiratory motor outputs were simultaneously recorded during natural REM sleep, a compilation of data from many such studies shows that the pattern of carbachol-induced depression of respiratory motor output in decerebrate cats is the same as that during natural REM sleep, albeit exaggerated. Interestingly, in contrast to the strong suppression of activity in their target spinal phrenic and intercostal motoneurons, central medullary respiratory neurons are minimally suppressed, and in some cases excited, during the carbachol-induced atonia (Fig. 1 D) (7). Such a disparity also occurs during natural REM sleep (13) and provides evidence that the suppression of respiratory motor activity cannot be explained by changes in the activity of premotor respiratory neurons in the medulla; rather, REM sleep-specific, nonrespiratory pathways must mediate the state-dependent changes in activity.

Despite the similarities, the respiratory changes during the carbachol-induced atonia in decerebrate cats differ from those characteristic of natural REM sleep in at least three ways. 1) The decerebrate model does not exhibit a highly variable respiratory rate and pattern. Indeed, the respiratory frequency is very regular and often reduced by 10–30% (5). This regularity appears to be due to the particular mode of action of carbachol on pontine neurons, since similar changes are seen when the REM sleeplike state is produced by carbachol injection in chronically instrumented cats (10). 2) Phasic “twitchlike” activity is rarely seen in either respiratory or postural nerve activity. 3) The depression of respiratory nerve activity (especially the phrenic) is stronger than in natural REM sleep. For the phrenic nerve, this exaggerated depression is largely due to the use of artificial ventilation and the elimination of feedback mechanisms that normally compensate for the centrally induced depression of the respiratory motor output; in spontaneously breathing decerebrate cats, carbachol produces only a transient reduction in phrenic nerve activity that quickly recovers as a result of the rise in arterial CO2.

In summary, the decerebrate carbachol model allows one to 1) produce and terminate a state having major postural and respiratory hallmarks of REM sleep, 2) dissociate the central effects exerted on the respiratory system in association with the REM sleep-like atonia from respiratory reflexes, and 3) record from, stimulate, and identify central neurons with greater ease and better control of the experimental conditions than is possible in studies of natural REM sleep in chronically instrumented behaving animals. The trade-off is that the model does not fully mimic the changes in respiratory and postural motor outputs (lack of respiratory variability and phasic muscle twitches). This necessitates caution in interpreting results and the need to determine the degree to which the new findings obtained using this model can be applied to natural REM sleep.

**Lessons from the carbachol model about the control of upper airway muscle activity: lack of evidence for inhibitory mechanisms**

Studies using the decerebrate carbachol model have led to new concepts about the neurochemical control of upper airway motor tone during REM sleep. As discussed above, the reductions in respiratory motoneuronal activity must be mediated by state-specific pathways. Such pathways must use one or both of the two mechanisms employed by the nervous system to reduce neuronal activity: activation of inhibitory inputs or withdrawal of excitatory inputs (disfacilitation). Because glycinergic inhibition plays a major role in the atonia of postural muscles of the hindlimb during both natural REM sleep and carbachol-induced atonia (3, 12), we assessed the possibility that the changes in upper airway motoneuronal activity might also be due to inhibition mediated by amino acids such as glycine or GABA. However, attempts to antagonize the carbachol-induced suppression of XII nerve activity by microinjections into the XII nucleus of antagonists of the two major inhibitory neurotransmitter receptors (strychnine for glycinergic receptors and bicuculline for GABAa receptors) did not support the inhibitory hypothesis (6). As a control...
for the effectiveness of the blockade, we determined that the reflex inhibition of XII motoneurons mediated by the two transmitters was abolished. In addition, in intracellular recordings from XII motoneurons during the carbachol-induced atonia, the motoneurons were indeed hyperpolarized, but large “state-specific” inhibitory postsynaptic potentials like those seen in lumbar motoneurons (see Ref. 3) occurred infrequently, and the decreases in membrane resistance that are typical of the inhibition mediated by glycine or GABA were small and inconsistent.

The failure to antagonize the REM sleeplike suppression of XII motoneurons with strychnine or bicuculline is important because it shows that different mechanisms must mediate the REM sleep-related suppression of activity in different motoneuronal pools. Additional motoneuronal groups in both the brain stem and spinal cord need to be studied to determine whether the revealed difference reflects a distinction between spinal vs. upper airway (or orofacial) or postural vs. respiratory-modulated motoneurons. However, the magnitude of the carbachol-induced suppression varies widely among populations of respiratory motoneurons having the same gross origin (e.g., spinal or vagal; see Fig. 1C), suggesting that the effects of REM sleep on motoneurons innervating distinct respiratory muscles may be specific for that muscle and there may be no simple, general rules.

Lessons from the carbachol model about the control of upper airway muscle activity: disfacilitation plays a major role

Because the nervous system uses only inhibition or disfacilitation to reduce neuronal firing, the absence of evidence for a postsynaptic inhibition in XII motoneurons during the carbachol-induced, REM sleep-like atonia suggested that disfacilitation was the predominant mechanism. Such a disfacilitation could be mediated by one or both of two important brain stem neuronal groups that show large decreases in activity during REM sleep, the serotonergic neurons of the midline raphe nuclei and the noradrenergic neurons of the locus ceruleus complex. Both neuronal groups 1) exhibit a gradually decreasing activity across wakefulness and slow-wave sleep and become almost inactive during REM sleep (4), 2) have extensive axonal projections throughout the neuraxis, including the XII nucleus, and 3) are excitatory to motoneurons. To date, only the potential role of the 5-HT-containing neurons has been specifically assessed in relation to the REM sleeplike atonia of XII motoneurons.

Four lines of evidence from the decerebrate cat model support the hypothesis that 5-HT is an important neurotransmitter that maintains the activity of XII motoneurons during wakefulness but is withdrawn during REM sleep.

1) Microinjections of 5-HT into the XII nucleus of decerebrate cats excite XII motoneurons, whereas microinjections of methysergide (a broad spectrum 5-HT receptor antagonist) reduce the spontaneous XII nerve activity to ~50% of control (9). The latter finding indicates that, at least in this model, 5-HT provides endogenous excitatory input to these motoneurons. This is supported by studies in chronically instrumented English bulldogs (a natural animal model of obstructive sleep apnea) in which systemic injections of an antagonist of the excitatory effects of 5-HT strongly reduced the activity of pharyngeal muscles and produced airway occlusions (14). In vitro studies show that 5-HT excites XII motoneurons by a direct action on the motoneuronal membrane (2). On the basis of the use of various agonists and antagonists of different specificities, the 5-HT receptors likely to be involved in this excitation are type 2, which are found at high density in the XII and other orofacial motor nuclei.

2) In a study of the properties of neurons in the caudal medullary raphe, cells were found that send their axons to, and ramify within, the XII nucleus and reduce or stop their activity during the carbachol-induced atonia (Fig. 2, A–C) (15). These neurons have low firing rates (0.5–3.5 Hz) and nonmyelinated axons and are inhibited by 8-OH-DPAT, a specific agonist of 5-HT1A autoreceptors. Because 5-HT-containing neurons in the pontine dorsal raphe nucleus have similar characteristics (4), these caudal medullary raphe cells are likely to be serotonergic. Interestingly, some of those neurons also arborize within the nucleus of the solitary tract, the site of termination of many visceral and somatic afferents, including cardiorespiratory: Thus the transmission of visceral afferent information may also be modulated, in a state-dependent manner, by serotonergic projections from the medullary raphe.

3) Consistent with the behavior of these serotonergic raphe neurons that project to the XII nucleus, the extracellular level of 5-HT in the XII nucleus region decreases following the onset of the carbachol-induced atonia and returns to control following the recovery from the atonia produced by atropine (Fig. 2D) (8).

4) Microinjections of 5-HT into the XII nucleus attenuate the decrease in XII nerve activity induced by carbachol, a result consistent with an exogenous source of 5-HT substituting for the decreased release of endogenous 5-HT that occurs during the carbachol-induced atonia (9).
This effect of 5-HT contrasts with the inability of inhibitory amino acid antagonists to significantly attenuate the suppression of XII nerve activity (6). Mechanisms other than the withdrawal of the excitatory effect of 5-HT also must contribute to the carbachol-induced depression of XII motoneuronal activity. This conclusion derives from the fact that microinjections of methysergide (an antagonist of the excitatory effects of 5-HT mediated by 5-HT2 receptors) reduces XII nerve activity to ~50% of control, significantly less than the magnitude of the carbachol-induced suppression (to ~10% of control; see Fig. 1C). Consequently, the withdrawal of excitation by other neurotransmitters released by neurons with REM sleep-related decreases in activity may be important. Such neurotransmitters include norepinephrine from locus ceruleus complex neurons and substance P and thyrotropin-releasing hormone (TRH), two peptides frequently colocalized with 5-HT in raphe neurons. These mediators are all excitatory to XII motoneurons (Fig. 3). Collectively, the state-dependent effects of these transmitters on respiratory motoneurons may represent one neurochemical substrate of what has been referred to as the “wakfulness stimulus for breathing” (13). However, for transmitters other than 5-HT, it remains to be assessed whether they normally exert a tonic, endogenous excitatory effect on XII and other upper airway motoneurons and to what degree state-dependent changes in their release and actions contribute to changes in motoneuronal activity.

Another potential disfacilitatory mechanism is suggested by intracellular recordings from XII motoneurons during the carbachol-induced atonia. Such recordings show not only a tonic hyperpolarization of motoneurons but also a large decrease in the phasic inspiratory modulation of their membrane potential. This decrease in the magnitude of inspiratory depolarizations contrasts with the behavior of most medullary bulbospinal respiratory neurons (see Fig. 1D and the related text). Thus the respiratory input to XII motoneurons is unlikely to result simply from a reduction of the excitatory drive from central respiratory neurons. Presynaptic modulation (transmitter-mediated changes in the release of other transmitters at the nerve terminal) could explain the phenomenon of reduced respiratory modulation of XII motoneurons during the atonia of REM sleep. Such a modulation may be exerted by neurons belonging to any one of the brain stem transmitter systems showing state-dependent changes in activity (5-HT, norepinephrine, ACh) and may act on both respiratory and nonrespiratory (tonic and reflex) inputs to upper airway motoneurons (Fig. 3). This remains to be studied. The carbachol model offers a unique tool with which one can begin to address this question.

FIGURE 2. Changes in activity of medullary serotonergic cells during the carbachol-induced atonia. A: transverse section through lower medulla showing schematically the location of serotonergic raphe neurons with axons in the XII motor nucleus and the viscerosensory nucleus of the solitary tract (NTS). P, pyramidal decussation. B: activity of a medullary raphe cell that became silenced in parallel with suppression of XII nerve activity during the carbachol (carb)-induced atonia [modified from Ref. 15.] C: cells like those shown in A and B consistently decrease their activity during the carbachol-induced atonia, recover when the atonia is reversed, and are silenced by a systemic administration of 8-OH-DPAT, a serotonergic 1A receptor agonist. Changes in mean firing rates of 13 such cells are shown (modified from Ref. 15). D: extracellular level of serotonin (5-HT) in the XII nucleus region follows a pattern consistent with the behavior of putative serotonergic cells shown in C [data from Ref. 8.]

“...the withdrawal of excitation by other neurotransmitters... may be important.”
Relevance of serotonergic control of upper airway muscles to sleep apnea

Most of the studies discussed so far were concerned with the effects of the REM sleeplike atonia on upper airway motoneurons in animals with no history of upper airway dysfunction. However, subjects with sleep apnea have two distinct features: an anatomically compromised upper airway and recurring airway occlusions during sleep. Both factors may cause alterations in the control of upper airway muscle activity specific for the disorder. For example, electromyographic recordings from selected upper airway muscles of patients with obstructive sleep apnea and English bulldogs show that the tone of upper airway dilating muscles is increased during wakefulness compared with control subjects (11,14). This is a positive adaptive change that helps maintain airway patency; it may result from modifications of the excitability of upper airway motoneurons and/or changes in neurotransmission along reflex pathways. Because, in bulldogs, systemic administration of a 5-HT₂ receptor antagonist greatly decreases upper airway motor tone and increases the incidence of breathing disorders during wakefulness (14), we presume that the brain stem 5-HT system plays a role in the adaptive changes in upper airway motor tone, maintaining normal airway patency in the face of anatomic obstructions.

Conclusions

We have emphasized that one major component of the REM sleeplike reduction in XII motoneuronal activity is mediated by reductions in their serotonergic excitation secondary to the decreased activity of medullary raphe neurons. It appears, however, that, at least qualitatively, this can be generalized to other upper airway motoneuronal pools. All upper airway motoneurons have a serotonergic, excitatory innervation that may, in part, originate from the same raphe neurons. Quantitatively, however, differences exist in the density and pattern of the serotonergic innervation among distinct motoneuronal populations. Our recent studies with the iontophoretic application of 5-HT onto XII and laryngeal motoneurons demonstrated stronger excitatory effects on the former than on the latter, in apparent contrast to neuroanatomic data suggesting that serotonergic synapses are denser and closer to the cell body in laryngeal motoneurons. However, serotonergic effects on motoneurons may be exerted through both classical synaptic contacts and so-called “volume transmission,” which relies on transmitter release into the extracellular space in the vicinity of the target and is not associated with specialized synaptic contacts (1). Conceivably, state-dependent modulatory effects may be exerted through volume transmission, whereas the effects of 5-HT that are related to specific motor tasks may use focal synaptic

"...subjects with sleep apnea have... recurring airway occlusions during sleep.

FIGURE 3. Summary of neurochemical mechanisms that are likely to contribute to rapid eye movement (REM) sleep-related changes in activity of upper airway motoneurons. Arrows, excitatory effects; , inhibitory effects. So far, evidence for disfacilitatory mechanisms mediated by serotonin (5-HT) and other transmitters and peptides is the strongest, whereas the state-dependent inhibition mediated by major inhibitory amino acids such as glycine and y-aminobutyric acid (GABA) appears to play a minimal role. Presence of a state-dependent modulation of those inputs to upper airway motoneurons that, in principle, are not themselves state dependent is only hypothetical. Subst P, substance P; NE, norepinephrine; TRH, thyrotropin-releasing hormone; ACh, acetylcholine.
contacts targeted to selected somatic or dendritic motoneuronal compartments, but this remains to be determined.

In this review, we presented new data about the control of upper airway motoneurons during REM sleep in both healthy subjects and those with sleep apnea and pointed to some uncertainties and questions that still need to be addressed. There is clearly much to be learned before we will be able to generate a comprehensive picture of the mechanisms that collectively determine the behavior of distinct pools of respiratory motoneurons during REM sleep. Further progress in this research should lead to a better understanding of both the basic neuronal mechanisms that control upper airway muscle tone and their relation to obstructive sleep apnea.

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