Medullary Control of the Upper Airway During REM Sleep

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Summary

In obstructive sleep apnea patients, upper airway muscle tone is depressed during rapid eye movement (REM) sleep parallel to the characteristic postural atonia. Previous electrophysiological, pharmacological and anatomical studies provided evidence that a withdrawal of excitation mediated by norepinephrine and serotonin as well as active inhibition may contribute to the REM sleep-related depression of hypoglossal (XII) motoneurons, important upper airway dilators. Using a new carbachol model, we determined that the combined antagonism of noradrenergic and serotonergic receptors in the XII nucleus region is necessary and sufficient to eliminate the depressant effects of the REM sleep-like state on XII motoneurons.

Introduction

Obstructive sleep apnea (OSA) patients maintain adequate ventilation during wakefulness, but during sleep they experience recurring episodes of upper airway obstruction that coincide with periods of decreased tone in upper airway dilating muscles [12,22]. Sleep-related airway obstructions are longest during the REM stage of sleep when upper airway tone reaches its nadir parallel to the postural atonia characteristic of this state [20]. The neuroanatomical and neurochemical basis of the central neural mechanisms by which REM sleep affects upper airway motor tone have been the subject of extensive studies, as this may help devise effective pharmacological treatments for OSA.

To assess the role of different pathways that mediate REM sleep-related decrements in upper airway tone, hypoglossal (XII) motoneurons are studied...
because they innervate the genioglossus, an airway dilator that protects the
airway from collapse [4,22]. There are at least four distinct medullary and
pontine sources of afferents to the XII nucleus that may mediate the effects
of REM sleep. The pathway that originates in the intermediate medullary
reticular formation (IRt) provides inspiratory drive to XII motoneurons. The
activity of inspiratory IRt neurons is not altered, or even increased, during the
REM sleep-like depression of XII motoneuronal activity [24]. In contrast, the
serotonin (5-HT)-containing cells of the medullary raphe and noradrenergic
cells of the pons are strongly state-dependent, with activity being highest
during wakefulness and lowest during REM sleep [9,23]. They send axons to
the XII nucleus [2,18] and excite XII motoneurons [1,15], suggesting that a
sleep-related withdrawal of excitation mediated by norepinephrine (NE) and
5-HT contributes to the sleep-related upper airway hypotonia [13]. Finally,
cells located in the REM sleep-triggering region of the medial pontine reticular
formation (mPRF) project to the ventro-medial medullary reticular formation
(v-mMRF), where they excite spinally-projecting neurons that may inhibit
motoneurons [3,10,23]. It is not known whether such cells also project to the
XII nucleus, but the concept of active inhibition is supported by the findings
that: (1) inhibitory postsynaptic potentials (IPSPs) occur in motoneurons during
the atonia of REM sleep [5,25]; (2) v-mMRF cells are activated during the
atonia [19,23]; and (3) stimulation of v-mMRF cells suppresses postural tone
[16].

Here, we summarize our recent studies that led us to conclude that the
depressant effect of REM sleep on upper airway motoneurons is mediated by
the combined withdrawal of noradrenergic and serotonergic excitation.

Material and Methods

Carbachol model of REM sleep-like state

In urethane-anesthetized, vagotomized, paralyzed and artificially ventilated
rats, carbachol injections (18.3 ng/10 nl) are made into a discrete region of the
dorsomedial pontine tegmentum (Fig. 1 in ref. [8] shows the effective sites).
They trigger 2-4 min REM sleep-like episodes that comprise reduced magni-
tude and frequency of inspiratory bursts in the XII nerve, silencing of pontine
NE neurons, and activation of both a theta-like rhythm in the hippocampus
and the cortical EEG in 6-12 Hz range [11]. The episodes can be elicited
repeatedly, with no adaptation, by injections made at the same site at ~30 min
intervals.

Drug microinjections into the XII nucleus

In urethane-anesthetized rats prepared for pontine carbachol injections and
XII nerve recording, microinjections of various combinations of antagonists
were made into one XII nucleus to assess their effects on the magnitude of
depression of XII nerve activity elicited by subsequent injections of carba-
chol. Three injections, 40 nl each spaced at 650 µm intervals along the rostro-
caudal extent of the XII nucleus, were made over a period of ~10 min. The
drugs used were the excitatory α-adrenergic receptor antagonist, prazosin (0.2 mM), a broad-spectrum serotonergic receptor antagonist, methysergide (1 mM), and a GABA<sub>α</sub> receptor antagonist, bicuculline (1 mM).

Combined anterograde and retrograde tracing of the pathways connecting the pontine REM sleep-triggering region with the XII nucleus

Nembutal-anesthetized rats received iontophoresic injections of the anterograde tracer, Phaseolus vulgaris leucoagglutinin (PHA-L); Vector; 3.0%, 1-5 µA, 40-72 µA min) into the pontine REM sleep-triggering region. After 7-10 days, the animals were re-anesthetized and the retrograde tracer, Fluoro Gold (FG; Fluorochrome, Inc.; 4%, 10-25 nl) was injected into the XII nucleus on the same side as the earlier PHA-L injection. After another 7-9 days,
the rats were anesthetized and perfused with saline and then 4% paraformaldehyde/0.25% glutaraldehyde. The brainstems were serially sectioned at 35 μm, and every 7th section processed to visualize PHA-L sites using biotinylated antibodies against PHA-L, avidin-biotin-horseradish peroxidase (A-B-HRP) histochemistry (Vector), diaminobenzidine (DAB), and Ni-ammonium sulfate; this yielded black staining of PHA-L-containing fibers and terminals. Subsequently, the sections were incubated with antibodies against FG, then with biotinylated secondary antibodies, and then subjected to A-B-HRP histochemistry with DAB only; this resulted in brown staining of FG-containing cells.

Results

We tested whether a putative inhibitory pathway analogous to that pro-

Figure 2: Antagonism of glycineergic inhibition in the XII nucleus is not required to abolish the depressant effect of the REM sleep-like state on XII motoneuronal activity. The record in A shows a control REM sleep-like response elicited by pontine carbachol injection (at the marker). In B, another response elicited in the same animal 50 min after three injections of an antagonist mix containing prazosin, methysergide and bicuculline (but not strychnine) into the XII nucleus. The traces from top are: power of the cortical EEG in 6-12 Hz range; power of the hippocampal activity in the theta-like range; Hipp - raw hippocampal signal; central respiratory rate; moving average (MA) of XII nerve activity (individual peaks show the magnitude of inspiratory activity in successive respiratory cycles). In B, note that the pre-carbachol level of XII nerve activity was strongly reduced by prior antagonist injections and that there was no depressant effect of pontine carbachol on XII nerve activity, whereas the carbachol-elicited cortical and hippocampal activations and slowing of the respiratory rate were intact. Data from ref. [7].
posed to mediate inhibition of spinal motoneurons during REM sleep also exists for XII motoneurons. Following combined injections of PHA-L into the dorsomedial pons (Fig. 1A) and FG into the XII nucleus (Fig. 1B), we found in the v-mMRF cells that were retrogradely labeled and had closely apposed terminals of axons descending from the pontine REM sleep-triggering region (Fig. 1C). Figure 1D shows all such cells found in every 7th section through the medullary reticular formation of 3 rats.

This finding was consistent with the concept that inhibition contributes to REM sleep-related depression of XII motoneuronal activity. However, we previously found that antagonists of amino acid-mediated inhibition (strychnine or bicuculline) injected into the XII nucleus did not prevent the REM sleep-like depression of XII nerve activity [14], indicating that active inhibition has little impact on the suppression of motoneuronal activity. In support of this interpretation, we recently determined that the REM sleep-like depression of XII nerve activity is eliminated by combined microinjections of the antagonists of adrenergic, 5-HT and GABA_A receptors, whereas antagonism of glycinegic receptors was not required (Fig. 2) [7].

In further studies using the same model, we established that the REM sleep-like atonia of XII motoneurons is eliminated by combined microinjections of just á_1-adrenergic and 5-HT receptor antagonist (Fig. 3) [8]. Thus, at least in the model used, the REM sleep-like depression of XII motoneuronal activity is mediated by the combined withdrawal of noradrenergic and serotonergic excitation.

Figure 3: In the rat carbachol model, combined antagonism of adrenergic and serotonergic receptors is sufficient to eliminate the depressant effect of the REM sleep-state on XII motoneuronal activity. A: the average effect of three injections of an antagonist mix containing only prazosin (Pz; 0.2 mM) and methysergide (Me; 1mM) into the XII nucleus on the baseline XII nerve activity. The injections (small arrows) caused a depression of XII nerve activity lasting over 3 h that, at its nadir, was of the same order as that typically caused by pontine carbachol. B: Examples of three responses to carbachol elicited at the times marked by the large arrows in A. Hipp - raw hippocampal activity; XII nerve MA - moving average of XII nerve activity (both shown at the same gains in all three panels). During the response elicited 42 min after the antagonists, there was no depression of XII nerve activity at the time of the characteristic hippocampal activation. During the next response to carbachol elicited 137 min after the antagonists, a depression of XII nerve activity partially recovered. Data from ref. [8].
Conclusions

While our tracing studies support the concept that an inhibitory pathway contributes to the REM sleep-related depression of XII motoneuronal activity, our antagonist microinjection studies demonstrate that the depression can be fully accounted for by a combined withdrawal of noradrenergic and serotonergic excitation. This result provides proof of the concept that the REM sleep atonia can be completely eliminated by antagonists of just the two aminergic receptor systems. It follows that the contribution of the IPSPs [25] to the depression of XII motoneuronal activity is negligible. The cells located in the v-mMRF that project to the XII nucleus and are contacted by axons descending from the REM sleep-triggering region may be inhibitory, but their effects on XII motoneurons must be small. Alternatively, they may mediate excitatory effects of REM sleep on XII motoneurons; such effects occur in the carbachol model [6] and in XII nerve-innervated muscles of behaving rats [17]. These findings need to be tested in other motoneuronal groups, both cranial and spinal.

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References

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