Preoptic hypothalamic warming suppresses laryngeal dilator activity during sleep

Dennis McGinty, Agnes Metes, Md. Noor Alam, David Megirian, Darya Stewart, and Ron Szymusiak. Preoptic hypothalamic warming suppresses laryngeal dilator activity during sleep. Am J Physiol Regul Integr Comp Physiol 286: R1129–R1137, 2004. First published February 26, 2004; 10.1152/ajpregu.00296.2003.—Upper airway dilator activity during sleep appears to be diminished under conditions of enhanced sleep propensity, such as after sleep deprivation, leading to worsening of obstructive sleep apnea (OSA). Non-rapid eye movement (NREM) sleep propensity originates in sleep-active neurons of the preoptic area (POA) of the hypothalamus and is facilitated by activation of POA warm-sensitive neurons (WSNs). We hypothesized that activation of WSNs by local POA warming would inhibit activity of the posterior criocartoynoid (PCA) muscle, an airway dilator, during NREM sleep. In chronically prepared unrestrained cats, the PCA exhibited inspiratory bursts in approximate synchrony with inspiratory diaphragmatic activity during waking, NREM, and REM. Integrated inspiratory PCA activity (IA), peak activity (PA), and the lead time (LT) of the onset of inspiratory activity in POA relative to diaphragm were significantly reduced in NREM sleep and further reduced during REM sleep compared with waking. Mild bilateral local POA warming (0.5–1.2°C) significantly reduced IA, PA, and LT during NREM sleep compared with a prewarming NREM baseline. In some animals, effects of POA warming on PCA activity were found during waking or REM. Because POA WSN activity is increased during spontaneous NREM sleep and regulates sleep propensity, we hypothesize that this activation contributes to reduction of airway dilator activity in patients with OSA.

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Non-rapid eye movement sleep; laryngeal airway dilator; preoptic hypothalamus; warm-sensitive neurons; posterior criocartoynoid; obstructive sleep apnea

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delta activity within sustained sleep (reviewed in Ref. 29). Ambient warming also increases subsequent sleep with delta activity in both humans (17) and rats (30) and increases sleep-related c-Fos expression in MnPN (13). Most POA WSNs are sleep-active neurons (1, 4). These studies support a hypothesis that activation of WSNs contributes to the hypnogenic output of the POA and regulates sleep pressure.

We hypothesized that POA WSNs would regulate UAD muscle function during sleep. To test this hypothesis we examined the effects of POA warming on the inspiratory activity of a laryngeal dilator muscle, the posterior cricoarytenoid (PCA), during waking and sleep in the cat.

METHODS

Animals and surgery. All experimental procedures were conducted in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals. Six adult mongrel female cats weighing 2.5–3.9 kg were used in this study. Cats were obtained from an on-site breeding colony and were 6–18 mo of age. Preoperatively each cat was given 5.5 mg/kg of ketamine and 0.27 mg/kg diazepam intramuscularly. The right femoral vein was catherized for the administration of fluids and drugs. The animal was intubated with a no. 3uffed endotracheal tube attached to the anesthesia machine and administered isoflurane (2.5–3%), balance O2. The animals breathed spontaneously during surgery. Vital signs were checked and recorded every 10 min until completion of surgery. Sterile technique was used throughout.

With the animal in the supine position, an abdominal incision below the right costal margin permitted exposure of the diaphragm for the implantation of four juxtaposed, gold-plated barbed electrodes constructed from dental broaches (Fig. 1). Lead wires were tunneled subcutaneously to the back of the head. The abdominal incision was closed in layers.

A ventral midline incision in the neck from the hyoid bone to the sternum permitted exposure of the larynx and trachea. After partial rotation of the larynx on its long axis, taking care to preserve the recurrent laryngeal nerve, the PCA could be directly viewed. The PCA muscle was implanted with an array of gold-plated, broach-type electrodes (Fig. 1). Lead wires were tunneled subcutaneously to the back of the head. The neck incision was closed with nylon sutures.

Next, the cat was turned to the prone position and placed in a stereotoxic frame (Kopf 1430 model). A midline incision was made in the skin over the skull from the frontal bones to the lambdoid ridge. EEG and electrooculogram (EOG) electrodes consisted of small gold-plated stainless steel screws threaded into 1-mm holes bored into the skull over frontal cortex and in the orbital wall. EMG electrodes were Teflon-insulated wires with bared gold-plated 3-mm tips that were inserted into dorsal neck muscles with a needle. A pair of stainless steel water-perfused thermodes (OD 0.76 mm), with adjacent thermocouples, were stereotaxically implanted (anteroposterior 14.5, lateral 2.5, height −4.0) so as to bracket the medial POA (Fig. 1B). Thermocouple tips were centered 2.0 mm from the center of the thermode, ~1.6 mm from the thermocouple walls. All the electrode wires were soldered to separate pins of a connector, which, along with the thermode-thermocouple assembly, were secured to the skull with sterile dental cement.

At the end of the surgery, all wound areas were cleaned and treated with an antibiotic cream. All cats awoke spontaneously at the end of the anesthesia. During the postoperative period they were placed in a warmed oxygenated chamber. Their vital signs and temperature were checked regularly. Antibiotic treatment (Cefazoline) and intravenous fluids were given for 5 days.

Data collection and analysis. Experiments started after the 12th postoperative day, when the cats were in stable condition. Cats were placed in a sound-attenuated recording chamber (ambient temperature ~25°C) and connected to counter-weighted cables for recording the neurophysiological variables and hypothalamic temperature. EEG, EOG, neck muscle EMG, diaphragm and PCA EMGs and their moving averages, and hypothalamic temperature were amplified (Grass Instruments), digitized (Cambridge Electronic Design), and displayed and recorded continuously. Thermodes were connected to a peristaltic pump via Silastic tubing through a Peltier device that was suspended ~12 cm above the animals’ heads. POA temperature could be rapidly elevated by 0.4–1.5°C by pumping water through this system.

Physiological data were acquired first during spontaneous waking, non-rapid eye movement (NREM) and REM states (Fig. 2). Waking,
NREM, and REM were identified based on EEG and EMG patterns using standard criteria (43). Briefly, NREM was identified by occurrence of sustained periods with high-amplitude spindles and slow waves. REM was identified by a low-amplitude, mixed frequency EEG combined with very low neck muscle tone and occurrence of rapid eye movements. NREM samples were obtained from episodes with high-amplitude EEG activity including delta activity and sleep spindles beginning 10–60 min after sustained sleep onset. These samples were obtained when EEG slow-wave activity was maximal. REM samples were obtained from sustained REM periods lasting at least 3 min. REM samples did not contain episodes of very rapid breathing. For all comparisons, samples were obtained from contiguous sleep periods in which posture was unchanged. Two sleep cycles were examined in each cat. Subsequently, we determined responses to local POA warming by 0.5–1.5°C measured at the thermocouple tips. A minimum effective temperature in NREM was chosen for consistent use in each cat (average 0.9°C). Warming periods lasted 75–90 s during sustained waking, NREM, and REM episodes. We used tests in which states remained stable without postural change for at least 60 s before and during warming trials. Analyses were done on all breaths during continuous 60-s samples (18–20 breaths each). The raw and moving average of the EMG signals (CWE; time constant 50 ms) of the PCA and diaphragm were analyzed offline with the Spike 2 program (Cambridge Electronic Design). Analyzed parameters included integrated inspiratory activity (IA) and peak integrated amplitude (PA) for both PCA and diaphragm and the inspiratory burst onset time difference between PCA and diaphragm or PCA lead time (LT). We also measured the total respiratory time (Ttot) and the inspiratory time (Ti). To assure that effects of POA warming did not reflect progressive changes within sleep epochs, we measured PA during successive 60 samples within NREM without POA warming.

Statistical analysis. The means and variances of IA, PA, and LT sample breaths recorded during baseline sleep and wake states were calculated, and percent changes during sleep states compared with awake were determined. The effects of state on these parameters were assessed by ANOVA followed by multiple paired comparison tests (Holm-Sidak) in both individual cats and in pooled data. Effects of warming in each state were evaluated statistically using the Student’s t-test in both individual cats and pooled data. For all comparisons, P < 0.05 was considered significant.

Histology. After completion of experiments, cats were deeply anesthetized and perfused transcardially with saline followed by 4% formaldehyde. Brains were removed, postfixed in 15 and 30% sucrose in formaldehyde, and subsequently sectioned through the POA (40 μm) and stained with thionin. We confirmed that thermode tips were localized in the lateral POA or adjacent diagonal band within 0.75 mm of the target site (Fig. 3).

RESULTS

In four of six cats, both the PCA and diaphragm EMG recordings exhibited inspiratory bursts that were stable for...
several weeks (Fig. 4). These four cats were used for all analyses. The ANOVAs showed an overall effect of state on measures of PCA activity as well as PCA-diaphragm lead time in each cat. Post hoc tests showed significant decreases in individual cats in both NREM and REM compared with waking (Table 1) as well as in pooled data. As shown in Fig. 5, in NREM compared with waking, a decrease was found in integrated IA (−19%), PA (−31%), and LT (−21%). PA decreased significantly in each cat, and IA decreased significantly in two of four cats.

Significant decreases were also observed in REM sleep compared with waking (IA, −63%, PA, −66%). These differences were significant in each cat as well as in pooled data (Table 1). REM sleep PA was also significantly reduced compared with NREM sleep in three of four cats. The onset of PCA IA preceded the onset of diaphragmatic activity (PCA lead time), averaging 0.55 s in waking. PCA lead time decreased significantly compared with waking in NREM (22%) and REM (40%, Fig. 4). Compared with quiet waking, breath-by-breath integrated diaphragmatic inspiratory activity exhibited only minimal and nonsignificant decreases, 5.5% in NREM and 4.5% in REM sleep.

Effects of POA warming. Local warming of the POA within NREM sleep further decreased the PCA IA from the NREM
significantly. During waking, POA warming decreased both PCA peak activity; LT, lead time; PCA, posterior cricoarytenoid. *Ti/Ttot was significantly reduced during warming during both NREM and REM compared with waking. The present study showed that the PCA vs. diaphragm lead time, most consistently in NREM (~35%). In two of four cats, POA warming also significantly decreased PCA activity in waking and REM. In addition, POA warming decreased the PCA vs. diaphragm lead time, most consistently in NREM (~35%). As it is likely that the activation of airway dilator muscles before onset of diaphragmatic inspiratory activity reduces airway resistance, it seems reasonable to hypothesize that a reduction in lead time could contribute to a potential for airway obstruction.

The effects of POA warming were most consistent among individual cats in NREM sleep. The response to POA warming depends on the sensitivity of the WSNs that is increased in

Table 1. Effects of sleep states on PCA function:
Holm-Sidak pairwise multiple comparisons

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<th>Cat</th>
<th>NREM vs. W</th>
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NREM, non-rapid eye movement; W, wake; IA, integrated activity; PA, peak activity; LT, lead time; PCA, posterior cricoarytenoid. *P < 0.05, †P < 0.025, ‡P < 0.01, §P < 0.001, NS: not significant.
Fig. 6. Polygraphic example of POA warming trial during NREM sleep. A horizontal line is provided to assist visualization of the warming effect. During warming, PCA integrated activity was mildly, but consistently, reduced. Expanded samples (A, B, and C) are shown below.
NREM (2). Increased neuronal response to warming in NREM probably accounts for the consistent responses during this state. Only two of the four cats exhibited responses to POA warming in waking and REM. We do not have a definitive explanation for differences among cats in responses to POA warming in waking and REM. The anatomical distributions of sleep-active neurons and WSNs in POA are not uniform (29), so slight variations in the position of thermodes within the POA could be important. The mild POA warming applied in this study did not significantly change respiratory rate or total integrated diaphragmatic activity in any state, but the duration of the inspiratory phase of breathing measured with the diaphragmatic EMG was changed differentially, depending on state. During waking, POA warming increased T_i/T_tot, but during both NREM and REM, T_i/T_tot was reduced. This suggests a differential effect of added thermal drive during waking vs. sleep. The reduction of T_i/T_tot during sleep and in response to POA warming suggests that the duration of the inspiratory phase could also be reduced under conditions of increased activation of WSNs.

We studied responses to mild POA warming ranging from 0.7 to 1.2°C measured 2.0 mm from the center of the thermode tip. The response reported here is distinct from thermoregulatory heat loss responses such as panting typically elicited by stronger POA warming of 2.0°C or more (33).

We showed previously that most POA WSNs exhibit strongly increased neuronal activity before and during spontane-ous NREM sleep (1, 3). Increased WSN in NREM was equivalent to that seen in response to POA warming during waking. As noted above, the amplitude of warm sensitivity, i.e., the magnitude of the response of POA WSNs to a specific warming stimulus, also increased during NREM sleep (2). In our studies, POA neuronal warm sensitivity was also maintained in REM sleep. Activation of WSNs during waking induces heat loss, body cooling, and reduced metabolic rate, processes that also occur during spontaneous NREM sleep (29). WSN activation in NREM may account for the thermoregulatory and metabolic changes associated with this state.

Inasmuch as we found that the activation of WSNs is sufficient to suppress PCA inspiratory discharge, and this activation occurs in spontaneous NREM and REM sleep, we can hypothesize that WSN activation could account for some of the suppression of PCA activity that occurs in NREM and REM sleep. It is worth noting that many OSA patients exhibit intense nocturnal sweating, a heat loss process (15). Sweating suggests that POA WSNs are strongly activated in these patients. Hypoxia also induces active downregulation of body temperature, probably as an element of a coordinated protective lowering of metabolic rate (32). Thus hypoxia associated with OSA could further increase activation of WSNs and contribute to the inhibition of airway dilator muscles.

Recent studies suggest possible pathways that could mediate the POA output from WSNs. Using the c-Fos and GABAergic marker immunostaining techniques, we showed that segregated subgroups of sleep-active neurons localized in the VLPO and MnPN are GABAergic (14) and that the numbers of sleep-active neurons are increased by ambient warming (13). Most GABAergic neurons in these sites express c-Fos during rebound sleep after sleep deprivation (12). Sleep deprivation increased sleep-related activity of VLPO neurons (41). The posterior hypothalamus (PH) is a potent cardiorespiratory excitatory region (reviewed in Ref. 47) that sends projections to pontine and medullary respiratory centers (5, 45). Sites within pontine and medullary reticular regions that generate either inhibition or excitation of PCA have been identified (34). Cardiorespiratory facilitation by the PH was shown to be regulated by inhibitory GABAergic input (46). POA GABAergic neurons send projections to the PH. We showed that local POA warming inhibits PH arousal-related neuronal activity (23). Thus activation of POA sleep-active GABAergic WSNs would be expected to inhibit PH-induced cardiorespiratory activation by suppressing excitatory influences from PH on medullary respiratory centers.

Our study may have relevance to OSA. Although in humans the critical level of airway obstruction is the pharyngeal airway rather than the larynx (18, 42), pharyngeal dilator muscles and the PCA exhibit similar sleep-related activity changes, timing with respect to the respiratory cycle, and reflex control (18).

Two related hypotheses can be considered. The baseline reduction in measures of PCA activity as well as PCA lead

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POA, preoptic area. *P < 0.05, †P < 0.025; †P < 0.01, §P < 0.001.

Table 2. POA warming-induced reductions in PCA activity: significance in individual subjects
time in spontaneous REM sleep are likely related to the general loss of muscle tone in this state (9) and may account for the initial emergence of OSA in REM sleep in the natural history of the disease seen in many patients. OSA may begin as REM-related airway obstruction and REM fragmentation, perhaps accompanied by NREM snoring. This initial sleep fragmentation would result in increased sleep drive, leading to increased activation of hypogenic POA WSNs and subsequent worsening of NREM as well as REM OSA through the mechanism shown in this report. The severity of OSA is modulated by sleep drive, and OSA patients exhibit signs of enhanced sleep drive, even after CPAP treatment (see introduction). This hypothesis implies that OSA is worsened through a positive feedback loop, in which sleep fragmentation (reduction) is enhanced sleep drive, even after CPAP treatment (see introduction). This hypothesis implies that OSA is worsened through a positive feedback loop, in which sleep fragmentation (reduction) is enhanced sleep drive, even after CPAP treatment (see introduction). This hypothesis implies that OSA is worsened through a positive feedback loop, in which sleep fragmentation (reduction) is enhanced sleep drive, even after CPAP treatment (see introduction). This hypothesis implies that OSA is worsened through a positive feedback loop, in which sleep fragmentation (reduction) is enhanced sleep drive, even after CPAP treatment (see introduction). This hypothesis implies that OSA is worsened through a positive feedback loop, in which sleep fragmentation (reduction) is enhanced sleep drive, even after CPAP treatment (see introduction). This hypothesis implies that OSA is worsened through a positive feedback loop, in which sleep fragmentation (reduction) is enhanced sleep drive, even after CPAP treatment (see introduction). This hypothesis implies that OSA is worsened through a positive feedback loop, in which sleep fragmentation (reduction) is enhanced sleep drive, even after CPAP treatment (see introduction). This hypothesis implies that OSA is worsened through a positive feedback loop, in which sleep fragmentation (reduction) is enhanced sleep drive, even after CPAP treatment (see introduction). This hypothesis implies that OSA is worsened through a positive feedback loop, in which sleep fragmentation (reduction) is enhanced sleep drive, even after CPAP treatment (see introduction). This hypothesis implies that OSA is worsened through a positive feedback loop, in which sleep fragmentation (reduction) is enhanced sleep drive, even after CPAP treatment (see introduction). This hypothesis implies that OSA is worsened through a positive feedback loop, in which sleep fragmentation (reduction) is enhanced sleep drive, even after CPAP treatment (see introduction). This hypothesis implies that OSA is worsened through a positive feedback loop, in which sleep fragmentation (reduction) is enhanced sleep drive, even after CPAP treatment (see introduction). This hypothesis implies that OSA is worsened through a positive feedback loop, in which sleep fragmentation (reduction) is enhanced sleep drive, even after CPAP treatment (see introduction). This hypothesis implies that OSA is worsened through a positive feedback loop, in which sleep fragmentation (reduction) is enhanced sleep drive, even after CPAP treatment (see introduction). This hypothesis implies that OSA is worsened through a positive feedback loop, in which sleep fragmentation (reduction) is enhanced sleep drive, even after CPAP treatment (see introduction).


