Effect of upper airway negative pressure on inspiratory drive during sleep

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Effect of upper airway negative pressure on inspiratory drive during sleep. J. Appl. Physiol. 84(3): 1063–1075, 1998.—To determine the effect of upper airway (UA) negative pressure and collapse during inspiration on regulation of breathing, we studied four unanesthetized female dogs during wakefulness and sleep while they breathed via a fenestrated tracheostomy tube, which was sealed around the permanent tracheal stoma. The snout was sealed with an airtight mask, thereby isolating the UA when the fenestration (Fen) was closed and exposing the UA to intrathoracic pressure changes, but not to flow changes, when Fen was open. During tracheal occlusion with Fen closed, inspiratory time \( (T_I) \) increased during wakefulness, non-rapid-eye-movement (NREM) sleep and rapid-eye-movement (REM) sleep \((155 \pm 8, 164 \pm 11, \text{ and } 161 \pm 32\% \text{ respectively,})\), reflecting the removal of inhibitory lung inflation reflexes. During tracheal occlusion with Fen open \( (\text{vs. Fen closed}) \): 1) the UA remained patent; 2) \( T_I \) further increased during wakefulness and NREM \((215 \pm 52 \text{ and } 197 \pm 28\% \text{ respectively,})\) but nonsignificantly during REM sleep \((196 \pm 42\% \text{ respectively,})\); 3) mean rate of rise of diaphragm EMG \( (\text{EMGdi}/T_I) \) and rate of fall of tracheal pressure \( (P_{tr}/T_I) \) were decreased, reflecting an additional inhibitory input from UA receptors; and 4) both \( \text{EMGdi}/T_I \) and \( P_{tr}/T_I \) were decreased proportionately more as inspiration proceeded, suggesting greater reflex inhibition later in the effort. Similar inhibitory effects of exposing the UA to negative pressure \( (\text{via an open tracheal Fen}) \) were seen when an inspiratory resistive load was applied over several breaths during wakefulness and sleep. These inhibitory effects persisted even in the face of rising chemical stimulus. This inhibition of inspiratory motor output is afferent within an inspiration and reflects the activation of UA pressure-sensitive receptors by UA distortion, with greater distortion possibly occurring later in the effort.

Obstructive apnea; genioglossus; geniohyoid; diaphragm; tracheal occlusion; fenestrated tracheostomy tube

Upper airway (UA) patency and the reflex mechanisms that regulate it are critical determinants of breathing and therefore of both obstructive and central apnea during sleep. A dual protective reflex triggered by UA negative pressure has been well described, namely, activation of hypoglossal motoneurons acting on UA muscle abductors, which influence the diameter of the UA, acting together with reduced phrenic nerve activity and therefore less negative “collapsing” intrathoracic pressures. The magnitude of these reflex responses varies markedly depending on the conditions under which the UA is exposed to negative pressure. Contributing factors to this variation include the sleep state \((7–9, 13, 18, 25, 27) \); anesthesia \((10) \); UA collapse or distortion \((5, 7) \); the timing and magnitude of pressure change \((5–9, 17, 18, 28) \); and the presence of flow \((6, 23) \), temperature \((24) \), or chemical stimuli \((3, 6) \). Although the results of these studies demonstrate an effect of UA negative pressure that is generally inhibitory to respiratory drive and excitatory to UA dilator muscle activity, it is difficult to apply them to normal or obstructed breathing in which the UA is exposed to negative intrathoracic pressure only during inspiration and in which UA pressure changes occur in a graded physiological profile, in proportion to central inspiratory drive.

Therefore, the purpose of this study was to examine the reflex ventilatory responses to UA negative pressure in a more physiological manner: by approximating the conditions in which the UA is normally exposed to negative pressure, that is, during inspiration, and with the timing and magnitude of UA negative pressure being spontaneously generated in proportion to inspiratory drive, in the absence of changes in UA flow, temperature, or \( \text{CO}_2 \). We studied these effects during wakefulness and sleep and also questioned whether UA collapse, initiated by spontaneously generated negative intrathoracic pressures, potentiated the responses.

METHODS

General

Studies were performed on four unanesthetized female mixed-breed dogs \((20–25 \text{ kg}) \) during wakefulness and sleep. The dogs were trained to sleep in an air-conditioned sound-attenuated chamber. Throughout all experiments the dogs’ behavior was monitored by an investigator seated within the chamber and also by closed-circuit television. The protocol for this study was approved by the Animal Care and Use Committee of the University of Wisconsin.

Animal Preparation

Sterile surgical techniques were used to create a permanent tracheostomy and to implant electrodes for recording of inspiratory and UA muscle activities and to permit staging of sleep state. The dogs were premedicated with acepromazine \((0.5 \text{ mg/kg sc}) \), induced with thiopental sodium \((20 \text{ mg/kg iv}) \) and maintained on a mechanical ventilator with \% halothane in balance \( \text{O}_2 \).

A midline cervical incision and removal of the ventral aspect of three to five cartilaginous rings was used to create the chronic tracheostomy. Bipolar Teflon-coated multistrand stainless steel wire electrodes were sewn into the crural diaphragm, genioglossus, geniohyoid, and parasternal intercostal \((\text{dog 4 only}) \) muscles for measurement of electromyogram \( (\text{EMG}) \) activity. The raw EMGs were filtered \((30–1,000 \text{ Hz}) \), amplified, rectified, and moving time averaged with a time constant of \(100 \text{ ms} \). Sleep state was determined with five wire electrodes implanted subcutaneously, consisting of an electroencephalogram \( (\text{EEG}) \), two electrooculograms \( (\text{EOGs}) \), a common reference electrode, and a ground electrode. The EEG
and EOGs were amplified (BMA-831, CWE) and filtered at 10 Hz for the EOGs and 1–50 Hz for the EEG. These methods have been described in detail previously (4). All electrodes were tunneled subcutaneously to the cephalad portion of the dog’s back, where they were exteriorized.

Analgesics (butorphanol, 0.3 mg/kg sc) and antibiotics (enrofloxacin, 2.2 mg/kg po, or trimethaprim sulfa, 24 mg/kg po) were administered postoperatively as required. At least 2 mo were allowed for recovery from surgery before any experiments were performed.

Experimental Setup and Measurements

The dogs breathed via a fenestrated cuffed tracheostomy tube (10.0 mm OD; Shiley, Irvine, CA), which was inserted into the permanent tracheostomy and then sealed around the stoma. A removable inner sleeve allowed the fenestration to be closed or open. Connected to the tracheostomy was a breathing circuit consisting of a pneumotachograph and a series of valves, which enabled inspiration and/or expiration to be unloaded or loaded (Fig. 1).

The thermostated (37°C) pneumotachograph system (model 3700, Hans Rudolph, Kansas City, MO; model MP-45-14-871, Validyne, Northbridge, CA) was used to measure airflow and was calibrated before each study with five known flows. A low-resistance two-way valve (model 1400, Hans Rudolph) was attached to the pneumotachograph. The inspiratory port of the two-way valve was connected to a circuit that, by inflating or deflating the appropriate balloons, could unload, add a flow-resistive load, or occlude inspiration. Inflation or deflation of a balloon on the expiratory port enabled expiration to be unloaded or occluded. The dead space of this system was ~40 ml.

A lightweight polyethylene mask with polymer-gel inserts (Silipos, Niagara Falls, NY) was placed over the dog’s snout to form an airtight seal around the mouth and nose (Fig. 1). Therefore, when the fenestration was open, the UA was exposed to negative intrathoracic pressures developed during eupneic breathing or during inspiratory efforts against tracheal occlusions or resistive loads, in the absence of UA flow or temperature changes. In contrast, when the fenestration was closed, the UA was effectively isolated from all intrathoracic pressure changes.

Airway pressure changes were simultaneously measured at three sites: mask pressure (Pm) was measured with a catheter passed through the mask near the nares, sublaryngeal pressure (Psl) was measured via a catheter affixed to the outer sleeve of tracheostomy tube and positioned ~0.5 cm above the fenestration, and tracheal pressure (Ptr) was measured from a catheter passed into the two-way valve (Fig. 1). Each catheter was connected to a pressure transducer (model MP-45-14-871, Validyne) and was calibrated before each study by applying eight known pressures. Tracheal PCO2 was continuously sampled via a catheter passed into the two-way valve (mass spectrometer, model MGA 1100, Perkin-Elmer, Pomona, CA). The mass spectrometer was calibrated daily with three gases of known concentrations.

Fig. 1. Schematic representation of experimental setup illustrating isolated upper airway (UA) preparation (fenestration closed) and valve system used to occlude the trachea or increase airway resistance. UA is exposed to intrathoracic pressure changes only when fenestration is open. Pm, mask pressure; Psl, sublaryngeal pressure; Ptr, tracheal pressure; PCO2, expired CO2 concentration.
All signals were collected on a 12-channel polygraph (Gould ES 2000, Rolling Meadows, IL) and were passed via an analog-to-digital converter and stored on the hard disk of a computer for subsequent analysis.

Experimental Protocol

Each dog was studied on 9 ± 4 separate days. On each day the tracheostomy tube was randomly occluded or resistive loaded during wakefulness, non-rapid-eye-movement (NREM) sleep, and rapid-eye-movement (REM) sleep, with the fenestration closed or open (see Discussion).

Inspiratory and expiratory occlusions (single breath). Each trial consisted of five unloaded control breaths followed by a single effort against an occluded trachea. The occlusion was applied by inflating both balloons on the inspiratory port during the expiratory phase of the fifth control breath, thereby occluding the subsequent inspiration. During this inspiratory effort, a balloon on the expiratory port was inflated so that the subsequent expiration was occluded. During the expiratory effort the inspiratory balloons were deflated, and during the subsequent unloaded inspiration the expiratory balloon was deflated. This technique ensured that all inspiratory efforts against the occlusion and all unloaded recovery breaths were initiated at the dog’s normal functional residual capacity.

Baseline eupneic breathing pattern (timing, volume, pressure generation (also see Discussion)).

Inspiratory flow-resistive loading (5 successive breaths). Each trial consisted of five unloaded control breaths followed by five breaths against an inspiratory flow-resistive load (80 cmH₂O·l⁻¹·s⁻¹ at 0.2 l/s), where the load was applied by inflating a balloon on the inspiratory port during the expiratory phase of the fifth control breath so that the subsequent inspiratory effort was loaded (Fig. 1). This inspiratory load was maintained for five successive breath cycles, and expiration remained unloaded. Trials were performed only during wakefulness and NREM sleep.

Diazepam administration. Diazepam was administered in three of the dogs (0.25–0.5 mg/kg) with the aim of preferentially depressing respiratory activity of the UA muscles relative to that of the diaphragm (2) to induce spontaneous UA collapse by the negative intrathoracic pressures developed during inspiratory efforts against an occlusion or resistance, with the fenestration open. For these experiments the dogs were fasted for 15 h before the study and diazepam was administered orally, as close as possible to the beginning of the study (within 10 min), and trials were performed as described in Inspiratory and expiratory occlusions (single breath) and Inspiratory flow-resistive loading (5 successive breaths).

Baseline eupneic breathing pattern (timing, volume, pressures, Pco₂, and EMGs) with the fenestration open or closed was similar on days with and without diazepam; thus these data were pooled for each condition. Similarly, the breathing pattern responses to tracheal occlusions or resistive loads were unaffected by diazepam and were also pooled for each condition (fenestration open and closed), except when comparing within-breadth changes, which were analyzed separately.

Data Collection and Analysis

Sleep staging. Sleep staging was determined from the paper record of each study by using previously described criteria (20). NREM sleep was defined as a synchronized low-frequency EEG associated with an absence of rapid eye movements. REM sleep was defined as a desynchronization of the EEG, eye movements occurring at a rate > 0.5 events per 2 s, and postural muscle atonia. Specific behavioral events such as frequent twitching movements of the nose, ears, lips, and limbs were also commonly observed in this sleep stage. Any trials in which the dog changed state or showed EEG evidence of arousal were excluded from analysis.

Respiratory timing and EMGs. Timing of all respiratory events was based on the moving-time-averaged EMGdi. Once inspiratory time (Ti) was defined, an in-house software package determined ventilatory variables, including expiratory time (Te); tidal volume (Vt); end-tidal Pco₂ (PETco₂); peak negative Pm, Psl, and Ptr; and peak and integrated inspiratory EMGdi and parasternal intercostal EMG.

Within-breath pressure and EMG profiles. We observed that pressure and EMG changes were often linear only during the initial portion of an inspiratory effort and were variably linear for the remainder of the effort (i.e., see Figs. 2 and 5).

UA collapse. During inspiratory efforts against an occlusion or during efforts against resistive loads, with the fenestration open, inspiratory UA collapse was defined as any deviation of Pm from Psl and Ptr (see Figs. 2C and 5C). In dogs 1–3, the UA remained patent during all inspiratory efforts on days when diazepam was not administered but collapsed on days when diazepam was administered (peak plasma levels: 100–200 ng/ml, TDX system, Abbott Laboratories, Irving, TX). In one dog (dog 4), which had previously been observed to snore when asleep, spontaneous UA collapse occurred in all trials (fenestration open) in the absence of diazepam.

Data Selection and Statistical Analyses

For any given trial, occluded or resistive-loaded efforts were compared with control values, which were obtained by averaging the values for the preceding five unloaded breaths. Comparisons were made between the means obtained via multiple trials within a single dog and the mean values obtained for all four dogs.

Eupneic control measurements in each sleep state were compared by using Student’s t-tests, as were the effects of diazepam on resting breathing pattern. In each sleep state the effect of UA negative pressure (fenestration closed vs. open) and UA collapse (fenestration open) were compared with analysis of variance (ANOVA) and for resistive-loaded trials with two-way repeated measures ANOVA. Post hoc tests were performed with t-tests, corrected for multiple comparisons. Statistical significance was inferred when P < 0.05.

RESULTS

Eupneic Breathing Pattern

Differences between eupneic breathing pattern variables, fenestration closed vs. open, were small and
nonsignificant. An exception, however, was a decrease in T\textsubscript{E} when the dogs were breathing with the fenestra-
tion open in NREM sleep (Table 1).

**Single-Breath Occlusions**

Figure 2 shows for one dog during NREM sleep representative polygraph records of the effects of tracheal occlusion maintained for a single respiratory cycle on respiratory timing, respiratory muscle EMGs, and airway pressures. The individual and mean data for each animal are summarized in Table 2.

During tracheal occlusions with fenestration closed (Fig. 2A), relative to eupneic breathing, Ti increased during wakefulness, NREM sleep, and REM sleep (155 ± 8, 164 ± 11, and 161 ± 32\%, respectively; P < 0.05 from eupneic control), reflecting the removal of inhibitory lung inflation reflexes. During tracheal occlusions with the fenestration open (Fig. 2, B and C), an additional inhibitory effect of the UA being exposed to the negative intrathoracic pressure changes was observed, because Ti was further prolonged during wakefulness and NREM sleep (215 ± 52 and 197 ± 28\% of eupneic control values, respectively; P < 0.05) but not during REM sleep (196 ± 42\%) where increased variability resulted in P values slightly below the required level of significance.

Relative to fenestration closed, both the mean rate of rise of EMGdi (peak EMGdi/Ti) and mean electrical activity of the diaphragm (integrated EMGdi/Ti) were decreased when occlusions were performed with the fenestration open (Table 2). Similar significant decreases in the mean rate of fall of Ptr (peak Ptr/Ti) were seen during wakefulness, NREM sleep, and REM sleep (Figs. 3 and 4). No additional inhibitory effects were seen on Ti, EMGdi, or Ptr when the UA spontaneously collapsed in the presence (dogs 1–3) or absence (dog 4) of diazepam (Fig. 2C).

Although these changes in EMGdi indicated a general-ized inhibitory effect of UA negative pressure, analysis of within-breath changes revealed that the degree of inhibition was greater as the inspiratory effort proceeded (Fig. 2, B and C). Ptr decreased linearly throughout most efforts when tracheal occlusions were performed with the fenestration closed, whereas EMGdi tended to depart from linearity (Figs. 3 and 4).

During occlusions with the fenestration open and UA exposed to negative intrathoracic pressures developed during the effort, a linear decrease in Ptr would be seen initially, followed by a departure from linearity (Fig. 2, B and C). The Ptr at which this departure occurred was similar regardless of whether the UA remained patent or collapsed during the effort. Beyond this point of inflection the pattern of pressure change was variable, ranging from no further change, such as the plateau in Ptr seen in Fig. 2C, to a decreased rate of change of Ptr, such as in Fig. 2B. The mean data for all dogs are shown in Fig. 3, and variability of the responses in one dog is shown in Fig. 4.

In dog 4, in which UA collapse occurred in all trials with the fenestration open in the absence of diazepam, similar changes were also seen in the parasternal intercostal EMG.

**Inspiratory Resistive-Loaded Breaths**

Representative polygraphs of the responses to inspiratory resistive loading in one dog with the fenestration closed and open are shown in Fig. 5, A–C. The results from all animals in NREM sleep are summarized in Fig. 6.

When dogs breathed with the fenestration closed, the initial resistive-loaded breath was characterized by a significant increase in Ti, PET\textsubscript{CO\textsubscript{2}}, and mean electrical activity of the diaphragm and by decreases in VT and rate of fall of Ptr (P < 0.05). With successive breaths Ti and PET\textsubscript{CO\textsubscript{2}} remained elevated, whereas both the rate of rise of EMGdi and the mean electrical activity of the diaphragm increased. TE was unchanged from control values.

When dogs breathed with the fenestration open and UA exposed to negative pressure, the patterns of changes of these variables were similar to those seen with the fenestration closed; however, the magnitudes of changes were significantly greater for Ti, EMGdi, and Ptr. Ti was prolonged and rate of rise of EMGdi was less during all breaths, and mean electrical activity of the diaphragm was decreased for the first breath only.

In all dogs UA collapse was clearly evident on the initial resistive-loaded breath (Fig. 5C). With successive efforts the airway became more resistant to inspiratory collapse, as seen by the more negative intrathoracic pressures developed before P\textsubscript{m} departed from P\textsubscript{sl} and Ptr and the tendency for the airway to transiently reopen and then collapse again (Fig. 5C). When dogs were breathing against the resistance with the fenestration open, changes in all variables were similar whether or not the UA collapsed.

### Table 1. Eupneic breathing pattern during wakefulness and sleep: effect of fenestration closed vs. open

<table>
<thead>
<tr>
<th>State</th>
<th>No. of trials</th>
<th>Ti, s</th>
<th>Te, s</th>
<th>V\textsubscript{T}, liter</th>
<th>Ve, l/min</th>
<th>No. of trials</th>
<th>Ti, s</th>
<th>Te, s</th>
<th>V\textsubscript{T}, liter</th>
<th>Ve, l/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Awake</td>
<td>16 ± 10</td>
<td>1.67 ± 0.23</td>
<td>4.40 ± 1.13</td>
<td>0.37 ± 0.06</td>
<td>3.84 ± 0.86</td>
<td>33 ± 20</td>
<td>1.71 ± 0.16</td>
<td>3.99 ± 0.64</td>
<td>0.35 ± 0.07</td>
<td>3.82 ± 0.58</td>
</tr>
<tr>
<td>NREM sleep</td>
<td>58 ± 20</td>
<td>1.55 ± 0.14</td>
<td>4.19 ± 1.12</td>
<td>0.35 ± 0.05</td>
<td>3.91 ± 1.00</td>
<td>121 ± 48</td>
<td>1.55 ± 0.14</td>
<td>3.84 ± 1.08*</td>
<td>0.33 ± 0.04</td>
<td>3.91 ± 1.00</td>
</tr>
<tr>
<td>REM sleep</td>
<td>10 ± 6</td>
<td>1.56 ± 0.21</td>
<td>3.76 ± 1.72</td>
<td>0.32 ± 0.06</td>
<td>4.21 ± 1.37</td>
<td>10 ± 5</td>
<td>1.57 ± 0.19</td>
<td>3.23 ± 0.69</td>
<td>0.36 ± 0.09</td>
<td>5.03 ± 1.39</td>
</tr>
</tbody>
</table>

Values are means ± SD of 4 dogs when upper airway (UA) was isolated (fenestration closed) or exposed (fenestration open) to intrathoracic pressures. Each trial represents average of 5 breaths preceding application of occlusion or resistance to trachea. Ti, inspiratory time; Te, expiratory time; V\textsubscript{T}, tidal volume; Ve, minute ventilation; NREM sleep, non-rapid-eye-movement sleep; REM sleep, rapid-eye-movement sleep. *Significantly different from fenestration closed, P < 0.05.
Fig. 2. Polygraph records from 1 dog (dog 1) of tracheal occlusions performed during non-rapid-eye-movement (NREM) sleep with fenestration closed (and UA isolated from intrathoracic pressure changes; A), fenestration open and intrathoracic pressures transmitted to a patent UA (B), and fenestration open and evidence of UA collapse (divergence of Pm from Psl and Ptr; C). EMGdi, diaphragm EMG (raw and integrated (int)); EMGgg, genioglossus EMG (raw and integrated); VT, tidal volume.
Inspiratory efforts against resistive loads were also accompanied by a linear decrease in Ptr when the fenestration was closed and alinear changes in Ptr and EMGdi when the fenestration was open (Fig. 5, A–C).

**DISCUSSION**

The results of this study demonstrate an inspiratory modulation of central drive by reflexes originating in the UA when negative pressure is spontaneously generated in response to occlusions or increased airway resistance in the awake and sleeping dog. This reflex inhibition of neural drive to the inspiratory pump muscles, and the resultant decrease in the inspiratory intrathoracic pressures generated, occurs throughout the inspiratory phase, although the magnitude of the inspiration is disproportionately greater later in the inspiratory effort. Activation of mechanoreceptors by negative pressure-induced distortion of the UA would appear to be a likely candidate to mediate this reflex. Collapse of the UA did not potentiate this inhibition of central drive.

**Eupneic Breathing Pattern**

The data from this study indicate that pressure-sensitive receptors in the UA have little influence on the eupneic pattern of breathing during wakefulness or sleep. These findings do not mean that UA receptors are not important to breathing pattern, because negative pressures of greater magnitudes than normally seen during eupneic breathing (see UA Negative Pressure Effects on Drive and Timing) or concurrent activation of these receptors by negative pressure and other inhibitory stimuli (i.e., flow, temperature, CO2) have been shown to influence eupneic breathing pattern.

Previous studies have demonstrated that square-wave negative pressure applied to the isolated UA will prolong Ti and decrease the motor output to the diaphragm (14–15, 18, 27, 28). Similar inhibitory effects have been reported in studies comparing eupneic nasal and tracheal breathing (13, 18), such that nasal breathing is associated with a prolonged Ti and decreased peak inspiratory flow, although these findings remain controversial because other investigators using similar techniques have reported no changes in breathing pattern (25). Differences between these studies may be due to the variable reflex effects of stimulation of other UA mechanoreceptors during nasal and tracheal breathing such as flow receptors (6, 23), local UA chemoreceptors (3, 6), or thermal receptors (24), the influences of

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**Table 2. Tracheal occlusion during wakefulness and sleep: effect of fenestration closed vs. open**

<table>
<thead>
<tr>
<th></th>
<th>Fenestration Closed</th>
<th>Fenestration Open</th>
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<tbody>
<tr>
<td></td>
<td>No. of trials</td>
<td>T1, s</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Awake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog 1</td>
<td>4</td>
<td>2.9 ± 0.5</td>
</tr>
<tr>
<td>Dog 2</td>
<td>8</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>Dog 3</td>
<td>23</td>
<td>2.7 ± 0.4</td>
</tr>
<tr>
<td>Dog 4</td>
<td>3</td>
<td>2.1 ± 0.5</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>10 ± 9</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NREM sleep</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog 1</td>
<td>41</td>
<td>3.0 ± 0.7</td>
</tr>
<tr>
<td>Dog 2</td>
<td>28</td>
<td>2.4 ± 0.4</td>
</tr>
<tr>
<td>Dog 3</td>
<td>26</td>
<td>2.5 ± 0.5</td>
</tr>
<tr>
<td>Dog 4</td>
<td>30</td>
<td>2.2 ± 0.4</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>31 ± 17</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REM sleep</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog 1</td>
<td>5</td>
<td>3.6 ± 1.1</td>
</tr>
<tr>
<td>Dog 2</td>
<td>8</td>
<td>2.5 ± 0.6</td>
</tr>
<tr>
<td>Dog 3</td>
<td>2</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td>Dog 4</td>
<td>9</td>
<td>2.1 ± 0.7</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>6 ± 3</td>
<td>2.5 ± 0.7</td>
</tr>
</tbody>
</table>

Values are means ± SD of each dog when UA was isolated (fenestration closed) or exposed (fenestration open) to intrathoracic pressure changes during a single effort against a tracheal occlusion. EMGdi(RR), mean rate of rise of diaphragm EMG; EMGdi(MEA), mean electrical activity of diaphragm. *Significantly different from fenestration closed, P < 0.05.
which are removed in the present study by use of the fenestrated UA technique.

Application of square-wave negative pressure to the UA severalfold in excess of that normally present during eupnea results in an inhibition of inspiratory drive (7, 14, 15, 18, 27, 28), the magnitude of which is proportional to the level of the applied pressure (7, 18, 28). However, when sustained square-wave negative pressures of similar magnitude to those developed during eupnea are applied to the UA of awake dogs, no changes in breathing pattern are seen (18). We extend these findings by demonstrating that UA negative pressure has no effect on the eupneic pattern of breathing during NREM or REM sleep when the UA pressure changes are generated by the spontaneously breathing dog during inspiration.

Loaded Breathing (Occlusions and Resistances)

By use of the isolated UA preparation and the fenestrated tracheostomy tube, it was possible to match the timing, magnitude, duration, and shape of pressure changes as would be seen during efforts against a spontaneously obstructed or narrowed airway. Matching of normal UA pressure pattern was deemed important because previous studies have demonstrated that the pattern of mechanical perturbation is critical to determining the effect (6, 7, 11, 17, 18, 28). Furthermore, study of different sleep states was thought to be important because 1) sleep minimizes behavioral effects present during wakefulness and 2) REM sleep is known to affect many types of feedback reflex effects (7, 26, 29).

UA Negative Pressure Effects on Drive and Timing

The most striking finding of this study was the marked modulation of within-breath inspiratory drive by UA negative pressure. The inhibition of drive when the UA was exposed to inspiratory intrathoracic pressure was reflected in decreases in the mean rate of rise and mean electrical activity of the EMGdi and was
accompanied by corresponding changes in Ptr. This inhibitory effect was apparent in all sleep states and was also present during resistive loading of the airway. Such an immediate depression of inspiratory drive has been reported previously in studies using square-wave negative pressures applied to the UA and maintained throughout the initial respiratory cycle (11, 17, 18, 27, 28) and in studies comparing the responses to first-breath nasal and tracheal occlusions (13, 18, 22).

During inspiratory resistive loading in NREM sleep, we observed that the rate of rise of EMGdi was consistently decreased when the fenestration was open vs. closed, regardless of whether it was the initial or subsequent resistive-loaded breaths. Thus the inhibitory effect of UA negative pressure persisted, even in the presence of increased chemical stimuli. There was a tendency for the rate of rise of EMGdi to increase toward control values with successive breaths. However, the fact that this compensatory effect was similar whether the fenestration was closed or open indicates that UA negative pressure reflexes, per se, were probably not adapting: rather, the increasing drive reflected the influence of increasing chemical stimuli (19). These findings differ from previous studies that have demonstrated adaptation of UA pressure-sensitive receptors in response to held, static negative pressures in the UA (11, 12, 16–18). Differences between these studies and the present one most probably represent methodological differences, i.e., transient (inspiration only) vs. held pressure and graded (in proportion to inspiratory drive) vs. square-wave pressure.

A potential contributing factor to the decreased rate of rise of EMGdi and prolongation of Ti during efforts with the fenestration open vs. closed may be the effect of added dead-space volume, and the subsequent increased compliance, that the UA adds to the respiratory system when the fenestration is open. However, we believe that this effect is negligible because we found no differences in the response to occlusions with the fenestration open and UA patent (in which case the entire UA contributes to the added volume) and with the fenestration open with UA collapsed (in which case the only added volume is that below the site of collapse). Furthermore, the finding in one dog that adding a dead space approximating that of the UA and mask to the tracheostomy tube had no effect on the responses to tracheal occlusions (see METHODS) adds further support to our conclusion that the difference in responses...
Fig. 5. Polygraph records from 1 dog (dog 1) of an inspiratory resistive load applied for 5 successive breaths during NREM sleep with fenestration closed (and UA effectively isolated from intrathoracic pressure changes; A), fenestration open and intrathoracic pressures transmitted to a patent UA (B), and fenestration open with evidence of UA collapse (divergence of Pm from Psl and Ptr; C).
The mechanical consequences of this inhibition of inspiratory drive by UA negative pressure were especially apparent during studies in NREM sleep in which resistive loads were applied to the airway with the fenestration closed and open. In both conditions load compensation was incomplete during all breaths, because VT, minute ventilation (V̇E), and the rate of rise of inspiratory neural activity, thus preserving VT and V̇E. These inhibitory effects were evident when the dog was awake and in NEM sleep, but not significantly so in REM sleep, most likely as a consequence of the inherent variability of breathing pattern in REM sleep, the
study because TE was unchanged from control after the effects of inspiratory negative pressure in the present study. Prolongation of the apnea, which outlasts the stimulus effect on breathing timing. These effects are seen as a expiration also appear to have an inhibitory "memory": Negative pressure pulses applied during late inspiration is observed when it is applied during inspiration. The effect of REM events interrupting Ti prolongation, and the smaller number of observations obtained in this state. The effects of negative pressure in the UA on ventilation depend on in which phase of the respiratory cycle the negative pressure occurs. During inspiration the effects are protective of ventilation: Ti is prolonged, the rate of rise of EMGdi is decreased, and VT and VE are preserved. In contrast, when applied during late expiration, negative pressure sufficient to collapse the UA will inhibit the onset of the subsequent inspiration and result in a central apnea (7). Thus a complete resetting of timing occurs when negative pressure is applied in expiration, whereas only a modulation of the inspiratory ramp is observed when it is applied during inspiration. Negative pressure pulses applied during late expiration also appear to have an inhibitory "memory" effect on breathing timing. These effects are seen as a prolongation of the apnea, which outlasts the stimulus duration (7). We observed no such persisting inhibitory effects of inspiratory negative pressure in the present study because TE was unchanged from control after inspiratory efforts against tracheal occlusions or resistive loads, even though inhibitory afferent stimuli from the UA would be gradually augmented throughout the inspiratory effort and be maximal at end inspiration.

Negative UA Pressure or UA Distortion

Whether the inhibitory effects were mediated by negative pressure itself or negative pressure-induced deformation of the laryngopharyngeal walls is debatable; however, the data from this study would indicate that the magnitude of the response to UA negative pressure is similar regardless of whether the UA collapses or remains patent throughout the inspiratory effort. It should be noted that the "degree" of collapse was variable throughout the efforts, ranging from complete collapse, which was maintained throughout the effort, to initial collapse followed by transient and repetitive reopenings and collapses (see Fig. 5C).

In either case it would seem reasonable to assume that the UA would be subjected to greater "distortion" during an inspiratory effort where the airway collapsed rather than if it remained patent. Thus, for a given intraluminal pressure, a larger number of distortion-sensitive receptors would be activated in the presence of collapse, particularly if the greatest density of these receptors was located at or near the site of collapse (7, 12, 23). It is theoretically possible, however, that the degree of UA distortion may actually be less when there is UA collapse because the portion of the airway above the site of collapse may be subjected to less negative pressure and smaller distorting forces than if the airway remained patent. This may be particularly relevant when the nares are open and the airways above the site of collapse are open to atmospheric pressure, such as occurs in obstructive sleep apnea.

In the present study, for any given negative pressure, the degree of inhibition of diaphragm activity was similar regardless of whether the UA collapsed. Although these data do not support the idea that UA collapse in some way potentiates the inhibitory afferent signals arising from the UA, the marked alinearity of pressure and EMG changes in the presence of UA negative pressure does suggest a role for UA deformation in modulating inspiratory drive. These findings differ from those of Harms et al. (7), who recently demonstrated that square-wave negative pressure pulses, applied during inspiration, had to be of sufficient magnitude to collapse the isolated UA before inspiratory drive was inhibited. The different findings may be attributable to the method of application of pressure (square-wave vs. graded decrease) and the effects of each perturbation on the activation patterns of the UA receptors mediating the response.

Of the receptors in the UA of the adult dog, those responsive to negative pressure have been shown to be the most common (23). Mediated by afferents in the superior laryngeal, glossopharyngeal, and trigeminal nerves, activation of these receptors can influence

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Table 3. UA muscle activation during tracheal occlusion in NREM sleep

<table>
<thead>
<tr>
<th></th>
<th>Fenestration Closed</th>
<th>Fenestration Open</th>
<th>Without UA collapse</th>
<th>With UA collapse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of trials %Activation</td>
<td>No. of trials %Activation</td>
<td>No. of trials %Activation</td>
<td></td>
</tr>
<tr>
<td>Genioglossus EMG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog 1</td>
<td>37</td>
<td>62</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>Dog 2</td>
<td>25</td>
<td>84</td>
<td>44</td>
<td>98</td>
</tr>
<tr>
<td>Dog 3</td>
<td>18</td>
<td>67</td>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td>Dog 4</td>
<td>21</td>
<td>5</td>
<td>44</td>
<td>47</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>25 ± 8</td>
<td>55 ± 34</td>
<td>21 ± 20</td>
<td>99 ± 1</td>
</tr>
<tr>
<td>Geniohyoid EMG</td>
<td></td>
<td></td>
<td>52 ± 12</td>
<td>91 ± 17</td>
</tr>
</tbody>
</table>

Percentage of trials in which UA muscle EMG activity was observed during inspiratory efforts against tracheal occlusions with fenestration closed or open (with and without UA collapse). UA collapse was evident in all trials in dog 4; therefore, no measurements were obtained in this dog with fenestration open and UA patent throughout the effort.
phrenic nerve discharge (12). Graded changes in UA negative pressure as a consequence of inspiratory efforts against an occlusion or resistive load may initially cause activation of low-threshold receptors (slowly adapting receptors found mainly in the surface mucosal wall) and the additional activation of high-threshold receptors (rapidly adapting receptors found deeper in laryngeal joints and muscles) as intraluminal pressure becomes more negative (12, 23).

**UA Muscles**

In contrast to the inhibitory effects of inspiratory UA negative pressure on drive to inspiratory pump muscles, increased activation of the genioglossus and geniohyoid muscles was observed during efforts against an occlusion or resistive load when the UA was exposed to intrathoracic pressure changes. Activation of these UA dilator muscles was seen during wakefulness and NREM sleep but not in “phasic” REM sleep in which UA muscle atonia persisted. These findings are consistent with previous studies that have demonstrated strong genioglossus muscle activation in response to square-wave negative pressures during wakefulness (9) and an attenuated response during NREM sleep (8, 27). Although UA muscles appear not to respond to static negative pressure stimuli during REM sleep (7), it is interesting to note that oscillating pressure waves in the isolated UA of the dog in REM sleep can produce significant activation of the genioglossus, a response Plowman et al. (21) attributed, in part, to rapidly adapting UA polymodal receptors sensitive to vibration.

We found differences in the activation patterns of the genioglossus and geniohyoid muscles in NREM sleep. Genioglossus activity was still noted in 55% of the trials during occlusions with the fenestration closed, that is, in the absence of negative pressure in the UA. In contrast, only 2% of these trials were accompanied by activation of the geniohyoid muscle. Factors that could potentially explain this finding include the preferential recruitment of genioglossus vs. geniohyoid muscle by UA “drive” receptors (23) or an effect of posture that is associated with UA muscle activity.

**Implications**

This study has demonstrated that in the unanesthetized, awake, and sleeping dog, negative pressure in the UA does not modulate the eupneic pattern of breathing when the UA is patent and intraluminal pressures are low. However, when airway resistance is increased, or during efforts against an occluded airway, in which case UA negative pressure is substantially more negative, feedback from UA afferents sensitive to pressure change will have a profound effect on the pattern of breathing and respiratory muscle activation.

Whereas the effect of negative UA pressure is excitatory to UA muscles during wakefulness and NREM sleep, but not REM sleep, the effect on central respiratory drive is inhibitory in all states. During occlusions in NREM sleep, it is apparent that the magnitude of inhibition is variable throughout the effort, such that greater inhibition is seen later in the effort. This is advantageous in that such a depression will decrease the collapsing pressure seen in the UA. This inhibitory effect on central drive does not appear to further compromise the capacity of the respiratory system to compensate for increased load during sleep, because the increase in Ti offsets the decreased rate of inspiratory neural output and ventilation is maintained at a level comparable to that seen in the absence of UA negative pressure. Although beneficial in terms of decreasing the collapsing pressure and maintaining airway patency during obstructive apneas, this reflex may be detrimental during central apneas, in which UA collapse during the apnea (1) may distort the UA and delay the onset of the subsequent inspiratory effort, thereby prolonging the apnea (7).

Our findings also have methodological implications for quantifying respiratory motor output. We found that linearity arises in neural respiratory output as inspiration progresses and airway narrowing intensified. This time course of modulation of neural respiratory output by upper airway deformation challenges conventional linear methods of defining the “neural drive” to breathe, which we believe must be dealt with by using many parameters of timing, total area, and “shape” to adequately define the linear effects of upper airway reflexes, which change throughout the breath.

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