Reflex Activities of the Upper Airway Muscles during Experimental Nasal Occlusion in Anesthetized Dogs

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ABSTRACT. Reflex responses of the upper airway muscles during experimental nasal and tracheal occlusions were studied in 21 anesthetized dogs breathing through the nose or a tracheostomy (tracheal cannula). Of the animals, five were examined for maintained negative and positive pressures applied to the isolated upper airway. When the nose was occluded at end-expiration during three consecutive breathings, the electromyographic (EMG) activity of alae nasi (AN) and posterior cricoarytenoid (PCA) muscle markedly increased. The upper airway was subjected to negative pressure, \(-2.41 \sim -3.04 \text{kPa} (n=34)\) by these nasal occlusions. The activity of upper airway muscles was also increased by tracheal occlusions. However, such augmentation of PCA activity was much less in tracheal occlusions than nasal occlusions, whereas there was the smaller difference in AN activity between both occlusions. During nasal occlusions, a prolongation of the inspiratory time (T_i) was observed. Furthermore, maintained negative pressure applied to the isolated upper airway provoked an augmentation of PCA activity. Such an augmented EMG activity of PCA and the prolongation of T_i were largely diminished by sectioning the superior laryngeal nerves. These results proves that the upper airway muscles play an important role to protect the upper airway from collapsing by nasal occlusions. In addition, it is suggested that a lack of lung volume feedback is substantially concerned with the activation of AN, while negative pressure in the upper airway is essential to stimulate PCA. — KEY WORDS: negative pressure, obstruction, superior laryngeal nerve, tracheal occlusion, upper airway.

The upper airway is a source of various respiratory reflexes such as sneezing, coughing, sniffing, glottal closure and bronchomotor actions which have been recognized as protective and defensive mechanisms in the respiratory tract [14]. The upper airway is also associated with some pathophysiological conditions such as snoring, sleep apnea, laryngeal paralysis or spasms and airway obstructions [7, 16].

In particular, the obstruction in the upper airway is important from the aspects of veterinary clinical medicine since there are many cases of the obstruction due to structural abnormalities of the nasophaqyngal-laryngeal regions in some dogs such as brachycephalic breeds [4]. The upper airway obstruction is also produced by various infectious diseases that cause nasal mucus secretion and swelling of mucous membrane, and by neoplasms in the upper airway. Such obstructions in the upper airway should produce respiratory distress which is manifested by serious respiratory effort. In such obstructive conditions, certain reflex mechanisms would be elicited to protect the airway from obstruction, and thus maintain the ventilation.

This study was performed to elucidate the reflex activities of upper airway muscles, which contribute to the upper airway patency, in experimental nasal and tracheal occlusions.

MATERIALS AND METHODS

Animals and anesthetics: Twenty-one mongrel dogs of either sex, weighing between 7.0 and 17.0 kg, were used. They were anesthetized with a mixture of urethane (25%) and alpha-chloralose (2.5%) injected intravenously, and placed on an operating table in the supine position. During experiments the same anesthesia was added up to a total dose of approximately 1 g/kg b.w. of urethane and 0.1 g/kg b.w. of alpha-chloralose through a cannula placed in the femoral vein.

Surgical procedure: A polyethylene cannula was inserted into the femoral artery and connected to a pressure transducer (GOULD Statham, P23Db) to monitor blood pressure. The longitudinal incision was made in the neck in order to expose the larynx and trachea. The right and left superior laryngeal nerves (SLNs) were identified in the vicinity of laryngeal cartilage and isolated from the surrounding structures. A tracheal cannula with two side
arms was placed into the trachea below the cricoid cartilage (Fig. 1). This cannula allowed the dog to breathe via a tracheostomy or nose by switching the ventilation route. A saline-filled polyethylene catheter (1.5-mm I.D.) was placed into the midportion of the esophagus and connected to a pressure transducer for recording esophageal pressure (Peso). A nosemask (volume = 35 ml) was constructed around the nose with quick-setting epoxy after the mouth was fastened with a thread and sealed with the same epoxy (Fig. 1).

The electromyogram (EMG) of the sternal portion of diaphragm (DIA), alae nasi (AN) and posterior cricoarytenoid muscle (PCA) were recorded with a pair of enamel-coated copper wires. These EMG activities were amplified with a biophysical amplifier and integrated (time constant = 0.1–0.2 sec).

Pressure changes in the nose mask or tracheal cannula were detected with a pressure transducer (Toyoda, PD104) connected to one of the side arms of the tracheal cannula or to the opening of the nose mask. These pressure signals were amplified with a DC-amplifier (Toyoda, AA6610).

**Nasal and tracheal occlusions:** Sixteen dogs spontaneously breathing through the nose were challenged with nasal occlusions by closing the frontal opening (Fig. 1, A) of the nose mask with a balloon catheter inflated at end-expiration for following three consecutive breathings. Tracheal occlusions were also examined in which the midportion (Fig. 1, B) of two side arms of the tracheal cannula was occluded at end-expiration for three consecutive breathings. These occlusions were repeated 4 or 5 times at an approximately 5 minute-interval.

**Application of maintained pressure to the upper airway:** In order to assess the effect of nasal pressure changes on respiratory activities of the DIA, PCA and AN, maintained positive (+0.5 ~ +1.8 kPa) and negative (~0.5 ~ −5.8 kPa) pressures were applied to the nasal cavity through the nose mask in the dog breathing through a tracheostomy, while the nasopharynx was isolated with occlusion by inflating a balloon.

These pressures were also applied to the whole area of the upper airway (nasal cavity, pharynx, larynx and mouth) while the midportion of two side arms of the tracheal cannula was obstructed by inflating a balloon.

**Section of SLNs:** In order to elucidate the contribution of the laryngeal afferent on respiratory reflexes during nasal and tracheal occlusions, the internal branch of the superior laryngeal nerve (SLN) was bilaterally sectioned. Nasal and tracheal occlusions were carried out after the cut of SLNs.

**Analysis by CT-image:** The movement and aperture of the glottis (vocal cord) was determined by CT (computed tomography; HITACHI MEDICAL CORPORATION, CT-W400) analysis during nasal and tracheal occlusions before and after the section of SLNs.

**Data analysis:** Several respiratory measurements were made as follows: (1) Duration of inspiration (T1) from the onset of the integrated diaphragmatic activity to its rapid fall which indicated the onset of expiration, (2) Peak EMG activities of DIA, PCA and AN from the baseline to the top in their integrated activities, (3) Mean inspiratory slope (Peak DIA/T1) calculated as the ratio between peak diaphragmatic activity and T1.

These measurements were performed for each of three breaths during both nasal and tracheal occlusions, and their values were compared with mean values of four breaths immediately before occlusions. In the experiments with application of maintained pressures, the same measurements were carried out for three to fifteen breaths during the application of negative or positive pressure. The results obtained from these analysis were expressed as percent change from the control. The data was tested for statistical analysis by Wilcoxon's t-test or U-test and the difference was considered significant if P<0.05.

**RESULTS**

The effect of diversion of breathing route: When breathing was diverted from a tracheostomy (tracheal cannula) to the nose, the magnitude of
negative pressure in Peso increased significantly (P<0.05), i.e., from -0.59 (tracheal breathing) to -0.71 kPa (nasal breathing). Peak DIA, PCA and AN activities were also increased by this diversion, showing 17.6, 6.0 and 65.2% increase.

**Nasal and tracheal occlusions:** The airway was occluded at end-expiration at the nose or trachea during three consecutive breathings. During these occlusions, the dog showed an inspiratory effort which was represented by increased diaphragmatic activity and a large swing of negative pressure in Peso. During nasal occlusions, the upper airway was subjected to negative pressure, i.e., -2.41, -2.75 and -3.04 kPa (n=34) at the 1st, 2nd and 3rd effort, respectively (P<0.05). Peak PCA and AN activities considerably increased, showing 164, 235, 308% and 257, 621, 1080% increases, respectively (Fig. 2 and Table 1). Peak DIA activity also increased, as 28.3, 48.5 and 68.3% increases were shown in each effort.

In these occlusions a prolongation of T1 (87.4, 70.4 and 58.7% increases) was observed. The mean slope of inspiration (Peak DIA/T1) showed 31.4% and 11.8% decreases in the 1st and 2nd effort, but 9.3% increase in the 3rd effort.

During tracheal occlusions Peak DIA activity increased (25.1, 36.4 and 50.3% above the control level) and Peso showed negative pressure (-1.82, -1.97 and -2.13 kPa) in each effort, whereas there was no significant difference in these changes between the tracheal and nasal occlusions. Peak PCA and AN activities were increased (47.0, 65.9 and 86.3% increases in PCA and 202, 378 and 760% increases in AN) by tracheal occlusions. However, these changes were much less than those in nasal occlusions mentioned above. Especially, there was a considerable difference in the peak activity of PCA between the tracheal and nasal occlusions (P<0.05). On the other hand, there was no significant difference of AN activity between these two occlusions (P>0.05). A prolongation of T1 (54.1, 38.2 and 30.1%) and a decrease or increase of Peak DIA/T1 (-20.2, -1.86 and +17.4%) were observed during tracheal occlusions. However, these changes were apparently smaller than those in nasal occlusions (P<0.05) (Table 1, Fig. 3).

The majority of augmentation of such respiratory reflexes during nasal occlusions was largely diminished by sectioning the SLN bilaterally (Figs. 2 and 3).

**Analysis by CT-image:** The CT-image analysis of the larynx indicated that both horizontal and vertical dimensions of the glottis (vocal cord) were enlarged during inspiratory effort by the occlusions, to the greater extent in nasal occlusions than tracheal occlusions (Table 2). Such an increased patency of the glottis in nasal occlusions was markedly reduced by sectioning the SLNs.

**The effect of maintained pressure in the isolated upper airway:** Maintained negative (-0.5 ~ -5.8 kPa) and positive (+0.5 ~ +1.8 kPa) pressures were applied to the isolated upper airway in the dog breathing through a tracheostomy (Fig. 4). The maintained negative pressure in the entire upper airway produced a significant increase in the peak activity of PCA with a wide range of 25.1~694.6% (mean, 211%). Such increase was markedly small in AN (mean, 15.1%). The augmentation in PCA activity was clearly associated with the magnitude of...
Table 1. Changes in the upper airway muscle activities and other respiratory parameters during three consecutive nasal and tracheal occlusions

<table>
<thead>
<tr>
<th>Nasal Occlusion</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ptr(kPa)</td>
<td>-2.41±0.12</td>
<td>-2.75±0.15</td>
<td>-3.04±0.17</td>
<td>34</td>
</tr>
<tr>
<td>Peso (kPa)</td>
<td>-1.72±0.07</td>
<td>-1.89±0.09</td>
<td>-2.06±0.11</td>
<td>30</td>
</tr>
<tr>
<td>Peak DIA(%)</td>
<td>+28.3±2.8</td>
<td>+48.5±4.0</td>
<td>+68.3±6.3</td>
<td>30</td>
</tr>
<tr>
<td>Peak PCA(%)</td>
<td>+163.6±17.9</td>
<td>+234.5±22.9</td>
<td>+307.9±25.2</td>
<td>34</td>
</tr>
<tr>
<td>Peak AN(%)</td>
<td>+256.7±40.3</td>
<td>+620.8±119.2</td>
<td>+1079.8±242.8</td>
<td>26</td>
</tr>
<tr>
<td>Tl(%)</td>
<td>+87.4±5.8</td>
<td>+70.4±5.5</td>
<td>+58.7±5.7</td>
<td>34</td>
</tr>
<tr>
<td>Peak DIA/Tl(%)</td>
<td>-31.4±2.8</td>
<td>-11.8±4.2</td>
<td>+9.28±6.7</td>
<td>30</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tracheal Occlusion</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ptr(kPa)</td>
<td>-2.64±0.13</td>
<td>-2.96±0.16</td>
<td>-3.27±0.19</td>
<td>34</td>
</tr>
<tr>
<td>Peso(kPa)</td>
<td>-1.82±0.07</td>
<td>-1.97±0.10</td>
<td>-2.13±0.14</td>
<td>30</td>
</tr>
<tr>
<td>Peak DIA(%)</td>
<td>+25.1±3.1</td>
<td>+36.4±4.2</td>
<td>+50.3±5.7</td>
<td>30</td>
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<tr>
<td>Peak PCA(%)</td>
<td>+47.0±7.6</td>
<td>+65.9±9.1</td>
<td>+86.3±10.7</td>
<td>34</td>
</tr>
<tr>
<td>Peak AN(%)</td>
<td>+202.0±38.8</td>
<td>+377.6±66.6</td>
<td>+759.5±148.1</td>
<td>26</td>
</tr>
<tr>
<td>Tl(%)</td>
<td>+54.1±4.1</td>
<td>+38.2±3.4</td>
<td>+30.1±3.7</td>
<td>34</td>
</tr>
<tr>
<td>Peak DIA/Tl(%)</td>
<td>-20.2±2.6</td>
<td>-1.86±4.6</td>
<td>+17.4±8.0</td>
<td>30</td>
</tr>
</tbody>
</table>

a) Differences are significant (P<0.05; Wilcoxon’s t-test, comparison with tracheal occlusion). Each value represents percent change (mean±S.E.) from the control.

Fig. 3. Changes in the upper airway muscle activity and some representative parameters of respiratory function during nasal and tracheal occlusions before and after the section of SLNs. Abbreviations are the same as in Figs. 1 and 2. *; Differences are significant (P<0.05; Wilcoxon’s U-test, comparison with nasal occlusion with intact SLNs).

Fig. 4. Reflex responses of upper airway muscles to maintained negative pressure applied to the isolated upper airway. Abbreviations and time marker are the same as in Fig. 1 and 2.

negative pressure applied to the upper airway (Fig. 5). A slowing of respiratory timing as well as a prolongation of duration of these muscles’ activities were also observed (Figs. 4 and 5). When maintained negative pressure was applied to only the
Table 2. Changes in patency of the vocal cord during nasal and tracheal occlusions

<table>
<thead>
<tr>
<th>Nasal Occlusion (SLNs intact)</th>
<th>Horizontal Plane</th>
<th>Vertical Plane</th>
<th>number</th>
</tr>
</thead>
<tbody>
<tr>
<td>+38.8±7.1&lt;sup&gt;o&lt;/sup&gt;</td>
<td>+7.6±1.3&lt;sup&gt;o&lt;/sup&gt;</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Nasal Occlusion (SLNs cut)</td>
<td>+29.4±4.7</td>
<td>+4.2±0.6&lt;sup&gt;o&lt;/sup&gt;</td>
<td>5</td>
</tr>
<tr>
<td>Tracheal Occlusion</td>
<td>+10.5±3.0</td>
<td>+4.9±0.5</td>
<td>8</td>
</tr>
</tbody>
</table>

a) Differences are significant (P<0.05; Wilcoxon’s U-test, comparison with tracheal occlusion). b) Difference is significant (P<0.05; Wilcoxon’s U-test, comparison with nasal occlusion with intact SLNs). Each value represents percent change (mean±S.E.) from the control.

Fig. 5. Changes in PCA activity during different negative pressures in the upper airway. Abbreviations and time marker are the same as in Figs. 1 and 2.

Fig. 6. Changes in PCA and AN activities by maintained negative pressure to the isolated upper airway. The negative pressure was applied to the nose (left column) and the entire upper airway (middle and right columns). The SLNs were cut at the right column. Abbreviations are the same as in Figs. 1 and 2. *: Differences are significant (P<0.05; Wilcoxon’s t-test, comparison with the control level before application of negative pressure).

nasal cavity, all these effects were minimum (Fig. 6). These reflexes by maintained negative pressure applied to the entire upper airway were mostly eliminated by sectioning the SLNs (Fig. 6). These respiratory activities were unchanged by maintained positive pressure applied to either the entire upper airway or nasal cavity.

DISCUSSION

The upper airway is constructed or surrounded by many intrinsic and extrinsic muscles, i.e., posterior cricoarytenoid (PCA), cricothyroid, thyroarytenoid, lateral cricoarytenoid, interarytenoid, sternothyroid, sternohyoid, genioglossus muscles and alae nasi (AN) [13]. The activity of these muscles play an important role in maintaining normal breathing [2, 3, 11]. Especially, AN and PCA have been known as main airway dilating muscles of which activities can decrease airway resistance by their abduction in humans and many animals such as dog, cat and rabbit [1, 2, 12, 13]. In humans the nasal resistance with voluntary activation of the alae nasi is 40-50% less than with voluntary inhibition of these muscles, while nasal resistance has been estimated to be two-thirds of the airway resistance during nose breathing [12]. PCA is the primary muscle that controls the laryngeal aperture [7, 14], and serious obstructive disorders are produced if this muscle is paralyzed in the dog as well as the other animals [4]. Therefore, it must be important that the activity of these upper airway muscles during airway occlusions is investigated in detail. In this study the EMG activity was recorded from AN and PCA together with diaphragm that is a representative muscle in respiratory function.

The respiratory activity of AN and PCA was increased by switching the breathing route from a tracheostomy to the nose. This increased activity might reflect the increase in airway resistance which was shown as an augmentation of diaphragmatic
activity and esophageal pressure change during nasal breathing.

The respiratory activity of AN and PCA markedly increased when the airway was occluded at the nose or trachea. Although this enhancement of PCA was observed in both the nasal and tracheal occlusions, it was significantly larger for PCA in nasal occlusion than tracheal occlusion (Fig. 3). The enhancement of AN activity was considerable even if the occluded airway did not include the upper airway, i.e., nose, larynx, pharynx and the mouth.

It is assumed that the augmentation of PCA activity in nasal occlusions was produced by negative pressure transmitted to the upper airway since the upper airway was subjected to large subatmospheric pressure during nasal occlusions. In fact, a large number of 'negative pressure' receptors have been recorded from the SLN in the dog [6, 9] and such receptors have been considered to play an important role on the reflex mechanism in the upper airway occlusions [8, 14, 15]. The difference in the strength of inspiratory effort during tracheal and nasal occlusions was small (Table 1) and therefore the respiratory 'drive' [9] would contribute to only a minor extent to the augmented PCA activity in nasal occlusions.

The augmented activity in PCA during nasal occlusions was largely diminished by sectioning both the SLNs, while the augmented AN activity was less diminished by such elimination of laryngeal afferents (Fig. 3). Therefore, the laryngeal afferent component is considerably important in activation of PCA, while it is not necessarily a predominant factor on AN. In the latter muscle, the lack of volume feedback in the lung might be a major factor to stimulate it because the tracheal occlusion produced a great augmentation of AN activity and there was no significant difference between the tracheal and nasal occlusions.

In this study, maintained negative pressure applied to the entire upper airway evoked the reflex augmentation of AN and PCA activities, where the increase in PCA activity was clearly greater than that in AN. This result confirms that the negative pressure in the upper airway was a strong and adequate stimulus for PCA, while it was not in case to AN. As for the AN activity in nasal occlusions, the stimulatory effect of negative pressure would be small since only 15.1% increase was provided by the application of maintained negative pressure to the entire upper airway, while in contrast the greater increase (more than 257%) was observed in PCA activity. These findings were partially in agreement with those reported in the experiments with the rabbit [5] and dog [11], although these studies emphasized the greater effect of negative pressure in the activation of the AN as well as PCA.

During nasal occlusions the duration of inspiratory time (T₁) prolonged markedly, whereas the increase in the peak diaphragmatic activity was present to the smaller extent. These changes resulted in a decrease in the mean inspiratory slope (Peak DIA/T₁), especially in the 1st effort, during nasal occlusions (Table 1). Hence, the upper airway has a mechanism to depress the velocity of inspiratory process in the early stage of nasal occlusions, mediating by the superior laryngeal nerve. Such a mechanism serves to protect the upper airway from a 'rapid' exposure to the negative pressure during upper airway occlusions, while the augmented activity of PCA and AN must help to facilitate the upper airway patency and thereby reduce the airway resistance [5, 7, 8]. The wide aperture of the glottis during nasal occlusions was also elucidated by CT-analysis in this study (Table 2). These changes would also contribute to stabilize the upper airway.

It has been reported to exist pressure receptors in the trigeminal nerve responding to negative pressure in the nose of the rat [10]. However, the maintained negative pressure to the nasal cavity caused only little change in the reflex, indicating that the nasal pressure receptors seem to play a slight role on the reflex mechanism in nasal occlusions in the dog.

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REFERENCES


