Characteristics of Glottic Closure Reflex in a Canine Model

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Purpose: The most important function of the larynx is airway protection which is provided through a polysynaptic reflex closure triggered by the receptors in the glottic and supraglottic mucosa, evoking the reflex contraction of the laryngeal muscles especially by strong adduction of vocal cords. Based on the hypotheses that central facilitation is essential for this bilateral adductor reflex and that its disturbance can result in weakened laryngeal closure, we designed this study to elucidate the effect of central facilitation on this protective reflex. **Materials and Methods:** Seven adult, 20 kg mongrel dogs underwent evoked response laryngeal electromyography under 0.5 to 1.0 MAC (minimum alveolar concentration) isoflurane anesthesia. The internal branch of the superior laryngeal nerve was stimulated through bipolar platinum-iridium electrodes, and recording electrodes were positioned in the ipsilateral and contralateral thyroarytenoid muscles. **Results:** Ipsilateral reflex closure was consistantly recorded regardless of anesthetic levels. However, contralateral reflex responses disappeared as anesthetic levels were deepened. Additionally, late responses (R2) were detected in one animal at lower level of anesthesia. **Conclusions:** Deepened level of anesthesia affects central facilitation and results in the loss of the crossed adductor reflex, predisposing to a weakened glottic closure response. Precise understanding of this effect may possibly provide a way to prevent aspiration in unconscious patients.

Key Words : Glottic closure reflex, anesthesia, central facilitation, aspiration

INTRODUCTION

The physiologic functions of the larynx can be classified into several systems such as respiratory, phonatory and protective reflex system. Although primitive, the protective reflex may be the most important in protecting the lung against aspiration. The larynx provides this protection through a reflex closure triggered by the receptors in the glottic and supraglottic mucosa, thereby evoking the reflex contraction of laryngeal muscles. This reflex is a polysynaptic brain stem response,[1-3] and the starting point is the input of signals into the internal branch of the superior laryngeal nerve (iSLN).

After electrical stimulation of one iSLN, the electromyographic potential can be recorded in the ipsilateral or contralateral thyroarytenoid (TA) muscle. Three categories of protective laryngeal responses have been observed. Firstly, an early response involves adduction of the ipsilateral vocal cord with a latency of approximately 10 to 18 msec in anesthetized cats, dogs, and pigs.[4-7] This short latency-evoked response, termed R1, has also been noted consistently in humans under anesthesia.[4] The second category of laryngeal responses involves simultaneous, contralateral adduction with a short-latency, also known as the crossed adductor reflex. Although this response has consistently been found in anesthetized cats, it is less consistently found in dogs and pigs, and rarely in anesthetized human subjects.[4] The third category of adductor response involving a longer latency reflex, termed R2, has been observed to produce bilateral responses, but its presence is most readily noted in awake human subjects with a latency of 50 to 80 msec.[8]

These observations indicate to us that we cannot demonstrate either the crossed R1 or R2 adductor reflex in fully anesthetized animals and humans. On the other hand, the presence of crossed reflexes in awoken subjects suggests the possibility that this response may be dependent on anesthesia.

When these reflexes are exaggerated or hypersensitive, it may be responsible for several disorders such as spasmodic dysphonia, idiopathic laryngospasm, reflex apnea, apnea of infancy, stuttering, sudden infant death syndrome, etc.[3,9-14] The loss of these reflexes is an equally or more important problem. Absence of these protective responses may place patients into a devastating situation, in which the chance of aspiration increases, resulting in an aspiration pneumonia fatal to the patient's life. Therefore, a precise understanding of this effect may improve the prevention of aspiration in patients at risk who are emerging from prolonged sedation[15,16] or under heavy psychotropic control.[17]

The purposes of this study are to confirm our hypothesis that when the supramedullary influences are abolished by general anesthesia, the crossed adductor response is also abolished[18] and to provide an explanation for increased risk of aspiration in unconscious patients through the understanding of this laryngeal reflex responses.

MATERIALS AND METHODS

Seven young mongrel dogs were used in this experiment. Subjects were approximately 6 to 8 months of age and their weights were at around 20 kg. A canine larynx model was selected because of its anatomic and physiologic similarities to humans.[4]

Atropine and Xylazine, 0.05 and 2 mg/kg, respectively, were administered together as induction agents. Anesthesia was maintained through an endotracheal intubation. electrocardiography (EKG), respiratory rate, and pulse oximetry were constantly monitored. And core body temperature was maintained at 38°C using a heating pad.

Sufficient amount of time, at least 90 minutes, was allowed for the pharmacological effects of induction agents to clear. During this period, preparatory procedures described below were carried out prior to the initiation of the study protocol.

After routine antiseptic preparation, a midline neck incision was made that ectended from the hyoid bone to the sternal notch. A low tracheotomy was done below the level of the fourth tracheal ring, and an endotracheal tube (internal diameter 6 mm) was inserted into the trachea and secured to the skin. Subsequent inhalational anesthesia with isoflurane was maintained through the tracheotomy. Both the right and left iSLNs were identified and sectioned proximal to the entrance to the thyrohyoid membrane. Sufficient portion of the thyroid cartilage was removed to allow full access to and visualization of both vocal cords.

MP 100 WSP data acquisition system (Biopac Systems, Inc., Santa Barbara, CA, USA) provided nerve stimulation and electromyogram (EMG) recording capabilities. Bipolar platinum-iridium stimulating electrodes were applied to the proximal end of each severed iSLN. Monopolar platinum recording electrodes were inserted into the mid-portion of TA muscles bilaterally. Reference electrodes were placed in the ipsilateral strap muscles, and a ground electrode was placed in the sternocleidomastoid muscle. Because the crossed adductor response may be obscured by bilateral iSLN stimulation, the iSLN was stimulated unilaterally in sequential fashion.

In Protocol 1, the left iSLN in each subject was electrically stimulated sequentially under varying depths of anesthesia. Each subject was exposed to five different anesthesia depths (less than 0.5, 0.5, 0.75, 1.0, and more than 1.0 MAC) and minimal alveolar concentration; 1 MAC is defined as 1.5% of inhaled isoflurane concentration,[19] and is a concentration to suppress electroencephalogram (EEG) waves characterized by increased activation of delta wave, and 2 MAC is a concentration to silent wave.[20,21] No other pharmacologic agent was used during the period of data collection. At least 20 minutes of equilibration time were allowed following change in each anesthetic level.

Stimulation parameters consisted of single, rectangular pulse of 0.1 mA intensity with 0.1 msec pulse duration. Stimulation intensity was increased incrementally by 0.1 mA until a consistent EMG response was seen in the TA muscle or until the maximum stimulation intensity was attained. An average of six trials were performed in each experimental paradigm.

In Protocol 2, identical procedures to Protocol 1 were repeated on the right side.

The ratios of R1, crossed R1 and R2 responses obtained in Protocols 1 and 2 were calculated and the latencies of each response were averaged. Statistical analysis was performed by Student's t-test.

RESULTS

Protocol 1

When the left iSLN was electrically stimulated, the average latencies of ipsilateral and contralateral evoked responses with their standard deviations are summarized in Table 1. We could calculate the percentage of responses with the number of evoked responses compared to stimulus presentations under each level of anesthesia. It is noted that ipsilateral R1 responses occured at all depths of anesthesia with almost 100 % efficiency (Fig. 1A, B). However, the number of contralateral R1 responses underwent a dramatic decline with anesthesia levels over 0.75 MAC. By 1 MAC, only 1 out of 17 stimulations resulted in contralateral R1 evoked
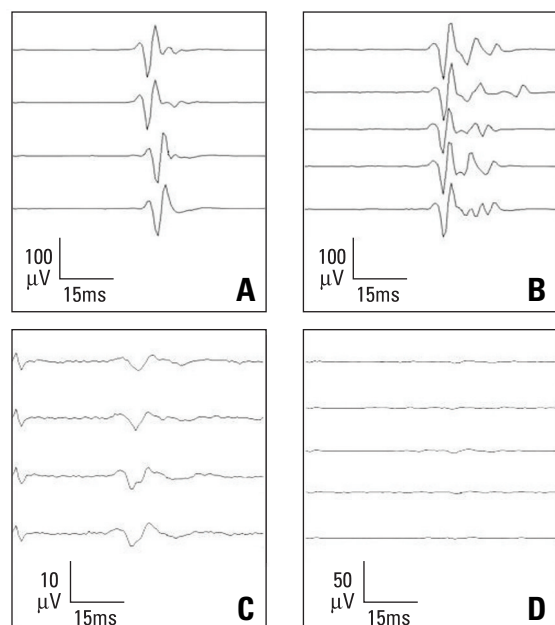
Fig. 1. Stimulation of the left internal branch of SLN. Compound muscle action potential (CMAP) recordings obtained from ipsilateral thyroarytenoid muscle at (A) 0.5 MAC, and (B) 1 MAC, from contralateral thyroarytenoid muscle at (C) 0.5 MAC, and (D) 1 MAC.

responses. Above 1 MAC, no contralateral responses were elicited ($p < 0.010$) (Fig. 1D). When the percent of contralateral responses obtained below 1 MAC was compared to those obtained above 1 MAC, the differences were statistically significant ($p < 0.010$) (Table 1).

Protocol 2

Results of Protocol 2 are shown in Table 2. Again, as with the results of Protocol 1, the number of contralateral response underwent marked declination with anesthesia levels above 0.75 MAC. No contralateral response could be elicited with anesthesia levels at or above 1 MAC. When the percent of contralateral responses obtained below 1 MAC was compared to those obtained above 1 MAC, the differences were again statistically significant ($p < 0.010$) (Table 2).

In one subject, R2 responses with the average latency of 84 msec (range 55.5-136 msec) were recorded at 0.5 MAC anesthesia level (Fig. 2).

DISCUSSION

As in our previous study with pig model, the canine model

Table 1. Left iSLN Stimulation

			MAC		
	< 0.5	0.5	0.75	1.0	> 1.0
Ipsilateral R_1					
Efficiency (%)	30 / 30 (100)	5 / 5 (100)	17 / 17 (100)	34 / 34 (100)	31 / 33 (94)
		52 / 52 (100)		65 / 67 (97)	
Latency (ms)	26.7 ± 1.2	26.2 ± 1.5	26.7 ± 1.4	24.7 ± 3.4	27.4 ± 0.9
Contralateral R_1					
Efficiency (%)	20 / 30 (67)	11 / 15 (73)	5 / 20 (25)	1 / 17 (6)	0 / 23 (0)
		36 / 65 (55)*		1 / 40 (2.5)*	
Latency (ms)	27.3 ± 0.7	29.4 ± 1.0	28.8 ± 0.7	28.0 ± 0.0	

MAC; minimal alveolar concentration.
Latency: average ± 1 SD.
*$p < 0.010$ (Student's t-test).

Table 2. Right iSLN Stimulation

			MAC		
	< 0.5	0.5	0.75	1.0	> 1.0
Ipsilateral R_1					
Efficiency (%)	19 / 19 (100)	6 / 6 (100)	15 / 15 (100)	33 / 33 (100)	32 / 34 (94)
		40 / 40 (100)		65 / 67 (97)	
Latency (ms)	21.4 ± 2.6	21.6 ± 0.4	21.8 ± 1.4	22.6 ± 2.4	26.1 ± 1.6
Contralateral R_1					
Efficiency (%)	24 / 27 (89)	19 / 21 (90)	13 / 26 (50)	1 / 15 (7)	0 / 16 (0)
		46 / 74 (62)*		1 / 31 (3.2)*	
Latency (ms)	22.3 ± 2.3	23.8 ± 0.8	24.0 ± 1.6	26.5 ± 0.0	

MAC; minimal alveolar concentration.
Latency: average ± 1 SD.
*$p < 0.010$ (Student's t-test).

also appears to support the notion that a facilitated adductor reflex is likely responsible for a crossed R1 in the awoken state, whereas its sensitivity to pharmacologic sedation would imply that relevant facilitatory mechanisms are located central to motor neurons of nucleus ambiguus.[22]

The reason why the overall latencies obtained in this study are bigger than those of previous studies with canine model[4,6] can be explained by the fact that we used younger dogs that are not fully mature.[23] Furthermore, the longer latency of the left rather than right ipsilateral evoked response understandably reflects the longer course of the left recurrent laryngeal nerve.[6]

After electrical stimulation of iSLN, we can expect the swallowing reflex as well as the laryngeal responses. The esophageal muscle of dogs is entirely composed of striated fibers, and therefore is controlled by cranial motoneurons.[24] Accordingly, we can expect the existence of esophageal motility during checking the laryngeal EMG when we stimulate the iSLN. However, a typical rhythmic pattern of swallowing was elicited during long-lasting repetitive stimulation of iSLN.[24] Thus, we could not only exclude the possibilities of electrical interruption of esophageal peristalsis, but also avoid the aspiration which could exaggerate the laryngeal responses mimicking the laryngospasm as happens in gastroesophageal reflux.

Interestingly, the latency of contralateral evoked response was about 1.5 to 2 msec longer than that of ipsilateral response. Assuming that nerve conduction velocity approximates 5 cm/msec,[25] that the average length of neural circuitry from larynx to brain stem is 20 cm, and that each synaptic delay is 1.5 msec,[25] the data support the following organizational model. In our model, we propose that the
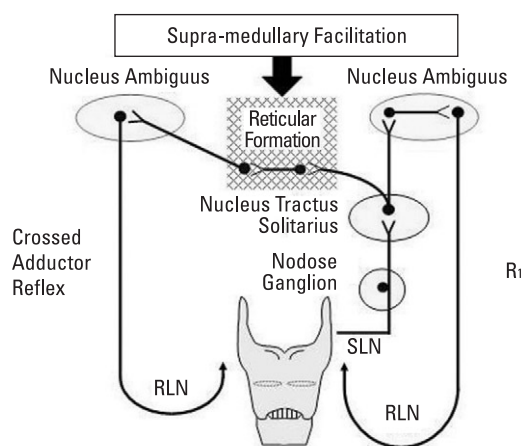
iSLN projects to motor neurons of nucleus ambiguus through at least two synapses, the first within ipsilateral nucleus tractus solitarius, and the second likely within nucleus ambiguus ipsilaterally and contralaterally within the reticular formation in a manner supported by Sessle's observations.[26] In recent studies, it was suggested that the pig model has equivalent numbers of ipsilateral and contralateral interneurons,[22] and that humans have 2 to 3 more contralateral interneurons compared to ipsilateral side.[27] However, in our current study with canine model, the fact that the contralateral latency was about 2 msec longer than ipsilateral one leads us to suggest the presence of one more interneuron possibly in the reticular formation (Fig. 3).

The presence of R2 found in our study, which has not been observed in anesthetized animal models,[1] can be a valuable data to investigate the level of awakening to affect the laryngeal reflex.

In summary, the glottic closure response appears bilaterally when the contralateral response is supported by central facilitation in awoken state, but anesthesia abolishes the crossed response, thus restricting the response to an ipsilateral one. Therefore, the anesthetic loss of crossed adductor response is expected to weaken the glottic closing force even under the normal conditions of physiologic stimulation when stimuli are delivered bilaterally and concurrently to each iSLN. A complete suppression of crossed response under anesthesia may exert a clinically important effect. Pharmacologic suppression of supramedullary facilitation gives us a clear explanation for several important clinical findings, including the increased incidence of life threatening aspiration among sedated ICU patients or institutionalized psychiatric patients under heavy psychotropic control where aspiration pneumonia represents a highly significant risk. The results of this study are expected to provide the neurophysiologic basis for preventive measures in high risk patients and could, for example, alter dosing schedules of psychotropic medications, improve respiratory

monitoring, institute lifesaving dietary modifications, or suggest cautious management of patients who are awakening from anesthesia after surgery. It might also stimulate the search for alternative pharmacologic agents that do not alter facilitation of the glottic closure response without losing their specific effects. A series of basic research using various animal species should be preceded before applying the precise mechanism of this reflex to human in the future.

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