Effects of Volatile Anesthetics on the Activity of Laryngeal ‘Drive’ Receptors in Anesthetized Dogs

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ABSTRACT. Effects of halothane, isoflurane and sevoflurane on laryngeal drive receptor activity were studied in the afferent activity of the superior laryngeal nerve in anesthetized spontaneously breathing dogs. Of 40 single units recorded, most of them (65%) responded to the volatile anesthetics applied to the isolated larynx at a concentration of 5%. The exposure to the anesthetics resulted in either an inspiratory increase (15%), both inspiratory and expiratory decrease (54%), or both inspiratory increase and expiratory decrease (31%) responses. The average discharge frequency of the receptors tended to be decreased on inhalation of the anesthetics, where significant decreases were observed in both respiratory phases for halothane and at expiration for isoflurane, but in neither respiratory phase for sevoflurane. These results support an advantage of sevoflurane over halothane and isoflurane for induction of anesthesia to minimize the influence of the activity of laryngeal drive receptors on the breathing pattern and airway stability. —KEY WORDS: canine, control of breathing, laryngeal afferent, laryngeal muscle, volatile anesthetic.

The larynx is a potent reflexogenic region of the upper airway which is rich in sensory receptors. In a recent study we suggested the activation of these receptors by application of volatile anesthetics into the larynx of dogs [10]. The induction of anesthesia in this manner promotes the exertion of upper airway reflexes such as inhibited breathing (apnea), breath-holding, coughing and laryngospasm [1, 4, 5, 6, 16]. The responses may not delay the induction but induce it in patients at risk for hypoxemia. The sensory innervation of the larynx is mainly supplied by the internal branch of the superior laryngeal nerve (SLN) [21, 23, 24]. It is well known that the initiation of the upper airway respiratory reflexes is under the control of non-respiratory-modulated laryngeal irritant and capsaicin-sensitive C-fibers. Besides these fibers, the SLN contains a substantial number of other receptors showing signs of strong respiratory modulation [21, 23, 24]. Three types of laryngeal respiration-modulated receptors which are affected by changes in transmural pressure, temperature, and laryngeal motion have been identified from single unit recordings of SLN afferents [21, 23, 24]. Among them, a prevalent effect of laryngeal ‘negative pressure’ receptors on the breathing pattern and upper airway patency during collapsing pressure has been reported in several studies [17, 18], but these receptors did not show responsiveness to volatile anesthetics [12]. Moreover, the ventilatory depressive effect is pronounced by cooling of the larynx in newborns, presumably through the stimulus of laryngeal cold receptors, but often completely absent in adult animals [21]. Nishino et al. [12] reported that some of the laryngeal cold receptors were stimulated by inhalation of halothane, but not by isoflurane and enflurane.

In the larynx, laryngeal muscle contraction and/or tracheal tug with respiration activates sensory receptors known as laryngeal ‘drive’ receptors [21, 23, 24]. Recent studies have clarified important roles of laryngeal drive receptors in maintaining upper airway patency and the breathing pattern: Curran et al. [3] suggested that respiratory frequency is increased after section of the SLN in dogs breathing through a tracheostomy, and the reflex prolongation of expiration time induced by upper airway collapse was greatly reduced. It is probable that feedback from the laryngeal ‘drive’ receptors acts as a kind of ‘sensor’ of larynx distortion which regulates the breathing pattern and airway patency.

Laryngeal drive receptors have been recognized as naked nerve endings in the airway muscle, acting like spindles [7]. In previous studies, slowly adapting pulmonary stretch receptors which are sensitive to stretching of the airway smooth muscle were greatly influenced by volatile anesthetics [11, 13]. It is possible that volatile anesthetics can also affect the laryngeal drive receptors, and thus affect ventilation and airway condition during the induction of anesthesia with volatile anesthetics, but the electrophysiological data on the endings are limited. The purpose of the present study is to evaluate the effects of recently available volatile anesthetics on the activity of laryngeal drive receptors in dogs.
MATERIALS AND METHODS

Animals and basal anesthesia: Fifteen healthy beagle dogs (8 males and 7 females) were used in this study. Their mean age was 13.6-months (ranging from 10 to 18 months) and mean body weight was 9.7 kg (ranging from 7.6 to 14.0 kg). Anesthesia was induced with thiopental sodium (25 mg/kg), then maintained with a mixture of urethane (500 mg/kg) and α-chloralose (50 mg/kg) injected intravenously. A supplemental dose of urethane (200 mg/kg) and α-chloralose (20 mg/kg) was injected hourly through an intravenous catheter placed into the cephalic or saphenous vein. All the dogs were euthanized after the experiment by an intravenous injection of pentobarbital sodium (50 mg/kg).

Animal preparation: The dogs were placed on an operating table in the supine position, and a laryngeal mask (LMA® Size-3, Intervent, UK) was introduced to cover the larynx, including the epiglottis (Fig. 1). Arterial blood pressure was monitored with a pressure transducer (DXT-300, Nihon Kohden, Japan) connected to a catheter inserted into the femoral artery. A thermal probe was inserted just below the epiglottis through the laryngeal mask to record laryngeal temperature. The cervical trachea was exposed in its entire length and cut longitudinally to insert a tracheal cannula with two side arms. A pressure transducer (PD 104, Toyota Machine Works, Japan) was attached to the most cranial sidearm of the cannula to measure upper airway pressure. A saline-filled polyethylene catheter (O.D. = 3 mm) was placed in the middle portion of the esophagus and connected to a pressure transducer (DX-300, Nihon Kohden, Japan) for recording esophageal pressure.

The internal branch of the SLN was transected bilaterally at the junction with the external branch, and the peripheral cut end on the left side was split into fine filaments in preparation for recording its electrical activity. The recurrent nerve was also cut bilaterally to avoid secondary respiratory-modulated reflexes mediated by the nerves.

Recordings of laryngeal afferent activity: Afferent activity of the nerve filament was recorded with a pair of platinum electrodes. The nerve trunk was dissected into several thin filaments until a single unit activity was clearly discriminated. These nerve preparations were produced within a pool of paraffin oil by using fine forceps with the aid of a binocular microscope (SZ 60, OLYMPUS, Japan). The signal was amplified by a low noise DC-amplifier (DPA 201, DIA Medical, Japan) and a biophysical amplifier (DPA 200, DIA Medical, Japan), and displayed on an oscilloscope (SS 5762, IWATSU, Japan) in parallel with a loudspeaker (Model 7747, NEC san- ei, Japan). All the signals were displayed on a thermal-array recorder (RT 3100N, NEC san- ei, Japan) and copied by a magnetic tape recorder (PC 204A, SONY, Japan). Once an acceptable single unit was found, the sensory mode of that particular ending was identified by the conventional method [19], from which respiration-modulated 'pressure', 'drive', and 'cold' receptors were characterized. Briefly, the identification was performed on the basis of recordings under four respiratory conditions: (1) upper airway breathing (U.A.B.), in which the dogs were allowed to breath via the oral cannula, with the two sidearms of the tracheal cannula occluded (Fig. 1, A open; B1 and B2 closed); (2) tracheostomy breathing (T.B.) in which the dogs breathed via the most cranial sidearm of the tracheal cannula (Fig. 1, B2 open; closed at C); (3) upper airway occlusion (U.A.O.), in which the dogs were subjected to occlusion of both the oral and tracheal cannula at either end-inspiration or end-expiration (Fig. 1, B1 and B2 closed; occluded at A); and (4) tracheal occlusion (T.O.), in which the caudal side of the trachea was occluded (Fig. 1, closed at C, occluded at B3).

The diversion of the breathing route and the occlusions during 3 consecutive breaths were made by inflating Foley catheters. In this study the type of receptor which had spontaneous discharges during T.B. and augmented activity during T.O. was categorized as a 'drive' receptor. The location of each receptive field was established by touching the laryngeal mucosa with the Foley catheter or the external surface of the larynx with a cotton stick. In addition, the larynx was manually displaced in both caudal and cranial directions.

Experimental protocol: The upper airway was functionally isolated by inflating the cuff of a Foley catheter at the middle position of the tracheal cannula, which allowed tracheostomy breathing (Fig. 1, B2 open; closed at C). After a control period of more than 1 min with 100% oxygen at a flow rate of 6 L/min through the isolated upper airway in the expiratory direction, all fibers were exposed to a 5% concentration of each of three anesthetics for 1 min: sevoflurane, halothane and isoflurane. The order of administration was random. If any response of the receptor was observed, 1 and 3% of each anesthetic were tried. The anesthetic vaporizer was the usual type for each anesthetic.
(FLUOTEC Mark 3 for halothane, Cyprane; FORAWIC for isoflurane, Muraco Medical; S-3 for sevoflurane, Kimura Medical) and was precalibrated for the intended anesthetic concentration (5% = dial vol.5, full dial setting; 3% = dial vol.3; 1% = dial vol. 1). The intralaryngeal anesthetic concentration was monitored with an infrared gas monitor (AGM-103 Capnomac, Datex, Finland) which was attached by means of a sampling tube connected to the uppermost sidearm of the tracheal cannula.

Data analysis: The discharge frequencies of single unit activity during each inspiratory and expiratory phase were counted with a spike height discriminator (DSE 425, DIA Medical, Japan). The mean discharge frequency was counted breath by breath to 3 breaths during the control and test periods at the maximum response after the onset of inhalation. In addition, the time when the receptor was stimulated or inhibited by the anesthetic was measured. Wilcoxon’s signed rank sum test or the Mann-Whitney U-test was employed for comparison of the data. To compare the differences among anesthetic groups, a one-way ANOVA test was run, followed, when necessary, by Tukey’s multiple comparison test. To determine concentration-related changes in the receptors, the correlation of peak values of individual receptor response to each anesthetic at different concentrations (1, 3 and 5%) were calculated by using simple linear regression analysis. All data were expressed as the mean \( \pm \) SE. \( P<0.05 \) was considered statistically significant.

RESULTS

Laryngeal ‘Drive’ receptors: Single unit activities were recorded from a total of 40 ‘drive’ receptors, which were stimulated by T.O. at either end-expiration [Fig. 2, T.O.(E)] or inspiration [Fig. 2, T.O.(I)]. Twenty-nine (29/40, 73%) of the inspiration-modulated receptors showed signs of tonic activity (Fig. 3 types ‘a’ and type ‘b’), and the rest (11/40, 27%) had phasic activity during U.A.B. and T.B. (Fig. 2). Thirty-two (32/40, 80%) of the ‘drive’ receptors responded to both intra- and extra-luminal probings at a level between the cricoid cartilage and epiglottis. Thirty drive receptors were stimulated by cranial (6/30, 20%) or caudal (24/30, 80%) tracheal displacement.

Effects of volatile anesthetics on the laryngeal drive receptors: Twenty-six drive receptors (26/40, 65%) showed an excitatory (Fig. 3, type ‘a’) or inhibitory (Fig. 3, type ‘b’) response to volatile anesthetics at a concentration of 5%. The exposure to the anesthetics, irrespective of their type, resulted in either an increased response (4/26, 15%) which was observed predominantly during the inspiratory phase, or a decreased response (14/26, 54%, Fig. 3 type ‘b’), observed in both inspiratory and expiratory phases. Abrupt and transient inhibition of the drive receptor activities (e.g., Fig. 3, type ‘b’) was observed for 5 receptors by halothane, 3 by isoflurane, and none by sevoflurane. In addition, 8 (31%) drive receptors with tonic activity had both an inspiratory increase and expiratory decrease (Fig. 3, type ‘a’). All of them (26/26, 100%) were sensitive to both intra- and extra-luminal probings at a level between the cricoid cartilage and epiglottis.

Significant decreases in receptor discharges were observed for both inspiratory and expiratory phases for halothane, but a significant decrease was recognized only at expiration in isoflurane and there were no significant changes in sevoflurane during either respiratory phase (Fig. 4). As a whole, the net discharge frequency from all three anesthetics at 5% decreased during inspiratory and expiratory phases. The effect of sevoflurane was significantly less at expiration than with the other two anesthetics, whereas no significant differences were found at inspiration.

The onset of inhibition or excitation of the receptor activity after administration of 5% volatile anesthetics was 30.1 \( \pm \) 3.7 sec in halothane, 37.0 \( \pm \) 4.5 sec in isoflurane, and 48.6 \( \pm \) 6.4 sec in sevoflurane. No statistically significant differences were found in the duration of responses among anesthetics. The excitatory or inhibitory response tended to be prolonged if the inhalation of anesthetics was sustained (data not shown). Some receptors, 7 in halothane (e.g., Fig. 5), 3 in isoflurane, and 1 in sevoflurane, showed increased or decreased discharges in a concentration-dependent manner after the inhalation of each anesthetic at three different concentrations.

![Fig. 2. Activities of a laryngeal drive receptor during upper airway breathing (U. A. B.), tracheal breathing (T. B.), and tracheal occlusions (T. O.) during both expiration (E) and inspiration (I). ENG = electroneurogram, P_{es} = esophageal pressure, P_{ua} = upper airway pressure, Temp. = laryngeal temperature.](image-url)
Afferent activity of laryngeal 'drive' receptors is mostly related to the contraction of intrinsic laryngeal muscles such as the posterior cricoarytenoid muscle (PCA) or passive movements of the larynx [21, 23, 24], and its response can be sustained in the absence of airflow and transmural pressure change across the larynx (e.g., Fig. 2). The results of this study suggest that volatile anesthetics affect the drive receptor activity of the larynx. It is important to note that a total of 65% of the drive receptors responded to volatile anesthetics applied to the larynx. Most of the drive receptors seem to be located relatively deep in the larynx because they required longer duration than other superficial endings to block their activities by application of local anesthesia to the internal surface of the larynx [20]. In this study, the effect of volatile anesthesia was observed 30.1 to 48.6 sec after the beginning of applications, indicating some delay in inducing functional changes in the nerve endings. In a previous study [20], 15% of the drive receptors were not blocked, but the remaining 85% were blocked by lidocaine applied to the laryngeal mucosa with a delay of 9 sec - 14 min. The drive receptors which were blocked by lidocaine or influenced by volatile anesthetics in this study were likely to be located at joints or ligaments close to the internal surface of the larynx. 

DISCUSSION

Afferent activity of laryngeal 'drive' receptors is mostly related to the contraction of intrinsic laryngeal muscles such as the posterior cricoarytenoid muscle (PCA) or passive movements of the larynx [21, 23, 24], and its response can be sustained in the absence of airflow and transmural pressure change across the larynx (e.g., Fig. 2). The results of this study suggest that volatile anesthetics affect the drive receptor activity of the larynx. It is important to note that a total of 65% of the drive receptors responded to volatile anesthetics applied to the larynx. Most of the drive receptors seem to be located relatively deep in the larynx because they required longer duration than other superficial endings to block their activities by application of local anesthesia to the internal surface of the larynx [20]. In this study, the effect of volatile anesthesia was observed 30.1 to 48.6 sec after the beginning of applications, indicating some delay in inducing functional changes in the nerve endings. In a previous study [20], 15% of the drive receptors were not blocked, but the remaining 85% were blocked by lidocaine applied to the laryngeal mucosa with a delay of 9 sec - 14 min. The drive receptors which were blocked by lidocaine or influenced by volatile anesthetics in this study were likely to be located at joints or ligaments close to the internal surface of the larynx.

Fig. 1. Examples of recordings from two laryngeal drive receptors when 5% halothane was inhaled. Type 'a' increased and type 'b' decreased inspiratory discharges after the onset of inhalation, while both types equally decreased expiratory discharges. The horizontal lines show the inhalation time of each volatile anesthetic. Abbreviations as in Fig. 1.

Fig. 2. Responses of laryngeal drive receptors (n = 26) to 5% volatile anesthetics. Each value (mean ± SE) was obtained from recordings of 3 consecutive breaths during each of the control and test (approximately 1 min after the onset of inhalation) periods. The data were expressed as percent change from the control period (before inhalation). * P<0.05 vs control, ** P<0.01 vs control, † P<0.05 vs sevoflurane. Hal = halothane, Iso = isoflurane, Sevo = sevoflurane. Inspi. = inspiratory phase, Expi. = expiratory phase.
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surface of the larynx. In fact, in the present study most of the drive receptors which were influenced by volatile anesthetics could be stimulated by inflation of a balloon catheter inside the larynx, as well as by external proffings.

In this study two types of response - sensitization and depression (Fig. 3, types ‘a’ and ‘b’) - were exhibited by inhalation of volatile anesthetics. It is noteworthy that the responses of the laryngeal drive receptors to volatile anesthetics were mostly consistent with those of slowly adapting tracheobronchial stretch receptors [11, 13]. The net inhibitory effect of halothane on the laryngeal respiration-modulated mechanoreceptors was reported in a previous study [12], which agrees with our results. Abrupt and transient inhibition of drive receptor activities (Fig. 3, type ‘b’) might be caused by the blocking effect of volatile anesthetics on the nerve endings. It is possible that the sensitization of drive receptor activity during inhalation of halothane and isoflurane is due to increased PCA contraction and/or a direct effect on the sensory endings. Curran et al. [3] pointed out that the removal of drive receptor activity increases the compliance of the upper airway, as with the effect of upper airway collapse on genioglossus electromyogram activation in tracheostomy-breathing dogs. On the other hand, several reports suggest that primary muscle spindle endings or arterial baroreceptors are sensitized by halothane [2, 15, 22].

The removal of feedback from the laryngeal drive receptors is a source of the alteration in the breathing pattern and stabilization of the upper airway [3]. Although the elicitation of the powerful airway defensive or protective reflexes such as coughing or laryngospasm by acute inhalation of volatile anesthetics is related to the stimulation of non-respiration modulated laryngeal irritant receptors [10, 12] and capsaicin-sensitive C-fibers [10], the inhibition of laryngeal drive receptors would also affect the regulation of breathing [3]. In fact changes in the breathing pattern, elicited by brief inhalation of halothane and isoflurane in humans, as represented by an increase in respiratory frequency and a decrease in tidal volume, may be a reflection of such responses [4].

Overall quality of the induction of anesthesia is generally higher for sevoflurane than for other anesthetics [25–27]. The breathing pattern during induction is less frequently changed by sevoflurane than by halothane or isoflurane [4]. In the present study the volatile anesthetics tended to decrease drive receptor activities, most remarkably for halothane, less for isoflurane, and least for sevoflurane at the full vaporizer dial setting (5%). Although the minimum alveolar concentration (MAC) of each anesthetic [9] might be considered for the quantitative comparison of anesthetics, the vaporizer percent concentrations were employed for the comparison of the drive receptor activities in this study, since a higher concentration such as 3–5% is generally required in any anesthetic for accelerating the induction period in humans [1, 25–27] and dogs [8, 14]. In view of the above, the results of the present study support an advantage of a high concentration of sevoflurane for the induction of anesthesia over halothane and isoflurane to minimize the effect of the activity of laryngeal drive receptors on the breathing pattern and airway stability.

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