Studies on the actions of volatile anesthetics to the sensory system in the respiratory tract of the dog

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Section 1 Preface

1. 1. Volatile anesthetics as general anesthesia

Recently, volatile anesthetics are used widely for the anesthetic management of animals. Rapid elimination of volatile anesthetics from the body through the lung induces the easy control of anesthetic level. The predictable and stable anesthetic conditions exerted by these drugs contributes their popularity among the anesthetics. In the history of inhalation anesthesia for over 150 years, many volatile anesthetics were developed. In those drugs, less than 20 agents have actually been introduced and approved for general use fro human (Eger, 1982) (Fig. 1) and only 5 are of current clinical importance in veterinary medicine (Steffey, 1996) (Table 1).

Table 1 Inhalation anesthetics

Group 1: Agents in current clinical use for animals	
Major use	Halothane
	Isoflurane
	Sevoflurane
Minor use	Enflurane
	Nitrous oxide (gaseous anesthetic)
Group 2: New agents	
	Desflurane
Group 3: Agents of historical interest	
	Methoxyflurane
	Chloroform
	Cyclopropane
	Diethyl ether
	Fluroxene
	Trichlorethylene

(Adapted in part from Steffey, 1996)

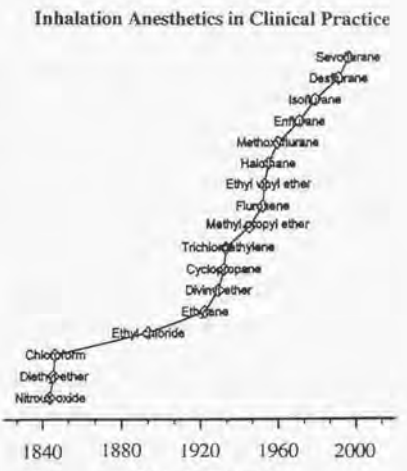


Fig. 1 Inhalation anesthetics introduced for widespread clinical practice. (From Eger, 1982)

The first group of volatile anesthetics listed in Table 1 includes halothane (Hal) ($CF_3CHBrCl$) and isoflurane (Iso) (CF_3-CHCl-O-CHF_2) and sevoflurane (Sevo) [CH_2F-O-$CH(CH_3)$], which have been used most widely in veterinary medicine. Hal has gained popularity for human and veterinary anesthesia in 1960s, and steadily increased in usage to become the predominant inhaled agent in veterinary anesthesia until Iso has been aveilable. Hal decreases arterial blood pressure and myocardial contractile force but has little or no effect on heart rate in dogs (Steffey and Howland, 1977). Respiratory depression of Hal is usually evident, but lesser than that of Iso or Enf in dogs (Steffey and Howland, 1977). Milder airway irritation at low concentration of Hal than that of Iso and Enf has been reported in humans (Doi and

Ikeda, 1993). Although a high incidence of cardiac dysrhythmia has been reported (Bednarski and Majors, 1986), Hal has still been used inhalants in most animals.

Iso has played a major role of anesthetic care in veterinary anesthesia. Iso provides stable cardiac performance, excellent muscle relaxation, but profound respiratory depression (Steffey and Howland, 1977). The lower incidence of cardiac arrhythmia of Iso than with Hal has been reported (Bednarski and Majors, 1986). Lower blood/gas partition coefficient (1.41 at 37°C) of Iso than that of Hal (2.36 at 37°C) provides rapid recovery from anesthesia, whereas airway irritation has been reported in humans during inhalation induction (Doi and Ikeda, 1993).

Sevo, a new halogenated volatile anesthetic recently approved for clinical use in Japan, United Kingdom ,and United States in humans, is a typical of potentially useful volatile anesthetics. Sevo has a low blood/gas partition coefficient (0.65 at 37°C), which is approximately half of that of Iso (1.41 at 37°C) (Eger, 1994). It causes less irritation of the airway mucosa than does Iso in human beings (Doi and Ikeda, 1993) and provides rapid induction of anesthesia in dogs (Mutoh *et al.*, 1995).

Enflurane (Enf) ($CFHCl\text{-}CF_2\text{-}O\text{-}CHF_2$) is still available in minor use for veterinary practice. Because of muscle movements that was produced in dogs (Steffey and Howland, 1977) and the questionable results of its trials in cats, the popularity of Enf in veterinary anesthesia was not reach the same level as that seen in humans. Ventilatory depression of Enf is very pronounced and appears to be greater than that seen with Hal in dogs (Steffey and Howland, 1977; Mutoh *et al.*, 1997a).

Desflurane (Des) ($CF_2H\text{-}O\text{-}CFH\text{-}CF_3$) has the lower blood/gas partition coefficient (0.42 at 37°C) than any other volatile anesthetic in Table 1. Cardiovascular effects of Des are similar to those of Iso in dogs (Merin *et al.*, 1991). In humans, rapid increases in Des concentration is associated with increases in heart rate and arterial blood pressure as a result of sympathetic stimulation (Weiskopf *et al.*, 1994, 1995). Des has higher vapor pressure of 669 mm of Hg and MAC value of 10.3% for the dog, requiring a specially designed and expensive vaporizer which at this time is not adapted in veterinary anesthetic units. Because of these characterestics, Des is unlikely to use for clinical anesthesia in veterinary medicine, and thus excluded from the scope of this study.

1. 2. Airway irritation of volatile anesthetics

As described above, volatile anesthetics provide a number of advantages over disadvantages for a general anesthesia when used properly. We, however, must pay careful attention for use on unpredictable adverse effects during induction of anesthesia. Induction of anesthesia with volatile anesthetics is sometimes complicated by the protective or defensive airway reflexes such as cough, apnea, laryngospasm, and secretion (Drummond, 1988, 1993). Clinically, these reflexes have been presumed to be caused by an irritation on airway mucosa, the degree of which varies with anesthetic and anesthetic depth (Doi and Ikeda, 1993). For example, Iso and Enf are known to cause relatively strong airway irritation when inhaled by face mask (Doi and Ikeda, 1993), and inhalation

induction of anesthesia is very difficult with Iso or Enf in spite of their lower blood/gas partition coefficient in humans (Sampaio *et al.*, 1989; Yurino and Kimura 1992; Doi and Ikeda, 1993). Hal is a slightly milder irritant to airways than Iso and Enf are, but it can produce similar reflex actions when inhaled at higher concentration (Doi and Ikeda, 1993; Yurino and Kimura, 1992, 1994). On the other hand, Sevo is relatively low-irritating to airways providing a rapid and smooth onset of anesthesia in humans coupled with a lower blood/gas partition coefficient (Yurino and Kimura 1992, 1993a, b, 1994; Muzi *et al.*, 1996).

1. 3. Sensory functions of the respiratory tract and lungs

The respiratory system can be divided into the respiratory tract, lungs, and the extrinsic respiratory muscles which are responsible for ventilating the lungs. The respiratory tract and lungs are essentially a tubular cul-de-sac, with quite different structural features in their various parts. In this paper we scoped on the sensory function in three major parts of the respiratory tract and lungs, i.e., nasal cavity, larynx, and lower airway and lungs, all of which being richly supplied with various sensory afferents to be sensitive for various inhaled chemical irritants (Sant'Ambrogio *et al.*, 1995).

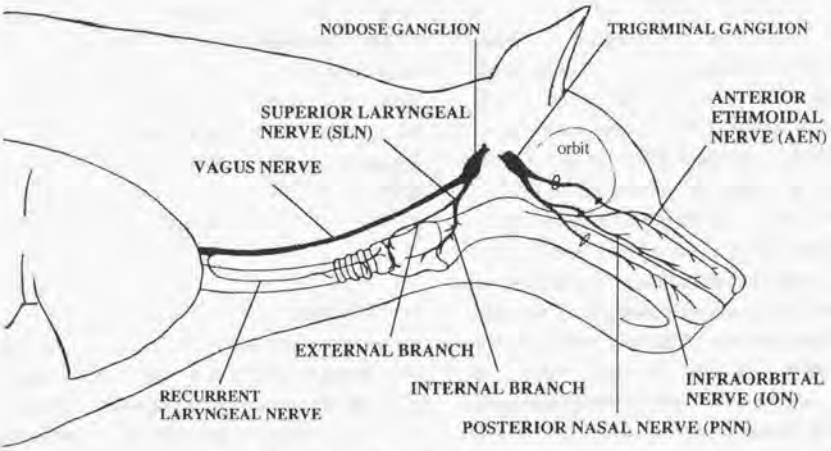


Fig. 2 Schematic illustration of the afferent innervation of the respiratory tract.

1.3.1 Upper airway

Nasal Cavity

It is well known that the nasal mucosa is a potent reflexogenic part of the upper airway. Nasal cavity is an initial pathway of the inhaled irritants, and nasal mucosa riches in sensory afferents which elicite various types of respiratory and circulatory reflex responses (Widdicombe *et al.*, 1988; Sant'Ambrogio *et al.*, 1995).

The sensory innervation of nasal mucosa is supplied by the ophthalmic and maxillary divisions of the trigeminal nerve, i.e. the anterior ethmoidal nerve (AEN), the posterior nasal nerve (PNN), and infraorbital nerve (ION), these trigeminal nerve branches convey a variety of stimuli arising from the nose (Sant'Ambrogio *et al.*, 1995) (Fig. 2). Among these nerve branches PNN has been reported to play a major role for the elicitation of airway reflexes. For example, sneeze reflex can be evoked by the electrical stimulation of PNN (Wallois *et al.*, 1991a), while intranasal cold/flow stimuli or irritant stimuli with ammonia vapor increases the activity of PNN (Wallois *et al.*, 1991b). In contrast to the laryngeal sensory endings of internal branch of the SLN, the definite category of the PNN afferents or the type of the fiber component is still unclear.

Larynx

The larynx has been the major subject of most studies on defensive and regulatory roles of the upper airway because of its anatomical position and powerful reflexogenic mechanisms. The larynx is innervated by the recurrent laryngeal nerve, and the internal and external branches of the superior laryngeal nerve (SLN) (Widdicombe *et al.*, 1988) (Fig. 2). Among them the internal branch of the SLN is the major pathway of afferent fibers from the cranial portion of the larynx. In most of the mammals studied, section of the internal branch of the SLN abolished the reflexes produced by the stimultion of the larynx (Sant'Ambrogio *et al.*, 1995). In dogs the ratio between myelinated and unmyelinated fibers of the internal branch of the SLN is close to unity (Chung *et al.*, 1993), while the cervical vagus nerve has a 1:4 ratio and the vagal bronchial branches 1:11 ratio (Jammes *et al.*, 1982).

With single unit action potential recordings of the internal branch of the SLN, two major groups of specialized receptors associated with or without respiratory-modulation or not (Sant' Ambrogio *et al.*, 1983).

The former category of laryngeal endings is activated by the changes in pressure (pressure receptor), temperature (cold receptor) and laryngeal motion (drive receptor) (Sant'Ambrogio *et al.*, 1983). The latter category of laryngeal endings has a scant or random spontaneous activity, generally unrelated to the respiratory cycle, and can strongly be activated by mechanical or chemical irritants (irritant receptors) and C-fiber receptors (Widdicombe *et al.*, 1988). The presence of unmyelinated C-fibers (C-fiber receptors) in the larynx which response to various noxious chemical stimuli has been clarified in immunological (Shin *et al.*, 1987; Haxhiu *et al.*, 1991) and electrophysiological studies (Tsubone *et al.*, 1991). The summary of the mechanoreceptors, its optimal stimulus, and resultant reflex responses are described in Table 2.

Table 2 Sensory function of laryngeal receptors

Receptors	Optimal stimulus	Reflexes
drive receptor	distortion, stretching	stabilizing airway, respiratory timing ?
pressure receptor	negative or positive pressure	upper airway patency
cold receptor	decrease in temperature (airflow), *l*-menthol (selective stimulant)	slowing of breathing, inhibition of inspiratory demand
irritant receptor	chemical substances, light mechanical touch of the mucosa, distilled water (lack of chroride anions)	cough, bronchoconstriction, glottis closure, secretion
C-fiber receptor	nociceptive chemical stimuli (capsaicin) (selective stimulant)	inhibition of breathing, cough, bradycardia, hypertension

(Adapted from Tsubone, 1994)

1.3.2. Lower respiratory tract and lungs

Sensory innervation from the lower respiratory tract and lungs is predominantly vagal (Fig. 2), and when the vagus nerves are cut, all airway reflexes from the lungs disappear (Widdicombe and Sant'Ambrogio, 1992). The vagi consist largely of unmyelinated fibers (Jammes *et al.*, 1982) like other nerves supplying the respiratory tract. Three types of sensory endings (slowly adapting pulmonary stretch receptor, rapidly adapting irritant receptor, and C-fiber endings) have thus far been recognized in the lower airway and lungs (Coleridge and Coleridge, 1984).

Slowly adapting pulmonary stretch receptors locating along the airway wall are the afferents responsible for the Hering-Breuer inflation reflex mediated by the vagal pathway. Their mounting discharge in inspiration accelerates an inspiratory off-switch mechanism that limits the depth and duration of breathing (Widdicombe and Sant'Ambrogio, 1992). Rapidly adapting receptors are responsive to a very wide range of chemical irritants and inflammatory mediators, and thus these receptors have been called 'irritant receptors'. Keller and Loeser identified it to show irregular or random discharges without respiratory modulation, which adapted rapidly to maintained inflation and deflation of the lungs. Selective stimulation of the irritant receptors by brief pressure pulses triggers an augmented breath and also cuts short the expiratory pause, and thus have been believed that they might mediate the cough reflex (Widdicombe and Sant'Ambrogio, 1992).

C-fiber endings in the lung parenchyma were studied by Paintal (1969). He described them as J-receptors (juxtapulmonary capillary receptors), a term now usually replaced by pulmonary C-fiber receptors. These receptors are distinguished from bronchial C-fiber receptors because the former are affected by the agents put into the pulmonary vascular bed and the latter by agents in the systemic (bronchial) circulation. C-fiber receptors respond to almost the same range of stimuli as that of irritant receptors, but generally C-fiber receptors are more sensitive to chemical and less sensitive to mechanical stimuli (Coleridge and Coleridge, 1984). Vigorous stimulation of the C-fiber receptors cause apnea followed by rapid shallow breathing, or only rapid shallow breathing when the

stimulus is weak (Coleridge and Coleridge, 1984; Widdicombe, 1986). C-fiber receptors are thought to underlie the axonal reflex inflammation seen in airway disease (neurogenic inflammation). These changes are mediated by neuropeptides such as substance P, neurokinin A and calcitonin gene-related peptide (CGRP) to induce vasodilation and edema of vascular beds and muscus secretion (McDonald, 1990).

The summary of the sensory receptors, its optimal stimulus, and resultant reflex responses are described in Table 3.

Table 3 Sensory function of bronchopulmonary receptors

Receptors	Optimal stimulus	Reflexes
slowly adapting pulmonary stretch receptor	stretching of airway wall	respiratory timing inspiratory off-switching
rapidly adapting irritant receptor	chemical substances (SO_2, ammonia vapor, cigarette smoke, histamine, prostaglandins, etc.), light mechanical touch of the mucosa	cough, secretion of muscus, bronchoconstriction
pulmonary and bronchial C-fiber receptors	nociceptive chemical stimuli (capsaicin [selective stimulant], SO_2, ammonia vapor, cigarette smoke, [pulmonary and bronchial C-fibers], serotonin [pulmonary], ozone, histamine, bradykinin [bronchial], prostaglandins [bronchial > pulmonary], etc.), Lung hyperinflation (bronchial < pulmonary)	inhibition of breathing (apnea), rapid shallow breathing, bradycardia, bronchoconstriction, vasodilation,muscus secretion, edema (axon reflexes)

(Widdicombe and Sant'Ambrogio, 1992)

1. 4. Purpose

It is presumed that inhalation of volatile anesthetics produce an airway irritation and reflexes as a result of the stimulation of the airway and pulmonary sensory receptors described above. In fact, Sant'Ambrogio *et al.* (1993) reported that Hal administered into the isolated upper airway remarkably depressed ventilation in newborn dogs, the effect of which was greatly diminished by the SLN section. Nishino *et al.* (1993) pointed out that 5% halogenated volatile anesthetics, especially Hal, activated laryngeal irritant and cold receptors and inhibited respiratory modulated mechanoreceptors in adult dogs. Nishino *et al.* (1994) have also shown that 5% Hal, Enf, and Iso inhibit the activities of slowly adapting pulmonary stretch and rapidly adapting irritant receptors in the tracheobronchial tree. Coleridge *et al.* (1968) demonstrated that pulmonary C-fiber afferents were activated just after inhalation with high concentration (5-20%) of Hal in dogs.

However, non of the studies mentioned above have succeeded in clarification of the source of

airway irritation by volatile anesthetics. Moreover, only a few have targeted the responses of unmyelinated C-fiber receptors in spite of numerous and extensive studies have been conducted on the pulmonary or bronchial C-fiber afferents to other chemical irritants (Coleridge and Coleridge, 1984, 1994). Besides this, the effect of Sevo, a new inhaled volatile anesthetic, on sensory functions has not been investigated in any of the study.

Therefore the purpose of the present study was to elucidate the actions of volatile anesthetics to the sensory system in the respiratory tract in dogs. This thesis consists of following 4 major parts (section 2 to 5). In section 2, as a preliminal study, pulmonary and cardiovascular effects of Hal, Enf, Iso and Sevo during induction of anesthesia were evaluated in dogs. In the following section, respiratory reflexes and afferent activities of bronchopulmonary (section 3), laryngeal (section 4), and nasal (section 5) receptors by Hal, Enf, Iso and Sevo were evaluated in anesthetized dogs.

Section 2 Incidence of airway irritation of volatile anesthetics in dogs

Rapid inhalation induction of anesthesia with volatile anesthetics in dogs

Abstract

Clinical usefulness of rapid induction of anesthesia (RII) with Hal, Enf, Iso and Sevo at 2.5 minimum alveolar concentration (MAC) was evaluated in 24 dogs. The most rapid and smoothest induction was observed by Sevo, followed by Iso and Enf. Hal took the longest induction period. Movements during RII were minimal in Sevo compared with other inhalants. Values of heart rate, cariac index and rate-pressure product were significantly increased after the beginning of inhalation in all inhalants except for Hal. These changes exceeded the physiological level just after the beginning of inhalation, however, rapidly reversed to the maintenance level (1.5 MAC) approximately 10 min after intubation. In conclusion, Sevo seems to be the best inhalational anesthetic for RII in dogs without significant cardiac and/or respiratory diseases, although there were a certain level of problems in cardiovascular functions. Iso also induced rapid induction with some degree of the movements.

1. Introduction

Ideal drugs for induction of anesthesia in small animals must be rapid and smooth onset and safe without any undesired side effects (Saywer, 1982). Mask induction is a choice for induction of anesthesia with volatile anesthetics. Mask induction can be smoothly shifted to maintenance anesthesia by using the same anesthetic apparatus. Recovery from anesthesia is rapid because there is no prolonged effect of intravenous anesthetics. Thus the method is suitable for patients with liver disease or the patients receiving examinations such as CT-scan or minor surgery that needs rapid recovery after the end of these procedures (Harvey, 1992; Paddleford, 1992).

There are two approaches for mask induction (Editorial, 1986; Drummond, 1988). The conventional technique is a slow inhalation induction, which induces anesthesia by an gradual increase in anesthetic concentration at intervals of 30-60sec. Although this technique aims for the patients to accustom to the pungency of anesthetic gas because inhalation anesthetics generally have an irritation on airways, it is impossible to avoid excitement while passing through light level of anesthesia during induction (Editorial, 1986; Drummond, 1988). Another new induction technique is rapid inhalation induction (RII). In RII patients inspire anesthetic gas at a higher concentration by the patient's vital capacity. RII was first described by Ruffe *et al.* (1982) and has been widely accepted in human medicine in the patients to whom an intravenous induction is not desired.

Although inhalation anesthetics are thought as safer and easily adjustable agents, RII has some practical problems. Body movement under restraint may reduce the gross volume of inhaled anesthetics, which will cause prolonged induction time. It is very difficult to force animals to inspire at the animal's vital capacity. The dog sometimes hates to inspire the gas presumably due to strong pungency and/or

airway irritation, and resists to mask induction. In addition, mask induction may cause the strong excitement of dogs which may affect the cardiopulmonary function.

The purpose of the present study is to evaluate the effects of mask induction with RII by Hal, Enf, Iso and Sevo and their cardiopulmonary effects in dogs.

2. Methods

2.1. Dogs—Twenty-four healthy Beagles (12 females and 12 males) were studied. Mean age was 13.4 (range, 10 to 19) months and mean body weight was 9.2 (range, 7.9 to 10.8) kg. Food was withheld at least 12 hours before experiments.

2. 2. Drugs and anesthesia equipment—Hal¥ (Halothane®, Hoechst Japan), Enf (Ethrane®, Dainabot) Iso (Forane®, Dainabot) and Sevo (Sevofrane®, Maruishi Medical) were the anesthetic agents used. A semi-closed circle anesthesia system (Model KA-3020, Kimura Medical), with the vaporizers for each anesthetic agent out of circle, was used.

2. 3. Animal preparation—At least 7 days before an experiment, a 14-gauge heparin-coated polyvinyl chloride catheter (Anthron, Toray Medical) was implanted in dogs under anesthesia with Iso in O_2. Approximately 2 hours before the experiment, a 6-F, 10-cm introducer (SI-5600, Arrow International) was implanted percutaneously into the right jugular vein of dogs by use of local anesthesia with 1 ml of 2% lidocaine.

2.4. Mask induction technique—Before inhalation of each anesthetic, O_2 at a flow rate of 5 L/min was delivered via the face mask. The anesthetic circuit was primed with each vapor at the desired concentration measured by the infrared gas analyzer (AGM-103 Capnomac, Datex), where the gas sample was collected from the connector attached to the face mask at a sternal position with their fore and hind limbs tying together. Then the face mask was fitted over the muzzle of the dog by one person and the inhalation of anesthesia was started. The inhalation of each anesthetic was continued keeping the dogs on the restrainting table until laryngeal reflex disappeared, then the dog was placed on the surgical table and intubated with a cuffed endotracheal tube keeping the animal in lateral recumbency.

2. 5. Experimental design—Dogs were assigned at random to the 4 anesthetic agent groups (n = 6/group). Each dog was positioned on a restraining table (Takeda-style canine restraining table, Clea Japan) in sternal recumbency, and a 5-F Swan-Ganz catheter (Model 93-132-5F, Baxter Healthcare) was inserted through the introducer and advanced into the pulmonary artery to monitor intravascular pressure. The Swan-Ganz catheter was then positioned at the proximal portion of the right atrium. After the dog's condition stabilized, all cardiopulmonary measurements were obtained as baseline values. After measurements in the conscious state (baseline) were obtained, anesthesia was induced, with each anesthetic agent delivered in O_2 at a flow rate of 5 L/min via face mask. Dogs were intubated after induction of anesthesia at 2.5 times the minimum alveolar concentration (MAC) of inspiratory anesthetic concentration, then maintained anesthesia under spontaneous ventilation at 1.5 MAC of end-tidal anesthetic concentration for 30 min. The MAC values of each anesthetic are 0.87% in Hal, 2.06% in Enf, 1.28% in Iso and 2.36% in Sevo, respectively (Quasha *et al.*, 1980; Kazama and Ikeda, 1988; Cheng *et al.*, 1992). Inspired and end-tidal anesthetic concentrations were collected through the sampling line placed at the tip of the cuffed endotracheal tube ($ETCO_2$ cuffed tracheal tube, Sheridan Catheter), and

were monitored by use of an infrared gas analyzer (AGM-103 Capnomac, Datex).

Cardiovascular measurements were repeated every minute during induction period until intubation. The durations from the beginning of inhalation until onset of movements, to end of movements, to loss of laryngeal reflex and to intubation were recorded. Induction was accomplised as the completion of endotracheal intubation. After intubation, all cardiopulmonary measurements were repeated every 10 min. During maintenance anesthesia, pulmonary arterial temperature was maintained at 38.0 ± 0.5°C, using a warming mat.

2. 6. Determination of cardiopulmonary measure-ments—Cardiopulmonary measurements were performed as follows. Heart rate (HR) and ECG were recorded by use of a multi-function electrocardiograph (CMO-104 Cardiocap, Datex). Systolic, mean and diastolic arterial blood pressures (SAP, MAP and DAP) were measured through the arterial catheter, using a calibrated pressure transducer (PR-AS123S, Terumo Co) connected to the multi-function electrocardiograph (CMO-104 Cardiocap, Datex). Right atrial pressure (RAP), mean pulmonary arterial blood pressure (MPAP) and pulmonary arterial wedge pressure (PAWP) were measured through the Swan-Ganz catheter, using the same equipment. Cardiac output (CO) was determined, using the thermodilution (Criti kit Model SP4500, Ohmeda Medical Devices Division Inc) technique, with injection of 3 ml of 0 ° C saline solution into the right atrium during end-expiration. Cardiac output determinations were performed in triplicate, and the mean value of the 3 determinations was used as data. From the aforementioned values, the following cardiovascular variables were calculated: cardiac index (CI) = CO/body weight (ml/min/kg); stroke index (SI) = CI/HR (ml/kg); systemic vascular resistance (SVR) = (MAP - RAP)/CO x 79.9 (dynes·s/cm^5); pulmonary vascular resistance (PVR) = (PAPm - PAWP)/CO x 79.9 (dynes·s/cm^5); Rate pressure product (RPP) = SAP x HR (mmHg/min). Arterial blood was collected in heparinized syringes and stored on ice for measurement of blood gas tensions and acid-base balance. The pH (pHa), PaO_2, and $PaCO_2$ were measured by the blood gas analyzer (IL-1303, Instrumentation Laboratory). Respiratory rate (RR) was monitored by use of the infrared gas analyzer (AGM-103 Capnomac, Datex).

2. 7. Data analysis—Within each anesthetic group, two-factor ANOVA for repeated measures was used to evaluate the effect of interaction among the following factors: time, dog, and time-by-dog interaction. When the interaction was statistically significant, the within-subject dose effects on the variables were evaluated by one-factor ANOVA for repeated measures followed by Sheffé's multiple comparison test performed for mean values. For comparisons of the changes between anesthetic groups, one-factor ANOVA followed by the Sheffé's test was used. Values of $P < 0.05$ were considered significant.

3. Results

3. 1. Mask induction with 2.5 MAC of Hal, Enf, Iso and Sevo—Time course-effects of mask induction with each anesthetic are summaried in Table 1-3. Among anesthetics, induction with Sevo were the shortest. Those with Iso were slightly longer than with Sevo, however there was no significant difference between the two agents. Induction time in these two anesthetics were significantly shorter than those in Hal and Enf groups. Movements during the inhalation procedure were similar in the anesthetics except for Sevo. Movements started at approximately 2 min

after the beginning of Enf or Iso, then continued for approximately 2 min in Enf and for 1.3 min in Iso, respectively. In Hal, however, movements started at approximately 3.5 min and continued for 4 min, which was significantly longer than in Enf or Iso groups. In Sevo, there were almost no movements in 5 of 6 dogs, and they were rapidly and smoothly induced to deep anesthesia.

Table 1 Comparison of the effects of RII associated with 2.5 MAC of Hal, Enf, Iso and Sevo in 24 dogs

Variable	Hal	Enf	Iso	Sevo
Induction (sec)	790.3±75.7* §¶	374.8±36.0 §¶	285.8±34.1	209.0±44.2
Onset of movements (sec)	217.2±38.5* §¶	125.5±40.0	117.2±25.0	48.0
End of movements (sec)	457.5±66.6* §¶	250.8±47.4	197.7±31.1	135.0
Duration of movements	240.3±95.6* §¶	125.3±40.9	80.5±35.0	87.0
Movements	6 / 6 (100%)	6 / 6 (100%)	6 / 6 (100%)	1 / 6 (16.7%)

* Significantly different from Enf ($P < 0.05$); § Significantly different from Iso ($P < 0.05$);
¶ Significantly different from Sevo ($P < 0.05$).
MAC = minimum alveolar concentration. Data are expressed as mean ± SD.

3. 2. Changes in cardiovascular parameters of Hal, Enf, Iso and Sevo associated with RII — Changes in cardio-vascular parameters during induction and maintenance after intubation were summarized in Table 2 and Table 3, respectively. There were no significant differences in any baseline values among all anesthetic groups.

During RII, Heart rate of Enf, Iso and Sevo markedly increased after the beginning of inhalation and reached to the maximum level in approximately 2 to 3 min. Then the HR values slightly decreased from the maximal level but maintained significantly higher values as compared to their base-line levels. In contrast a slight but not significant increase was observed in Hal. Mean arterial pressure mildly but not significantly increased just after the beginning of inhalation in all inhalants, then it mildly but not significantly decreased until intubation. Systemic vascular resistance tended to decrease in all inhalants after the beginning of inhalation, but any significances were not observed. In all inhalants the changes of CI and RPP were similar to those of HR.

During maintenance anesthesia, significant increases in HR compared with the base-line values were observed at intubation in Enf and at 10 min after intubation with Iso and Sevo, respectively. Mean arterial blood pressure and SVR significantly decreased compared with the base-line values after intubation in all inhalation groups. Cardiac index showed little changes throughout this period in any anesthetic inhalation groups. Rate pressure product mildly but not significantly increased at intubation, but declined to the base-line values thereafter.

Table 2
Changes in cardiovascular parameters during RII associated with 2.5 MAC of halothane (Hal), enflurane (Enf), isoflurane (Iso) and sevoflurane (Sevo) in 24 dogs

Variable		Base-line	Time after onset of inhalation						
			60 sec	120 sec	180 sec	240 sec	300 sec	420 sec	540 sec
Heart rate (beats/min)	Hal	97±4	103±16	114±12	122± 7	127±10	127±12	124±8	112±12
	Enf	95±8	140±23*	182±25*	174±20*	153±13*	148±10*	ND	ND
	Iso	95±5	110±15	159±14*	178±20*	161±19*	ND	ND	ND
	Sevo	97±13	149±15*	171±10*	158±13*	ND	ND	ND	ND
Mean arterial pressure (mm of Hg)	Hal	118±12	131±10	126±12	122±11	116±13	114±12	108±12	95±10
	Enf	119±11	125±23	119±25	111±27	91±23	83±22	ND	ND
	Iso	116± 9	129±14	134±13	132± 5	110±13	ND	ND	ND
	Sevo	117± 5	121±16	111±18	101±18	ND	ND	ND	ND
Systemic vascular resistance (dynes·sec/cm^5)	Hal	4264±768	4552±720	4376±632	3840±424	3400±456	3400±496	3176±616	3184±472
	Enf	4312±750	3680±704	3312±808	3176±688	2944±736	2824±488	ND	ND
	Iso	4352±680	4336±920	3536±240	3552±360	3424±536	ND	ND	ND
	Sevo	4368±432	3592±832	3056±648	3352±680	ND	ND	ND	ND
Cardiac index (ml/min/kg)	Hal	219±21	226±22	225±17	247±24	264±31	277±31	264±16	231±13
	Enf	218±25	263±17*	279±24*	269±21*	239±10*	221±27	ND	ND
	Iso	212±24	235±29	294±25*	292±31*	250±23	ND	ND	ND
	Sevo	211±24	266±29*	284±20*	237±25	ND	ND	ND	ND
Rate-pressure product (mm of Hg/min)	Hal	15634±1207	17465±3901	18446±3493	18779±2426	18846±2590	18271±2555	16803±1694	13354±2007
	Enf	14659±1844	21969±5512*	27220±7145	24637±6520	17985±4927	17045±3351	ND	ND
	Iso	14383±901	18448±4218*	26676±4347*	29507±4052	23539±3168	ND	ND	ND
	Sevo	14884±2556	23103±3047*	23737±4080	20064±4158	ND	ND	ND	ND

* Significantly different from the base-line value ($P < 0.05$).
MAC = minimum alveolar concentration; ND = Not done. Data are expressed as mean ± SD.

Table 3 Changes in cardiovascular parameters after RII associated with 2.5 MAC of Hal, Enf, Iso and Sevo in 24 dogs

Variable		Base-line	Intubation	Time after intubation		
				10 min	20 min	30 min
Heart rate (beats/min)	Hal	97±4	118±10	117±12	114±14	115±13
	Enf	95±8	137±16*	120±11	117±11	114±11
	Iso	95±5	142±19*	133±14*	133±13*	128±8*
	Sevo	97±13	149±8*	134±15*	128±17*	125±17*
Mean arterial pressure (mm of Hg)	Hal	118±12	92±7*	72±7*	67±9*	67±7*
	Enf	119±11	78±20*	62±9*	61±10*	59±11*
	Iso	116±9	96±13*	71±8*	68±7* §	66±6* §
	Sevo	117±5	94±19*	73±10*	67±10*	65±10*
Systemic vascular resistance (dynes·sec/cm^5)	Hal	4,264±768	3,136±352	2,656±400*	2,592±392*	2,600±312*
	Enf	4,312±824	2,728±936	2,392±392* §	2,328±360* §	2,328±384* §
	Iso	4,352±680	3,464±554	2,632±376*	2,576±296*	2,504±240*
	Sevo	4,368±432	3,352±632	2,832±432*	2,728±584*	2,592±423*
Cardiac index (ml/min/kg)	Hal	219±21	226±19	206±20	197±16	196±17
	Enf	218±25	222±36	198±29	198±37	189±30
	Iso	212±24	215±22	205±13	198±16	198±10
	Sevo	211±24	216±14	200±16	191±22	192±22
Rate-pressure product (mm of Hg/min)	Hal	15,634±1,207	13,524±1,600	11,633±1,561	10,784±2,106	10,455±1,945
	Enf	14,659±1,844	14,592±2,721	10,370±1,862	9,462±1,964	8,982±2,203
	Iso	14,383±901	18,204±2,584	12,795±549	12,331±869	11,526±1,076
	Sevo	14,884±2,556	17,136±3,256	12,602±3,088	11,223±3548	10,461±3,243

§ Significantly different from the value at intubation ($P < 0.05$).
See Table 2 for key.

3. 3. Changes in respiratory parameters of Hal, Enf, Iso and Sevo associated with RII—Changes in respiratory parameters of Hal were summarized in Table 4. There were no significant differences in any baseline values among all anesthetic groups.

Respiratory rate significantly increased and maintained the steady level after intubation. In Sevo RR was also significantly higher at intubation compared with the base-line value, but reversed to the base-line value thereafter. On the other hand, RR of Enf decreased after intubation and showed significantly lower levels than the base-line value from 20 to 30 min after intubation. Respiratory rate of Iso did not change significantly from the base-line value. Pa_{O_2} increased during RII in all anesthetics, and maintained steady levels during the maintenance period. Pa_{CO_2} increased at intubation from the base-line level, and maintained the higher levels throughout maintenance period. In relation to the increased Pa_{CO_2}, pHa significantly decreased during RII.

Table 4 Changes in respiratory parameters after RII associated with 2.5 MAC of Hal, Enf, Iso and Sevo in 24 dogs

Variable		Base-line	Intubation	Time after intubation		
				10 min	20 min	30 min
Respiratory rate (breaths/min)	Hal	13.7±2.7	29.0±5.7*	24.3±3.7*	28.0±3.6*	28.8±3.1*
	Enf	13.3±2.1	14.2±2.5	8.7±2.5	8.2±2.6*	7.2±1.8*
	Iso	15.0±4.7	18.5±4.8	15.3±3.4	14.5±2.9	14.5±3.1
	Sevo	14.0±2.2	25.8±4.3*	15.0±2.0§	15.8±3.1§	14.5±3.3§
PaO_2 (mm of Hg)	Hal	104±13	496±24*	491±9*	488±12*	500±19*
	Enf	100±11	460±26*	472±22*	473±29*	465±27*
	Iso	103±8	484±14*	496±14*	496±18*	500±17*
	Sevo	108±12	486±23*	510±12*	508±12*	510±16*
$PaCO_2$ (mm of Hg)	Hal	36.9±4.7	42.2±3.4	47.9±4.9*	45.0±5.5	45.2±5.4
	Enf	37.4±5.2	49.3±4.7*	46.9±7.9	54.5±5.0*	53.5±2.8*
	Iso	37.7±3.5	46.2±2.8*	49.2±4.0*	49.7±3.3*	48.9±4.0*
	Sevo	39.9±5.6	45.2±5.4	52.3±6.8*	49.1±7.1*	51.3±6.3*
pHa	Hal	7.30±0.01	7.24±0.01*	7.21±0.02*	7.20±0.02*	7.18±0.03*
	Enf	7.28±0.02	7.19±0.05*	7.17±0.03*	7.16±0.04*	7.16±0.03*
	Iso	7.29±0.03	7.22±0.02*	7.19±0.03*	7.19±0.02*	7.19±0.03*
	Sevo	7.30±0.03	7.24±0.02*	7.19±0.02*	7.20±0.02*	7.20±0.01*

§ Significantly different from the value at intubation ($P < 0.05$).
See Table 2 for key.

4. Discussion

An important factor for the rapid increase in anesthetic level is blood/gas partition coefficient which defines the uptake of anesthetic (Muir *et al.*, 1995). Sevo has the lowest blood/gas partition coefficient among the anesthetics used in this study. RII with Sevo induced anesthesia most rapidly and smoothly among the anesthetics at an equianesthetic concentration. Iso also induced anesthesia rapidly, although the movements were stronger than Sevo. In human patients, Iso and Enf, and high concentration (≥ 2 MAC) of Hal, are not used for inhalation induction because of the strong irritation of the airway mucosa (Doi and Ikeda, 1993). Nishino *et al.* (1993) reported that laryngeal irritant receptors, which are recognized as a 'cough' receptors (Widdicombe and Sant'Ambrogio, 1992), are consistently activated by Hal, Enf and Iso in dogs. In the present study, most of the dogs except for Sevo group showed a strong body movements, although no presence of airway reflexes were found. Thus it is possible that the airway irritation of Hal, Enf and Iso might have induced the exaggeration of excitement period.

Cardiovascular changes during RII were remarkable in Enf, Iso and Sevo. Heart rate showed an approximately 85% increase after the beginning of the inhalation. Cardiac output also showed a rapid increase, which was mainly caused from the increased HR and partly from the mild decrease in afterload represented by SVR. Myocardial oxygen demand represented by RPP steeply increased, accompanied by the rapid increase in HR. Arterial blood pressures initially increased mildly along with the increase in CO, but tended to decrease, which might be caused by the decrease in SVR. These changes initially exceeded the physiological level just after inhalation, however, rapidly reversed to the normal level, thereafter. Thus, the RII by those anesthetics may be safe in the patients without significant cardiovascular diseases.

On the contrary, the degree of changes in cardiac parameters in Hal was milder compared with other anesthetic groups. In this group, the increase in HR was mild and CO did not change significantly, suggesting changes in afterload in this group was minimal. Hal is known to have less depressive effects on baroreceptor reflex function compared with other anesthetics (Wilkinson *et al.*, 1980), which may be one of the causes of the less cardiovascular changes. Further study will be needed to clarify the mechanism.

In spite of the dramatic changes in cardiovascular function during induction by inhalation of Enf, Iso or Sevo, the stabilization after intubation was fast in all anesthetics. The stable states in circulatory and respiratory system could be obtained in 10 min after intubation in all the dogs of any anesthetic groups. The adjustment of end-tidal anesthetic concentration was completed in 5 min, which suggests that alveolar and blood anesthetic concentration was stabilized in 5 min after intubation. These observations in dogs were similar to those in human (Shigematsu and Kobayashi, 1993). The level of circulatory and respiratory measurements at 10 min after intubation corresponded to those at 1.5 MAC of each anesthetic previously reported (Haskins, 1992), which suggests any 'hangover' (Editorial, 1986; Drummond, 1988) effect was not produced by RII.

Throughout the RII, dogs were able to breath spontaneously with any anesthetics used in the present study. This is a great advantage over the injectable anesthetics because rapid induction using ultrashort barbiturates such as thiobarbiturates often causes a certain period of apnea (Raulings and Kolata, 1983; Ilkiw, 1992). It is reported that the inhalational anesthetics, except for Enf, depress ventilation only slightly at the light (1 MAC) through middle (1.5 MAC) anesthetic level (Haskins, 1992). These anesthetics may gradually depress ventilation when deeper anesthetic level (2 MAC) is maintained, however, most of surgeries may be performed at an end-tidal anesthetic concentration less than 2 MAC, thus the respiratory depression will not be a problem in this type of anesthetic method. The increase in respiratory rate at and after intubation was the highest in Hal, as previously reported (Steffey and Howland, 1978), which suggests that the depressant effect of halothane on the respiratory center was least among anesthetics used in this study.

In conclusion, Sevo seems to be the best inhalational anesthetic for RII in dogs. Although there were a certain level of problems in cardiovascular functions, Sevo seems to be the best inhalational anesthetic for RII in dogs without significant cardiac and/or respiratory diseases. Iso could also induce rapid induction with more movements during induction. Considering the longer movements and prolonged induction, Hal may not be recommended for rapid inhalation induction. Advantages of cardiovascular stability of Hal may not compensate for these disadvantages.

Section 3 Effects of volatile anesthetics on the lower respiratory tract and lungs

3. 1. Responses of slowly adapting pulmonary stretch receptors to volatile anesthetics in anesthetized dogs

Abstract

The aim of this study was to evaluate the effects of volatile anesthetics on slowly adapting pulmonary stretch receptor (SAR) activity in dogs. Eight beagles were anesthetized with an intravenous injection of a mixture of urethane and α-chloralose as a basal anesthesia, then vagotomized, artificially ventilated, and chest-opened. Single afferent activities from SARs were recorded from the peripheral nerve cut end of the left vagus. Changes in SAR activities with inhalation of Hal, Enf, Iso and Sevo at 1, 1.5, and 2 MAC were measured, and differences in the discharges within and among four anesthetics were evaluated. As a result, two different types of SARs—low-threshold SARs and high-threshold SARs—were detected in this study. In all anesthetics, expiratory discharges of low-threshold SARs decreased significantly in a dose-dependent manner, while inspiratory discharges did not change significantly at any anesthetic level. Discharges of high-threshold SARs tended to decrease with increasing anesthetic level; however, no statistical significances were observed at all anesthetic levels. Only one exception to these changes was observed at 1 MAC of Hal where no significant decrease in expiratory discharge of low-threshold SARs and significant increase in discharge of high-threshold SARs were induced against a control value. In conclusion, all the volatile anesthetics evaluated in this study , except for Hal at the light anesthetic level, tended to decrease SAR activities depending on the anesthetic level, suggesting attenuation of the Hering-Breuer inflation reflex.

1. Introduction

As described in the preface, the respiratory reflex actions induced by SARs play an important role in the regulation of breathing in animals (Adrian, 1933).

Volatile anesthetics have effects on respiratory regulation mechanisms through their central and peripheral actions. Clark *et al.* (1972) suggested that the difference of respiratory timing between inhalation anesthetics may depend on the difference in modulation of strength of the Hering-Breuer inflation reflex by those drugs. The ventilatory effects of inhalation anesthetics have been reported from the aspect of reflex actions in several studies (Drummond, 1984; Kochi *et al.*, 1990, 1991; Canet *et al.*, 1994); however, only a few studies have been done regarding the effects of volatile anesthetics on vagal inputs originating from SARs.

The purpose of this study is to compare the effects of halothane, enflurane, isoflurane, and sevoflurane on SARs in dogs by recording the vagal nerve afferents electrophysiologically.

2. Methods

2. 1. Animals and basal anesthesia—Eight healthy beagle dogs (4 males and 4 females) were used in this study. Their mean age was 13.5-months-old (ranging from 10 to 18 months) and their mean body weight 10.2 kg (ranging from 6.5 to 11.4 kg). Dogs were induced to anesthesia by thiopental sodium (25 mg/kg) and were maintained under anesthesia by a mixture of urethane (500 mg/kg) and α-chloralose (50 mg/kg) injected intravenously. A supplemental dose of urethane (500 mg/kg) and α-chloralose (50 mg/kg) was injected hourly through an intravenous catheter placed into the cephalic or saphenous vein.

2. 2. Surgical procedure for recordings single unit activity of SARs—The dogs were placed on an operating table in the spine position, endotracheally intubated, and ventilated with room air by a ventilator (Kimura Medical, KV-1+1, Kimura Medical) to maintain tidal volume of 10-15 m*l*/kg at a frequency of 14-20 cycles/min, which was continuously monitored by a respirometer (Bear Medical Systems, NVM-1). A thoracotomy was performed and the chest was widely opened to identify each receptive field of SARs. A skin incision was made in the neck to expose the vagus nerve. The left vagus nerve was transected just caudal the nodose ganglion, and its peripheral cut end was separated to record its electrical activity. The right vagus nerve was also cut to eliminate possible secondary reflexes. A pressure transducer (Toyoda, PD104) was attached to the tracheal cannula in order to measure intratracheal pressure (P_{TR}). Arterial blood pressure was monitored by a pressure transducer (Nihon Kohden, DX-300) connected to a catheter inserted into the femoral artery.

Afferent activity of the nerve was recorded by a pair of platinum electrodes. Single unit activity was clearly discriminated by dissecting the nerve trunk into several thin filaments. These nerve preparations were performed in a pool of paraffin oil using fine forceps and a binocular microscope (OLYMPUS, SZ 60). The signal of the single unit activity was amplified by a low noise DC-amplifier (DIA Medical, DPA 201) and a biophysical amplifier (DIA Medical, DPA 200), and displayed on an oscilloscope (IWATSU, SS 5762) and a loud speaker (NEC san-ei, Model 7747). All the signals were also displayed on a thermal-array recorder (NEC san-ei, RT 3100N) and recorded by a magnetic tape recorder (SONY, PC 204A). Slowly adapting receptors were identified by their regular increasing discharge synchronized with each breath and by long-lasting adaptation to the maintained inflation of the lung (Fig. 1). These procedures have been fully described in a previous study (Sano *et al.*, 1992).

Fig. 1. Discharge pattern of a slowly adapting pulmonary stretch receptor. The horizontal line shows the maintained lung inflation. ENG = electroneuropram, P_{TR} = intratracheal pressure.

2. 3. Drugs and anesthetic instruments—In this study, all SARs were challenged with Hal, Enf, Iso and Sevo. Inhaled intratracheal anesthetic concentrations were 1, 1.5, and 2 MAC of end-tidal concentration. The MAC values of the anesthetics were: 0.87% for Hal, 2.06% for Enf, 1.28% for Iso and 2.36% for Sevo (Quasha *et al.*, 1980; Kazama and Ikeda, 1988). In the preliminary study, each vaporizer setting was precalibrated in order to perform the intended intratracheal anesthetic concentration within 1 min. The order of inhaled anesthetics and anesthetic concentrations were randomly selected. Intratracheal anesthetic concentration was continuously monitored with an infrared gas monitor (AGM-103 Capnomac, Datex, Finland).

2. 4. Evaluation of the effects of each volatile anesthetic on SARs—After a control period of more than 1 min, SARs were challenged with an inhalation of each anesthetic at intended concentration for 1 min. After the SAR activity recovered to the control level, the next anesthetic was presented. During this experimental period, the lungs were ventilated with 100% oxygen at a flow rate of 6 L/min. At the end of the procedure, the location of the receptive field of the fiber and its excitation properties were determined by gently probing the external surface of the lungs with a cotton stick.

2. 5. Dataanalysis—The number of action potentials of SAR during each ventilatory cycle (inspiratory and expiratory phase) was counted with a spike height discriminator (DIA Medical, DSE-425). The mean discharge frequency was taken from 3 consecutive breaths during each of the control, peak (approximately 1 min after inhalation), and recovery periods. The latency of each SAR response (time interval and firing-threshold) by inhalation of anesthetics was evaluated from the experimental record displayed on a thermal-array recorder (NEC san-ei, RT 3100N).

Statistical analysis was performed using a statistical software package (Abacus Concepts, StatView®4.5). For the comparison between the data, Wilcoxon's signed rank sum test or Mann-Whitney's U-test was used. To compare the differences among anesthetic groups, a one-way ANOVA test was run, followed, where appropriate, by Dunnet's multiple comparison test. All data were expressed as mean ± SE.

3. Results

3. 1. Subtypes and pulmonary location of SARs—Single unit activities were record-ed from a total of 40 SARs. From their discharge pattern synchronized with the respiratory cycle, the SARs observed in this study were divided into two types: 17 low-threshold (LT) SARs and 23 high-threshold (HT) SARs (Figs. 2 and 3). LT-SARs showed spontaneous discharge at functional residual capacity (FRC) (Fig. 2), whereas HT-SARs fired only above FRC (Fig. 3).

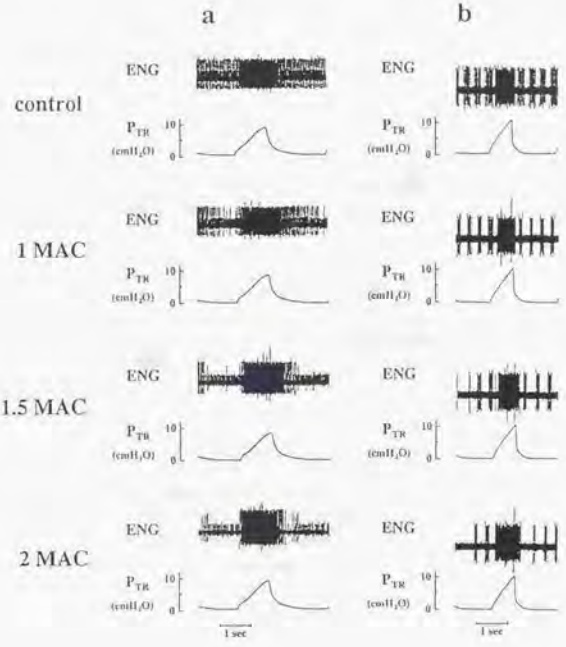


Fig. 2. Experimental records illustrating responses of two types of LT-SARs to sevoflurane at control, 1, 1.5, and 2 MAC. Columns a and b show subtypes of LT-SARs with tonic discharge and cardiac modulation, respectively. MAC = minimum alveolar concentration. Abbreviations as in Fig. 1.

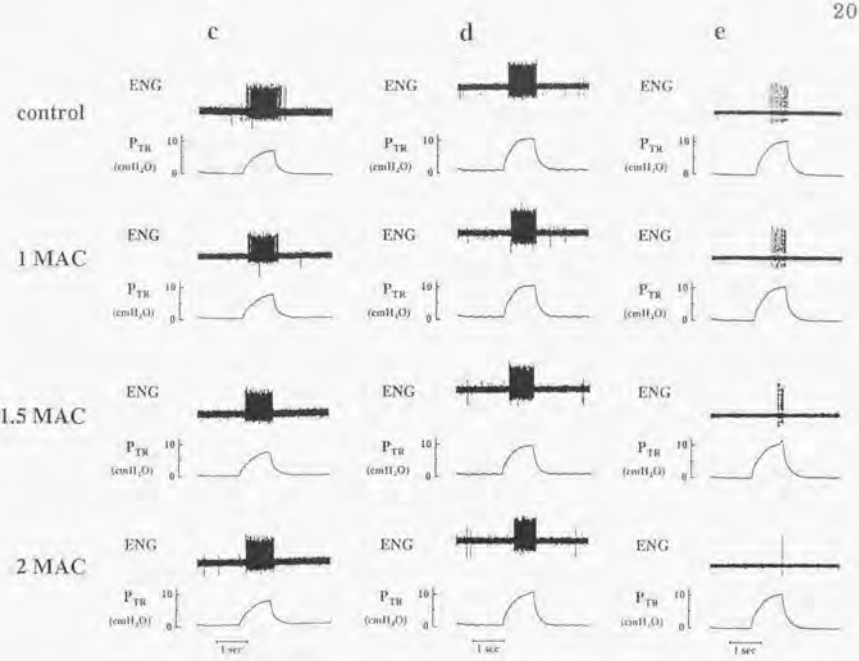


Fig. 3. Experimental records illustrating responses of three types of HT-SARs to halothane at control, 1, 1.5, and 2 MAC. Columns c, d, and e show subtypes of HT-SARs. Type-e increased discharges significantly at all anesthetic levels from the control value. Type-d did not change discharges significantly at all anesthetic levels. Type-e decreased discharges significantly in a dose-dependent manner. In type-d and e receptors, elevation of firing-threshold were observed. Abbreviations as in Fig. 1.

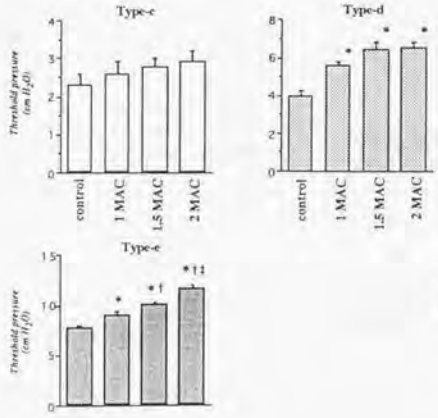


Fig. 4. Changes in firing-threshold pressure of HT-SARs by volatile anesthetics. Threshold-pressure was assessed at 20 sec after the onset of inhalation. * $P < 0.05$ vs control; † $P < 0.05$ vs 1 MAC; ‡ $P < 0.05$ vs 1.5 MAC. Threshold pressure was significantly higher in type-e receptor than that of type-c and d receptors in all anesthetic levels.

Moreover, LT-SARs were classified into two subtypes of discharge patterns according to whether they showed tonic discharge (type-a; Fig. 2a) or cardiac modulation (type-b; Fig. 2b) at FRC. HT-SARs were divided into three subtypes depending on their firing threshold (types c-e; Fig. 3c-e), the averaged values of which were 2.3 ± 0.3 cm H_2O for type-c receptors, 4.0 ± 0.3 cm H_2O for type-d receptors, and 7.8 ± 0.2 cm H_2O for type-e receptors, respectively (Fig. 4).

The pulmonary location of each subtype of each SAR was determined in 21 SARs (Fig. 5). All SARs were found in the left lung, which was also used for nerve recording. A majority of LT-SARs were located in the proximal part of the lung, and those with cardiac modulation were typically located close to the heart. On the other hand, most HT-SARs (7/10, 70%) were located in the peripheral part of the lung. All subtype-e receptors (5/5, 100%) were in the peripheral site.

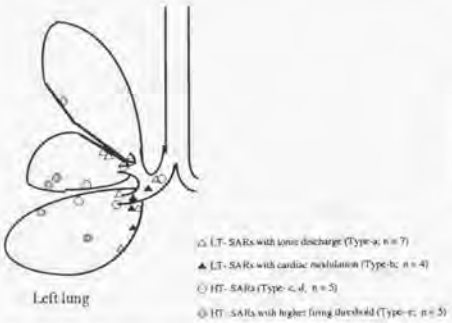


Fig. 5. Distribution of SARs in the left lung.

3. 2. Responses of SARs to inhalation of anesthetics—Peak percent changes of the SAR activity by each anesthetic are shown in Figs. 6 and 7. Similar changes of discharges were equally observed in both subtypes of LT-SARs with tonic and cardiac modulation. In all anesthetics, expiratory discharges of LT-SARs decreased significantly in a dose-dependent manner (Fig. 6). At 1 MAC, significant decreases in expiratory discharges of LT-SARs were observed for Enf, Iso and Sevo as compared with the decrease in Hal. At 1.5 and 2 MAC, a similar degree of decrease in expiratory discharge was observed for all anesthetics (Fig. 6).

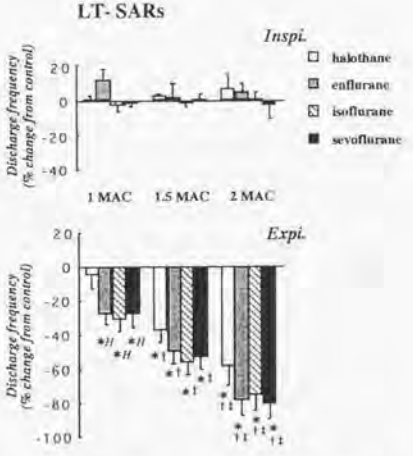


Fig. 6. Peak percent changes of LT-SARs from the control values by halothane, enflurane, isoflurane, and sevoflurane at 1, 1.5, and 2 MAC. * Significantly different from the control value ($P < 0.05$); † Significantly different from 1 MAC ($P < 0.05$); ‡ Significantly different from 1.5 MAC ($P < 0.05$); ^{H} Significantly different from halothane ($P < 0.05$). *Inspi.* = inspiratory discharge; *Expi.* = expiratory discharge.

Responses of HT-SARs were classified into three types: c) responses in which receptor discharges significantly increased at 1-2 MAC from the control values (n = 10, Fig 2c); d) responses in which discharges did not change significantly at all anesthetic levels with the exception of the abrupt and considerable inhibition at 2 MAC of 3 receptors for Iso and 2 receptors for Enf, sevoflurane, and halothane (n =7, Fig 2d); and e) responses in which a dose-dependent decrease in their discharges was observed (n = 6, Fig. 3e).

In each subtype of HT-SARs as described above, changes in threshold P_{TR} to fire during inspiration were assessed at 20 sec after the beginning of inhalation (Fig. 4). The firing-threshold of type-e receptors before inhalation of each anesthetic was significantly higher than those of type-c and d receptors. By inhalation of anesthetics, significant increases in the firing-threshold were observed in type-d and e receptors at all anesthetic levels compared with the control values, while no significant change was found in type-c SARs. The firing-threshold increased in a dose-dependent manner in type-e receptors.

Averaged discharge frequencies of all types of HT-SARs tended toEnf, Iso and Sevo, although no statistically significant difference from the control values was observed at all anesthetic levels (Fig. 7). Although the averaged discharge frequency significantly increased from the control values at 1 MAC of Hal, no statistical significance was recognized between the four anesthetics (Fig. 7). The latency of SAR response to inhaled anesthetics was not altered by different anesthetics (data not shown).

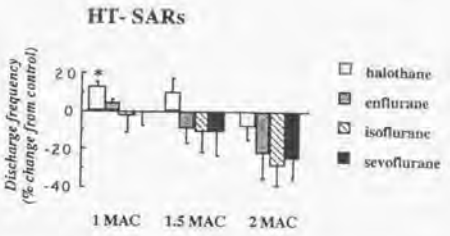


Fig. 7. Peak percent changes of HT-SARs from the control values by halothane, enflurane, isoflurane, and sevoflurane at 1, 1.5, and 2 MAC. * Significantly different from the control value ($P < 0.05$).

4. Discussion

Volatile anesthetics, such as chloroform, trichloroethylene, ethyl ether, cyclo-propane, divinil ether, and Hal, are known to increase the sensitivity of SAR activities without increasing airway pressures (Coleridge *et al.*, 1968; Paintal, 1964). Nishino *et al.* (1994) have also reported that the prevalent response of halogenated volatile anesthetics such as Hal, Enf and Iso, consisted of an elevation of excitation threshold and an increase in SAR activity. In this study, however, sensitization was not observed for most SARs by any anesthetics except for Hal at 1 MAC. This is because an elevation of firing-threshold that occurred during inhalation possibly overshadowed the increase in receptor activities. Dose-dependent depression of both HT-receptors with higher threshold (Figs. 3 and 4) and spontaneous discharge of LT-receptor at the FRC level (Fig. 2) appeared to be typical of the effect of increasing threshold. It is unclear why our results different from those of Nishino *et al.* (1994); however, one reason might lie in the difference between anesthetic concentrations applied. Namely, we evaluated the responses of SARs at several times the MAC values of end-tidal concentration of each inhalant, while Nishino *et al.*

measured them at 5% of inspiratory concentrations. Therefore, at least in the study of Nishino *et al.* (1994), it is possible that the intended anesthetic concentration was not sufficiently delivered to each SAR site in the airway and lungs, and the different SAR responses might be produced. In fact, they have also pointed out that some SARs with higher threshold decreased their discharges due to the elevation of firing-threshold. Moreover, in the larynx similar type of respiration-modulated stretch receptors have been recognized, the activities of which were consistently inhibited by Hal, Enf and Iso (Nishino *et al.*, 1993), partly supporting the prevalent inhibitory responses of SARs in our study.

The spontaneous activities, discharge patterns, and excitation thresholds of SARs are mostly influenced by changes in lung volume or transpulmonary pressure, which are in turn related to airway smooth muscle tension (Sant'Ambrogio, 1982; Widdicombe and Sant' Ambrogio, 1992). Results of this study showed that the response of two different types of SARs—low-threshold and high-threshold receptors—were involved in the vagal afferents. The former were located mostly in the larger airways near the hilum and the latter were located mostly in more peripheral airways (Fig. 5). The variations in the discharge patterns of such receptors can be accounted for by the morphological properties of the receptor site: the extrapulmonary airway walls are supported by a series of U-shaped cartilaginous rings, which tend to expand and exert a transverse stretch force on the posterior wall, even at zero transpulmonary pressure (Sant'Ambrogio, 1982). This is the reason why most SARs in the extrapulmonary airways are active at zero transmural pressure as LT-receptors. On the other hand, intrapulmonary airways, such as terminal bronchioles, the smallest airways on which SARs exist, are not affected by the expanding force of the rings, and thus their activities are mainly influenced by the volumes of the respective lobes of the lung. Accordingly, the activity of SARs that locate peripheral site tends to cease at end-expiration, and to be limited to HT-receptors during inspiration.

In the present study, the dogs were chest-opened and artificially ventilated with a fixed tidal volume that produced a constant transpulmonary pressure, and therefore, a constant stimulus at the receptor site. Further, the intra tracheal pressure did not change during inhalation of all anesthetics change significantly. Variations in the intra-airway delivery of anesthetic gas and the associated difference in the airway location of each receptor site had no significant influence on the latency of the response between HT and LT-receptors. Therefore, the changes in the SAR activities were most likely to the direct effect of inhaled anesthetics on the nerve endings.

Several factors in addition to the lung volume, transpulmonary pressure, and morphological properties of airways, serve to influence the activities of SARs. Bartlett *et al.* (1976) suggested that transverse (circumferential) tension is a direct stimulus for SARs. Sant'Ambrogio (1982) pointed out that SARs are sensitive not only to the forced tension, but also to any dynamic change in the rate of pressure change (dP/dt). Changes in the SAR activities by inhaled anesthetics may also be due to changes in lung volume and transpulmonary pressure.

The considerable depression of SARs observed in some type-d HT-receptors at 2 MAC might be caused by the blocking effect of volatile anesthetics on the nerve endings, since chloroform, trichloroethylene, ethyl ether, and divinil ether at the high doses used to induce anesthesia, usually block the nerve endings, although they can also sensitize the endings at maintenance doses

(Whitteridge, 1958; Sant' Ambrogio, 1982). Younes *et al.* (1978) reported that phasic vagal influence was markedly depressed at 2% Hal, which equates to 2.3 MAC of Hal, supporting our results.

On the whole, recent inhalation anesthetics have tended to decrease SAR activities with increasing anesthetic level except for 1 MAC of Hal. Although the resultant reflex actions caused by the effects of the inhalants on SAR activity were not evaluated in this study, it is possible that the negative volume-feedback mechanism by the Hering-Breuer inflation reflex was reduced by the net decrease in SAR activities. This might induce a slowing of respiratory timing and an increase in tidal volume. In contrast, increased SAR activity for 1 MAC of Hal might contribute to an increase in respiratory frequency and a decrease in tidal volume for light Hal anesthesia (Doi and Ikeda, 1988; Mutoh *et al.*, 1997a).

3. 2. Responses of bronchopulmonary capsaicin-sensitive C-fiber and irritant receptors to volatile anesthetics in anesthetized dogs

Abstract

Effects of Hal, Enf, Iso, and Sevo on vagal capsaicin (CAPS)-sensitive C-fibers were elucidated in anesthetized dogs. The CAPS-sensitive C-fibers were significantly stimulated by all volatile anesthetics with a significantly greater response to Hal than with Sevo. A significant increase in respiratory frequency (f_R) and a significant decrease in tidal volume (V_T) were observed with Hal and Iso, and a significant increase in f_R was observed with Sevo. In contrast, a significant decrease in f_R was induced by Enf. The tachypnea induced by Hal, Iso, and Sevo was significantly reduced or no longer observed after perineural CAPS-treatment or bilateral vagotomy, whereas the slowing of respiration observed with Enf was not affected by either of these treatments. These results suggest that vagal C-fibers play an important role in the reflex tachypnea that occurs with Hal, Iso, and Enf.

1. Introduction

Among the three types of sensory endings (slowly adapting stretch receptor, rapidly adapting irritant receptor, and C-fiber afferents) recognized in the lower airways, the rapidly adapting irritant receptors (RARs) and capsaicin (CAPS)-sensitive C-fibers are known to be responsible for the sensitivity to various chemical substances (Coleridge and Coleridge, 1984). Several studies have been performed on the responses of these vagal sensory endings to inhalation of volatile anesthetics: Coleridge *et al.* (1968) have pointed out that pulmonary CAPS-sensitive C-fibers are stimulated by ether, chloroform, trichloroethylene, and halothane. Nishino *et al.* (1994) have reported that the discharge of tracheobronchial RARs is consistently inhibited by Hal, Enf, and Iso.

Considering the above, it is possible to speculate that vagal C-fibers and RARs are play an important role in the elicitation of airway reflexes during the inhalation of volatile anesthetics. However, electrophysiological data on these sensory endings, especially regarding the effects of new volatile anesthetics such as Sevo on vagal C-fibers, has been limited. Therefore, the aim of this study was (1) to elucidate the effects of the volatile anesthetics on vagal CAPS-sensitive C-fiber activities, and (2) to evaluate the role of the C-fibers in the elicitation of cardiorespiratory reflexes in response to the anesthetics.

2. Methods

2. 1. General—Twenty-seven beagle and 10 mongrel dogs of either sex were used in this study. Their mean body weight was 11.4 kg (ranging from 7.2 to 17.0 kg). Dogs were induced anesthesia with a thiopental sodium (25 mg/kg), followed by maintained anesthesia with a mixture of urethane (500 mg/kg) and α-chloralose (50 mg/kg) injected intravenously. Supplemental dose of urethane (500 mg/kg) and α-chloralose (50 mg/kg) was injected hourly through the intravenous catheter placed into the cephalic or saphenous vein.

2. 2. Experimental protocol

2. 2. 1. Protocol 1: Recordings of the afferent activity—Nineteen beagle and 4 mongrel dogs were used in this experiment. The dogs were placed on an operating table in the supine position, endotracheally intubated, and ventilated spontaneously with room air. A skin incision was made in the neck to expose the vagus nerve. The left vagus nerve was transected just caudal to the nodose ganglion, and its peripheral cut end was separated to record its electrical activity. The right vagus nerve was also cut to eliminate possible secondary vagal reflexes. A pressure transducer (Toyoda, PD 104) was attached to the tracheal cannula in order to measure the intratracheal pressure (P_{TR}). Arterial blood pressure was monitored by a pressure transducer (Nihon Kohden, DX-300) connected to a catheter inserted into the femoral artery. End-tidal P_{O_2}, ($PETO_2$) and P_{CO_2} ($PETCO_2$) were sampled through the line attached to the endotracheal tube and monitored by a gas analyzer (Respina 1H26, NEC san-ei, Japan).

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 performed within a pool of paraffin oil using fine forceps with the aid of a binocular microscope (OLYMPUS, SZ 60). The signal was amplified by a low noise DC-amplifier (DIA Med., DPA 201) and a biophysical amplifier (DIA Med., DPA 200), and displayed on an oscilloscope (IWATSU, SS 5762) in parallel with a loudspeaker (NEC san-ei, Model 7747). All the signals were displayed on a thermal-array recorder (NEC san-ei, RT 3100N) and recorded by a magnetic tape recorder (SONY, PC 204A). Once an acceptable single unit was found, CAPS-sensitive C-fibers and RARs were identified by their characteristic patterns of discharge, responses to lung inflation (2-3 V_T) and deflation, and to right and left atrial injections of CAPS (10 μg/kg) according to the previous methods (Coleridge and Coleridge, 1977, 1984). The CAPS injections were performed either (1) through a 5-French Swan-Ganz catheter (Baxter Healthcare, Model 93-132-5F) positioned at the right atrium via the left jugular vein or (2) through a 14 G polyvinyl catheter (Toray Medical, Anthron) advanced into the left atrium via the common carotid artery.

After a control period of more than 1 min with 100% oxygen at a flow rate of 6 L/min, each fiber was challenged with 5% Hal, Enf, Iso, and Sevo for 1 min each. The order of administration of each anesthetic was random.

At the end of the experiment, the dogs were chest-opened and artificially ventilated by a ventilator (Kimura Medical, KV-1+1) to investigate each receptive field by exploring the external surface of the lower airways with a cotton stick.

2. 2. 2. Protocol 2: Perineural CAPS-treatment— Eight beagle and 6 mongrel dogs were used in this experiment. To evaluate the reflex contribution of C-fibers by inhalation of volatile anesthetic, a method that had previously been used successfully in dogs (Schelegle et al., 1995) for the perineural CAPS treatment of the vagus nerves was applied in this study. Briefly, the midcervical segment of each vagus nerve was isolated, and cotton pledgets soaked in a digestive solution consisting of Krebs solution (136.9 mM NaCl, 5.4 mM KCl, 5.5mM glucose, 23.8 mM $NaHCO_3$, 1.5 mM $CaCl_2$, 1.0 mM $MgCl_2$, and 0.001-0.01 mM EDTA) containing collagenase (1000 U/ml) and hyaluronidase (1000 U/ml) were placed on each segment for 20 min to remove connective tissue and to increase segment permeability. The digestive solution was removed by washing with saline after 20 min, and cotton pledgets containing a 1% CAPS solution in a vehicle of

10% ethanol, 10% Tween 80, and 80% saline were then placed on the vagal segments for 15-20 min to block C-fiber activity. Each anesthetic was then administered according to the procedure described in *Protocol 1*. Changes in tidal volume (V_T), respiratory frequency (f_R), and ventilation ($\dot{V}_E$) were recorded by a respirometer (Bear Medical Systems, NVM-1). The efficacy of C-fiber blockade was evaluated by the preservation of responses to lung inflation (Hering-Breuer reflex) and lack of response to the right atrial injection of CAPS (10 μg/kg) before and after the trial.

2. 3. Dataanalysis—The discharge frequencies of single unit activity were counted every second for a period of 1 min before the trial with a spike height discriminator (DIA Medical, DSE425), then the data were averaged for every 10 sec. The averaged discharge frequency for 1 min before the trial was taken as the control value, and the maximum or minimum discharge frequency out of the values averaged every 10 sec after start of the trial was taken as the peak value. The latency (sec) of each receptor's response was evaluated as the point when the action potential began to increase. For the receptors which decreased action potentials, the duration (sec) of onset of inhibition was recorded in the same way. Changes in heart rate (HR) and mean arterial blood pressure (ABP) were obtained from the tracings before and after the onset of each trial. The ABP was calculated as the sum of diastolic pressure and one-third of pulse pressure. The HR was calculated from the arterial blood pressure tracings. In all the cardiorespiratory parameters, control values averaged for 1 min before the trial and peak values after onset of each trial were taken into account.

Statistical analysis was the same as described in experiment 3. 1.

3. Results

3. 1. Subtypes and pulmonary location of CAPS-sensitive C-fibers and RARs—Single units were recorded from a total of 21 pulmonary C-fibers and 15 bronchial CAPS-sensitive C-fibers and 15 RARs. Changes in the discharge pattern of both types of endings in response to the right atrial injection of CAPS are shown in Fig. 1. Stimulation of CAPS-sensitive C-fibers by right atrial injection of CAPS coincided with an inhibition of breathing (apnea), followed by hypotension and bradycardia, while the activity of the RARs was unaffected (Fig. 1).

The CAPS-sensitive C-fibers observed in this study were divided into two types of receptors according to the criteria reported by Coleridge and Coleridge (1977, 1984). The latency in response to the right atrial injection of CAPS was 2.7 ± 0.5 sec for pulmonary C-fibers and 7.4 ± 1.8 sec for bronchial C-fibers. The pulmonary C-fibers were not stimulated by the left atrial injection of CAPS, whereas the bronchial C-fibers were activated with a latency of 3.5 ± 1.2 sec.

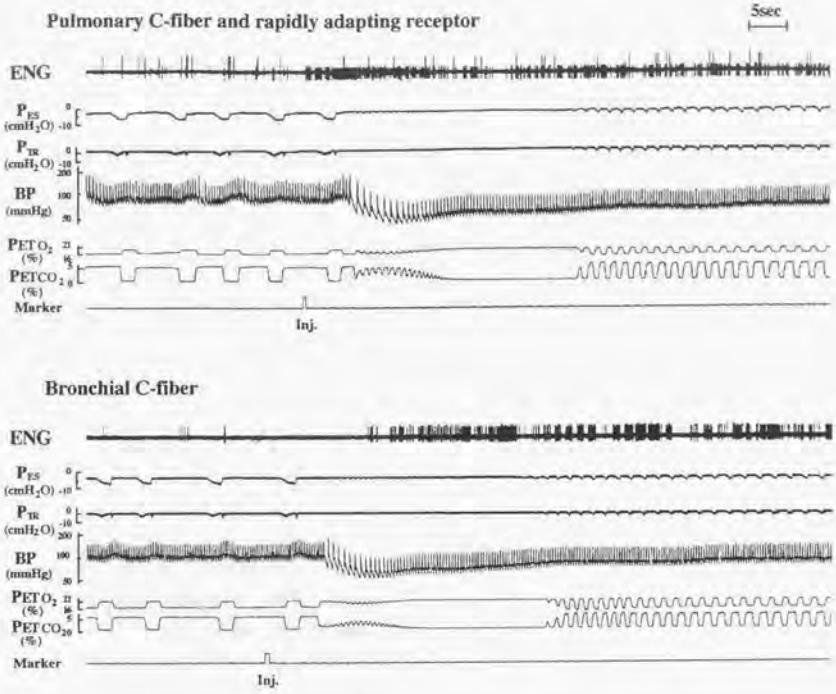


Fig. 1. Response of a pulmonary C-fiber (smaller spikes) and a rapidly adapting receptor (larger spikes) (upper panel) and a bronchial C-fiber (lower panel) to the right atrial injection of capsaicin (10 μg/kg) in an anesthetized spontaneously breathing dog. Note the pulmonary C-fiber was activated immediately after the capsaicin injection but the bronchial C-fiber had a longer latency, whereas in both cases apnea was followed by hypotension and bradycardia. Rapidly adapting receptor activity was unaffected by the capsaicin injection. The pulmonary C-fiber ending was located in the central part of the left lower lobe and the rapidly adapting receptor ending was in the left main bronchus. The bronchial C-fiber ending was located at the hilum of the left lower lobe. ENG = electroneurogram, P_{ES} = esophageal pressure, P_{TR} = intratracheal pressure, BP = arterial blood pressure. $PETO_2$ = End-tidal P_{O_2}, $PETCO_2$ = End-tidal P_{CO_2}. The injection time is marked on the bottom trace.

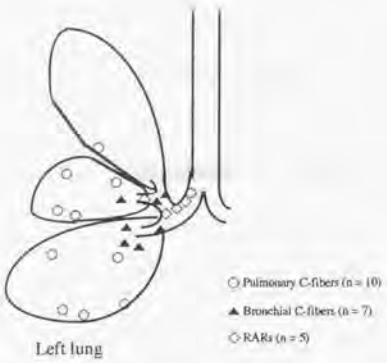


Fig. 2. Distribution of pulmonary and bronchial C-fibers and RARs in the left lung.

The pulmonary location of each ending was determined in 17 CAPS-sensitive C-fibers (10 pulmonary C-fibers and 7 bronchial C-fibers) and 5 RARs. In this study, all the RARs (5/5, 100%) and 3 bronchial C-fibers (3/7, 43%) were located in the extra-pulmonary bronchi. On the other hand, all pulmonary C-fibers (10/10, 100%) and 4 bronchial C-fibers (4/7, 57%) were found within the left lung (Fig. 2). Most pulmonary C-fibers were located in the central or peripheral part of the lung, while the majority of bronchial C-fibers were located in the proximal part.

3. 2. Effects of volatile anesthetics on CAPS-sensitive C-fibers and RARs—The discharge frequency of C-fibers was markedly increased after the onset of inhalation of all the anesthetics (Figs. 3 and 4), while that of the RARs was inhibited (Fig. 3). The increase in both pulmonary and bronchial C-fiber activity was significantly greater with Hal than with Sevo ($P < 0.05$, Fig. 5). The effects of Enf and Iso were midcourse between Hal and Sevo. The latency of pulmonary C-fibers was 18.5 ± 2.1 sec for Hal, 29.5 ± 2.4 sec for Enf, 27.3 ± 1.9 sec for Iso, and 42.5 ± 3.1 sec for Sevo. The latency of bronchial C-fibers was 7.3 ± 1.0 sec for Hal, 11.2 ± 1.8 sec for Enf, 9.8 ± 1.6 sec for Iso, and 16.5 ± 2.2 sec for Sevo. With all anesthetics, a significantly shorter latency was observed in bronchial C-fibers than in pulmonary C-fibers ($P < 0.05$). The latency of each C-fiber response was significantly shorter with Hal than with Sevo ($P < 0.05$).

There was no statistically significant difference among anesthetics with regard to the discharge of RARs ($P = 0.17$, Fig. 5). The delay of the onset of inhibition was 5.7 ± 0.9 sec for Hal, 12.8 ± 1.6 for Enf, 8.6 ± 1.5 sec for Iso, and 14.3 ± 2.2 sec for Sevo. No statistical significances were observed among anesthetics as to these latencies ($P = 0.09$).

The peak percent changes of f_R, V_T, and $\dot{V}_E$ after the inhalation of each anesthetic in dogs with non-treatment (n = 14), CAPS-treatment (n = 7), and bilateral vagotomy (n = 7) are shown in Fig. 6. A significant increase in f_R and a significant decrease in V_T was observed during the inhalation of Hal ($P < 0.01$) and Iso ($P < 0.05$), and a significant increase in f_R was observed with Sevo ($P < 0.05$). In contrast, a significant decrease in f_R was observed during the inhalation of Enf ($P < 0.05$) (Fig. 6).

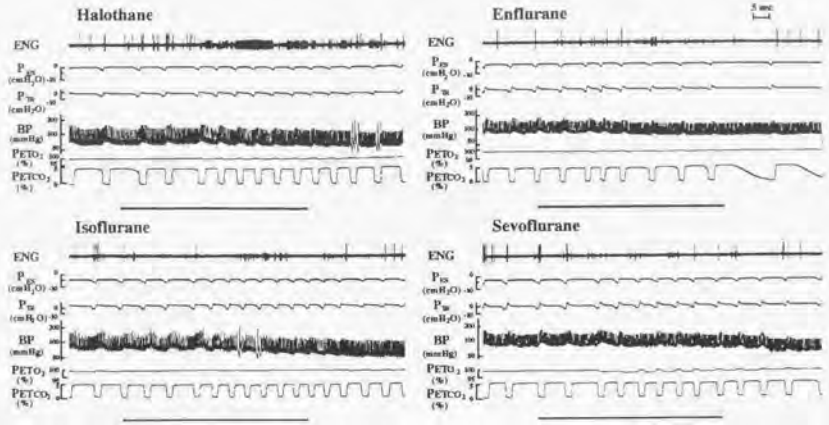


Fig. 3. Responses of a pulmonary C-fiber and a rapidly adapting receptor (the same endings as in Fig. 1, upper panel) to 5% volatile anesthetics in an anesthetized spontaneously breathing dog. The pulmonary C-fiber (smaller spike) was stimulated especially by Hal, whereas the rapidly adapting receptor (larger spike) was inhibited by all anesthetics. Shallow breathing was introduced by the inhalation of Hal, Iso, and Sevo, but slowing of breathing was observed during the administration of Enf. The horizontal lines show the inhalation time of each volatile anesthetic. Abbreviations as in Fig. 1.

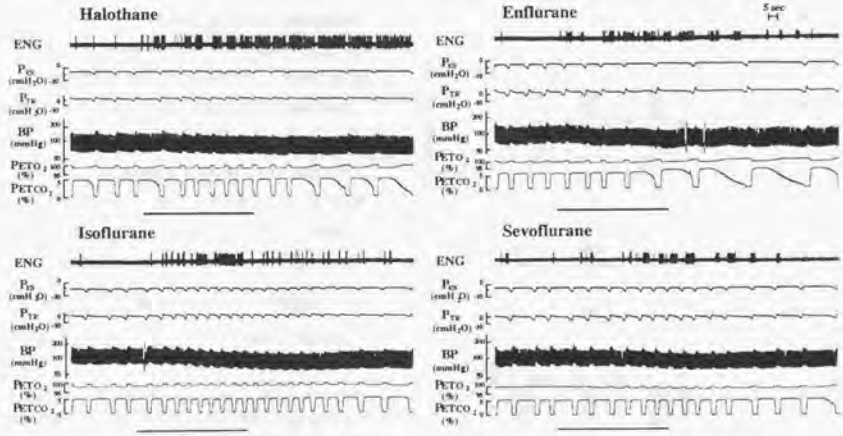


Fig. 4. Responses of a bronchial C-fiber (the same ending as in Fig. 1, lower panel) to 5% volatile anesthetics in an anesthetized spontaneously breathing dog. The bronchial C-fiber was stimulated by all anesthetics, most strongly by Hal. The horizontal lines show the inhalation time of each volatile anesthetic. Abbreviations as in Fig. 1.

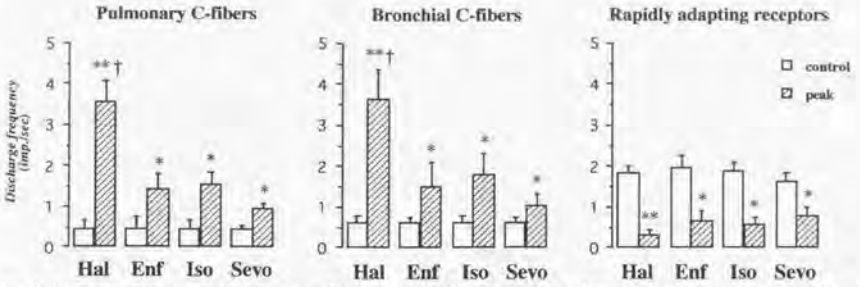


Fig. 5. Responses of pulmonary C-fibers (n = 21), bronchial C-fibers (n = 15), and rapidly adapting receptors (n = 15) to 5% volatile anesthetics. Control and peak values (mean ± SE) were obtained from recordings for 1 min before inhalation and for 10 sec at maximum response during inhalation. $^{*}P<0.05$, $^{**}P<0.01$ *vs* control, $^{\dagger}P<0.05$ *vs* Sevo.

The latency of the respiratory responses was 15.6 ± 1.8 sec for Hal, 26.3 ± 2.2 sec for Enf, 21.5 ± 2.5 sec for Iso, and 28.4 ± 2.5 sec for Sevo. No statistically significant differences were found among these latencies ($P = 0.26$). The increase in f_R and the decrease in V_T were significantly reduced by both perineural CAPS-treatment and bilateral vagotomy only for Hal ($P < 0.05$) (Figs. 6 and 7). Significant changes in these parameters from the control level were no longer observed during the administration of either Iso or Sevo after perineural CAPS-treatment or bilateral vagotomy. In contrast, the effect of Enf on f_R and V_T are the opposite of those of the other three anesthetics. No significant differences were found during Enf administration with non-treatment, CAPS-treatment and bilateral vagotomy. No statistically significant changes were found in $\dot{V}_E$ during the administration of any of the anesthetics (Fig. 6).

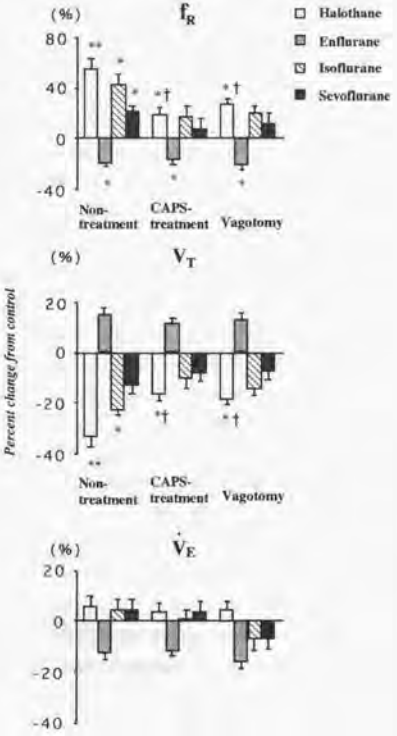


Fig. 6. Summary of ventilatory responses to volatile anesthetics with or without capsaicin (CAPS)-treatment and bilateral vagotomy in 14 anesthetized spontaneously breathing dogs. The increase in f_R and the decrease in V_T with Hal were significantly depressed after CAPS-treatment and vagotomy. Significant changes in these respiratory parameters with Iso and Sevo were no longer observed after the treatments. In contrast, no significant changes were observed with Enf among treatments. Each value is expressed as the peak percent change from control (mean ± SE).
$^{*}P<0.05$, $^{**}P<0.01$ *vs* control, $^{\dagger}P<0.05$ *vs* non-treatment.

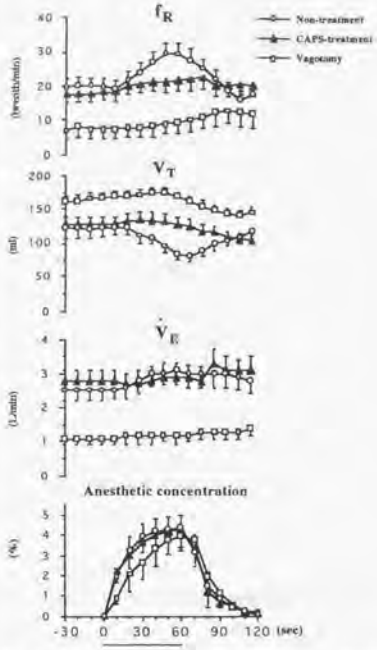


Fig. 7. Time-course of changes in ventilation during the inhalation of Hal with or without capsaicin (CAPS)-treatment and bilateral vagotomy in 14 anesthetized spontaneously breathing dogs. Values are expressed as mean ± SE. The horizontal lines show the onset of the inhalation of Hal.

In all groups (non-treatment, CAPS-treatment, and bilateral vagotomy), ABP decreased significantly with all anesthetics ($P < 0.05$), while HR increased significantly only with Iso ($P < 0.05$) (Table 1). No statistically significant differences were found in HR and ABP values among the anesthetics within and between groups, although HR was higher in dogs with vagotomy (Table 1).

4. Discussion

A rapid shallow breathing pattern represented by an increase in f_R and a decrease in V_T was consistently observed after the onset of inhalation of Hal, Iso, and Sevo, which coincided with an increase in the number of action potentials for the bronchial and pulmonary C-fibers. Moreover, most of the effects on breathing pattern produced by these inhalants were reduced or eliminated by either perineural CAPS treatment or bilateral vagotomy. Perineural CAPS-treatment provides a complete abolition of cardiorespiratory reflexes via the vagal CAPS-sensitive C-fibers without affecting the Hering-Breuer inflation reflex via the slowly adapting pulmonary stretch receptors (Schelegle *et al.*, 1995). Such reflex responses do not include any upper airway reflex because the anesthetics were administered by method that bypassed the larynx. These observations are consistent with the findings that the reflex tachypnea, at least that evoked by the inhalation of Hal, Iso, and Sevo observed in the present study, is mediated primarily by the stimulation of vagal C-fibers.

In this study, a greater stimulation of the vagal C-fibers was observed with Hal, accompanied by a greater tachypnea than that induced by other anesthetics, which is consistent with the significant reduction in response after CAPS-treatment or vagotomy (Fig. 6). This finding, along with clinical observations that the administration of Hal results in an increased incidence of tachypnea as compared with Enf, Iso, and Sevo during the induction and maintenance of anesthesia (Mutoh *et al.*, 1995, 1997a), suggests the important role of the C-fibers during the inhalation of Hal.

Table I
Changes in heart rate (HR) and mean arterial blood pressure (ABP) by inhalation of volatile anesthetics

		HR (beats/min)		ABP (mm Hg)	
		control	peak	control	peak
Hal	Non-treatment	125 ± 9	116 ± 9	115 ± 9	93 ± 8*
	CAPS-treatment	122 ± 8	118 ± 10	121 ± 10	101 ± 7*
	Vagotomy	141 ± 10	136 ± 12	128 ± 10	108 ± 8*
Enf	Non-treatment	120 ± 11	124 ± 8	114 ± 8	89 ± 7*
	CAPS -treatment	128 ± 11	131 ± 12	118 ± 9	92 ± 8*
	Vagotomy	144 ± 10	146 ± 11	122 ± 11	108 ± 10*
Iso	Non-treatment	125 ± 9	133 ± 8*	118 ± 10	80 ± 8*
	CAPS -treatment	123 ± 8	132 ± 10*	115 ± 11	83 ± 7*
	Vagotomy	138 ± 9	148 ± 7*	126 ± 9	97 ± 8*
Sevo	Non-treatment	123 ± 11	129 ± 10	112 ± 9	87 ± 8*
	CAPS -treatment	130 ± 8	135 ± 10	110 ± 9	89 ± 7*
	Vagotomy	143 ± 10	149 ± 12	125 ± 11	99 ± 10*

* Significantly different from control value ($P < 0.05$). n = 14.
No statistical significant differences among anesthetics were found in HR and ABP within and between treatments. All data were expressed as mean ± SE.

In contrast to the tachypnea which occurs in response to the inhalation of these three inhalants, a slowing of respiration is observed during the inhalation of Enf. Because these respiratory responses to Enf were equally observed in all the groups with or without CAPS-treatment and bilateral vagotomy, it is possible that the reflex responses mediated by vagal C-fiber afferents might have been masked or blocked by the depression of the central nervous system via the anesthetic absorbed into the systemic circulation. Halogenated volatile anesthetics generally depress medullary inspiratory and expiratory neurons by blocking synaptic transmission, the effect of which is greater with Enf than Hal (Kasaba *et al.*, 1987).

In the present study, differences in the latencies between pulmonary and bronchial C-fibers to the right atrial CAPS injection were recognized, the results of which mostly agree with those of a previous study (Coleridge and Coleridge, 1977, 1984). In contrast, the latencies of each C-fiber to inhalation of volatile anesthetics were reversed. This is because the preferential stimulation of bronchial C-fibers by inhaled substances is more likely due to the greater accessibility of the agents. In any event, it is still unclear which type of C-fibers are more likely to be associated with the tachypnea that occurs during Hal, Iso, and Sevo administration; it can be presumed, however, that the bronchial C-fibers make an important contribution to the reflex tachypnea based on the coincidence of the latencies between the two responses.

The inhibitory effects of volatile anesthetics on RARs in the present study were not consistent with findings for other inhaled chemical irritants such as NH_3 (Mills *et al.*, 1969; Bergren and Sampson, 1982; Matsumoto, 1989) and cigarette smoke (Sellick and Widdicombe, 1971; Kou and Lee, 1990), which induced a stimulation of RARs in various species. The inhibition of tracheobronchial

RARs by the inhalation of volatile anesthetics has also been reported in a previous study (Nishino *et al.*, 1994), as well as the inhibition of some laryngeal irritant receptors (Mutoh *et al.*, 1998). It is, however, still unclear from the results of the present study whether the removal tonic RAR activity has any impact on the dog's breathing pattern.

The remainder of the tachypnea to Hal after vagotomy might originate from the central nervous system. In fact, the hyperpnea during the inhalation of Hal is eliminated by decerebration (Gautier *et al.*, 1987). Moreover, a transient increase in the activity of some medullary respiratory neurons can be induced by the brief inhalation of Hal (Ebata and Aoki, 1977).

We did not find any significant differences in the cardiovascular responses to the volatile anesthetics with or without treatment. This suggests that the cardiovascular change in response to the anesthetics is more likely attributable to direct peripheral vasodilation (Bernard *et al.*, 1990; Pagel *et al.*, 1991), than the stimulation of vagal C-fibers. Higher HR and slightly lower ABP values of isoflurane might be due to the direct action on the sympathetic output and vasodilator motor neurons. In fact, the stimulation of efferent sympathetic activities, rather than the stimulation of vagal sensory afferents, is primarily responsible for the cardiovascular responses to a rapid increase in isoflurane concentration (Okamoto *et al.*, 1996). The vasodilator action of Iso is known to be greater than other anesthetics (Bernard *et al.*, 1990; Pagel *et al.*, 1991)

Clinically, various complications associated with the induction of anesthesia with volatile anesthetics have been presumed to be caused by an irritation of the airway mucosa (Doi and Ikeda, 1993). In our previous study, laryngeal CAPS-sensitive receptors were consistently stimulated by halogenated volatile anesthetics (Mutoh *et al.*, 1998), and the degree of change in discharge frequency observed for volatile anesthetics clearly corresponded to the degree of airway irritation by volatile anesthetics as experienced clinically in humans, i.e., a higher incidence of complications such as coughing, laryngospasm, inhibition of breathing (apnea), and excessive secretions with Hal, Enf, and Iso than with Sevo during the induction of anesthesia (Yurino and Kimura, 1992, 1993a,b, 1994; Funk *et al.*, 1996).

The present study demonstrates the important role of vagal CAPS-sensitive C-fibers in the reflex hyperpnea induced by Hal, Enf, and Iso. Such findings may be an indicative of the functional importance of these fibers in the defensive or protective airway reactions to volatile anesthetics.

Section 4 Effects of volatile anesthetics on the larynx

4. 1. Responses of laryngeal capsaicin-sensitive and irritant receptors to volatile anesthetics in anesthetized dogs

Abstract

The responses of laryngeal CAPS-sensitive receptors to Hal, Enf, Iso and Sevo were evaluated in anesthetized spontaneously breathing dogs from the recording of action potentials of internal branch of the superior laryngeal nerve. The CAPS-sensitive receptors were clearly distinguished from irritant receptors by their prevalent responsiveness to CAPS and their lack of responsiveness to water. All the CAPS-sensitive receptors were significantly stimulated by all volatile anesthetics in a concentration-related manner, and the activation by Hal, Enf and Iso was significantly greater than by sevoflurane. In contrast, responses of irritant receptors to the volatile anesthetics were divided into three types (stimulation, inhibition, or non-response), to which no significant differences were found among anesthetics. In conclusion, the present study demonstrated that the CAPS-sensitive receptors were consistently stimulated by halogenated volatile anesthetics and especially by Hal, Enf and Iso, and that these responses were dissimilar to the variable responses in irritant receptors.

1. Introduction

The larynx is a potent reflexogenic region of the upper airway that is rich in sensory afferents and that elicits various reflexes to protect the lower airway and lung (Widdicombe *et al.*, 1988; Sant'Ambrogio et al., 1995). Several studies have revealed that the larynx is a possible source of airway irritation by volatile anesthetics. Sant'Ambrogio *et al.* (1993) suggested that Hal administered into the isolated upper airway remarkably depressed ventilation in newborn dogs, the effect of which was greatly diminished by superior laryngeal nerve (SLN) section. Nishino *et al.* (1993) pointed out that halogenated volatile anesthetics, especially Hal, stimulated laryngeal irritant and cold receptors in adult dogs, whereas they little influenced on laryngeal pressure receptors.

In the larynx, as described in preface 3.3., there is a type of receptor which can be activated by a nociceptive chemical stimuli such as CAPS, which produced marked cardiorespiratory responses like apnea, cough, bradycardia, and hypertension in dogs (Mutoh *et al.*, 1997b), rats (Palecek et al., 1990; Hishida *et al.*, 1996), and guinea pigs (Tsubone *et al.*, 1991). Numerous and extensive studies have been conducted on the pulmonary or bronchial CAPS-sensitive C-fiber receptors to chemical irritants including volatile anesthetics (Coleridge and Coleridge, 1984, 1994). However, only a few of these attempts have targeted the larynx, and laryngeal CAPS-sensitive C-fiber receptors have thus far been identified only in guinea pigs (Tsubone *et al.*, 1991).

The aims of this study are to 1) identify the laryngeal CAPS-sensitive receptors; and 2) evaluate their responses to volatile anesthetics in the dog. In this study the responses of the laryngeal CAPS-

sensitive receptors were compared with those of irritant receptors.

2. Methods

2. 1. General—Fifteen healthy beagle dogs (8 males and 7 females) were used in this study. Their mean age was 13.6-months-old (ranging from 10 to 18 months) and their mean body weight 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.

Dogs were placed on an operating table in the supine position, then a laryngeal mask (Intervent, LARYNGEAL MASK Size-3) was introduced to cover the larynx, including the epiglottis. Arterial blood pressure was monitored by a pressure transducer (Nihon Kohden, DX-300) 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 for tracheostomy breathing. This cannula allowed the diversion of breathing from the upper airway to the trachea as well as the occlusion of the airway either at the level of the upper airway or the trachea by inflating the cuff of Foley catheters (Sant'Ambrogio *et al.*, 1983). A pressure transducer (Toyoda, PD104) was attached to the uppermost sidearm of the cannula in order to measure intratracheal 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 (Nihon Kohden, DX-300) for recording esophageal pressure.

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

2. 2. Recordings of the 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 performed within a pool of paraffin oil using fine forceps and the aid of a binocular microscope (OLYMPUS, SZ60). The signal was amplified by a low noise DC-amplifier (DIA Med., DPA201) and a biophysical amplifier (DIA Med., DPA200), and displayed on an oscilloscope (IWATSU, SS5762) in parallel with a loudspeaker (NEC san-ei, Model 7747). All the signals were displayed on a thermal-array recorder (NEC san-ei, RT3100N) and recorded by a magnetic tape recorder (SONY, PC204A). Once an acceptable single unit was found, identification of the sensory modality of that particular ending was established according to the conventional method (Sant'Ambrogio et al., 1983), from which respiration-modulated 'pressure,' 'drive,' and 'cold' receptors were characterized. Briefly, the respiratory-modulated receptors were identified by comparing their activity during (1) upper airway breathing, (2) tracheostomy breathing, (3) upper airway occlusion, and (4) tracheal occlusion. All fibers with random activities except the respiration-modulated receptors were used in the following experiments.

2. 3. Experimental protocol—Upper airway is functionally isolated by inflating the cuff of a Foley catheter inside the tracheal cannula at its middle position through tracheostomy breathing. 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 challenged with 5% of Hal, Enf, Iso and Sevo for 1 min, respectively. If any visible response was observed in the receptor discharge at 5% of concentration, 1 and 3% of each anesthetic were tried thereafter for the evaluation of the concentration-relationship. Intralaryngeal anesthetic concentration was monitored with an infrared gas monitor (Datex, Capnomac) which was sampled through a sampling tube connected to the uppermost sidearm of the tracheal cannula.

After the volatile anesthetics challenges described above, the individual receptor was stimulated by a light mechanical probing of the laryngeal lumen using a Foley catheter inserted through a sidearm of the cannula. Then, 10 ml of CAPS solution (10 μg/ml, a diluted solution of CAPS 100 μg/ml, in a solution containing 0.9% NaCl, 1% ethyl alcohol, and 0.1% Tween 80) or the same volume of distilled water at a temperature of 37°C was topically instilled into the laryngeal lumen through a catheter with multiple holes at the distal port. The order of each challenge was random. Warmed isotonic NaCl solution (0.9%) at 37°C was used for rinsing the laryngeal lumen after each trial. Each challenge was performed at an interval of 20 min or more.

2. 4. Dataanalysis—The discharge frequencies of single unit activity were counted every second for a period of 30 sec before the trial with a spike height discriminator (DIA Medical, DSE 425), then the data were averaged every 10 sec. The averaged discharge frequency for 30 seconds before the trial was taken as the control value, and the maximum or minimum discharge frequency out of the values averaged every 10 sec after onset of the trial was taken as the peak value. The latency (sec) of each receptor's response was evaluated visually as the point when the number of action potential began to increase. For the receptors which decreased the number of action potentials, the duration (sec) of onset of inhibition was recorded in the same way. Statistical analysis was the same as described in experiment 3.1.

3. Results

3. 1. Laryngeal 'CAPS-sensitive' receptors and 'water-responsive' irritant receptors—Single unit activities were recorded from a total of 30 receptors with an irregular discharge which did not have a respiratory modulation. Fifteen receptors (15/30, 50%) were stimulated by CAPS; 11 receptors were stimulated by only CAPS ('CAPS-sensitive' receptors: Table 1; Fig. 1), and 4 receptors were clearly stimulated by water and slightly by CAPS (Table 2 #1-4; Fig. 2a). The remaining 15 receptors were stimulated by only water and had no response to CAPS (Table 2 #5-19; Fig. 2b).

Table 1

Responses of laryngeal capsaicin-sensitive receptors before and after administration of 5% volatile anesthetics, water, and capsaicin

Number of	Halothane		Enflurane		Isoflurane		Sevoflurane		Water		Capsaicin	
receptor	before	after	before	after	before	after	before	after	before	after	before	after
#1	0.5	5.5 (+5.0)	0.7	5.2 (+4.5)	0.6	7.4 (+6.8)	0.2	3.4 (+3.2)	0.2	0.1	0.1	20.2
#2	0.3	3.6 (+3.3)	0.5	3.2 (+2.7)	0.3	7.0 (+6.7)	0.8	2.2 (+1.4)	0.1	0.3	0.3	23.5
#3	0	2.8 (+2.8)	0	0.8 (+0.8)	0	0.4 (+0.4)	0	1.0 (+1.0)	0	0	0	10.3
#4	0.2	1.2 (+1.0)	0.5	1.3 (+0.8)	0	2.4 (+2.4)	0.5	1.6 (+1.1)	0.1	0	0.1	15.0
#5	0.2	2.2 (+2.0)	0.2	2.8 (+2.6)	0	1.6 (+1.6)	0.2	0.6 (+0.4)	0.3	0	0	14.5
#6	0	2.4 (+2.4)	0.4	3.2 (+2.8)	0	1.0 (+1.0)	0.2	1.4 (+1.2)	0	0	0	16.4
#7	0.4	3.6 (+3.2)	0.5	2.4 (+1.8)	0.2	2.2 (+2.0)	0.4	1.2 (+0.8)	0.2	0.1	0.1	22.2
#8	0.8	6.4 (+5.6)	0.8	6.5 (+5.7)	1.2	5.0 (+3.8)	1.6	4.3 (+2.7)	1.5	1.7	1.3	20.5
#9	1.8	7.2 (+5.4)	1.4	5.2 (+3.8)	1.2	7.9 (+6.7)	1.6	2.0 (+0.4)	1.0	1.3	1.2	24.5
#10	2.0	4.0 (+2.0)	1.3	2.5 (+1.2)	1.3	1.8 (+0.5)	2	2.2 (+0.2)	1.4	1.3	1.8	22.8
#11	1.0	6.6 (+5.6)	1.4	7.0 (+5.6)	1.0	5.4 (+4.4)	1.2	2.8 (+1.6)	1.3	1.1	1.0	18.0
average	0.7	4.1 (+3.4)	0.7	3.6 (+2.9)	0.5	3.8 (+3.3)	0.8	2.1 (+1.3)	0.6	0.5	0.5	18.9

nc: not challenged.

The data are shown as mean discharge frequencies (imp./sec). The number in the parenthesis represents the number of impulses increased or decreased by each volatile anesthetics.

Table 2

Responses of water-responsive irritant receptors before and after administration of 5% volatile anesthetics, water, and capsaicin

Number of	Halothane		Enflurane		Isoflurane		Sevoflurane		Water		Capsaicin	
receptor	before	after	before	after	before	after	before	after	before	after	before	after
#1	0	1.6 (+1.6)	0	1.0 (+1.0)	0	1.2 (+1.2)	0	1.8 (+1.8)	0	25.5	0	2.3
#2	2.4	5.0 (+2.6)	2.9	5.0 (+2.1)	3.5	4.8 (+1.3)	2.6	3.0 (+0.4)	2.4	22.5	2.8	4.3
#3	1.7	0.1 (-1.6)	2.0	0.1 (-0.9)	1.5	0 (-1.5)	2.1	0 (-2.1)	1.6	35.2	1.6	2.8
#4	0.9	0 (-0.9)	1.8	0 (-1.8)	1.3	0 (-1.3)	0.8	0 (-0.8)	0.6	28.3	0.8	4.2
#5	0.2	2.8 (+2.6)	0.3	2.6 (+2.3)	0.2	1.8 (+1.6)	0.5	3.0 (+2.5)	0.4	16.7	0.3	0.4
#6	1.0	5.5 (+4.5)	1.2	5.8 (+4.6)	1.5	6.2 (+4.7)	1.2	3.2 (+2.0)	1.4	20.2	1.5	1.1
#7	0.5	3.2 (+2.7)	0.4	2.8 (+2.4)	0.2	4.0 (+3.8)	0.5	2.4 (+1.9)	0	24.5	0.2	0.2
#8	1.0	2.6 (+1.6)	0.7	1.4 (+0.7)	0.4	1.0 (+0.6)	0.8	1.4 (+0.6)	0.6	18.0	1.1	0.8
#9	0.3	2.3 (+2.0)	0.5	1.3 (+0.8)	0.3	1.2 (+0.9)	0.8	1.7 (+0.9)	0.7	35.0	0.5	0.6
#10	1.4	4.5 (+3.1)	1.6	3.8 (+2.2)	0.8	3.0 (+2.2)	1.4	2.7 (+1.3)	1.7	29.0	1.4	1.8
#11	0.2	1.4 (+1.2)	0.5	1.0 (+0.5)	0.3	0.8 (+0.5)	0.3	1.2 (+0.9)	0.3	19.8	0.2	0.4
#12	1.0	3.0 (+2.0)	0.8	2.4 (+1.6)	0.8	1.5 (+0.7)	0.8	2.0 (+1.2)	0.6	24.8	1.3	0.7
#13	1.1	0 (-1.1)	0.8	0 (-0.8)	0.6	0 (-0.6)	0.5	0.1 (-0.4)	0.8	17.5	0.6	1.1
#14	1.2	0 (-1.2)	1.2	0.1 (-0.9)	1.0	0.3 (-0.7)	0.8	0 (-0.8)	1.0	15.0	0.8	0.4
#15	1.8	0.4 (-1.4)	1.5	0.2 (-1.3)	2.3	0.9 (-1.4)	1.8	0.3 (-1.5)	2.0	15.1	1.4	2.0
#16	1.1	0 (-1.1)	0.6	0 (-0.6)	0.7	0 (-0.7)	0.9	0 (-0.9)	1.3	21.5	1.0	0.7
#17	0.7	0.1 (-0.6)	1.0	0.1 (-0.9)	1.5	1.0 (-0.5)	1.5	0 (-1.5)	1.7	31.5	2.0	2.3
#18	0.1	0 (-0.1)	0.1	0 (-0.1)	0	0 (0)	0	0 (0)	0.1	14.4	0	0
#19	0.4	0.5 (+0.1)	0.4	0.4 (0)	0.7	0.5 (-0.2)	0.4	0.3 (-0.1)	0.8	16.4	0.5	0.5
average	0.9	1.7 (+0.8)	1.0	1.5 (+0.5)	0.9	1.5 (+0.6)	0.9	1.2 (+0.3)	0.9	22.7	0.9	1.2

#1-4: greatly activated by water and slightly by capsaicin; #5-19: predominantly stimulated by water and little or no response to capsaicin. The data are shown as mean discharge frequencies (imp./sec). The number in the parenthesis represents the number of impulses increased or decreased by each volatile anesthetics. nc: not challenged.

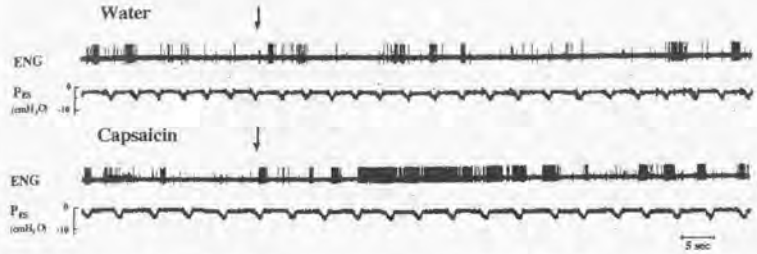


Fig. 1. An example of recording from a capsaicin-sensitive receptor (Table 1 #9). This receptor responded to distilled water to only a minor extent but was strongly activated by the capsaicin instillation.
ENG = electroneurogram, P_{ES} = esophageal pressure.

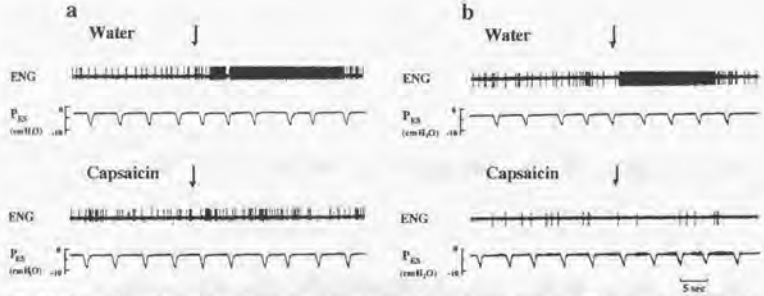


Fig. 2. Examples of recording from two 'water-responsive' irritant receptors. The arrows indicate the intralaryngeal instillation of distilled water or capsaicin. Type-a (Table 2 #4) predominantly responded to water but only weakly to capsaicin, while type-b (Table 2 #9) was activated only by water and showed no response to capsaicin. Abbreviations as in Fig. 1.

All but 3 of the CAPS-sensitive receptors (8/11,73%) failed to respond to the mucosal mechanical probing, while all the water-responsive receptors (19/19, 100%) were activated by the probing. All the CAPS-sensitive and water-responsive receptors with mechanical sensitivities showed a rapid adaptation to the probing. In this study, the 19 rapidly adapting receptors that were stimulated by water were categorized as 'irritant' receptors. Neither CAPS-sensitive receptors nor irritant receptors were activated by saline solution.

The latency of CAPS-sensitive receptor by the CAPS instillation was 9.6 ± 8.6 sec. The CAPS-sensitive receptors showed a long-lasting discharge to CAPS instillation of 30.6 ± 7.1 sec. On the other hand, all the irritant receptors showed a latency of 2.4 ± 0.5 sec (n = 19). The duration of continuous discharge of the irritant receptors to water was 44.2 ± 7.4 sec (n = 19, Table 2), whereas that to CAPS was only 7.8 ± 5.5 sec (n = 4, Table 2).

3. 2. Responses of laryngeal CAPS-sensitive and irritant receptors to volatile anesthetics—The administration of oxygen flow for the control recording lowered the laryngeal temperature to as much as 27.7 ± 0.9 °C (ranging from 25.4 to 30.0 °C) from the value of room air at 34.4 ± 0.8 °C (ranging from 32.2 to 36.5 °C); however, discharges of both CAPS-sensitive and irritant receptors were unchanged.

A large number of CAPS-sensitive receptors significantly increased their discharges in all anesthetics at 5% of concentration compared with the control value ($P < 0.01$; Fig. 3). The increase in discharge frequency at 5% was significantly greater in Hal, Enf and Iso than in Sevo ($P < 0.05$; Figs. 3, 4).

The latency of the CAPS-sensitive receptor response to administration of each volatile anesthetic was 17.2 ± 2.8 sec in Hal, 17.5 ± 4.2 sec in Enf, 19.0 ± 4.0 sec in Iso, and 24.4 ± 3.4 sec in Sevo, respectively. No statistical significance was observed in these latencies ($P = 0.35$). All the receptors showed a concentration-related increase in discharge frequency with increasing anesthetic concentrations, as represented in Fig. 5.

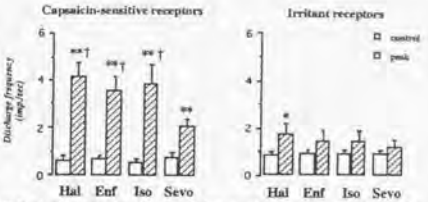


Fig. 3. Responses of capsaicin-sensitive receptors (n = 11) and irritant receptors (n = 19) to 5% volatile anesthetics. Each value (mean ± SE) was obtained from the recordings of both the control discharge frequency before and peak discharge frequency after the onset of the trial. * $P < 0.05$, ** $P < 0.01$ *vs* control, † $P < 0.05$ *vs* Sevo.

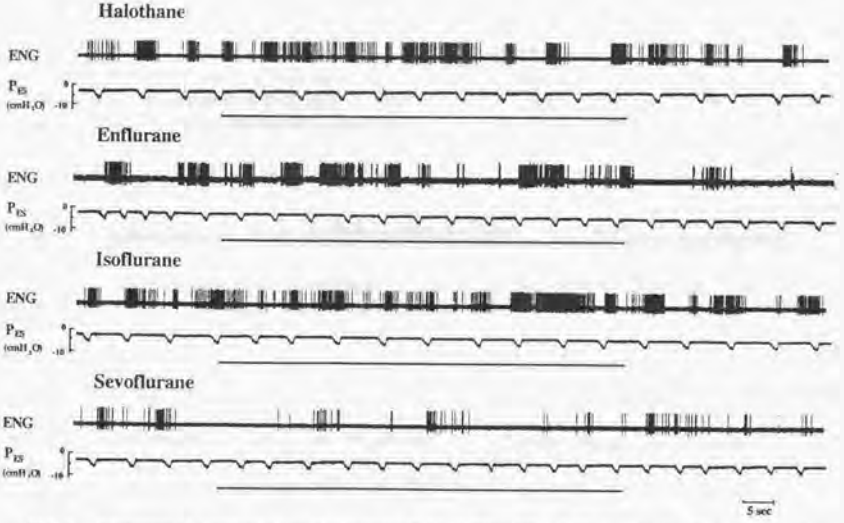


Fig. 4. Responses of a capsaicin-sensitive receptor (Table 1 #9) to 5% volatile anesthetics. The horizontal lines show the intralaryngeal administration of each volatile anesthetic. This fiber was stimulated by Hal, Enf, and Iso, but not by Sevo. Abbreviations as in Fig. 1.

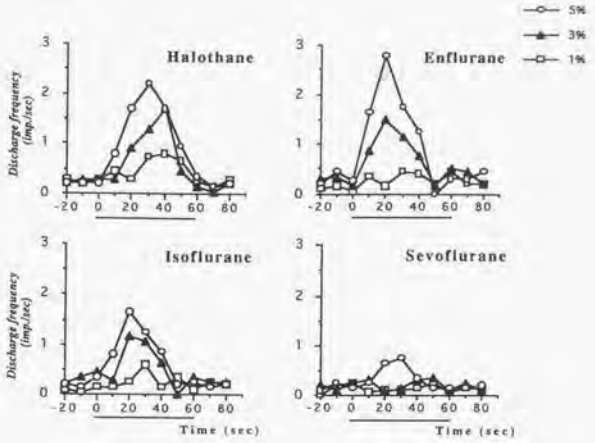


Fig. 5. Time-course changes in a capsaicin-sensitive receptor (Table 1 #5) to 1, 3, and 5% volatile anesthetic. This receptor was stimulated in a dose-dependent manner by each volatile anesthetic except for Sevo. The abscissa and ordinate represent time (sec) and discharge frequency (imp./sec) measured every 10 sec. The horizontal lines indicate the intralaryngeal administration of each volatile anesthetic.

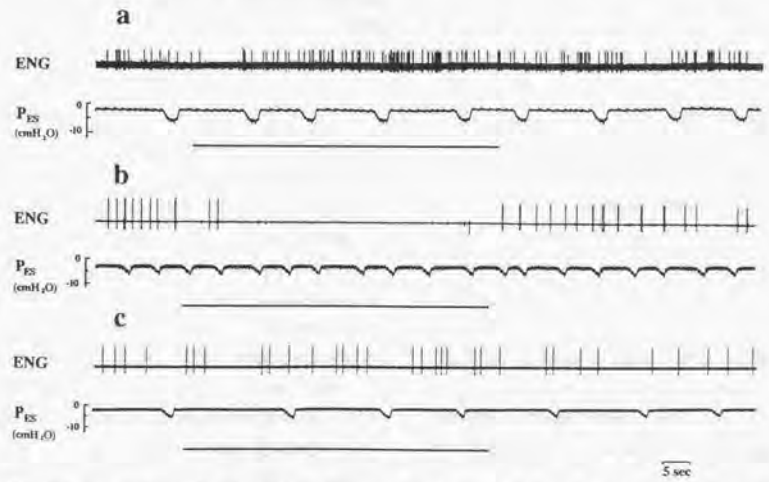


Fig. 6 Examples of recording from irritant receptors (a, #10; b, #14; c, #19 in Table 2) to 5% volatile anesthetics. The response of each receptor was similar regardless of the type of anesthetic agent. Type-a receptor was stimulated, type-b was inhibited, and type-c failed to respond to volatile anesthetics. Abbreviations as in Fig. 1.

Responses of irritant receptors to volatile anesthetics were divided into three types as follows: 1) activated (Table 2 #1, 2, 5-12; Fig. 6a), 2) inhibited (Table 2 #3,4, 13-17; Fig. 6b), and 3) little or not changed (Table 2 # 18,19; Fig. 6c) by administration of anesthetics. The latency of activation in irritant receptors (13.3 ± 1.2 sec) was significantly longer than the onset time of inhibition (6.7 ± 0.8 sec; $P < 0.01$) in the inhibition type of receptor.

In all the inhalants at 5% the averaged discharge frequencies of all irritant receptors tended to increase as compared to the control value, but the increase was significant only in Hal ($P < 0.05$; Fig. 3). There were no significant differences in discharge frequencies among the anesthetics ($P = 0.85$).

The mean latencies to increase the discharge in administration of all anesthetics at 5% were 18.8 ± 1.9 sec for CAPS-sensitive receptors and 13.3 ± 1.2 sec for irritant receptors, respectively. No statistical significance was observed between those latencies ($P = 0.29$).

4. Discussion

4. 1. Laryngeal CAPS-sensitive and irritant receptors in the dog—In this study, the presence of laryngeal CAPS-sensitive receptors was ascertained in dogs through the topical application of CAPS into the laryngeal lumen. The characteristics of their activation were clearly distinguished from those of irritant receptors by their predominant responsiveness to CAPS and lack of water response. These findings well coincided with those of CAPS-sensitive receptors in guinea pigs (Tsubone *et al.*, 1991). Capsaicin is known to be a selective stimulant for C-fiber endings and a part of A-δ fiber endings (Holzer, 1991), and the use of CAPS has been an advantageous method for identifying C-fiber endings (Coleridge and Coleridge, 1984). An *in vitro* study of single vagal afferents innervating guinea pigs trachea showed a marked response of C-fibers, but no response of A-δ fibers to CAPS application (Fox *et al.*, 1993). Although not all the unmyelinated C-fiber afferents are CAPS sensitive, and a part of small myelinated afferents may be stimulated by CAPS (Holzer, 1991), the results of the present study indicated a high possibility of the presence of unmyelinated C-fibers in the larynx of dogs. In fact, the histological study on the internal branch of the superior laryngeal nerve in the dog demonstrated that 46% of afferents were unmyelinated fibers (Chung *et al.*, 1993).

In this study, most of the CAPS-sensitive receptors (73%) failed to respond to the mucosal mechanical stimuli, indicating the predominant nociceptive chemosensitivity in this receptor. As to the mechanical susceptibility of the CAPS-sensitive C-fibers, the presence of fundamentally different properties is presumed among airways since vagal CAPS-sensitive C-fibers could respond to mechanical stimuli with low threshold (Fox *et al.*, 1993).

We regarded all the water-responsive receptors having irregular discharges as 'irritant' receptors, since a lack of chloride anions (Anderson *et al.*, 1990) and a rapid adaptation to mechanical probing (Widdicombe and Sant'Ambrogio, 1992; Sant'Ambrogio *et al.*, 1995) are identifiable with irritant receptors. In this study, some irritant receptors were also activated to a lesser extent by CAPS application. It is doubtful, however, that these receptors were identical with unmyelinated C-fiber endings. The long-lasting discharges, i.e., 30.6 sec for CAPS-sensitive receptors to CAPS and 44.2 sec for irritant receptors to distilled water, as well as their short latency to their respective substance, could confirm the clear difference in exciting property between the CAPS-sensitive and irritant receptors.

4. 2. Responses of the CAPS-sensitive and irritant receptors to volatile anesthetics—It was demonstrated that all the CAPS-sensitive receptors were equally stimulated by volatile anesthetics in a concentration-dependent manner. Several studies have been demonstrated as to the stimulus effect of volatile anesthetics on CAPS-sensitive C-fibers. Pulmonary C-fiber afferents can be activated just after inhalation with a high concentration (5-20%) of halothane in dogs (Coleridge and Coleridge, 1984). In a very recent study, Hal has been used as a stimulant for pulmonary C-fiber endings in monkeys (Ravi and Singh, 1996). In addition, Hal, Enf and Iso showed an excitatory property of C-fiber afferents from the *in vitro* rabbit cornea (MacIver and Tanelian, 1990). These previous studies and the present study evidenced that those volatile anesthetics, including Hal, share a common ability to stimulate to C-fiber endings. It is important to note that the degree of changes in discharge frequency observed for volatile anesthetics in the present study clearly corresponded to the degree of airway irritation by volatile anesthetics as experienced clinically in humans, i.e., higher incidence of complications such as cough, laryngospasm, apnea, and excessive secretion in Hal, Enf and Iso compared to Sevo during induction of anesthesia (Yurino and Kimura, 1992, 1993a, 1994; Doi and Ikeda, 1993).

In contrast to the uniform action of volatile anesthetics on CAPS-sensitive receptors, the response of irritant receptors was variable. Similarly, an *in vitro* study by MacIver and Tanelian (1990) revealed uniform response of C-fibers and variable responses of A-δ fibers to volatile anesthetics in rabbit cornea. The variety of responses (stimulation, inhibition, or non-response) of irritant receptors may partly correspond to the differences in the location and/or morphology of nerve endings. Namely, the type of irritant receptors with short onset of inhibition may locate in the most superficial site of the laryngeal mucosa, and their activities may be blocked by a prompt blocking effect as a local anesthesia (Camporesi *et al.*, 1979; Widdicombe *et al.*, 1988); some non-response type of irritant receptors are presumed to lie in the deeper site, where the anesthetic gas cannot sufficiently prevail. As regards the distribution and morphology of nerve endings in dog larynx, a recent study clearly showed the presence of both laminar and glomerular nerve endings as well as submucosal networks of neurons and intrinsic nerve cell bodies (Yamamoto *et al.*, 1997). We, however, cannot conclusively determine which type of these endings corresponds to the receptor type electrophysiologically identified and which type is inhibited or stimulated by the volatile anesthetics. Moreover, stimulatory mechanisms, especially membrane mechanisms, of the anesthetics to some irritant receptors and C-fibers is unknown.

On the other hand, the different anesthetics induced similar overall responses from irritant receptors, with only Hal inducing a relatively greater stimulation. These results were mostly consistent with those of a previous study in the larynx of dogs (Nishino *et al.*, 1993); however, such findings appear to be in poor agreement with the prevalent characteristics of airway irritation of volatile anesthetics (Doi and Ikeda, 1993).

On the whole, it is conceivable that the stimulation of CAPS-sensitive rather than irritant receptors can be associated with degree of airway irritation clinically observed in humans during inhalation of the volatile anesthetics. Topical application of CAPS into the larynx evoked marked cardiorespiratory reflexes such as apnea, bradycardia, and hypertension through the central reflex mechanisms in dogs (Mutoh *et al.*, 1997b). Moreover, the presence of substance P containing afferents has been described within the laryngeal

mucosa of dogs (Shin *et al.*, 1987), and the intravenous substance P produces an increase in submucosal gland secretion in the dogs larynx and pharynx via the axonal reflexes (Haxhiu *et al.*, 1991). Considering above, the stimulation of CAPS-sensitive receptors by volatile anesthetics might mediate at least some cardiorespiratory responses via the central mechanisms or local actions via the axonal reflexes in dogs.

In conclusion, the present study described the electrical activities of laryngeal CAPS-sensitive receptors in dogs, the responses of which were consistently stimulated by halogenated volatile anesthetics and especially by Hal, Enf and Iso, and that these stimulations were dissimilar to the variable responses in irritant receptors.

4. 2. Possible cardiopulmonary reflex responses of laryngeal capsaicin-sensitive and irritant receptors to volatile anesthetics in anesthetized dogs

Abstract

Possible cardiopulmonary reflex responses of the laryngeal CAPS-sensitive C-fiber and irritant receptors to volatile anesthetics were elucidated in anesthetized chronic tracheostomized dogs through the topical instillation of CAPS and distilled water into the larynx, and the responses evaluated to Hal, Enf, Iso and Sevo. Capsaicin and water instillations induced a prolongation of T_E and a decrease in V_T, followed by an increase in arterial blood pressure and a decrease in heart rate. The responsiveness to CAPS was reduced by 2nd CAPS trial, indicating the desensitization of CAPS-sensitive endings. All the anesthetics increased T_E and decreased $\dot{V}_E$ significantly from the control level, the effects of which were significantly greater in Hal, Enf, Iso than in Sevo. Approximately 50% of the responses reduced by the CAPS desensitization. Topical anesthesia with lidocaine eliminated all the reflex responses. These results suggested that the various cardiopulmonary reflexes could be elicited by the stimuli to laryngeal CAPS-sensitive and irritant receptors, the effects of which are associated with the laryngeal reflexes of volatile anesthetics especially Hal, Enf and Iso.

1. Introduction

It is well known that marked cardio-respiratory reflexes such as apnea, cough, bradycardia, and hypertension can be provoked by various chemical stimulus such as distilled water or CAPS instilled or aerosolized into the larynx in newborn dogs, rats, and guinea pigs (Mortola and Fisher, 1988; Palecek *et al.*, 1990; Sant'Ambrogio *et al.*, 1995); however, less recognized such reflex responses from the larynx of adult dogs (Palecek *et al.*, 1990). These might be due to maturational changes of the central nervous system, including the terminal site of the sensory nerves around the nucleus tractus solitarius (NTS) decreased with development (Donnelly, 1990). However, it does not mean the absence of reflex contribution of unmyelinated C-fibers, since a 46 % of the internal branch of the superior laryngeal nerve belonged to unmyelinated fibers (Sant'Ambrogio *et al.*, 1995).

It is our hypothesis that these effects may be due to a 'hangover' effect of the basal anesthesia used to attain adequate surgical anesthesia for the experimental setting and maintenance of anesthesia under tracheostomy. In fact, a mixture of urethane-chloralose produces poor analgesia for surgical procedures such as tracheostomy in dogs, whereas the combination provides minimal cardiorespiratory depression and maintenance of the autonomic nervous system (Harvey and Walberg, 1987). However, no study has been performed to study these effects.

The purpose of this study is to (1) elucidate the reflex role of the laryngeal water-responsive and CAPS-sensitive receptors, and (2) evaluate their contribution to the laryngeal reflexes by application of volatile anesthetics in dogs. In the present study we presented an *in vivo* isolated upper

airway model using chronic tracheostomized dogs, the technique for which has been successfully applied clinically to veterinary patients with either life-threatening upper respiratory tract obstruction or the anticipation of their development (Nelson, 1993).

2. Methods

2. 1. General

2.1.1. Animals—Sixteen healthy beagle dogs (7 males and 9 females) were used in this study. Their mean age was 13.5-months-old (ranging from 10 to 18 months) and mean body weight was 11.5 kg (ranging from 7.5 to 14.0 kg).

2.1.2. Permanent tracheostomy—All dogs were subjected to permanent tracheostomy 2 weeks before the experiments. Dogs were induced anesthesia with thiopental sodium (25 mg/kg) intravenously and endotracheally intubated, followed by maintained anesthesia with Iso in 100% oxygen. A window surgical technique for permanent tracheostomy (Nelson, 1993) was used in this study. Briefly, dogs were placed on an operating table in the supine position, and a midline oval-shaped skin incision was made. The sternohyoid muscles were separated, and the ventral aspect of the trachea was exposed. Then the medial edges of the sternohyoid muscles were sutured together at the dorsal end to the trachea for elevation to the skin by horizontal mattress sutures with 3-0 non-absorbable polypropylene monofilament. The ventral sections of three tracheal rings were removed just midportion of the trachea between the cricoid cartilage and the thoracic wall, leaving the tracheal mucosa intact. After the tracheal rings were completely removed, the mucosa was completely dissected with approximately 5 mm margin left from the trachea. Then the skin and trachea including the mucosa were sutured with 3-0 polypropylene monofilament. At the end of general anesthesia, butorphanol (0.2 mg/kg) was injected intramuscularly for postoperative analgesia. During the adjustment period after surgery, tracheostomy site was carefully cleaned and nebulized with saline solution if necessary. Dogs were fed in an isolated room and received ampicillin (20 mg/kg × 2/day) for at least a week after surgery for the prevention of bacterial infection.

2.1.3. Basal Anesthesia—Dogs were anesthetized with thiopental sodium (25 mg/kg), followed by a mixture of urethane (200 mg/kg) and α-chloralose (20 mg/kg) injected intravenously over 15 min. The supplemental dose of urethane and α-chloralose mixture (150 mg/ml urethane and 15 mg/ml α-chloralose) was infused at a rate of 1 ml/kg/hour with an infusion pump (Terumo, Model STC-531) through an intravenous catheter placed into the cephalic or saphenous vein. Lactated Ringer's solution was also infused at a rate of 10 ml/kg/hour through the intravenous catheter placed into the other side of the cephalic or saphenous vein. At least 1 hour's adjustment period was allowed after the induction of anesthesia to obtain suitable basal anesthetic level for the experiment.

2.1.4. Animal preparation—Dogs were placed on an operating table in the supine position, and then a laryngeal mask (The Laryngeal Mask Company, LM1003, I.D = 10 mm) was introduced to cover the epiglottis. Cuffed tracheostomy tubes (Nihon Medico, Portex, I.D = 7.0-8.0 mm) were introduced into the upper and lower trachea through a tracheostomized airway. The tip end of the upper tracheostomy tube was carefully positioned at the level of cricoid cartilage in order to isolate the larynx functionally. Arterial blood pressure was monitored by a pressure transducer (Nihon Kohden, DX-300) connected to a 20-G catheter inserted into the femoral artery. A thermal probe was inserted just below the epiglottis through the laryngeal

mask to record laryngeal temperature. End-tidal PO_2 ($PETO_2$) and PCO_2 ($PETCO_2$) were sampled through the line attached to the endotracheal tube and monitored by a gas analyzer (NEC, Respina 1H26). Intratracheal pressure was measured with a pressure transducer (Toyoda, PD104) through a side arm attached to the lower tracheostomy tube. Respiratory airflow ($\dot{V}$) was recorded with a differential pressure transducer (Toyoda, DD-102A) through two sidearms connected to the lowermost tube, and it was integrated with an A-D converter (BRC, Mac Lab Scope) to give tidal volume (V_T). Minutes ventilation ($\dot{V}E$) was calculated from the value of V_T and the total cycle duration. A saline-filled polyethylene catheter (O.D. = 3 mm) was placed in the middle portion of the esophagus and connected to a pressure transducer (Nihon Kohden, DX-300) for recording esophageal pressure. All the signals were displayed on a thermal-array recorder (NEC, RT 3100N) and recorded by a magnetic tape recorder (Sony, PC 208).

2. 2. Experimental protocol

2. 2. 1. Evaluation of reflex cardiopulmonary effects of volatile anesthetics—After a control period for more than 1 min with 100% oxygen at a flow rate of 6 L/min, 5% concentrations of Hal, Enf, Iso, and Sevo were topically inhaled into the larynx for 1 min. If any change was observed in the experimental tracing displayed on the thermal-array recorder, 1% and 3% of each anesthetic were inhaled.

2. 2. 2. Evaluation of reflex roles of CAPS-sensitive and irritant receptors— After the challenge of volatile anesthetics, 10 ml of CAPS solution (100 μg/ml of CAPS, in a solution containing 0.9% NaCl, 1% ethyl alcohol, and 0.1% Tween 80) or the same volume of distilled water was instilled into the isolated upper airway through a Foley catheter with multiple holes at the distal port to evaluate the reflex responses to the stimuli of either 'CAPS-sensitive' or 'water-responsive' irritant receptors (Mutoh et al., 1998). The order of each challenge was random. Isotonic NaCl solution (0.9%) at 37°C was used as a control and for rinsing the laryngeal lumen before and after each trial. The CAPS instillation (100 μg/ml) was repeated to evaluate the desensitization of CAPS-sensitive endings. In addition, *l*-menthol was added to the O_2 flow at a flow rate of 6 L/min. *l*-menthol was administered into the larynx for 1 minute to evaluate the reflex contribution of laryngeal cold receptors to the reflex responses (Sant'Ambrogio et al., 1988). The *l*-menthol stimulus was applied by passing through the bottle (200 ml) containing 1.0 g of *l*-menthol crystals. After the second CAPS trial (100 μg/ml), the volatile anesthetics and distilled water were applied again according to the protocol described above, and we evaluated the differences in measurements before and after the CAPS-treatment.

2. 2. 3. Topical anesthesia —At the end of the experiment, 5 ml of 2% lidocaine solution (Astra, Xylocaine®) was aerosolized with an ultrasonic nebulizer (Omron, NE-U12) driven by the airflow (15 L/min, output 2.5 ml/min) producing particles approximately 5 μm in size, and passed through the isolated upper airway for 2 min. Then the nebulizer was turned off, and volatile anesthetics, CAPS, and distilled water were applied again.

2. 3. Data analysis—The measurements of inspiration time (T_I), expiration time (T_E), heart rate (HR), and systolic arterial blood pressure (ABP) were obtained from the tracings before and after onset of each trial. Dynamic lung compliance (C_{dyn}) was calculated as the ratio of V_T and the change in P_{ES} from the end-expiratory level to the point corresponding to peak V_T. Lower airway resistance (R_{aw})

was obtained as the ratio of ΔP_{ES} and ΔV_T from the end-expiratory level to the point corresponding to peak V_T. The delay of respiratory responses to each challenge was measured from the delay of T_E between the onset of the challenge and the initial sign of its effect. Apnea was defined as an absence of breathing for a period of 3 consecutive breaths before the challenge (Boggs and Bartlett, 1982; Fisher *et al.*, 1985). Control values were averaged over three consecutive breaths before each challenge. The maximum responses were taken into account after onset of each trial. Statistical analysis was the same as described in experiment 3. 1.

3. Results

3. 1. Reflex cardiopulmonary effects of CAPS and water—Intralaryngeal instillation with CAPS and distilled water induced a transient inhibition of breathing, followed by a marked increase in ABP (Δ = 27.2 ± 5.5% for CAPS; 23.9 ± 2.8% for distilled water) and a decrease in HR (Δ = -15.0 ± 4.8% for CAPS; -15.7 ± 2.0% for distilled water) from the pre-instillation values, as described in Fig. 1. The inhibition of breathing as represented by a significant decrease in $\dot{V}_E$ was mainly due to a prolongation of T_E and a decrease in V_T (Table 1, Fig. 2). Dynamic lung compliance decreased significantly and lower airway resistance increased significantly from the pre-instillation levels in the case of water instillation ($P < 0.05$). The delay of response after the onset of instillation was 1.3 ± 0.5 sec for CAPS and 0.9 ± 0.3 sec for water. The duration of response to reach its maximum level compared to the initial response was 5.1 ± 0.9 sec for CAPS and 3.3 ± 0.5 sec for water. Apnea was caused in 5 dogs (5/16, 31%) by CAPS and in 8 dogs (8/16, 50%) by water. Saline solution did not produce any significant change in the measurements from the pre-instillation level (Table 1, Fig. 1).

The second CAPS instillation considerably depressed all the responses observed in the first CAPS challenge, whereas the response to water were still preserved by the first water instillation level even after the CAPS instillations (n = 8) (Table 1, Fig. 2). Topical anesthesia with lidocaine eliminated all the cardiorespiratory responses to CAPS and distilled water (Table 1, Fig. 2)

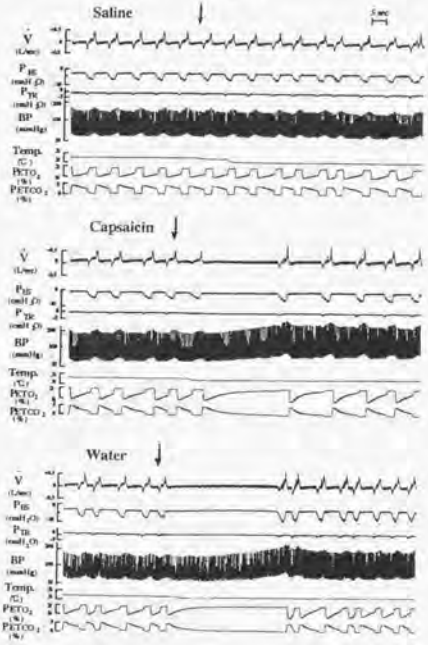


Fig. 1. Representative records illustrating topical instillation of saline, capsaicin, and distilled water into the larynx in an anesthetized dog. The arrows indicate the intralaryngeal instillation of each solution. Note that apnea was induced by both capsaicin and water instillations.

Table 1 - Resperatory variables of topical instillation of saline, capsaicin (CAPS), and distilled water into the larynx in dogs

Variables	V_T	$\dot{V}_E$	T_I	T_E	T_{TOT}	C_{dyn}	R_{aw}
Non-treatment (n =15)							
Saline	97.4±9.6	96.2±4.8	105.3±4.5	115.6±7.2	113.0±5.1	93.2±4.5	100.0±2.2
CAPS	45.4±5.6* [a]	23.3±7.9* [a]	140.4±8.2* [a]	298.0±52.8* [a]	241.7±35.2* [a]	80.4±10.9	129.2±13.5
Water	48.4±8.9* [a]	31.3±10.7* [a]	154.4±10.3* [a]	407.9±85.1* [a]	326.5±58.8* [a]	61.5±10.8*	144.7±15.0*
CAPS-treatment (n=8)							
Saline	103.8±6.7	96.0±7.2	99.7±3.8	107.5±4.3	105.0±4.5	102.5±3.6	99.4±1.8
CAPS	94.6±7.0 †	85.7±10.7 †	100.0±2.9 †	127.4±7.9* †	117.9±5.3 †	92.1±8.2 †	104.8±3.9 †
Water	60.9±15.9* [b]	26.7±13.2* [b]	135.5±8.1* [b]	385.0±40.7* [b]	304.8±31.5* [b]	66.3±9.0*	137.5±10.8*
Lidocaine anesthesia (n =15)							
Saline	105.3±8.0	98.3±8.0	100.4±3.5	97.8±4.7	98.6±3.9	101.3±2.5	100.0±1.6
CAPS	98.0±5.4 †	95.8±7.3 †	104.0±1.2 †	106.2±2.2 †	104.5±1.4 †	100.0±4.5 †	98.1±6.4 †
Water	94.0±6.3 †	93.3±7.2 †	102.8±2.2 †	112.0±6.3 †	109.5±4.4 †	89.8±7.1 †	108.7±9.3 †

* $P < 0.05$ compared with pre-instillation; † $P < 0.05$ compared with non-treatment;

[a] $P < 0.05$ compared with saline; [b] $P < 0.05$ compared with CAPS.

All data were expressed as percent change from control (mean ± SE).

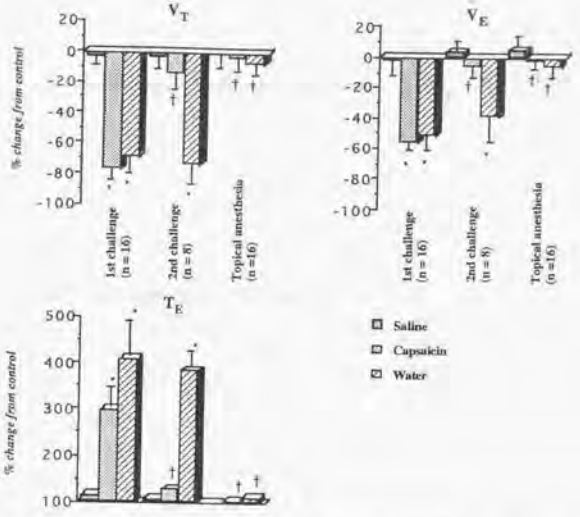


Fig. 2. Summary of maximal responses of topical instillation of saline, capsaicin, and distilled water into the larynx on ventilation in anesthetized dogs.
* $P < 0.05$ *vs* control, † $P < 0.05$ *vs* 1st challenge.

3. 2. Reflex cardiopulmonary effects of volatile anesthetics— The laryngeal temperature was lowered as much as 27.5 ± 0.6 °C by administration of oxygen flow when we obtained the control recording for the value at room temperature (33.9 ± 0.5 °C). The cold stimuli by O_2 flow significantly increased T_E to 126.5 ± 4.1% (n = 16) from the pre-inhalation level at room air ($P < 0.05$). The *l*-menthol stimulus increased T_E slightly (133.4 ± 3.4%, n = 6), but no statistical significances were found as compared to the value of O_2 flow without *l*-menthol ($P = 0.29$).

After each dog attained the static laryngeal temperature induced by O_2 flow, each volatile anesthetic was administered into the larynx at 5% concentration. All the inhalants significantly increased T_E and T_{TOT} and significantly decreased $\dot{V}_E$ from the control level ($P < 0.05$) (Table 2, Fig. 4). Hal showed a significant decrease in V_T from the control level ($P < 0.05$). The inhibition of breathing was significantly greater with Hal, Enf, and Iso than with Sevo ($P < 0.05$). Apnea was induced in 2 dogs (2/16, 13%) only by inhalation of 5% Hal. The delay of response to each inhalant was 11.9 ± 2.2 sec for Hal, 16.0 ± 2.1 sec for Enf, 13.1 ± 2.5 sec for Iso, and 26.0 ± 2.4 sec for Sevo, respectively. The delay was significantly longer for Sevo than for other anesthetics ($P < 0.05$).

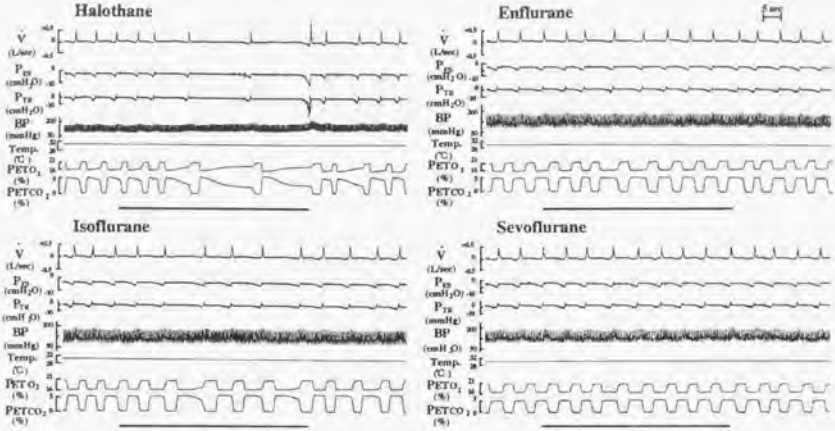


Fig. 3. Representative records illustrating effects of volatile anesthetics inhaled into the larynx in an anesthetized dog. The horizontal lines show the intralaryngeal administration of each volatile anesthetic.

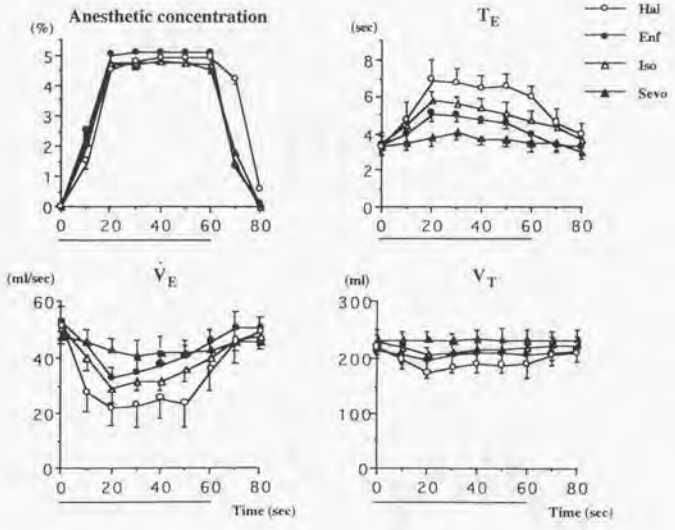


Fig. 4. Time-course changes in ventilation by inhalation of each anesthetic into the larynx in anesthetized dogs (n=16). The horizontal lines indicate the intralaryngeal administration of each anesthetic.

Table 2
Maximal cardiopulmonary responses of topical application of volatile anesthetics into the laryngeal lumen in dogs

Variables	V_T	$\dot{V}_E$	T_I	T_E	T_{TOT}	C_{dyn}	R_{aw}
Non-treatment (n =15)							
Halothane	80.1±5.5*	43.7±12.3*	109.9±5.9	209.1±12.3*	177.3±9.4*	92.0±8.0	113.3±9.7
Enflurane	92.5±4.9	62.0±4.8*	102.5±2.5	151.6±5.1*	132.8±4.1*	98.5±7.2	109.9±6.0
Isoflurane	90.4±4.5	57.0±3.9*	100.6±2.9	164.9±4.0*	140.2±3.8*	103.7±9.6	100.5±6.3
Sevoflurane	100.4±7.9 *H*	85.2±3.0* *HEI*	102.1±2.1	124.7±3.9* *HEI*	112.9±2.4* *HEI*	108.5±4.9	99.0±8.6
CAPS-treatment (n=8)							
Halothane	89.0±5.2*	65.0±4.3* †	98.0±2.2	165.0±9.1* †*E*	136.0±9.9* †	96.0±5.2	109.0±4.5
Enflurane	95.7±4.4	78.7±4.4* †	97.2±2.4	130.1±4.2* †	121.3±3.2* †	102.9±5.7	101.5±6.3
Isoflurane	95.2±5.5	73.6±3.6* †	100.5±1.7	137.8±3.8* †	128.5±3.2* †	102.0±3.6	97.0±4.0
Sevoflurane	99.1±3.2 *H*	91.7±4.9 *HEI*	100.0±2.3	112.4±4.9 *H*	107.5±3.4 *H*	97.7±5.9	101.0±6.2
Lidocaine anesthesia (n =15)							
Halothane	98.0±2.2	94.3±3.3	102.0±1.2	110.1±4.2	108.7±4.8	97.0±8.6	104.5±4.4
Enflurane	101.3±2.5	98.1±6.5	103.0±2.8	107.1±4.4	103.9±7.0	103.4±5.9	97.0±3.8
Isoflurane	99.6±1.5	97.9±4.8	98.5±3.5	107.4±2.1	105.2±3.6	99.1±4.5	103.0±6.7
Sevoflurane	100.0±1.5	98.0±1.5	100.0±0.4	103.7±2.4	101.8±1.8	100.0±2.7	100.0±0.9

* $P < 0.05$ compared with control; † $P < 0.05$ compared with non-treatment;
H $P < 0.05$ compared with halothane; *E* $P < 0.05$ compared with enflurane; *I* $P < 0.05$ compared with isoflurane.
See Table 1 for abbreviation.

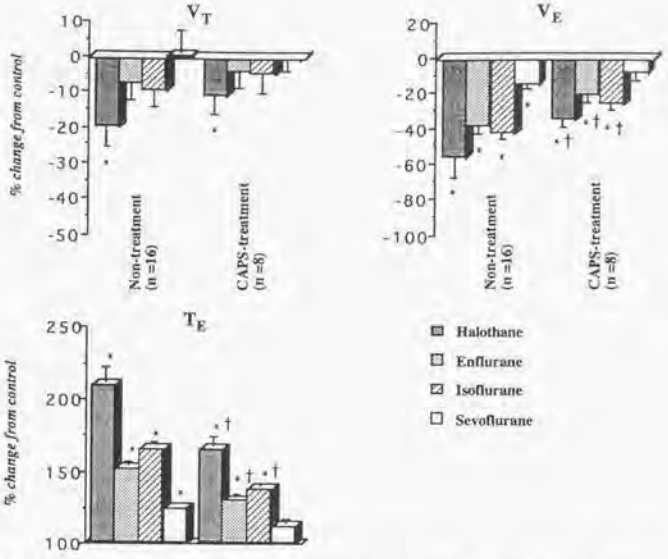


Fig. 5. Summary of maximal respiratory responses to each anesthetic topically applied to the larynx in anesthetized dogs. A significant increase in T_E and a significant decrease in $\dot{V}_E$ were observed in responses to all the anesthetics before CAPS treatment (non-treatment, left panel in each graph). The responses to Hal, Enf, and Iso were reduced significantly by the CAPS treatment (CAPS-treatment, right panel). Abbreviations as in Fig. 2.

The inhibitory effects tended to last until the offset of inhalation. Although no statistical significances were found in the averaged values of respiratory parameters at 3% and 5% concentrations, 4 dogs (4/16, 25%) showed an increase in T_E and T_{TOT} and a decrease in $\dot{V}_E$ in response to 3% halothane and 2 dogs had the same responses to both 3% Enf and 3% Iso (2/16, 13%). No response was found in any of the dogs to any of the anesthetic at 1% concentrations.

The reflex responses to volatile anesthetics tended to decline after CAPS treatment (Table 2, Fig. 5). Significant reduction of T_E prolongation and depression of $\dot{V}_E$ were observed with the introduction of Hal, Enf, and Iso as compared to the values before CAPS treatment ($P < 0.05$). The inhibition of breathing which remained after the CAPS treatment was significantly greater with Hal than with other anesthetics ($P < 0.05$). Neither Cdyn nor Raw varied significantly among the anesthetics. The delay of response to each inhalant after the CAPS treatment was 14.6 ± 2.3 sec for Hal, 18.5 ± 2.2 sec for Enf, 15.0 ± 1.6 sec for Iso, and 32.4 ± 3.6 sec for Sevo, all responses being prolonged compared to responses before CAPS treatment. The delay was significantly longer with Sevo than with other anesthetics ($P < 0.05$), which is similar to the

case before CAPS treatment.

Topical anesthesia with lidocaine considerably depressed ($P < 0.05$) all cardiorespiratory responses to all volatile anesthetics (Table 2, Fig. 5).

4. Discussion

4. 1. Possible cardiopulmonary reflex responses of laryngeal CAPS sensitive and irritant receptors in the dog— There are several reports on the cardiorespiratory responses to various fluids being introduced into the larynx or hypopharynx in animal species including sheep, pigs, rats, dogs, and humans (Mortola and Fisher, 1988). However, findings from all these studies have been limited to newborn stages. Boggs and Bartlett (1982) reported the apneic reflex in puppies with low concentration of NaCl with extremely low or high pH. Moreover, Fisher *et al.* (1985) described the apneic response to laryngeal flow stimulus, where the response occurred only in newborns puppies (1-14 days old) but never occurred after the puppies 29 days old. In the present study, however, the marked cardiorespiratory responses to distilled water were observed in adult dogs, which has not been reported in previous studies. Therefore, our findings were evidence that water-induced apnea occurred in adult dogs. This is not inconsistent with the finding that many respiratory modulated or non-modulated laryngeal receptors can be stimulated by distilled water in adult dogs (Anderson et al., 1990). The weak cardiorespiratory reflexes to laryngeal water stimuli in mature animals might be due to the anesthetic condition, since the apneic response is generally greater during sleep than in the awake state (Mortola and Fisher, 1988).

The results of the present study demonstrate that a clear inhibition of breathing, bradycardia, and hypertension can be induced by CAPS as well as distilled water, even in adult dogs, indicating the importance of noxious chemical stimuli of unmyelinated endings as well as those of water-responsive endings for the defensive reflexes in the dog's larynx. The CAPS-sensitive receptors reduced their responses to subsequent CAPS trial, indicating the occurrence of 'desensitization' of unmyelinated C-fiber endings. These findings well coincided with those obtained in rats and guinea pigs (Palecek *et al.*, 1990; Tsubone *et al.*, 1991). On the other hand, the responses to distilled water were sustained even after the CAPS desensitization, indicating the presence of different functional properties of the nerve endings. In fact, CAPS-sensitive receptors showed little or no response to water, and water-responsive receptors vice versa in our previous study of dogs (Mutoh *et al.*, 1998).

The similar reflex cardiorespiratory responses to both CAPS and water stimuli suggest that the central processing associated with afferent stimuli has a common final pathway to exhibit the reflexes via the somatic and autonomic nervous system. The afferents from the vagal pathway generally input to the nucleus tractus solitarius (NTS), and the electrical activity influenced the medullary respiratory neuron groups and dorsal nucleus of the vagus to elicit various cardiorespiratory responses (Richerson and Getting, 1992). The cardiovascular reflex responses are quite similar to the finding of the so-called 'diving reflex' when distilled water is instilled into the nasal cavity in dogs and other animals (Angell-James and Daly, 1972; Elsner and Gooden, 1983).

In this study, a clear decrease in Cdyn and an increase in Raw were the result of water instillation, suggesting the presence of bronchoconstriction. Such finding has also been reported in a study where cigarette smoke was inhaled into dog's larynx (Boushey *et al.*, 1972). It is, however, still unclear whether the irritant endings cause bronchoconstriction, because as several

investigators noted, the laryngeal osmoreceptors are also likely to mediate the reflex nature of the bronchoconstriction (Eschenbacker *et al.*, 1984; Higenbottam, 1984).

Several mechanoreceptors, i.e., the pressure, drive, cold, irritant, and CAPS-sensitive receptors, have been identified in the larynx of the dog (Sant'Ambrogio *et al.*, 1995; Mutoh *et al.*, 1998). In the present study, we applied the topical instillation of each selective stimulant into the laryngeal lumen to evaluate the reflex contribution of each sensory ending (Sant'Ambrogio *et al.*, 1995). Capsaicin is known to be a selective stimulant of unmyelinated C-fibers and a small number of A-δ fibers (Coleridge and Coleridge, 1984). On the other hand, the lack of chloride anions is associated with stimuli to the irritant type of endings (Anderson *et al.*, 1990). In this study, we cannot define whether the responsiveness to water was due to the lack of chloride anions and/or hyposmolality, because respiratory-modulated drive and pressure receptors can also show sensitivity to hyposmolality (Anderson *et al.*, 1990; Sant'Ambrogio *et al.*, 1991). However, these osmoreceptors are characterized as exhibiting long latency (2.6 sec) and long duration discharges (30.5 sec) (Sant'Ambrogio *et al.*, 1991), which is inconsistent with our results represented by the short latency (0.9 sec) and the short duration needed to reach maximum reflex responses (3.3 sec). All the reflex responses were eliminated by the application of topical anesthesia in the larynx, indicating the superficial location of the nerve endings and confirming the contribution of the intralaryngeal sensory endings on the reflex responses to CAPS and water. This finding is in agreement with those in previous studies that suggest that all the water-responsive receptors were able to be blocked within 50 sec of application of topical anesthesia with 2% lidocaine while most non-responsive receptors had longer blocking times (1-30 min) (Sant'Ambrogio *et al.*, 1991). Considering these facts, we regarded the laryngeal CAPS-sensitive endings and water-responsive 'irritant-type' endings to be the most probable source of the laryngeal reflex responses observed in this study.

In any event, the chronic tracheostomy model provided significant implications for the evaluation of laryngeal reflexes in adult dogs. The most important drawback with the successful elicitation of reflex responses appeared to be the result of a lack of surgical stimulation, which requires a deep anesthetic level. Indeed, the laryngeal induced-apnea is enhanced with increasing anesthetic depth (Donnelly and Haddad, 1986). It also depresses the efferent outflows at the level of the central nervous systems (Harvey and Walberg, 1987). Moreover, the necessary dose of anesthetic varies with the metabolic rate of animals, thus the larger the animal, the smaller the dose per unit of body weight necessary for anesthesia (Thurmon *et al.*, 1996). In fact, the dose of basal anesthesia used in this study was apparently less than that used in a study with newborn dogs (Sant'Ambrogio *et al.*, 1993).

4. 2. Possible cardiopulmonary reflex responses of CAPS sensitive and irritant receptors to volatile anesthetics— Induction of anesthesia with volatile anesthetics is sometimes interrupted by the exertion of excessive protective or defensive upper airway reflexes such as coughing, apnea, inhibition of breathing, laryngospasm, and secretion (Drummond, 1993). Whereas these strong responses were not introduced in this study since the animals were already anesthetized, results of the present study clarified, even in adult dogs, reflex inhibition of breathing similar to that in newborns. Sant'Ambrogio *et al.* (1993) pointed out that Hal inhaled into the isolated upper airway of newborn dogs profoundly depressed ventilation, whereas it

produced only a marginal change in ventilation in 26-28-day-old dogs and adult dogs, the change being mainly a rise from the SLN. They supposed that laryngeal irritant and cold receptors are associated with the responses since Hale showed stimulus effects on both types of endings (Nishino *et al.*, 1993); however, such an hypothesis, especially for the response to stimulus of cold receptors, is not applied to adult dogs, since neither laryngeal cooling by the O_2 flow nor by the application of *l*-menthol, a selective stimulant to the cold receptors (Sant'Ambrogio *et al.*, 1995), showed anything other than small inhibition of breathing in the present study. Depression of ventilation, mostly due to an increase in expiration time, by laryngeal cooling has also been reported to be especially pronounced in newborn animals (Sant'Ambrogio *et al.*, 1995).

We regarded the differences in the responsiveness to inhaled anesthetics before and after CAPS desensitization as a reflex contribution to laryngeal CAPS-sensitive receptors. Moreover, we considered the responsiveness to the anesthetics that still remained after desensitization to be due to the effects of 'water-responsive' receptors. In fact, the latency of the responses to each volatile anesthetic was mostly synchronized with the responses of both receptors in our previous study (Mutoh *et al.*, 1998). In this study, approximately 50% of the reflex responses were eliminated by the CAPS desensitization, indicating the importance of the reflex contribution of CAPS-sensitive receptors as well as the water-responsive receptors to the reflex responses in adult dogs.

In this study, the lack of significant change in the lung dynamic compliance and the lower airway resistance indicated that the reflex inhibitory effects of volatile anesthetics were limited to a transient inhibition of respiratory pumping muscles. In contrast to responses to the stimuli of CAPS and water, cardiovascular responses, such as bradycardia and hypertension, were not observed with any of the anesthetic inhaled. These observations may be due to the weaker stimuli of the anesthetics to the sensory endings and/or the milder responses being masked by basal anesthesia. Absence of the reflex cardiovascular responses to volatile anesthetics has also been demonstrated in anesthetized newborn dogs (Sant'Ambrogio *et al.*, 1993).

Clinically, airway irritation in response to volatile anesthetics varies with the type of anesthetic and anesthetic depth (Doi and Ikeda, 1993). In the present study, Hal inhaled into the larynx showed a greater inhibition of breathing than did Sevo, which is in harmony with the previous clinical findings that suggest that Hal causes greater irritation to airway mucosa than does Sevo (Doi and Ikeda, 1993). On the other hand, the degree of responses brought about by inhalation of Iso and Enf was midway between those of Hal and Sevo, which indicates that Iso and Enf produce relatively strong airway irritation when induced via a mask (Doi and Ikeda, 1993).

Results of the present study indicate that volatile anesthetics except for Sevo have a higher risk for causing laryngeal reflexes during induction of anesthesia. It is conceivable that low anesthetic concentrations (2-3%) are generally applied to patients for the inhalation induction with Hal and Iso to avoid possible undesirable airway reflexes (Yurino and Kimura, 1992, 1993). In contrast, induction of anesthesia with Sevo produces the minimum undesirable side effect even when inhaled at a 7% concentration both in dogs (Mutoh *et al.*, 1995) and humans (Muzi *et al.*, 1996), supporting our results.

In conclusion, results of the present study suggest that various cardiopulmonary reflexes, i.e., inhibition of breathing, bradycardia, and hypertension, can be elicited by the stimulus of

laryngeal CAPS-sensitive and irritant receptors in adult dogs, the effects of which are associated with the laryngeal reflex responses to volatile anesthetics, especially Hal, Enf, and Iso.

Section 5 Effects of volatile anesthetics on the nasal cavity

Cardiopulmonary reflexes and trigeminal nerve activity in response to nasal application of volatile anesthetics in anesthetized dogs

Abstract

Cardiopulmonary reflexes by intranasal application of Ha, Enf, Iso and Sevo were elucidated in anesthetized dogs breathing spontaneously through a tracheostomy. A significant increase in T_E and a significant decrease in $\dot{V}_E$ were observed following application of each anesthetic, where such changes were considerably greater for halothane than for sevoflurane. An increase in arterial blood pressure and a decrease in V_T were also elicited for halothane. All the reflex responses were considerably inhibited by topical anesthesia with lidocaine into the nasal cavity or by bilateral section of the posterior nasal nerve (PNN). The properties of stimulation of PNN afferents to volatile anesthetics coincided with the magnitude of reflex responses by the anesthetics. All the PNN afferents which responded to the anesthetics were markedly stimulated by capsaicin instillation, but not by distilled water. These results indicate that the volatile anesthetics evaluated in this study can stimulate the trigeminal afferents, at least capsaicin-sensitive endings, and evoke cardiopulmonary changes of which strength is largely associated with stimulatory effects on the sensory nerve.

1. Introduction

Nasal mucosa is abundant in sensory afferents and a potent reflexogenic part of the upper airway, and elicitable various types of respiratory and circulatory reflex responses in rats and guibea pigs (Widdicombe *et al.*, 1988; Sant' Ambrogio *et al.*, 1995). However, we don't have much information about the reflex responses induced by the volatile anesthetics applied to nasal mucosa nor even about the sensory afferents of nasal cavity in dogs. Although the effects of volatile anesthetics were investigated on the larynx in several studies (Sant'Ambrogio *et al.*, 1993; Nishino *et al.*, 1993; Mutoh *et al.*, 1997c), very few studies have been conducted on the nasal cavity and therefore we do not know what cardiopulmonary reflexes are induced and what extent the nasal receptors are stimulated or inhibited by the anesthetics applied to the nasal cavity.

The aim of the present study was to elucidate the cardiopulmonary reflexes originated from the nasal mucosa, and to evaluate the afferent activities of the posterior nasal nerve (PNN), by application of different volatile anesthetics in anesthetized dogs.

2. Methods

2. 1. General

2. 1. 1. Animals and basal anesthesia—Thirty-one healthy beagle dogs (14 males and 17 females) were used in this study. Their mean age was 13.5-months-old (ranging from 10.5 to 18.0 months) and their mean body weight 9.6 kg (ranging from 7.5 to 14.2 kg). Anesthesia was induced with thiopental sodium (25 mg/kg), then maintained with a mixture of urethane (200 mg/kg) and α-chloralose (20

mg/kg) injected intravenously slowly over 15 min. A supplemental dose of urethane and α-chloralose mixture (200 mg/kg urethane and 20 mg/kg α-chloralose) was infused at a rate of 1 ml/kg/hour with an infusion pump (Terumo, Model STC-531) through an intravenous catheter placed into the cephalic or saphenous vein. Lactated Ringer's solution was infused at a rate of 10 ml/kg/hour through the intravenous catheter placed into the other side of cephalic or saphenous vein. The dogs were allowed for at least one hour adjustment period after the induction of anesthesia.

2. 1. 2. Animal preperation— After the induction of anesthesia, dogs were placed on an operating table in the lateral recumbency, endotracheally intubated, and ventilated spontaneously with room air. Arterial blood pressure was monitored by a pressure transducer (Nihon Kohden, DX-300) connected to a 20-G catheter inserted into the femoral artery. End-tidal PO_2 ($PETO_2$) and PCO_2 ($PETCO_2$) were sampled through the line attached to the endotracheal tube and monitored by a gas analyzer (NEC, Respina 1H26). Intratracheal pressure was measured with a pressure transducer (Toyoda, PD104). Respiratory airflow ($\dot{V}$) was recorded with a differential pressure transducer (Toyoda, DD-102A) through two sidearms connected to the endotracheal tube, and integrated with an A-D converter (BRC, Mac Lab Scope) to give tidal volume (V_T). Minutes ventilation ($\dot{V}_E$) was calculated from the value of V_T and total cycle duration. A saline-filled polyethylene catheter (O.D. = 3 mm) was placed in the middle portion of the esophagus and connected to a pressure transducer (Nihon Kohden, DX-300) for recording esophageal pressure. All the signals were displayed on a thermal-array recorder (NEC, RT 3100N), and recorded by a magnetic tape recorder (SONY, PC 208). Arterial blood gas variables (pH, P_{O_2}, P_{CO_2}, bicarbonate concentration, and base excess) were measured at regular intervals by the blood gas analyzer (Instrumentation Laboratory, IL-1640).

2. 2. Experimental protocol

2. 2. 1. Protocol 1: Respiratory reflexresponses of nasal mucosa by inhalation of volatile anesthetics— Sixteen dogs were used in this experiment. A small longitudinal skin incision was made in the mandibular at the base of the tongue to introduce a cuffed tracheal tube (Nihon Medico, PORTEX, I.D. = 4.5-5.0 mm) into the nasopharynx. A nasal cannula with a pair of cuffed tubes was inserted into both nostrils to functionally isolate the nasal cavity. A thermal probe was inserted just onto the nasal mucosa through the nasal cannula to record nasal temperature.

After a control period of more than 1 min with 100% oxygen at a flow rate of 6 L/min through the isolated nasal cavity in the inspiratory direction, each dog was challenged with 5% of Hal, Enf, Iso, and Sevo for 1 min, respectively. If any visible response was observed in the cardiopulmonary measurements at 5% of concentration, 1 and 3% of each anesthetic were tried thereafter for the evaluation of the concentration-relationship.

In 10 dogs, bilateral zygomatic bones were removed carefully with the electric dental microengine (Osada, BL-F2) to identify PNN and infraorbital nerve (ION), both of which are nasal branches of the trigeminal nerve. Then, the bilateral nerve sections were performed, and measured the cardiopulmonary reflexes by inhalation of volatile anesthetics in the same manner as described above. As for the nerve sections, the ION was initially dissected at the infraorbital foramen, followed by the sections of PNN at the sheno-palatin foramen because of the location of each bundle. In another 6 dogs, 2% of lidocaine was instilled into

the nasal cavity, and evaluated the cardiorespiratory reflex responses by inhalation of volatile anesthetics.

2. 2. 2. Protocol 2: Recordings of the afferent activity of nasal mucosa by inhalation of volatile anesthetics—Fifteen dogs were used in this study. Before the experiment the tracheostomy and exposure of the trigeminal nerve branch was performed in each dog under Iso anesthesis. The cervical trachea was exposed in its entire length and cut longitudinally to insert a cuffed tracheostomy tube (Nihon Medico, PORTEX,) into the lower respiratory tract for tracheostomy breathing. A cuffed tracheal cannula was inserted into the nasopharynx through the uppermost tracheostomy site. The superior laryngeal nerve, recurrent laryngeal nerve, and ION were bilaterally cut to avoid secondary respiratory modulated reflexes mediated by the nerves.

Afferent activity of the nerve filament was recorded with a pair of platinum electrodes. The PNN was sectioned centrally at the junction of maxillary nerve and its peripheral cut end was separated from surrounding connective tissues with the aid of a binocular microscope (OLYMPUS, SZ60) from which whole PNN and single unit afferent activities were recorded. The signal was amplified by a low noise DC-amplifier (DIA Med., DPA201) and a biophysical amplifier (DIA Med., DPA200), and displayed on an oscilloscope (IWATSU, SS5762) in parallel with a loudspeaker (NEC san-ei, Model 7747). All the signals were displayed on a thermal-array recorder (NEC san-ei, RT3100N) and recorded by a magnetic tape recorder (SONY, PC204A).

The effects of volatile anesthetics on the whole or single PNN activity were evaluated according to the protocol as described in experiment 1. When the receptor showed a responsiveness to any of the anesthetic, their chemical sensitivity was evaluated by instillation of 10 ml of CAPS solution (100 μg/ml of CAPS, in a solution containing 0.9% NaCl, 1% ethyl alcohol, and 0.1% Tween 80) or the same volume of distilled water at a temperature of 37°C into the nasal cavity. Warmed isotonic NaCl solution (0.9%) at 37°C was used for rinsing the laryngeal lumen after each trial. In the receptors that responded to the CAPS, a subsequent CAPS (100 μg/ml) was repeated to evaluate the effect of desensitization. Each challenge was performed at an interval of 20 min or more. The individual receptor was also stimulated by a light mechanical probing to the nasal mucosa using a cotton stick.

2. 3. Data analysis—The measurements of inspiration time (T_I), expiration time (T_E), heart rate (HR), and systolic arterial blood pressure (ABP) were obtained from the tracings before and after onset of each trial. Dynamic lung compliance (C_{dyn}) was calculated as the ratio of V_T and the change in P_{ES} from the end-expiratory level to the point corresponding to peak V_T. The delay of respiratory responses to each challenge was measured from the delay of T_E between the onset of the challenge and the initial sign of its effect. Apnea was defined as an absence of breathing for a period of 3 consecutive breaths before the challenge (Boggs and Bartlett, 1982; Fisher *et al.*, 1985). Control values were averaged over three consecutive breaths before each challenge. The maximum responses were taken into account after onset of each trial.

The discharge frequencies of whole nerve and single unit activities were measured every second for a period of 30 sec before the trial with an window discriminator (DIA Medical, DSE 425), then the data were averaged every 10 sec. The averaged discharge frequency for 30 seconds before the trial was taken as the control value, and the maximum or

minimum discharge frequency out of the values averaged every 10 sec after onset of the trial was taken as the peak value. In case of the measurement of whole nerve activity, basal noise was cut off with the discriminator and the number of discharges during control period was adjusted to a constant level (approximately 100 imp./sec). The latency (sec) of each receptor's response was evaluated visually as the point when the number of action potential began to increase. Statistical analysis was the same as described in experiment 3.1.

3. Results

3. 1. Effects of volatile anesthetics on respiratory reflexes—The administration of oxygen flow for the control recording lowered the intranasal temperature to as much as 28.8 ± 0.9 °C (ranging from 26.1 to 33.7 °C) from the value of room airflow at 34.4 ± 0.8 °C (ranging from 32.0 to 35.7 °C). No difference, however, in the cardiopulmonary responses were observed between the oxygen and room airflow. After the constant intranasal temperature was obtained, each volatile anesthetic at 5% was administered into the nasal cavity. All the anesthetics significantly increased T_E and decreased $\dot{V}_E$ after the start of inhalation ($P < 0.05$) (Table 1, Figs. 1, 2).

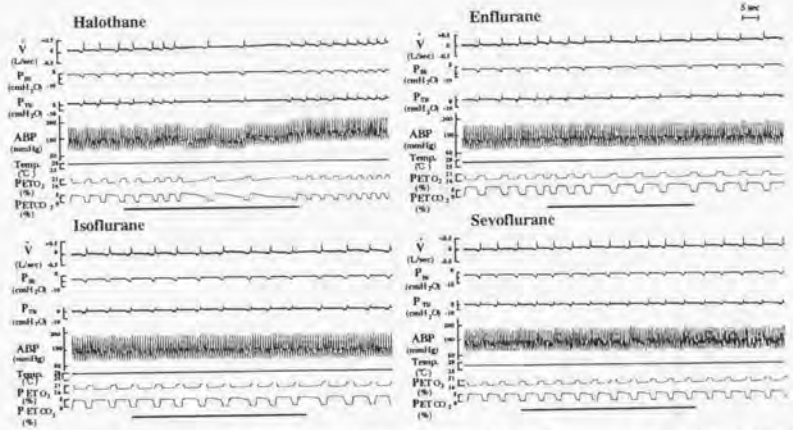


Fig. 1. Representative records illustrating cardiopulmonary effects of volatile anesthetics applied into the nasal cavity. The concentration was 5% in each anesthetic. Note that Hal caused an inhibition of breathing followed by a rapid shallow breathing and an increase in arterial blood pressure, whereas other anesthetics only showed an increase in exspiration time. The horizontal lines show the topical application of each volatile anesthetic into the nasal cavity. $\dot{V}$ = respiratory airflow, P_{ES} = esophageal pressure, P_{TR} = intratracheal pressure, BP = arterial blood pressure, Temp. = intranasal temperature.

Table 1.

Maximal cardiopulmonary responses to topical application of volatile anesthetics into the nasal cavity in dogs

Variables	V_T	$\dot{V}_E$	T_I	T_E	C_{dyn}	HR	ABP
Nerve-intact (n =16)							
Halothane	78.5 ± 10.1* §	46.5 ± 14.2* §	114.9 ± 6.5	218.5 ± 17.5* §	97.5 ± 9.4	93.5 ± 10.1	119.1 ± 4.3*
Isoflurane	92.5 ± 7.9	66.5 ± 10.6*	100.2 ± 3.5	164.9 ± 15.1*	100.0 ± 4.2	101.9 ± 4.3	98.6 ± 4.6
Enflurane	94.9 ± 8.3	72.0 ± 11.8*	100.0 ± 2.3	151.6 ± 14.9*	100.9 ± 7.1	99.5 ± 5.9	102.5 ± 3.5
Sevoflurane	98.7 ± 5.5	83.6 ± 7.5*	101.3 ± 3.2	124.7 ± 11.5*	100.1 ± 3.0	99.8 ± 4.5	100.0 ± 3.9
ION-section (n=10)							
Halothane	84.3 ± 9.6*	68.1 ± 11.2* §	103.5 ± 2.6	190.0 ± 15.4* §	98.5 ± 6.3	95.5 ± 7.2	100.3 ± 4.2
Isoflurane	99.6 ± 7.5	78.5 ± 11.5*	100.0 ± 3.6	145.1 ± 12.8*	101.4 ± 3.9	99.8 ± 3.6	98.0 ± 3.5
Enflurane	102.1 ± 8.5	80.7 ± 9.4*	99.5 ± 1.5	139.6 ± 10.5*	98.5 ± 3.2	98.5 ± 4.7	97.9 ± 5.7
Sevoflurane	106.5 ± 7.4	90.0 ± 7.7	100.0 ± 1.1	117.0 ± 10.9	99.2 ± 2.8	98.2 ± 5.4	100.7 ± 2.9
ION- and PNN-section (n=10)							
Halothane	95.5 ± 6.3 †	88.5 ± 7.9* †	96.7 ± 5.5	139.9 ± 11.5* † §	103.0 ± 8.2	97.2 ± 7.3	99.0 ± 2.2
Isoflurane	101.8 ± 7.4	95.2 ± 8.6 †	98.5 ± 4.0	112.0 ± 9.7 †	104.6 ± 6.5	101.5 ± 3.6	101.5 ± 2.5
Enflurane	98.6 ± 7.4	93.9 ± 8.1 †	99.3 ± 2.4	109.7 ± 10.4 †	99.3 ± 4.5	98.8 ± 4.9	100.0 ± 1.7
Sevoflurane	100.4 ± 5.5	100.2 ± 6.3 †	100.0 ± 1.8	102.4 ± 6.2 †	100.0 ± 2.8	100.0 ± 3.2	100.3 ± 1.4
Lidocaine anesthesia (n = 6)							
Halothane	98.3 ± 3.2 †	102.5 ± 3.1 †	100.0 ± 3.4	107.0 ± 4.5 †	99.8 ± 5.6	100.0 ± 3.5	99.4 ± 2.6
Isoflurane	100.0 ± 1.5	99.8 ± 2.4 †	98.5 ± 1.7	96.0 ± 7.1 †	100.2 ± 3.9	99.7 ± 2.8	100.7 ± 1.5
Enflurane	99.9 ± 2.9	100.8 ± 3.8 †	99.0 ± 1.7	98.5 ± 3.8 †	98.9 ± 5.5	100.2 ± 3.9	98.5 ± 2.9
Sevoflurane	100.0 ± 1.6	101.0 ± 1.9 †	99.5 ± 1.5	102.5 ± 3.4 †	100.0 ± 1.6	99.7 ± 3.5	103.8 ± 2.7

ION = infraorbital nerve; PNN = posterior nasal nerve; HR = heart rate; ABP = systolic arterial blood pressure.

* $P < 0.05$ compared with control; † $P < 0.05$ compared with nerve intact; § $P < 0.05$ compared with sevoflurane.

Each value was expressed as a percent of control (mean ± SE).

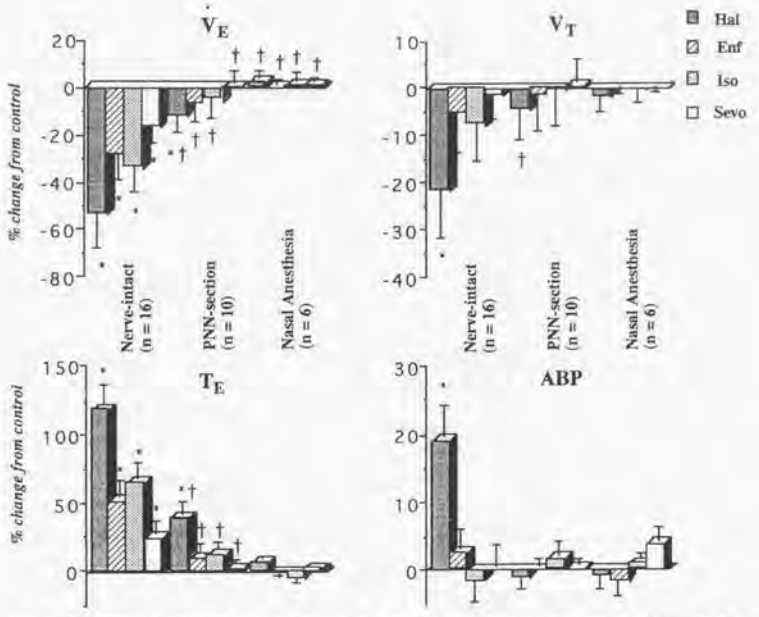


Fig. 2. Summary of cardiopulmonary changes at maximal responses by application of 5% volatile anesthetics into the nasal cavity. The value (mean ± SE) was expressed as a percent change from control in each column. * $P < 0.05$ *vs* control, † $P < 0.05$ *vs* Nerve-intact.

The mean T_E prolongation was 218.5, 151.6, 164.9 and 124.7% for Hal, Enf, Iso and Sevo, respectively. In addition, halothane showed a significant decrease in V_T and a significant increase ABP from the control level ($P < 0.05$) (Table 1; Figs. 1, 2). The reflex inhibition of breathing by Hal was significantly greater than by Sevo ($P < 0.05$) (Table 1). In 6 dogs (6/16, 38%) breathing cycle became shorter after the occurence of prolongarion of T_E (apnea) during the inhalation of Hal (Fig. 1). The apnea characterized by the absence of breathing for at least 3 consecutive breaths (Boggs and Bartlett, 1982; Fisher *et al.*, 1985) occurred in 4 dogs (4/16, 25%) by Hal.

The delay of response to each inhalant was 20.6 ± 1.8 sec for Hal, 25.1 ± 4.6 sec for Enf, 26.5 ± 3.2 sec for Iso, and 39.5 ± 3.1 sec for Sevo, respectively. The delay was significantly shorter in Hal than in Sevo ($P < 0.05$).

Four dogs (4/16, 25%) showed an increase in T_E and a decrease in $\dot{V}_E$ by inhalation of 3% Hal, whereas no statistical significant changes were found in the averaged cardiorespiratory variables as compared to the control level. None of the dogs

showed any cardiopulmonary change to each anesthetic challenged at 1%.

Bilateral section of ION did not significantly change the ventilatory responses to the volatile anesthetics as compared to the non-denervated group. Bilateral PNN section, however, significantly reduced all the reflex responses by Enf, Iso and Sevo (Table 1; Fig. 2). The increase in T_E and decrease in $\dot{V}_E$ by inhalation of Hal were still present, but to a lesser extent, after the denervation of ION and PNN. Topical nasal anesthesia with lidocaine eliminated all the cardiopulmonary responses to volatile anesthetics (Table 1; Fig. 2).

3. 2. Effects of volatile anesthetics on afferent activities of PNN—Averaged afferent activities recorded form the whole PNN by application of 5% volatile anesthetics were shown in Fig. 3. The increase in discharge frequency was the greatest in Hal and the smallest in Sevo, where such a tendency of responsiveness coincided with that shown by cardiopulmonary reflexes.

In this study, a total of 24 single unit fibers, most of which showed a scant or irregular discharge without respiratory modulation during control periods,were examinedfor their responsiveness to the volatile anesthetics.

Out of them, 15 fibers (15/24, 63%) showed clear response to the anesthetics. A large number of the receptors (10/15, 67%) were significantly activated by all the anesthetics at 5% of concentration compared with the control value ($P < 0.01$.; Figs. 4, 5). Three receptors (3/15, 20%) were activated by Hal, Enf and Iso in the absence of responsiveness to sevoflurane. Another 2 receptors (2/15, 13%) were stimulated by only Hal. The increase in discharge frequency at 5% was significantly greater by Hal than by Sevo ($P < 0.05$; Fig. 4). The latency of the activation to administration of each volatile anesthetic was 12.8 ± 1.9 sec in Hal, 22.8 ± 3.5 sec in Enf, 19.5 ± 3.0 sec in Iso, and 33.8 ± 3.7 sec in Sevo, respectively. The latency was significantly shorter to Hal than to Sevo ($P < 0.05$).

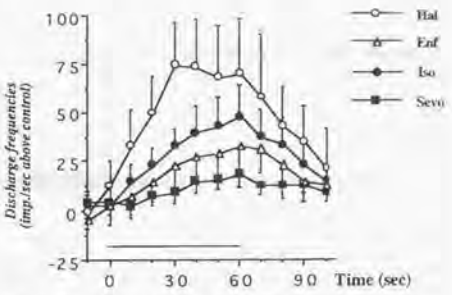


Fig. 3. Time-course changes of the afferent activity of the whole PNN in response to 5% volatile anesthetics. The abscissa and ordinate represent time (sec) and discharge frequency (imp./sec above control), respectively. The data were expressed as mean ± SE recorded from 15 nerve preparations of 15 dogs.

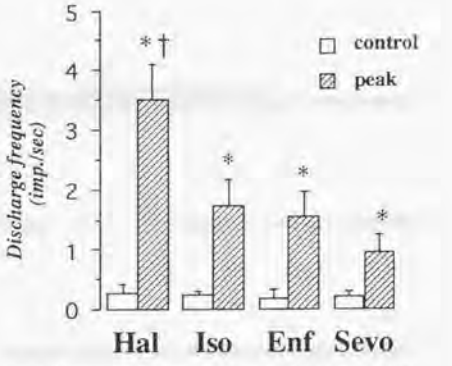


Fig. 4. Changes in discharge frequencies of single unit PNN by application of volatile anesthetics. The concentration was 5% in each anesthetic. Each value (mean ± SE) was obtained from the recordings (n = 15) during the control or application period of each anesthetic.

* $P < 0.05$, ** $P < 0.01$ *vs* control, † $P < 0.05$ *vs* Sevo.

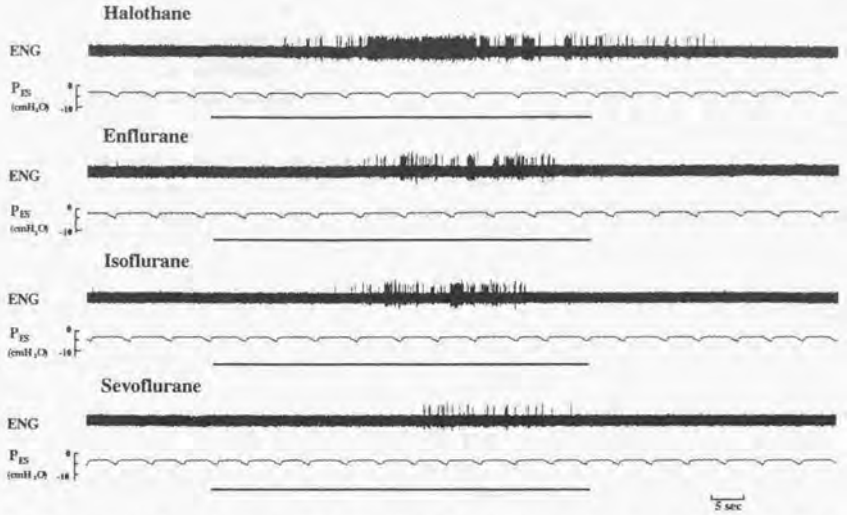


Fig. 5. An example of recordings from a PNN afferent fiber. The horizontal lines show the topical application of each volatile anesthetic into the nasal cavity. This fiber was markedly stimulated by Hal, followed by Enf and Iso, and slightly to Sevo. ENG = electroneurogram, P_{ES} = esophageal pressure.

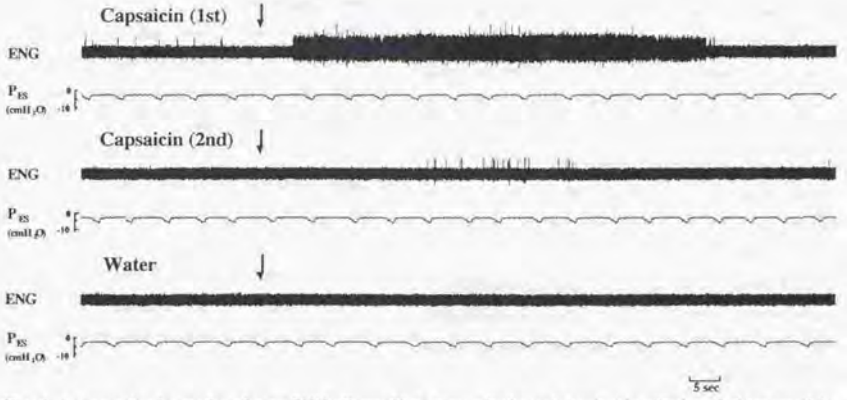


Fig. 6. An example of recordings from a PNN afferent fiber. Arrows indicate the topical instillation of either capsaicin or distilled water into the nasal cavity. This receptor was strongly activated by the first instillation of capsaicin but only slightly by the second capsaicin trial, and showed no response to distilled water. Abbreviations as in Fig. 5.

All the receptors (15/15, 100%) which responded to volatile anesthetics were consistently stimulated by CAPS (duration of discharges, 61.5 ± 9.7 sec; latency 4.5 ± 1.8 sec) but little activated by distilled water (Fig. 6) nor saline solution. The second CAPS instillation resulted in substantially no responses of their ractivities (Fig. 6). Most of them (11/15, 73%) showed irregular and short burst of discharges to the intranasal mechanical probings.

4. Discussion

The early studies on the nasal reflexes suggested that stimulation of nasal mucosa may initiate pronounced respiratory and circulatory reflexes resulting in apnea, glottal closure, mucus secretion, bradycardia, bronchoconstriction, and sneezing in many species, most of which have been supposed to be mediated by the stimulation of sensory receptors involved in the trigeminal nerves (Allen, 1936; Allison and Powis, 1971; Angell-James and Daly, 1969, 1972, 1975; White and McRitchie, 1973; White et al., 1975; Robleto and Peterson, 1981). In more recent studies, the presence of nasal mechanoreceptors in response to cooling (Glebovsky and Bayev, 1984; Tsubone, 1989; Wallois *et al.*, 1991a), pressure (Tsubone, 1990), drive (Tsubone, 1987; Wallois *et al.*, 1991a), and chemical substances (Tsubone and Kawata, 1991; Tsubone and Sekizawa, 1993; Wallois *et al.*, 1991a; Sekizawa and Tsubone, 1994) have been demonstrated in the ethmoidal nerve of cats, rats, and guinea pigs.

Results of the present study demonstrated that the reflex changes in respiratory and circulatory functions can be induced by the local application of volatile anesthetics into the nasal cavity in the anesthetized adult dog. Respiratory reflex responses to volatile anesthetics were mainly recognized by a prolongation of T_E and a decrease in $\dot{V}_E$. Such changes are essentially similar to those observed in the upper airway, including the larynx, of newborn (5~14-day-old) dogs, where 5% Hal decreased $\dot{V}_E$ to 37.6% of control due to a decrease in both frequency and V_T, and the decrease in $\dot{V}_E$ was abolished by the SLN section (Sant'Ambrogio *et al.*, 1993). However, such inhibitory effect on respiration was not induced in adult dogs (Sant'Ambrogio *et al.*, 1993), being inconsistent with the result in the present study. The reason for the discrepancy appeared to be due to differences in experimental settings of both studies: Namely, the flowing direction was in an inspiratory or expiratory direction, the stimulatory site was either nasal cavity or not, or the difference in basal anesthetic condition during experiments.

In this study, the marked reflex responses were also observed in the circulatory system, i.e., an increase in ABP, by Hal. The inhibitory effect on cardiopulmonary functions by nasal stimuli has been commonly observed in experiments with irritants such as cigarette smoke and ammonia vapor (Eccles, 1982; Widdicombe, 1986; Lee, 1988), where the transient inhibition of breathing and a rise in arterial blood pressure were reported. In general, afferent information from the trigeminal nerve inputs to the nucleus tractus solitarius (NTS) of which electrical activities influence the medullary respiratory neuron groups and dorsal nucleus of the vagus, possibly eliciting various cardiopulmonary responses (Warren Cottle and Calaresu, 1975; Richerson and Getting, 1992).

There is no doubt that in the present study the respiratory and circulatory changes by volatile anesthetics were mediated by chemical stimuli to the

afferents located in the nasal mucosa since the effects of the inhalants were considerably reduced by topical intranasal anesthesia with lidocaine or by sectioning the trigeminal nerve branches.

Since the sensory innervation of the nasal mucosa and nostril is supplied by the ophthalmic and maxillary branches of the trigeminal nerve, i.e., the anterior ethmoidal nerve (AEN), PNN, and ION, these trigeminal nerve branches convey a variety of stimuli arising from the nose (Sant' Ambrogio *et al.*, 1995). Among these nerve branches, an important role of PNN on the reflex control of breathing has been reported in previous studies. For example, sneeze reflex can be evoked by the electrical stimulation of PNN (Wallois *et al.*, 1991a), and the intranasal cold/flow stimuli or irritant stimuli with ammonia vapor increases the activity of PNN in cats (Wallois *et al.*, 1991b). In the present study, the whole PNN afferent activity was clearly increased by the volatile anesthetics (Fig. 3), where the tendency for stimulation among the volatile anesthetics was clearly related to that observed in cardiopulmonary reflex responses (Fig. 1). Although it is still unclear to what extent the volatile anesthetics influences the sensory endings of AEN and ION, the results of our study strongly suggest that the encoding of volatile anesthetic-induced reflex responses are mainly related to the stimulation of PNN afferents.

In the present study, a large number of receptors were able to be stimulated by volatile anesthetics to the greatest extent by Hal, to the least extent by Sevo, and intermediately by Iso and Enf. In addition, these receptors invariably responded to the CAPS application, being similar to our previous result in the larynx of dogs (Mutoh *et al.*, 1998). The stimulation of CAPS-sensitive fibers by volatile anesthetics has also been described in the lung (Coleridge *et al.*, 1968). Such a constant stimulatory response in nasal CAPS-sensitive fibers is apparently inconsistent with the variable responses represented by stimulation, inhibition, or non-response in the larynx (Nishino *et al.*, 1993; Mutoh *et al.*, 1998), or a consistent inhibitory effect on irritant receptors in the tracheobronchial tree (Nishino *et al.*, 1994).

It is important to note that the degree of changes in discharge frequency observed for 5% volatile anesthetics in the present study clearly corresponded to the degree of airway irritation by volatile anesthetics as experienced clinically in humans, i.e., higher incidence of complications such as cough, laryngospasm, apnea, inhibition of breathing, and excessive secretions in halothane, enflurane and isoflurane compared to sevoflurane during induction of anesthesia with high concentrations (Doi and Ikeda, 1993; Yurino and Kimura, 1992, 1993a, b, 1994). Moreover, the degree of airway irritation has also been reported to vary by the anesthetic depth (Doi and Ikeda, 1993). In fact, the earlier study reported that nasal reflexes were easily abolished with an increasing level of general anesthesia (Allen, 1936). In the present study we could not describe such a powerful defensive reaction as observed during the induction of anesthesia (Doi and Ikeda, 1993; Yurino and Kimura, 1992, 1993a, b, 1994) even inhaled at high concentration since the animals were already anesthetized and thus modified the reflex responses. Therefore, it has a highly possibility exists that the noxious stimuli of volatile anesthetics to the nasal mucosa activate the CAPS-sensitive fibers and induce various cardiopulmonary reflexes in unanesthetized patients especially for a high concentration of Hal. The minor reflex responses by Sevo might be due to the least irritation of nasal mucosa.

In this study, all the single fibers which responded to the volatile anesthetics were consistently stimulated by CAPS, demonstrating the presence of CAPS-sensitive receptors in PNN afferents. The marked reduction of the discharges of the CAPS-sensitive receptors following the second trial of CAPS can be accounted for by 'desensitization' (Lundblad *et al.*, 1985; Sekizawa and Tsubone, 1994). The noxious chemical stimuli by CAPS can induce sneezing, nasal mucus secretion, vasodilation, and plasma extravasation, where the latter three phenomena are associated with the axonal reflex of C-fibers (Lundblad *et al.*, 1985). These findings are consistent with morphological studies indicating the presence of unmyelinated C-fibers that terminate in the nasal epithelium and nerves containing substance P in the nasal mucosa (Widdicombe *et al.*, 1988). In the cat, the AEN, another branch of the trigeminal nerve, has 600-700 Remak's bundles containing unmyelinated fibers and 1200-1500 thin myelinated fibers within the category of A-δ fiber (Sant'Ambrogio *et al.*, 1995), which implies the presence of unmyelinated fibers also in the PNN.

Despite the consistent stimulation of the nasal receptors to CAPS, none were responsive to distilled water, indicating that these fibers cannot be applied to the category of 'water-responsive' receptors which can be stimulated by a lack of chloride anion and/or by hyposmolality such as non-respiratory modulated irritant receptors as described in the larynx (Anderson *et al.*, 1990).

It is concluded in the present study that the volatile anesthetics applied into the nasal cavity can produce marked reflex changes in respiratory and circulatory functions which are mediated by stimulation to presumably C-fiber endings of the PNN.

Section 6 Summary and conclusion

Summary

Recently, volatile anesthetics are use widely for the general anesthetics in animals. Rapid elimination of the anesthetics from the body through the lung induces the easy control of anesthetic level. The predictable and stable anesthetic conditions exerted by these drugs contributes their popularity in clinical use. We, however, must pay careful attention on the undesirable adverse effects of the volatile anesthetics on the respiratory and cardiovascular functions especially during induction of anesthesia. Induction of anesthesia with volatile anesthetics is sometimes associated with defensive or protective reflexes such as cough, apnea, inhibition of breathing (apnea), laryngospasm, and secretion. Clinically, these have been presumed to be caused by an irritation of airway mucosa, the degree of which varies with anesthetic and anesthetic depth. In the respiratory tract, especially in the nasal cavity, larynx, and lower airway and lungs, there are various sensory afferents to be sensible for various inhaled chemicals. Therefore, it was hypothesized that the airway reflexes of volatile anesthetics could be mediated by the stimulation of the airway sensory receptors. The purpose of this study was to elucidate the effects of volatile anesthetics on the sensory system in the respiratory tract and consequent cardiorespiratory reflexes in dogs.

In section 1, as introduction of this thesis, general information of volatile anesthetics, clinical observation of the airway irritation of these anesthetics, sensory function of the respiratory tract, and the purpose of this study is described.

In section 2, as a preliminal study, the incidence of complications including cardiopulmonary effects during rapid inhalation induction (RII) with halothane (Hal), enflurane (Enf), isoflurane (Iso) and sevoflurane (Sevo) were evaluated. As a result, body movements during RII with Sevo were minimal and induction was most rapid and smoothest among the anesthetics. Such finding is not inconsistent with the clinical observation in human patients indicating Sevo is the best inhalant for induction of anesthesia, although it is still unclear whether the least excitements of the dogs was due to its less airway irritation and/or lower blood/gas partition coefficient.

To clarify these aspects observed in the section 2, the respiratory tract was functionally isolated to three major parts, i.e., broncho-pulmonary, laryngeal and nasal region, then the respiratory and cardiovascular reflexes and afferent activity of sensory receptors to each part to topical inhalation of volatile anesthetics were evaluated in the following section.

In the section 3, responses of broncho-pulmonary receptors (SARs, RARs and bronchial and pulmonary CAPS-sensitive C-fiber receptors) to volatile anesthetics were evaluated from the recording of action potentials of vagus nerve. As a result, the discharges of SARs in all anesthetics except for Hal at low concentration

decreased significantly depending on the anesthetic level. These results suggests that the inhalation of the volatile anesthetics attenuate the Hering-Breuer inflation reflex and induce a slowing of respiratory timing and an increase in tidal volume. In contrast, increased SARs activities observed in Hal at low concentration might contribute to an increase in respiratory frequency and a decrease in tidal volume during maintenance of anesthesia. The discharges of RARs also decreased significantly irrespective of the type of anesthetic, suggesting less association of RARs in the lower airways toward the airway reflexes in dogs.

On the other hand, discharges of bronchial and pulmonary C-fiber receptors were remarkably increased by inhalation of all anesthetics. The degree of increase in both bronchial and pulmonary CAPS-sensitive C-fiber activities was significantly greater in Hal as compared to Sevo. The hyperpneic reflex responses for Hal, Iso and Sevo were considerably reduced or no longer observed after perineural CAPS-treatment and bilateral vagotomy, whereas no significant change was observed for Enf compared with non-treatments. These results suggested that the presence of reflex contribution of CAPS-sensitive C-fiber receptors in the lower airway and lungs to the reflex hyperpnea by inhalation of Hal, Iso and Sevo.

In the section 4, the responses of laryngeal CAPS-sensitive presumed C-fiber and RARs to volatile anesthetics were evaluated from the recording of action potentials of internal branch of the superior laryngeal nerve. The CAPS-sensitive receptors were clearly distinguished from irritant receptors by their prevalent responsiveness to CAPS and their lack of responsiveness to water. All the CAPS-sensitive receptors were significantly stimulated by all volatile anesthetics in a concentration-related manner, and the activation by Hal, Enf and Iso was significantly greater than by Sevo. In contrast, responses of RARs to the volatile anesthetics were not constant and divided into three types, stimulation, inhibition, or non-response, to which no significant differences of the RAR activities were found among anesthetics. Such findings suggested that the CAPS-sensitive presumed C-fiber endings were consistently stimulated by halogenated volatile anesthetics and especially by Hal, Enf and Iso, and that these responses were dissimilar to the variable responses in RARs.

In this section, possible cardiopulmonary reflex responses of the laryngeal CAPS-sensitive C-fiber and RARs to volatile anesthetics were also evaluated. All the anesthetics increased T_E and decreased $\dot{V}_E$ significantly from the control level, the effects of which were significantly greater in Hal, Enf and Iso than in Sevo. Approximately 50% of the responses reduced by the CAPS desensitization. Topical anesthesia with lidocaine eliminated all the reflex responses. It was suggested that various cardiopulmonary reflexes can be elicited by the stimuli to laryngeal CAPS-sensitive C-fiber and water-responsive RARs, the effects of which are associated with the laryngeal reflexes of volatile anesthetics especially Hal, Enf and Iso.

In the section 5, cardiopulmonary reflexes and trigeminal nerve activity in response to volatile anesthetics into the nasal cavity were evaluated. As a result, a significant increase in T_E and a

significant decrease in $\dot{V}_E$ were observed following application of each anesthetic, where such changes were considerably greater for Hal than for Sevo. An increase in arterial blood pressure and a decrease in V_T were also elicited for Hal. All the reflex responses were considerably inhibited by topical anesthesia with lidocaine into the nasal cavity or by bilateral section of the posterior nasal nerve (PNN). The responsiveness of PNN afferents to volatile anesthetics coincided with the magnitude of reflex responses by the anesthetics. All the PNN afferents which responded to the anesthetics were markedly stimulated by capsaicin instillation, but not by distilled water. These results demonstrate that the volatile anesthetics can stimulate the trigeminal afferents, at least capsaicin-sensitive endings, and evoke cardiopulmonary changes of which strength is largely associated with stimulatory effects on the sensory nerve.

Considering above, it was indicated that the airway irritation of volatile anesthetics during inhalation induction of anesthesia was largely mediated by the stimulation of CAPS-sensitive C-fiber receptors and a part of laryngeal RARs. Furthermore, it was suggested that the deference in the strength of the stimulation of CAPS-sensitive endings, especially in the larynx, rather than that of RARs can be greatly associated with the airway irritation of volatile anesthetics. Results have also suggested that the functional importance of the upper airway to induce various airway reflexes by inhalation of volatile anesthetics as a result of the stimulation of these sensory receptors. It is because the airway reflexes of the volatile anesthetics induced by the stimulation of the fibers in the airway seemed to play a minor role.

In the present study, such airway reflexes of volatile anesthetics by stimulation of the CAPS-sensitive C-fiber receptors were mainly represented by the cardiopulmonary reflexes though the autonomic nervous reflexes. Such noxious stimulus of C-fibers sometimes include vasodilation and edema, mucus secretion mediated by neuropeptides such as substance P, neurokinin A, and carcitonin gene-related peptide (CGRP) through axon reflexes. Therefore, it was concluded that we must pay careful attention to the concurrent stimulation of C-fiber receptors during induction of anesthesia with volatile anesthetics.

Conclusion

Airway receptors located within and/or between the airway mucosa, laryngeal intrinsic muscles, tracheobronchial smooth muscles, and alveolar walls are mainly responsible for the sensory function of the respiratory tract (Widdicombe and Sant'Ambrogio, 1992). Recently, large amount of sensory receptors were identified in the nasal, laryngeal, and bronchopulmonary mucosa and these findings indicated the important role of airway mucosa in the airway reflexes (Sant'Ambrogio *et al.*, 1995). Airway mucosa riches in sensory receptors such as RARs and CAPS-sensitive C-fiber receptors). One of the major role of the sensory receptors in the airway mucosa is its elicitation mechanism of various airway reflexes which defense or protect the body from inhaling irritant substances such as extrinsic or intrinsic chemicals or inspiration of foreign bodies or gastric contents.

On the other hand, sensory receptors in the airway smooth muscle such as SARs take part in the regulation of breathing (inspiratory off-switching; respiratory frequency and depth) in animals. Although it is still unclear how these receptors modulate or interact each other, it is possible that the resultant reflexes by one sensory receptors can secondary stimulate other fibers to exaggerate the strength of the responses: for example, chemical or mechanical induced cough reflexes are primarily mediated by the stimulus of RARs and/or CAPS-sensitive C-fibers, however, inspiratory phase of cough cannot be completed in the absence of consequent stimulation of the SARs (Coleridge and Coleridge, 1984, 1994).

The airway reflex mechanisms by volatile anesthetics clarified in this study were relatively simple in contrast to other chemical or mechanical induced airway reflexes (Fig. 1); i.e., the volatile anesthetics consistently stimulated the discharges of nasal, laryngeal, and bronchopulmonary CAPS-sensitive C-fibers, whereas they depressed those of SARs and RARs with the exception of the stimuli of some RARs in the larynx. Moreover, the consistent stimuli of CAPS-sensitive receptors in the upper airway caused an inhibition of breathing (apnea) and the stimulation of the receptors in the lower airway caused an increase of breathing (tachypnea), indicating the presence of two fundamentally different properties of CAPS-sensitive C-fibers in the airways. The afferents of CAPS-sensitive C-fibers through the vagal or trigeminal pathway generally input to the nucleus tractus solitarius (NTS), of which electrical activity influences on the medullary respiratory neuron groups and dorsal nucleus of the vagus, eliciting various cardiorespiratory responses. It is believed that the greater stimulus conveyed to NTS causes an inhibition of breathing (apnea) or coughing when stimulated vigorously, whereas only an increase of breathing (rapid shallow breathing) when the stimulus is weaker (Widdicombe and Sant'Ambrogio, 1992). Moreover, the stronger stimuli in the upper airways also exhibits cardiovascular reflexes represented by bradycardia and hypertension or bradycardia and hypotension by the stimulation in the lower airways via the autonomic nervous system, as well as the respiratory changes (Coleridge and

Coleridge, 1984, 1994; Widdicombe and Sant'Ambrogio, 1992; Coleridge and Coleridge, 1984, 1994; Mutoh *et al.*, 1997b).

In the present study, greater airway reflexes by inhalation of volatile anesthetics were elicited in the upper airway, while the anesthetics had less effects on the lower airways. It is difficult to clarify the interaction between the from the present study, however, it is reasonable to suppose that the sensory receptors in the initial pathway such as nasal cavity and larynx played the major role in protecting or defending to inhale the 'volatile anesthetics as an irritants'. On the other hand, the reflex roles of the bronchopulmonary CAPS-sensitive C-fibers were overshadowed by the systemic respiratory responses of volatile anesthetics absorbed into the systemic circulation (section 3).

Considering the functional and morphological importance of the upper airway, it is well assumed that the larynx should be the most reflexogenic part to protect the lower airway. In fact, it was indicated by the degree of the airway irritation of volatile anesthetics concurred the strength of the laryngeal CAPS-sensitive receptors and the stimulus of RARs by volatile anesthetics was observed only in the larynx. In humans, the larynx has been a most susceptible source of airway irritation by volatile anesthetics (Nishino *et al.*, 1985). In the present study, the importance of the upper airway, especially of the larynx, has also been demonstrated in dogs.

Clinically, induction of anesthesia with volatile anesthetics in humans are associated with airway reflexes such as cough, sneezing, laryngospasm, and broncho-constriction; however, such reflexes were not pronounced in any dog applied in this study. The reason might be due to the depth of basal anesthesia used in this study: in general, cough or sneeze reflexes are easily depressed by the basal anesthetic level, while the reflex inhibition of breathing (apnea) are not so greatly influenced (Mortola and Fisher, 1988). The species differences and modality of induction between dogs and humans might also cause less airway reflexes. The internal branch of the SLN in the dog has a relatively lower number of unmyelinated fibers than in other species (Sant'Ambrogio *et al.*, 1995). In addition, lower concentration of anesthetics might have been exposed to the airway mucosa can be presumed in dogs than in humans because dogs inhaled anesthetics with spontaneously breathing (Mutoh *et al.*, 1995), while humans generally induced anesthesia by their vital capacity (Yurino and Kimura 1992, 1993a, 1993b, 1994).

Apart from the respiratory and cardiovascular reflexes via the central nervous system, the stimulation of CAPS-sensitive receptors elicits regional reflexes so-called 'neurogenic inflammation' via the axon (Widdicombe and Sant'Ambrogio, 1992). In clinical practice, fortunately, such regional reflexes appears to be less evident of anesthesia with volatile anesthetics (Yurino and Kimura 1992, 1993a, 1993b, 1994, Mutoh *et al.*, 1995) probably because of relative higher firing-threshold of axonal reflexes. In any case, we had better to bear in mind the axonal reflexes for induction of anesthesia with volatile anesthetics in animals which suspected diseases in respiratory functions.

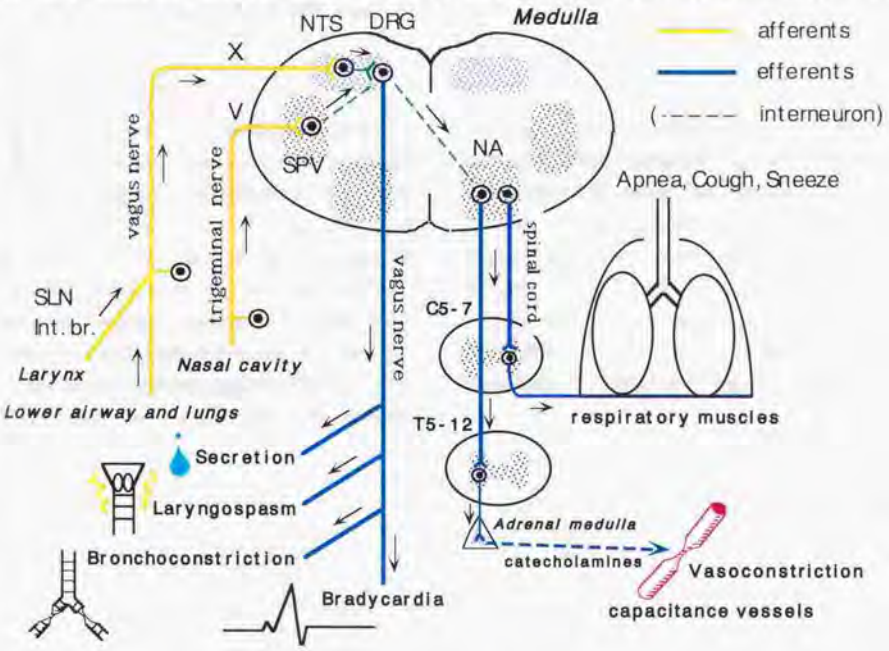
In clinical practice, therefore, we should consider how to block or depress these upper airway reflexes induced by the noxious chemical stimulus of CAPS-sensitive C-fibers in the nasal cavity and larynx and a part of RARs in the larynx when induced anesthesia with volatile anesthetics especially Hal, Enf, and Iso. The best way to reduce airway reflexes induced by the sensory receptors is to block the afferent pathway completely at its origin. Application of local anesthesia to the mucosa can be proposed to block or paralyze the nerve endings located within the epithelium. In the previous experimental study, local anesthesia on the mucosa with nebulized lidocaine completely eliminated the airway reflexes by noxious stimuli of CAPS into the laryngeal lumen or the nasal cavity (Eccles, 1982; Mutoh *et al.*, 1997b). In clinical practice, we usually apply local anesthetic (*e.g.*, lidocaine) directly onto the larynx to prevent the laryngeal reflexes against the noxious mechanical stimuli during endotracheal intubation; such a premeditation might also be necessary before induction of anesthesia with volatile anesthetics. Recently, pretreatment with nebulized lignocaine before induction of anesthesia has been successfully used for human patients to reduce the upper airway irritation of Iso or Des (Bunting *et al.*, 1995), though it may be not practical for animals because of struggle or movement against the therapy.

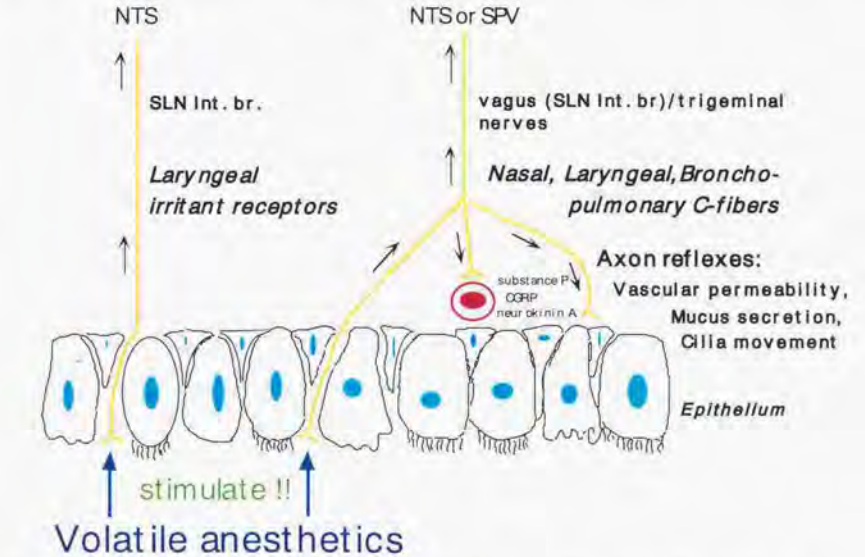
To prevent the airway reflexes via the CNS such as bradycardia and secretion, premedication with atropine sulfate might be useful to block cholinergic pathway. Of course, assisted ventilation with a bag and mask would reduce ventilatory depression and accelerate inhalation of volatile anesthetics. Moreover, premedication of patients with mild sedatives and/or tranquilizers might reduce the concentration of volatile anesthetics in need for accomplish of faster and smoother induction of anesthesia.

The better understanding of the mechanisms of airway irritation of volatile anesthetics (Fig. 1) would improve induction of anesthesia safer and smoother. At present, however, we only know the physiological aspects of the sensory functions of the volatile anesthetics since the morphological findings of them are still unclear, and there is a large amount of question to be clarified. For example, how and where the sensory endings translate the irritant stimuli into the electrophysiological signals ? How the signals input to NTS convey and output as reflexes ? Moreover, is it true that all the receptor sites were the nerve endings of afferent fibers ? Morphologically, the presence of structures such as taste buds at the end of sensory nerves within the epithelium have been demonstrated (Yamamoto *et al.*, 1997), indicating the presence of the receptor site. Further investigation of my research underlies the clarification of these neurological mechanisms

Fig. 1.

Possible reflex mechanism of airway irritation of volatile anesthetics





Abbreviations NTS: nucleus tractus solitarius; DRG: dorsal respiratory neuron group; SPV: spinal nucleus of the trimeminal nerve; NA: nucleus ambiguus.

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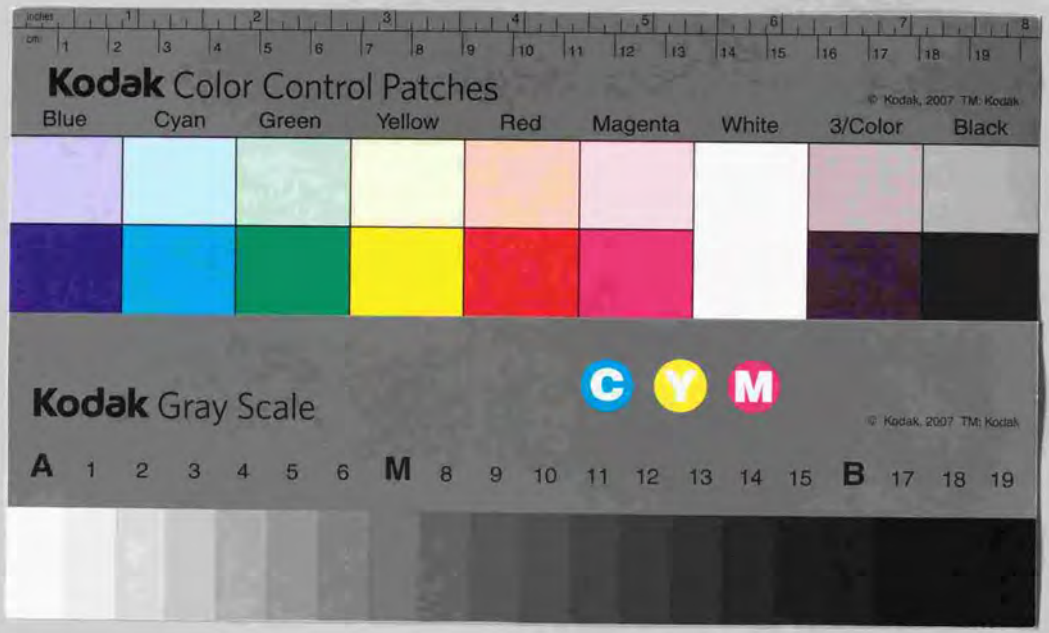
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