Cardiovascular Reflex Mechanisms by Topical Instillation of Capsaicin and Distilled Water into the Larynx in Anesthetized Dogs

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ABSTRACT. Cardiovascular reflex mechanisms by topical laryngeal instillation of capsaicin (CAPS) or distilled water were evaluated in anesthetized chronic tracheostomized dogs. Both CAPS (10 μg/m*l*) and water instillation into the isolated upper airway caused a significant decrease in heart rate ($P<0.05$) and a significant increase in blood pressure ($P<0.05$) from the values before instillation under both spontaneous and controlled ventilation. The bradycardia was significantly reduced by atropine pretreatment ($P<0.05$) and the hypertension was significantly decreased by phentolamine and propranolol pretreatments ($P<0.01$). A higher concentration of CAPS (100 μg/m*l*) instillation considerably reduced the response to subsequent CAPS (100 μg/m*l*) instillation, whereas the response to water was sustained, indicating the desensitization of laryngeal CAPS-sensitive endings. All the reflex responses to CAPS and water were eliminated by topical anesthesia with lidocaine. It was concluded that the laryngeal cardiovascular reflex responses were mediated by the afferents such as the laryngeal CAPS-sensitive presumably C-fiber endings or water-responsive receptors and by both the parasympathetic and sympathetic nervous systems as efferents. — KEY WORDS: autonomic nervous blockade, canine, cardiovascular reflex, capsaicin, distilled water.

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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 lungs [24, 29]. However, the excessive reactions of such reflex responses can be an incidence of increased risk for induction of anesthesia. For example, light mechanical touch on the laryngeal lumen at endotracheal intubation, inhalational induction of anesthesia with high concentration of volatile anesthetics, and aspiration of pharyngeal or gastric contents with full stomach induction of anesthesia occasionally induce reflex inhibition of breathing, apnea, cough, bronchoconstriction, bradycardia, and hypertension in various species [4, 7, 8, 12].

Recent electrophysiological studies have revealed that such reflexes are associated with the stimulus effects of water-responsive irritant receptors and/or capsaicin (CAPS)-sensitive unmyelinated C-fiber endings located in the laryngeal mucosa via the afferent vagal pathway [15, 21, 26]; however, the elicitation mechanisms of their cardiovascular responses are less recognized in contrast to the clarification of the respiratory defensive or protective reflexes.

The purpose of this study is to elucidate the reflex cardiovascular effects of topical instillations of CAPS and distilled water on the laryngeal lumen and their reflex mechanisms through experiments with autonomic nervous blockades in dogs.

MATERIALS AND METHODS

Animals: Sixteen healthy beagle dogs (6 males and 10 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). Food was withheld at least 12 hours before each experiment.

Surgical procedure for in vivo isolated upper airway model: All the dogs underwent permanent tracheostomy 2–3 weeks before experiments. Dogs were induced anesthesia with a thiopental sodium (25 mg/kg; RAVONAL®, Tanabe, Japan) intravenously, endotracheally intubated, followed by maintained anesthesia with isoflurane in 100% oxygen. An Window surgical technique for permanent tracheostomy was used in this study [14, 19]. Briefly, dogs were placed on an operating table in the spine position, and midline skin incision was made with an oval piece of the skin. Sternohyoid muscles were separated and the ventral aspect of the trachea was exposed. Then the medial edges of sternohyoid muscle were sutured together at the dorsal to the trachea for the elevation to the skin by horizontal mattress sutures with 3–0 non-absorbable polypropylene monofilament. The ventral aspect of three tracheal rings just mid portion of the trachea between cricoid cartilage and thoracic wall leaving the tracheal mucosa intact. After the tracheal rings were completely removed, the mucosa was completely dissected approximately 5 mm margin left from the trachea. Then the skin and trachea including mucosa were sutured with 3–0 polypropylene monofilament. Butorphanol (0.2 mg/kg; STADOL®, Bristol-Myears Squibb Co., Japan) was injected intramuscularly as postoperative analgesia. During the adjustment period after surgery, tracheostomy site was carefully cleaned and nebulized with saline solution if necessary. Dogs fed in an isolated room and received ampycillin (20 mg/kg × 2/day; PENTREX®, Banyu, Japan) at least for a week after surgery for the prevention of postoperative bacterial infection.

Basal anesthesia: Dogs were induced anesthesia with a

thiopental sodium (25 mg/kg), followed by a mixture of urethane (200 mg/kg) and α-chloralose (20 mg/kg) was injected intravenously slowly over 15 min. The supplemental dose of urethane (100 mg/m*l*) and α-chloralose (10 mg/m*l*) mixture was infused at a rate of 1 m*l*/kg/hr with an infusion pump (Model STC-531, Terumo Co., Japan) through a 22-G intravenous catheter placed into the cephalic or saphenous vein. Lactated Ringer's solution was also infused at a rate of 10 m*l*/kg/hr through the intravenous catheter placed into the other side of cephalic or saphenous vein. At least 1 hr's adjustment period was allowed after induction of anesthesia to obtain sufficient basal anesthesia for the experiment.

Animal preparation: Dogs were placed on an operating table in the spine position, then a laryngeal mask airway (LARYNGEAL MASK Size-3, Intervent) was introduced to cover the larynx. Cuffed tracheostomy tubes (I.D=7.0–8.0 mm; PORTEX, Nihon Med Co., Japan) were placed into the upper and lower trachea respectively through a tracheostomized airway. Arterial blood pressure was monitored by a pressure transducer (DX-300, Nihon Kohden, Japan) 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. Expired PO_2 (PEO_2) and PCO_2 ($PECO_2$) were monitored by a gas analyzer (Respina 1H26, NEC san-ei, Japan). All the signals were displayed on a thermal-array recorder (RT 3100N, NEC san-ei, Japan), and recorded by a magnetic tape recorder (PC 208, SONY Co., Japan).

Experimental protocol: A 10 m*l* of CAPS solution (10 μg/m*l*, a diluted solution of CAPS 100 μg/m*l*, 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 to avoid desensitization.

These experiments were performed under both spontaneous and controlled (tidal volume of 10–15 m*l*/kg at a frequency of 14–20 cycles/min) ventilations in all dogs. Then, 12 dogs were divided into 3 pretreated groups of 4 dogs each with autonomic nervous blockades of atropine (0.1 mg/kg i.v.; ATROPINE®, Tanabe, Japan; n=4), phentolamine (1 mg/kg i.v.; Regitin®, Chiba-Geigy, Japan; n=4), and propranolol (1 mg/kg i.v.; Inderal®, Zeneka, Japan; n=4) under controlled ventilation, and evaluated the responses to CAPS and water instillation again. Each pretreatment was performed at 10 min before the experiment.

In 8 dogs, a higher concentration of CAPS (100 μg/m*l*) was pretreated into the laryngeal lumen to evaluate the subsequent responses to CAPS (100 μg/m*l*) and distilled water. These challenges were performed at intervals of 10 min. At the end of the experiment, a 5 m*l* of 2% lidocaine solution (Xylocaine®, ASTRA, Japan) was aerosolized with an ultrasonic nebulizer (NE-U12, OMRON Co., Japan) driven by the airflow (15 L/min, output 2.5 m*l*/min) producing approximately 5 μm in size, and passed through the isolated upper airway for 2 min. Then the nebulizer was turned off, and the CAPS and distilled water were challenged again.

All the dogs were euthanatized after the end of the experiment by an intravenous injection of pentobarbital sodium (50 mg/kg, Nembutal®, Dainabot Co., Japan).

Data analysis: The systolic arterial blood pressure (SBP) and the heart rate (HR) were obtained from the tracings at 0, 10, 20, 40, 60, and 80 sec after the onset of instillation. The HR was calculated from the number of pulse pressure for each 5 sec before the recording points. Statistical analysis was performed using a statistical software package (StatView® 4.5J, Abacus Concepts Inc., U.S.A.). The raw data were statistically analyzed by Wilcoxon's signed rank sum test and the percent transformed data were by Mann-Whitney's U-test. P values less than 0.05 were taken as statistically significant. All data were expressed as mean ± SEM.

RESULTS

The intralaryngeal instillation with CAPS (10 μg/m*l*) and water induced a transient inhibition of breathing, followed by a marked increase in arterial blood pressure and a mild decrease in HR under spontaneous ventilation as represented in Fig. 1. Similar cardiovascular responses were also observed under artificial ventilation (Fig. 2). The peak changes in SBP and HR by CAPS or water were observed at 10 sec after the onset of instillation in both ventilatory conditions, and returned to the pre-instillation level at 20 sec (Fig. 2). Whereas significant differences in pre-instillation values of SBP and HR were found between both ventilatory conditions ($P<0.05$), the peak percent changes after the instillations of CAPS and water were not statistically significant (Fig. 2).

In both CAPS and water instillations the decrease in HR was completely inhibited by pretreatment with atropine, whereas SBP was still elevated from the pre-instillation values ($P<0.05$) (Fig. 2). The pretreatment with either phentolamine or propranolol showed no significant change in HR and SBP from the pre-instillation values ($P<0.05$) (Fig. 2).

Peak percent changes by CAPS and water instillations were compared between the treatment and non-treatment groups with atropine, phentolamine or propranolol (Fig. 3). In both CAPS and water instillations as compared to the non-treatment group, a significantly higher HR value was observed in atropine pretreatment group ($P<0.05$) and a significantly lower SBP values were observed in phentolamine or propranolol group ($P<0.01$), although pre-instillation values were significantly lower in phentolamine and propranolol pretreatment groups than in non-treatment and atropine pretreatment groups ($P<0.05$).

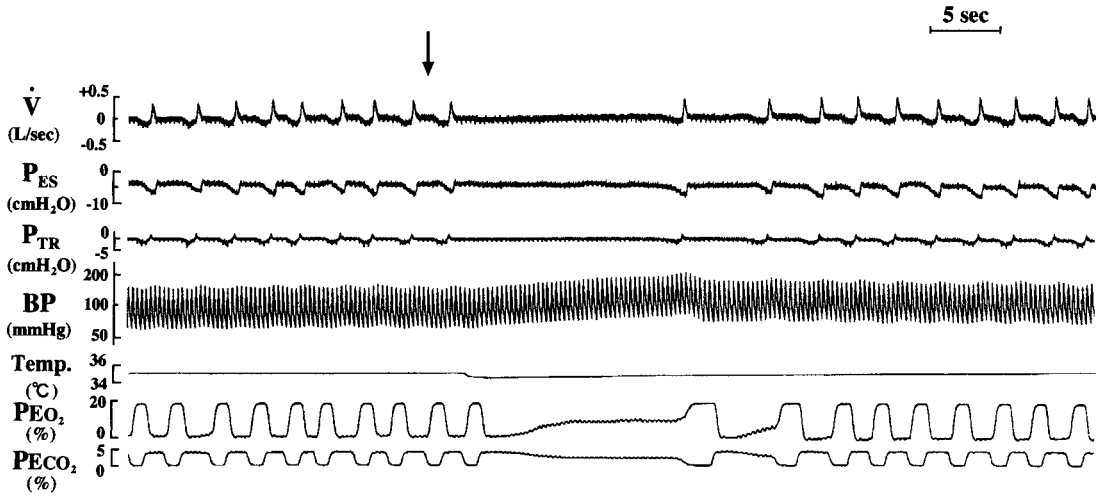

Fig. 1. An example of laryngeal cardiorespiratory reflexes elicited by capsaicin instillation into the larynx in a spontaneously breathing dog. A clear inhibition of breathing and reflex hypertension were observed immediately after the onset (the arrow) of instillation. $\dot{V}$=respiratory airflow; P_{ES}=esophagel pressure; P_{TR}=intratracheal pressure; BP=arterial blood pressure; Temp.=laryngeal temperature; PE_{O2}=expired P_{O2}; PE_{CO2}=expired P_{CO2}.

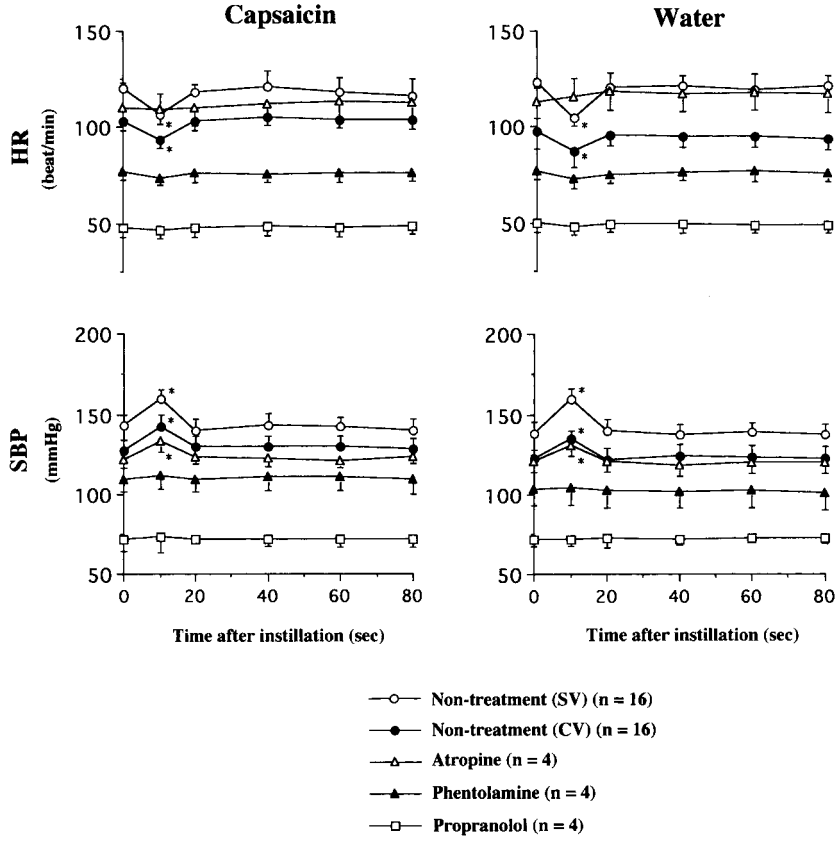

Fig. 2. Time-course changes in heart rate (HR) and systolic blood pressure (SBP) elicited by topical instillations of capsaicin and distilled water into the larynx under spontaneous ventilation (SV) and with or without pretreatments of autonomic blockades (atropine, phentolamine, and propranolol) under controlled ventilation (CV). * $P<0.05$ compared with pre-instillation value.

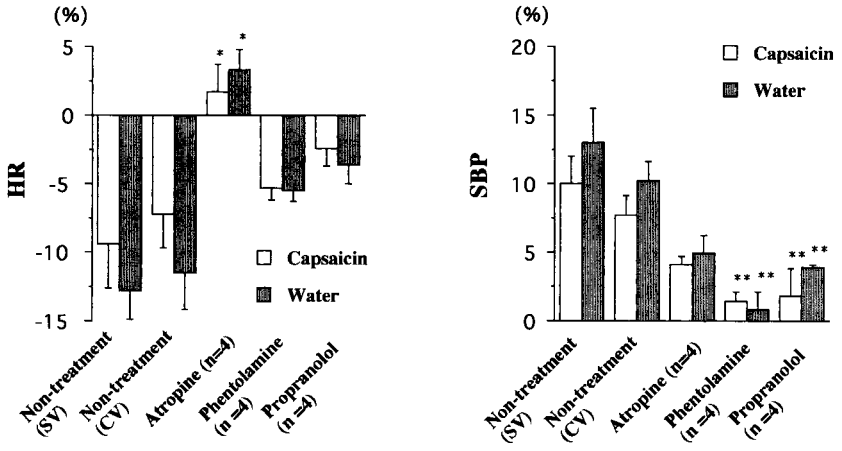

Fig. 3. Maximum changes in heart rate (HR) and systolic arterial blood pressure (SBP) by instillation of capsaicin and distilled water into the larynx under spontaneous ventilation (SV) and with or without pretreatments of autonomic blockades (atropine, phentolamine, and propranolol) under controlled ventilation (CV). The values represent mean ± SEM of percent changes from pre-instillation values. ** $P<0.01$; * $P<0.05$ compared with non-treatment group under CV.

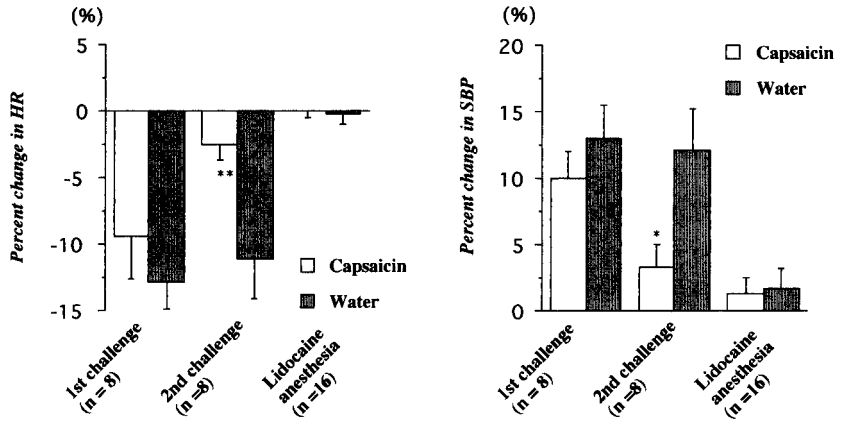

Fig. 4. Changes in heart rate (HR) and systolic arterial blood pressure (SBP) at first and second challenges with capsaicin and distilled water instillations into the larynx and after the intralaryngeal anesthesia with lidocaine. ** $P<0.01$; * $P<0.05$ compared with the value at the first challenge.

A higher concentration (100 μg/m*l*) of CAPS instillation significantly reduced the cardiovascular responses to the 2nd challenge with CAPS (100 μg/m*l*) ($P<0.01$, HR; $P<0.05$, SBP), while the response to water sustained almost the same level as the 1st water instillation (Fig. 4). No changes in the cardiovascular measurements were observed for the CAPS and water instillations after the topical anesthesia with lidocaine applied into the larynx (Fig. 4).

DISCUSSION

Capsaicin is widely accepted as a selective stimulant to unmyelinated C-fiber endings [5], while a lack of chloride anions as represented by the composition of distilled water to be a factor in the stimulation of laryngeal irritant endings [24]. In such circumstances, topical application of each selective stimulant into the laryngeal lumen offers good implication to evaluate the contribution of each endings to the reflex responses [24].

In the present study, the hypertension and bradycardia was clearly elicited by the topical instillation of both CAPS and distilled water into the larynx being independent of the inhibition of breathing. It is well known that the hypertension and resultant bradycardia can be produced by relatively brief asphyxia [9, 16]; however, the results from the present study demonstrated that such a secondary effect of asphyxia for inducing bradycardia and hypertension was not a major, since substantially the same degree of HR and SBP changes were elicited under both spontaneous and controlled ventilations.

The cardiovascular reflex elicitation appeared to have qualitatively similar efferent mechanisms irrespective of the type of stimuli, since in both CAPS and water challenges the hypertension and bradycardia were markedly inhibited by the adrenergic and cholinergic blockades. No change in heart rate in phentolamine pretreatment group might be due to a decrease in baroreflex in response to hypertension. Furthermore, no increase in systemic blood pressure in propranolol pretreatment group suggested that the blood pressure response could be exhibited only at normal level of blood pressure before CAPS or water challenges because its initial level was considerably as low as 72 mmHg. However, the bradycardia might be attributed to some extent by the concurrent increase in the sympathetic output which can reduce the parasympathetic effect. These cardiovascular reflex responses are quite similar to the finding that recognized as so-called 'diving reflex' when distilled water is instilled into the nasal cavity in dogs and other animals [2, 10, 27]. Similar cardiovascular changes have also been induced by the topical application of CAPS to the nasal mucosa in guinea pigs [17] and into the laryngeal lumen of the rat [15]. The introduction of hypertension from the nose and larynx associated with the sympathetic nervous system activation appears to be a common characteristic, whereas the hypotension and bradycardia are consistently elicited by phenyldiguanide and CAPS to the lung through the right atrial injection of CAPS [5, 20] and the intratracheal instillation of CAPS in rats and dogs [22]. Moreover, irritant stimuli such as distilled water to the lower airway is thought to have little or no influence on the cardiovascular function [6].

The higher concentration of CAPS application greatly reduced its subsequent response to CAPS. Such effect has been known as the 'desensitization' of the C-fiber endings due to a lack of nerve response to CAPS [17, 25, 26] presumably by a prolonged and irreversible depolarization of membrane potentials [30]. In contrast, the reflex responses to distilled water was not decreased even after the CAPS-desensitization, indicating the presence of different functional properties of the nerve endings. Therefore, the cardiopulmonary response to CAPS in the present study can be considered as being mediated by the unmyelinated C-fiber endings in the larynx.

The similar cardiovascular responses between CAPS and water stimuli suggest a possibility that the central processing associated with afferent stimuli processes common pathway to exhibit the reflexes via the autonomic nervous system. The afferents from the vagal pathway generally input to the nucleus tractus solitarius (NTS), of which electrical activity influenced on the medullary respiratory neuron groups and dorsal nucleus of the vagus and, elicit various cardiorespiratory responses [23, 28]. At present, we cannot describe whether the afferent informations associated with CAPS and water stimuli which arose from the larynx in the dog terminate polymodal neurons in the NTS as observed in the spinal dorsal horn neurons [13].

There are several reports on the cardiopulmonary response to various fluids into the larynx or hypopharynx in some animal species such as sheep, pigs, rats, dogs and humans [18]. However, findings from all these studies were limited to only newborn stages. Boggs and Bartlett [3] reported the apneic reflex in puppies with low concentration of NaCl on extremely low or high pH. Moreover, Fisher *et al*. [11] described the apneic response by laryngeal flow stimulus, where the response occurred at only newborns puppies (1–14 days old) but never occurred at least after 29-day-old. In the present study, however, the marked cardiopulmonary responses by water were observed in adult dogs, which has not been reported in the previous studies. Therefore, our findings evidenced that the 'water'-induced apnea remained firmly in also adult dogs. This is not inconsistent with the finding that many respiratory modulated or non-modulated laryngeal receptors can be stimulated by distilled water [1]. The weak cardiopulmonary 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 that in awake state [18].

All the reflex responses were eliminated by the topical anesthesia into the larynx, indicating superficial location of the nerve endings and confirming the contribution of the intralaryngeal sensory endings on the reflex responses in the present study.

In conclusion, the present study demonstrated that both CAPS and water instillations into the larynx could stimulate different sensory endings but produce similar reflex cardiovascular responses in which were mediated by the sympathetic and parasympathetic nervous systems in the anesthetized dog.

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