Effects of Perineural Capsaicin Treatment on Compound Action Potentials of Superior Laryngeal Nerve Afferents in Sevoflurane-Anesthetized Dogs

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ABSTRACT. Effects of perineural capsaicin (CAPS) treatment on compound action potentials of the superior laryngeal nerve (SLN) afferents were studied in 6 sevoflurane-anesthetized dogs. Perineural CAPS (100 μg/ml) to the bilateral SLNs reduced ($P<0.01$) the peak and integral amplitudes of the C-wave of the compound action potential. By contrast, the perineural CAPS had no effect on the A-wave component ($P>0.05$). Removal of the perineural CAPS recovered the C-wave to pretreatment level. The perineural CAPS treatment selectively blocks C-wave compound action potentials of the SLN afferents, providing a useful tool for studies of laryngeal C-fibers in respiratory physiology.—KEY WORDS: canine, conduction velocity, laryngeal C-fiber.

The larynx is a potent reflexogenic region of the upper airway that is rich in sensory afferents and elicits various defense reflexes to protect the lower airways and lungs [1, 2]. Apnea, coughing, glottal closure, mucus hypersecretion, bronchoconstriction, bradycardia and hypertension are characterized by stimulation of the laryngeal mucosa in various species [12, 14]. Previous reflex studies have demonstrated that topical laryngeal instillation of capsaicin (CAPS) produced marked cardiopulmonary reflex responses from the larynx of dogs. It is known that CAPS is a potent stimulator of unmyelinated C-fibers and the afferent pathway of the superior laryngeal nerve (SLN) plays an important role on the elicitation of such reflexes [5, 6]. Morphologically, 48% of the SLN composition in dogs is unmyelinated C-fibers [1].

Still lacking, however, is the evidence to support exclusive contribution of laryngeal C-fibers to the reflex elicitation to CAPS, since local application of CAPS also stimulates a portion of the myelinated Aδ-fibers, as well as the unmyelinated C-fibers [11, 14]. In a recent report, Schelegle *et al.* [13] succeeded in blocking vagal C-wave compound action potential and the C-fiber chemoreflexes by using perineural CAPS treatment to the vagus nerves in dogs. The purpose of this study was to determine whether the perineural CAPS treatment to the SLNs blocked the laryngeal C-fiber afferents in dogs.

Animals, anesthesia and animal preparation: Six healthy beagles (3 females and 3 males) were studied. Mean age

was 14 (range, 10 to 20) months and mean body weight was 12.3 (range, 10 to 14.2) kg. They were housed in individual runs at constant temperature and humidity, and fed commercial dry dog food once daily, with water available *ad libitum*. Food was with held for 12 hr before experiments. Dogs were premedicated with a mixture of acepromazine (0.1 mg/kg) and butorphanol (0.05 mg/kg) administered intravenously. Anesthesia was induced with thiopental (3.5–10.5 mg/kg, i.v.). Dogs were endotracheally intubated with a cuffed endotracheal tube (7.0–8.0 mm I.D.), then maintained with sevoflurane at an end-tidal concentration of 2.5–3.2% (equivalent to 1.2–1.5 times minimum alveolar concentration [MAC] [4]) in O_2 delivered at a flow rate of 1.5 L/min. All the dogs were paralyzed with gallamine (5 mg/kg, i.v.) and mechanically ventilated with a ventilator (KV-1+1, Kimura Medical, Japan) to maintain end-tidal PCO_2 ($PETCO_2$) within 35 ± 5 mmHg. A semi-closed circle anesthesia system (model KA-3020, Kimura Medical, Japan), with the vaporizers for sevoflurane (S-3, Kimura Medical, Japan) out side the circle, was used. End-tidal anesthetic concentration was collected through the sampling line connected to the endotracheal tube and was monitored by use of an infrared gas analyzer (AGM-103 Capnomac, Datex, Finland), the data from which were simultaneously downloaded into the Macintosh computer (PowerBook 5300 cs, Apple Computer Inc., U.S.A.) and calculated every 5 seconds. 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. During the experiment, rectal temperature was maintained at 36–38°C, using a warming blanket. Lactated Ringer's solution was infused at a rate of 10 ml/kg/hr into the cephalic vein through the needle-catheter.

Preparation of the SLN: A longitudinal skin incision was made at the aspect of the larynx, then left side of the SLN

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was exposed by separating the sternohyoides muscles bluntly. The SLN were isolated and desheathed entirely from the merge of the laryngeal lumen to the junction of the vagus nerve with a pair of iridectory scissors and fine-forceps, with the aid of a binocular microscope. The SLN was cut just below the junction of the vagus nerve (nodose ganglion), then a bipolar recording electrode was mounted in petroleum jelly mixed with mineral oil. A bipolar stimulating electrode was placed on the internal branch of the SLN, the main sensory nerve innervating the larynx, immerged from the laryngeal lumen. The external branch of the SLN, a major efferent nerve of the SLN, was cut at the junction of the internal branch in order to avoid any antidromically-evoked secondary response. The length between the recording and stimulating electrodes was 25–35 mm. The middle section of the SLN was prepared for perineural CAPS treatment as described below. Orthodromic compound action potentials were evoked with supramaximal rectangular pulses at a rate of 0.2 Hz (DPS-06, DIA Medical, Japan). The A-wave of the compound action potential was evoked with a 10 to 70 V pulse of 0.1 ms duration. The C-wave of the compound action potential was evoked with a 70 to 90 V pulse of 1 ms duration. These nerve preparations were performed using fine forceps with the aid of a binocular microscope (SZ 60, OLYMPUS, Japan). The signal was amplified by a low noise DC-amplifier (DPA 201, DIA Medical, Japan) and a biophysical amplifier (DPA 200, DIA Medical, Japan), and displayed on an oscilloscope (SS 5762, IWATSU Electronic, Japan) in parallel with a loudspeaker (Model 7747, NEC san-ei, Japan).

Perineural CAPS treatment: A method [7,13] used successfully in dogs for the perineural CAPS treatment of the vagus nerves was applied in this study. Cotton pledgets soaked in a digestive solution consisting of Krebs solution (136.9 mM NaCl, 5.4 mM KCl, 5.5 mM 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/m*l*) and hyaluronidase (1000 U/m*l*) were placed on the SLN between the electrodes for 20 min to remove connective tissue and to increase segment permeability. Then the digestive solution was removed by washing with saline, and cotton pledgets containing a 100 μg/m*l* capsaicin (Sigma, U.S.A.) solution in a vehicle of 10% ethanol, 10% Tween 80, and 80% saline were placed on the nerve segments for 10–15 min in order to block CAPS-sensitive C-fibers.

Experimental protocol: We employed three sampling periods: 1) before perineural CAPS (control), 2) during perineural CAPS (SLN CAPS) and 3) after removal of the perineural CAPS (recovery). Control compound action potential was measured just before the application of CAPS to the SLN. Test compound action potential was measured at 10–15 min during the CAPS treatment. Recovery compound action potential was measured 20 min after removal of the CAPS treatment. For each sampling period, at least 5 waves were recorded by a magnetic tape recorder (PC 204 A, Sony Co., Japan) for off-line analysis. The data

were averaged with a MacLab/2e data-acquisition system (BRC Inc, Japan) and were stored on the Macintosh computer. The stored compound action potentials for each sampling period were averaged and the peak amplitudes (mV), onset latency (ms) and integral under the wave (mV(ms) were determined using a Mac Lab *Scope* (BRC Inc, Japan). Conduction velocity (m/s) of each wave was calculated from the onset latency and distance between recording and stimulating electrodes. At the end of the experiments, all the dogs were euthanatized by administration of an overdose of pentobarbital (50 mg/kg, i.v.).

Data analysis: To compare the differences within each wave (A- or C-wave), a one-way ANOVA for repeated measures test was run, followed, where appropriate, Tukey-type multiple comparison test. Values of $P<0.05$ were considered statistically significant. All data were expressed as means ± SE.

An example of the effect of C-wave block by perineural CAPS treatment (100 μg/m*l*) to the SLN is shown in Fig. 1. The grouped data of each variable in A- and C-waves are shown in Table 1. Perineural CAPS treatment had no effect on the A-wave component of the SLN afferents ($P>0.05$, Fig. 1; Table 1). By contrast, the CAPS treatment reduced C-wave of compound action potential significantly from the control (F=75.4; $P<0.01$), suggesting a block of the C-fiber component (Figs. 1 and 2; Table 1). The mean conduction velocity of each wave (Table 1) was within the range of vagal A- and C-fibers as reported previously [13].

Because airway C-fibers are sensitive to various chemical and mechanical stimuli such as cigarette smoke, inhalation anesthetics, bradykinin, capsaicin, mucosal probing and lung hyperinflation [3, 7, 8], several techniques have been employed to block sensory afferents to evaluate the role of C-fibers. Topical application of a high concentration of CAPS (100 μg/m*l*) directly to the airway mucosa is sometimes successful in blocking the subsequent response to CAPS in dogs [5]; however, it is unclear whether this result is due to desensitization of the CAPS-sensitive C-fiber endings or to neural toxicity of the high concentration of CAPS [2, 3]. Graded vagal cooling by 6–7°C is known to block respiratory reflexes mediated by myelinated afferents such as slowly adapting pulmonary stretch receptors (SARs) and rapidly adapting 'irritant' receptors (RARs) with less influence on those by unmyelinated C-fibers [10]; however, significant changes in the breathing pattern as a result of removal of the Hering-Breuer inflation reflex make it difficult to interpret the role of C-fibers in the reflex control of the dogs' breathing.

The results of this study suggest that perineural CAPS treatment which its proper concentration to the SLN is a good tool for blocking the laryngeal C-fiber afferents with less influence on the A-fiber components. The most important drawback of perineural CAPS treatment may be its limited effectiveness (i.e., duration of the C-fiber block) and doses of perineural CAPS and applied stimuli [9, 13]. There are several reports with regard to the selective block
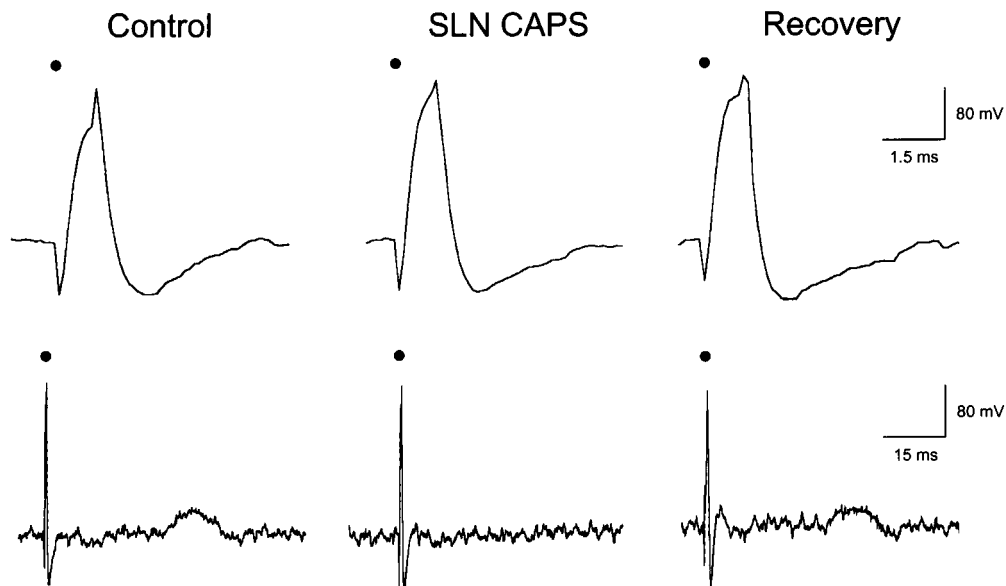
Fig. 1. An example of C-fiber block on compound action potentials in a sevoflurane-anesthetized dog. Note that a clear reduction of the C-wave (lower middle panel, SLN CAPS), by perineural CAPS treatment to the SLN, while the A-wave (upper panels) remained unaffected. The effect was recovered near control level after removal of the CAPS treatment (lower right panel, recovery). The mean peak onset latency of each wave was 1.1 ms (A-wave) and 38 ms (C-wave), respectively.　● = stimulus artifact.

Table 1. Effect of perineural capsaicin (CAPS) treatment on compound action potentials

	Control	Perineural CAPS	Recovery
A-wave			
peak ampritude (mV)	348 ± 32	323 ± 42	323 ± 38
integral (mV·ms)	290 ± 26	273 ± 30	286 ± 25
conduction velocity (m/s)	48.7 ± 8.2	47.9 ± 9.1	50.3 ± 9.8
C-wave			
peak ampritude (mV)	58.6 ± 8.2	8.7 ± 3.2*	49.5 ± 7.1
integral (mV·ms)	51.5 ± 7.9	4.8 ± 2.3*	42.6 ± 6.3
conduction velocity (m/s)	1.32 ± 0.28	ND	1.25 ± 0.33

Values are expressed as mean ± S.E. n=6/each group. ND=not determined. * $P<0.01$ from control.

of C-fiber by perineural CAPS treatment: Schelegle et al. [13] demonstrated that perineural CAPS selectively blocked both the C-wave components and C-fiber chemoreflexes evoked by CAPS (20 μg/kg) in dogs. Naida et al. [9] showed that an expiratory prolongation caused by topical laryngeal CAPS application (20 μg/ml) was blocked by perineural CAPS treatment (100 μg/ml) to the bilateral SLNs in rats. Given the observation, it is presumed that the dose of perineural CAPS treatment (100 μg/ml) used in this study may work on blocking C-fiber's reflexes at least up to 20 μg/ml.

Regarding potential contribution of the laryngeal C-fibers in cardiorespiratory reflexes, we suggested previously that laryngeal CAPS instillation (10 μg/ml) caused a marked prolongation of expiration (apnea), a decrease in heart rate (bradycardia) and an increase in arterial blood pressure (hypotension) in dogs [5]. Further studies are required to determine the magnitude of these responses by stimulation of laryngeal C-fibers using the perineural CAPS treatment.

In summary, our data suggest that the perineural CAPS blocks C-wave compound action potentials of the SLN afferents, providing a useful tool for various physiological studies on laryngeal C-fibers.
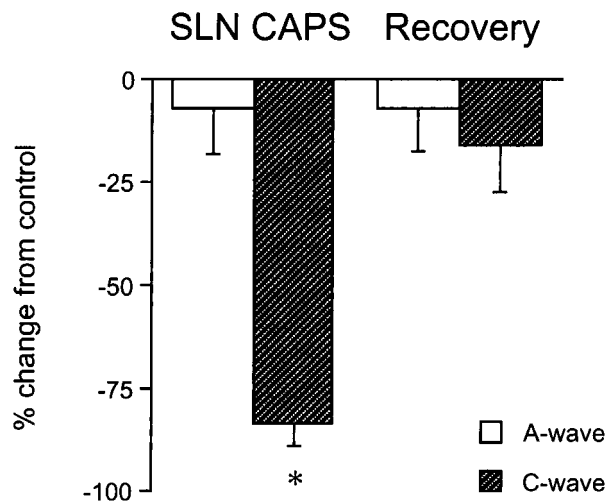
SLN CAPS   Recovery

Fig. 2.   Effect of perineural CAPS treatment to the SLN on peak amplitudes of compound action potentials. Significant reduction of the C-fiber component by perineural CAPS treatment (SLN CAPS) is evident. Data were expressed as a percent change from control. * $P<0.01$ from control.

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