The Posterior Nasal Nerve Plays an Important Role on Cardiopulmonary Reflexes to Nasal Application of Capsaicin, Distilled Water and l-Menthol in Anesthetized Dogs

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ABSTRACT. The sensory innervation of the cardiopulmonary reflexes to nasal application of capsaicin (CAPS), distilled water (DW) and l-menthol (LM) was studied in anesthetized dogs breathing through tracheostomy. A marked cardiopulmonary reflex was observed by CAPS and DW into the nasal cavity, while a prolongation of expiration was induced by LM. All these reflexes were significantly decreased by bilateral section of the posterior nasal nerve (PNN) and completely abolished by topical nasal anesthesia with lidocaine. Responses of the whole nerve activity of the PNN to these substances corresponded to the magnitude of the reflexes. These results indicate that PNN afferents play an important role on the reflex elicitation of the noxious, water and cold stimuli from the nasal cavity. — KEY WORDS: canine, control of breathing, trigeminal nerve afferent.

Among the upper respiratory tract, the nasal cavity and the larynx are known to be the potent reflexogenic regions which are rich in sensory afferents and can elicit various defensive or protective reflexes such as inhibition of breathing (apneoa), cough, sneeze, laryngospasm, bronchoconstriction and secretion accompanied by sensations of touch, pain, itch or cooling. The sensory innervation of the larynx is mainly supplied by the internal branch of the superior laryngeal nerve (SLN). Recently, the presence of sensory receptors which can respond to capsaicin (CAPS: noxious stimulus), distilled water (DW: water stimulus) and l-menthol (LM: cold stimulus) have been clarified in the dog’s larynx from the recordings of the afferent activity of the SLN [4, 7]. In a previous report, we described the elicitation of inhibition of breathing followed by rapid shallow breathing, bradycardia and increase in blood pressure by laryngeal instillation of CAPS or DW in dogs. Sant’Ambrogio et al. [6] indicated that laryngeal application of LM or cooling can induce an inhibition of breathing in newborn dogs.

On the other hand, in the nasal cavity, the sensory functions besides olfaction are mediated by the afferents of the trigeminal nerve. The ethmoidal nerve (EN), posterior nasal nerve (PNN) and infraorbital nerve (ION) are the branches of the trigeminal nerve which innervate the nose. Wallois et al. [11] suggested that sneezing in cats could be elicited by electrical stimulation of the EN, PNN or ION. They have also pointed out that eupnea induced by passing airflow into the nasal cavity is mainly related to the stimulation of the PNN afferents [11]. It is, however, still unclear which trigeminal nerve is a major incidence to influence the reflex elicitation to the stimulus applied into the nasal cavity. The aim of the present study is to investigate the sensory innervation of the nose by topical application of CAPS, DW and LM into the nasal cavity in anesthetized dogs.

Animals and basal anesthesia: Twelve healthy Beagles (6 females and 6 males) were used in this study. Their mean age was 14.5 (range, 10 to 18) months and mean body weight was 8.6 (range, 7.0 to 10.5) kg. Food was withheld at least 12 hr before experiments. Dogs were premedicated with a mixture of medetomidine (20 µg/kg) and midazolam (0.3 mg/kg) injected intramuscularly. 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, slowly over 15 min. A supplemental dose of urethane and α-chloralose mixture (150 mg/kg urethane and 15 mg/kg α-chloralose) was given hourly through an intravenous catheter placed into the cephalic or saphenous vein. All the dogs received an intravenous injection of pentobarbital sodium (50 mg/kg) for euthanasia at the end of each experiment.

Animal preparation: After the induction of anesthesia, the dogs were endotracheally intubated, and ventilated spontaneously with room air. Bilateral zygomatic bones were removed carefully with the electric dental microengine (BL-F2, Osada Medical, Japan) to identify the ION and PNN, both of which are the maxillary division of the trigeminal nerve. Then the dogs were placed on an operating table in a supine position and tracheostomy was performed just below the larynx and a tracheostomy tube was inserted into the lower respiratory tract to allow tracheal breathing. Respiratory airflow, intratracheal pressure, end-tidal P O 2 (PETO 2 ) and (PETO 2 ) were measured with a differential pressure transducer (DD 102A, Toyoda Machine Works, Japan), a pressure transducer (PP 104, Toyoda Machine Works, Japan), and the gas analyzer (Respina 1H26, NEC san-ei, Japan) connected to the tracheostomy tube. A saline-
filled polyethylene catheter (O.D.=3 mm) was placed into the middle portion of the esophagus and connected to a pressure transducer (Nihon Kohden, DX-300) to record esophageal pressure. Auffed tracheal tube (I.D.=4.5–5.0 mm) was introduced into the nasopharynx through the tracheostoma, and a nasal cannula with a pair of cuffed tubes was inserted into both nostrils to functionally isolate the nasal cavity. A fine thermal probe (IT-21, Sensortec, Clifton, NJ) was placed just onto the nasal mucosa through the nasal cannula to record nasal temperature (BAT-12, Physitemp Instruments, Clifton, NJ). 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. All the signals were displayed on a thermal-array recorder (RT 3100N, NEC san-ei, Japan), and recorded by a magnetic tape recorder (PC 204A, SONY, Japan).

**Reflex study:** Seven dogs were used in this study. After a control period for more than 1 min with 100% oxygen at a flow rate of 5 l/min, LM was administered by passing through the bottle (200 ml) containing 0.5 g of LM crystals into the isolated nasal cavity in the inspiratory direction, and the cardiopulmonary reflexes were measured. Then, a 15–20 ml of DW was topically instilled into the nasal cavity through the nasopharyngeal catheter with multiple holes at the distal port. The nasal cannula inserted into the nostril was closed in order to fill the whole nasal cavity with water. Finally, the same volume 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) was instilled through the catheter. Isotonic NaCl solution (0.9%) at 37°C was used for rinsing the nasal cavity between DW and CAPS trials. Each trial was performed at an interval of 15 min or more to minimize the tachyphylaxis to each substance. The order of each trial, time interval and the dose of CAPS were determined by preliminary study. These trials were repeated after bilateral nerve sections of the trigeminal nerves. As for the nerve sections, the ION was initially dissected at the infraorbital foramen, followed by initially dissected at the infraorbital foramen, followed by sectioning of PNN at the sheno-palatin foramen because of the location of each bundle. In this study, the EN remained intact because of the difficulty to approach in the absence of additional amount of basal anesthesia which affects respiration. Finally, 5 ml of 2% lidocaine solution (Xylocaine®, Astra, Japan) was aerosolized with an ultrasonic nebulizer (NE-U12, Omron, Japan) driven by the airflow (5 l/min, output 2.5 ml/min) producing particles approximately 5 µm in size, and passed through the isolated nasal cavity for 2 min. Then the nebulizer was turned off and the cardiopulmonary measurements to each substance were performed again.

**Electrophysiological study:** Afferent whole nerve activity of the trigeminal nerve was recorded in 5 dogs. To avoid the secondary effects on the activity mediated by the respiratory reflexes, 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 PetCO₂ within 35 ± 5 mmHg. Afferent activity of the nerve filament was recorded with a pair of platinum electrodes. The PNN was sectioned at the junction of the maxillary nerve and its peripheral cut end was separated from surrounding connective tissues with the aid of a binocular microscope (SZ 60, OLYMPUS, Japan) from which PNN afferent whole nerve activities were recorded. 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).

**Data analysis:** Respiratory airflow was integrated with an A-D converter (Mac Lab Scope, BRC, Japan) to give tidal volume (Vₜ). The measurements of inspiration time (Tᵢ), expiration time (Tₑ), heart rate (HR), and systolic arterial blood pressure (SBP) were obtained from the tracings before and after the onset of each trial. Control values were averaged over three consecutive breaths before each challenge. The maximum responses after the onset of each trial in each breath were taken into account, and were shown as peak percent changes. The discharge frequencies of whole nerve activities were measured every sec for a period of 30 sec before the trial and with an window discriminator (DSE 425, DIA Medical, Japan), then the data were averaged every 10 sec. The averaged discharge frequency for 30 sec 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 the onset of the trial was taken as the peak value. In the 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). Statistical analysis was performed using a statistical software package (StatView® 4.5J, Abacus Concepts, Berkeley, CA). For the comparison between the data, Wilcoxon’s signed rank sum test or Mann-Whitney’s U-test was used where appropriate. All data were expressed as mean ± SE. Values of P<0.05 were considered as statistically significant.

In the present study we used three different substances, i.e., CAPS, DW and LM, in order to elicit various nasal cardiopulmonary reflexes. CAPS is known to be a potent stimulant of unmyelinated C-fibers and a part of A-δ fibers [2]. DW is a stimulant for some mechanoreceptors such as rapidly adapting ‘irritant’ receptors and respiration-modulated receptors associated with either the lack of chloride ion or hypoosmolarity in the larynx [1]. LM is known as a selective stimulant for cold receptors in both the nasal cavity and larynx, although a decrease in temperature accompanied by upper airway airflow depresses other types of receptors [5]. The application of those substances, therefore, allows to disclose the reflex nature mediated by each nasal receptor type.

Nasal instillations of CAPS (Fig. 1) and DW induced an inhibition of breathing represented by an increase in Tₑ (CAPS, 248 ± 23%, P<0.01; DW, 188 ± 24%, P<0.01) and...
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a decrease in $V_T$ (CAPS, 78 ± 10%, $P<0.05$; DW, 89 ± 16%, $P=0.21$) followed by a rapid breathing, an increase in SBP (CAPS, 127 ± 5%, $P<0.01$; DW, 118 ± 6%, $P<0.05$) and a decrease in HR (87 ± 6%, $P<0.05$, 96 ± 8%, $P=0.18$). Inhalation of LM inhibited a breathing represented by an increase in $T_e$ (124 ± 15%, $P<0.005$). The nasal temperature was decreased from 33.8 ± 0.9°C to 27.5 ± 1.3°C. The prolongation of $T_e$ was greater with CAPS ($P<0.01$) and DW ($P<0.05$) than with LM. No significant changes were observed by saline solution for rinsing. These cardiopulmonary reflexes are in agreement with the results of the previous studies which applied those substances into the nose and the larynx of the dog, guinea pig, rat and newborn animals [3, 7].

Such reflex responses were not significantly changed by bilateral section of the ION, but significantly ($P<0.05$ in measurements described above) inhibited by bilateral section of the PNN (Fig. 1). Although a mildly rapid breathing and a small increase in SBP remained even after the section of the PNN (Fig. 1), they were completely abolished by topical anesthesia with lidocaine into the nasal cavity. These findings indicate that the major afferent information is supplied by the PNN afferents in the nasal cavity of the dog. The remainder of reflex responses to CAPS and DW after the PNN section might be arisen from the EN afferents, because we left the EN intact in this study and the increased activity in the EN afferents responding to various nasal stimuli has been reported in guinea pigs [8], rats [9] and cats [11]. In guinea pigs, the inhibitory reflex of ventilation by nasal cooling was solely inhibited by bilateral section of the EN [10], and the increased EN afferent activity by nasal application of LM was abolished by local anesthesia of the cranial part of the nose [8]. The results from the previous and the present study suggest the presence of species difference on the role of nasal trigeminal branches in response to nasal stimuli.

In the present study, the afferent activity of the PNN was recorded because of the considerable reduction of reflex responses to nasal stimuli by denervation of the PNN. The electrical activity of the PNN was significantly increased by all three substances. The increase in discharge frequency was the greatest with CAPS, followed by DW and the least by LM (Figs. 2 and 3). The strength of activation of the PNN afferents was well in accordance with the magnitude of the reflex responses to each substance. In conclusion, the present study demonstrates that the PNN plays important roles on the induction of reflex cardiopulmonary responses to noxious, water and cold stimuli in dogs.

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REFERENCES

Fig. 3. The discharge frequencies increased and reached to peak values after 30 sec in each application. CAPS induced the greatest peak response on the PNN afferents in these application. The values represent mean ± SE (n=5).

*Significantly different from control (P<0.05).

Fig. 2. A representative recording from the whole PNN activity. Note the increase in discharge frequency was the greatest with CAPS instillation, followed by DW instillation and the least with LM inhalation.