Nasal Sensory Receptors Responding to Capsaicin, Water and Tactile Stimuli in Sevoflurane-Anesthetized Dogs

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ABSTRACT. Responses of nasal receptors to capsaicin and water were studied from afferent recordings of the posterior nasal nerve (PNN) in 12 anesthetized dogs. Out of 12 non-respiration-modulated nasal receptors, 7 responded only to capsaicin, 3 responded to both water and capsaicin, and 2 to neither of them. All the fibers showed a rapid adaptation to mechanical probing of the nasal mucosa. These results indicate that the presence of sensory receptors responding to capsaicin and water are involved in PNN afferents of the dog.—KEY WORDS: breath control, canine, trigeminal nerve afferent.

Nasal mucosa endows with sensory afferents to elicit pronounced airway reflexes such as apnea, glottal closure, mucus secretion, bronchoconstriction and sneezing in various species [12, 17]. As described previously, marked cardiopulmonary reflex responses to capsaicin (CAPS) and distilled water can be elicited from the nose and larynx of dogs, in which the afferent pathway of the posterior nasal nerve (PNN), a maxillary branch of the trigeminal nerve, plays an important role [5, 9]. The presence of CAPS-sensitive and water-responsive sensory afferents has recently been demonstrated in the larynx from the single nerve unit recordings of the superior laryngeal nerve (SLN) afferents in dogs [10]; however, to the authors’ knowledge, the electrophysiological data of the nasal sensory receptors activated by these substances are still obscure. The aim of the present study was to identify the nasal ‘CAPS-sensitive’ or ‘water-responsive’ sensory receptors from afferent recordings of the PNN by topical application of CAPS and water into the nasal cavity in anesthetized dogs. Animals and basal anesthesia: Twelve healthy Beagles of either sex were used in this study. Their mean age was 15 (range, 11 to 19) months and mean body weight was 10.3 (range, 9.7 to 11.0) kg. Food but not water was withheld at least 12 hr before the experiments. Dogs were premedicated with a mixture of medetomidine (20 µg/kg of body weight) and midazolam (0.3 mg/kg) administered intramuscularly. Anesthesia was induced with thiopental (3.5–7.5 mg/kg, i.v.), endotracheally intubated, then maintained with sevoflurane at an end-tidal concentration of 2.5–3.2% (1.2–1.5 times minimum alveolar concentration [MAC]) [8], in O2 delivered at a flow rate of 3 l/min during animal preparation. A semi-closed circle anesthesia system (Model KA-3020, Kimura Medical Co, Japan), with the vaporizers for sevoflurane (S-3, Kimura Medical Co., Japan) out of circle, was used. End-tidal anesthetic concentration was collected through the sampling line connected to the tracheostomy tube and monitored by use of an infrared gas analyzer. Sevoflurane concentration was reduced by an end-tidal concentration of 2.1–2.4% (1.0–1.2 MAC) then maintained as basal anesthesia for the experiment. At least 15 min’s interval was allowed to attain a steady level of anesthesia. All the dogs received an overdosed injection of pentobarbital sodium (50 mg/kg, i.v.) for euthanasia at the end of each experiment.

Animal preparation: After the induction of anesthesia, the dogs were endotracheally intubated, and ventilated under spontaneous ventilation. Then the dogs were placed on an operating table in a supine position. The cervical trachea was exposed and the ventral aspect of 8–10 cartilaginous rings was longitudinally cut to allow the introduction of a cuffed tracheostomy tube (I.D = 7.0–8.0 mm, Portex, Nihon Medico, Japan) into the lower respiratory tract. End-tidal PCO2 (PETCO2) in the lower airway was measured with a 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 (DX-300, Nihon Kohden, Japan) to record esophageal pressure. A cuffed tracheal tube (I.D. = 4.5–5.0 mm) was introduced into the nasopharynx through the tracheostoma to functionally isolate the nasal cavity. A thermal probe (IT-21, Sensortec, U.S.A.) was inserted into the nasal cavity via the nasopharyngeal cannula to record nasal mucosal temperature (BAT-12, Physitemp Instruments, U.S.A.). 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 37.5 ± 0.5°C, using a warming mat. 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 Co, Japan).

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Recording of the PNN afferent activity: Afferent activity of the PNN, a branch of the trigeminal nerve, was recorded. Bilateral zygomatic bones were removed carefully with an electric dental microengine (BL-F2, Osaka Medical, Japan) to identify the PNN according to the method described previously [5]. The PNN was sectioned at the junction of the maxillary nerve and its peripheral cut end, from which PNN afferent unit activity was recorded with a pair of platinum electrodes, was separated from surrounding connective tissues. The nerve trunk was dissected into several thin filaments until 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 (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). To avoid the secondary effects on the activity mediated by the respiratory reflexes, 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 PETCO₂ within 35 ± 5 mmHg.

Experimental protocol: All fibers with random activities except the respiration-modulated receptors were tested, since nasal sensory receptors which respond to CAPS or water generally show irregular or scant discharge patterns under tracheostomy breathing in the absence of airflow or pressure change in the upper airway [10, 12, 13]. To ascertain the mechanical sensitivity, individual receptors were stimulated by mechanical probing of the nasal mucosa with a thin cotton stick. The sensitivity of each fiber to cold stimuli was also tested by passing O₂ at a flow rate of 5 l/min for 15 sec via the nasopharyngeal cannula through the isolated nasal cavity.

After a control period of more than 1 min, 15~20 ml of distilled water was topically instilled into the nasal cavity using a Foley catheter with multiple holes at the distal port through the nasopharyngeal cannula. Then, 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 in the same way. Warmed isotonic NaCl solution (0.9%) at 37°C was used for rinsing the nasal cavity between water and CAPS trials. Each trial was performed at an interval of 15 min or more to minimize the tachyphylaxis to each substance.

Data analysis: The discharge frequencies of unit activity were counted every second for a period of 30 sec before the trial with a 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 discharge frequency out of the values averaged every 10 sec after the 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 a number of action potentials began to increase after the onset of instillation. For individual receptors, a positive response was designated as a peak response of at least a 50% increase over baseline activity or at least 1 action potential/sec evoked when the baseline activity for 1 min was zero. The adaptation index (A.I.) of each receptor in response to the mechanical stimuli was calculated as (impulse frequency in the 1st sec – impulse frequency in the 2nd sec)/impulse frequency in the 1st sec [6]. Statistical analysis was performed using a statistical software package (StatView 4.51, Abacus Concepts Inc., U.S.A.). For the comparison of the data, student’s paired or unpaired t-test was used where appropriate. All data were expressed as mean ± SE. Values of P<0.05 were considered as statistically significant.

Afferent activities were recorded from a total of 12 receptors with irregular or scant discharge patterns which did not have a respiratory modulation. Seven receptors (7/12, 58%) were stimulated by only CAPS (‘CAPS-sensitive receptors’, Figs. 1 and 2 a), 3 receptors (3/12, 25%) were stimulated consistently by water and CAPS (Figs. 1 and 2 b), and 2 receptors (2/12, 17%) were not activated by either of them. No significant differences were observed in discharge frequencies between CAPS and water for the 3 receptors responding to both substances (Fig. 2 b). All the receptors had mechanical sensitivities and showed a rapid (8/12, A.I.= 0.89 ± 0.02, range: 0.84 to 0.98, Fig. 1 left inset) to intermediate (4/12, A.I.= 0.58 ± 0.05, range: 0.47 to 0.66, Fig. 1 right inset) adaptation to the mechanical probing of each receptive field. The latency of receptor response to CAPS was 2.3 ± 1.2 sec (n=7, CAPS-sensitive receptors) and 2.3 ± 1.5 sec (n=3), and the latency to water was 3.6 ± 1.9 sec (n=3). There was no statistically significant difference between the latencies for CAPS and water. All the responses to both substances tended to show a long-lasting discharge after onset of activation (Fig. 1).

The administration of O₂ flow lowered the intranasal temperature to as little as 27.9 ± 1.2 °C from the value of room air at 33.5 ± 0.6°C; however, no fibers recorded in this study were activated by the cold stimuli (data not shown), suggesting they were not at least subtypes within the category of ‘cold receptors’ as observed in the superior laryngeal nerve of the dog [12].

In our previous study [5], the presence of nasal sensory receptors responding to CAPS and water was predicted by the whole nerve recording of the PNN. The results of this study clarified the hypothesis from the single unit recordings of the PNN afferents. It is interesting to note that most of the non-respiration modulated nasal receptors with irregular or scant discharge were CAPS-sensitive, some of which can also be categorized as water-responsive type of endings. These results, however, were not in agreement with the characteristics of CAPS-sensitive as described in the larynx, i.e., laryngeal CAPS-receptors were clearly distinguished from laryngeal water-responsive receptors by their predominant responsiveness to CAPS and lack of water response [10]. Rapid to intermediate adaptation of the nasal
receptors in response to mechanical stimuli is consistent with the finding of CAPS-sensitive and rapidly adapting ‘irritant’ receptor responses to probing or hyperinflation in the larynx and lower airway [1, 10, 12]. In fact, most of the PNN afferents of the cat have rapid to intermediate adaptation [15]. In the present study, specific water-responsive receptors that respond only to water as observed in the larynx of dogs were not detected, suggesting the nasal receptors activated by water are polymodal endings that can also respond to CAPS.

The prevalent distribution of substance-P containing C-fiber endings in the nasal and laryngeal mucosa of guinea pigs, dogs, and humans has been reported histologically [2, 7, 14]. The CAPS-sensitive receptors are presumed to be unmyelinated C-fiber endings since CAPS is a potent stimulant of unmyelinated CAPS-sensitive C-fibers and a part of A-δ fibers [3, 4]. On the other hand, the other type of receptors that responded to both CAPS and water could be thin myelinated fibers within the category of A-δ fibers. In fact, most of rapidly adapting and intermediate adapting receptors within the range of A-δ fibers located in the trachea and main bronchus are stimulated by CAPS and water [3, 11]. It is well known that these rapidly adapting and intermediate receptors are commonly associated with defense reflexes of the respiratory tract [16].

As a whole, this study suggested the involvement of many
nasal receptors in the PNN afferents that respond to nociceptive stimuli, the activation of which possibly encode various cardiopulmonary reflex responses in the dog [5].

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REFERENCES