Respiratory and Cardiovascular Reflexes Elicited by Nasal Instillation of Capsaicin to Anesthetized, Spontaneously Breathing Dogs

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ABSTRACT. Cardiopulmonary reflexes elicited by capsaicin (CAPS) instilled into the nasal passages were determined in 6 anesthetized dogs breathing spontaneously. Nasal instillation of CAPS (10 µg/ml, 10 ml) induced: 1) apneic response characterized by an increase in expiration time; 2) bronchoconstrictor response characterized by an increase in lung resistance and a decrease in dynamic compliance; and 3) cardiovascular response characterized by a decrease in heart rate and an increase in arterial blood pressure. These reflex responses to CAPS were attenuated by pretreatment with a higher dose of CAPS (100 µg/ml, 10 ml), suggesting desensitization of CAPS-sensitive endings. These results suggest that marked cardiopulmonary reflexes are produced by nasal CAPS instillation, which may result, at least in part, from stimulation of nasal CAPS-sensitive sensory afferents.

KEY WORDS: canine, cardiopulmonary reflex, nasal afferent.

The nasal passages are rich in sensory afferents that are thought to be responsible for producing various airway defense reflexes to protect the lungs and lower airways from injury. The sensory function of the nasal passages, except for olfaction, is mediated by afferent branches of the trigeminal nerve, including the posterior (caudal) nasal nerve (PNN), infraorbital nerve and ethmoidal nerve [18]. Among several types of mechanoreceptors described in the upper airway, unmyelinated C-fiber afferents are primarily responsible for eliciting cardiopulmonary reflexes in response to irritants, playing a key role in neurogenic inflammation via an axon reflex pathway [18]. In a previous study, we demonstrated that an apneic response in dogs elicited by nasal instillation of capsaicin (CAPS), a potent stimulator of the C-fiber afferents, was considerably reduced by sectioning PNN and was abolished by local anesthesia with lidocaine of the nasal passages [6], suggesting the existence of the CAPS-sensitive C-fibers in the trigeminal nerve afferents. The finding was confirmed by single unit recordings of the sensory receptors responding to CAPS from the PNN afferents of dogs [7].

Considering the above, it is presumed that there is an important mechanism in the nasal passages mediated presumably via the C-fibers to provide airway reflexes in response to chemical irritants: however, the reflex data to support the contribution of C-fibers are still lacking. Thus, the aim of this study was to determine and characterize the cardiopulmonary reflexes elicited by topical nasal instillation of CAPS in anesthetized, spontaneously breathing adult dogs via a tracheostomy.

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Animals: Six healthy beagles (3 females and 3 males) were studied. Their mean age was 17.5 (range, 13 to 20) months and mean body weight was 9.5 (range, 6.7 to 12.5) kg. They were housed in individual runs at constant temperature (24°C) and humidity (50–60%), and fed commercial dry dog food once daily, with water available ad libitum. Food was withheld for 12 hrs before experiments.

Animal preparation: At least 2–3 weeks before experiments, dogs were subjected to permanent tracheostomy according to the method described previously [9, 11, 13]. On the day of each experiment, dogs were premedicated with a mixture of acepromazine (0.05 mg/kg) and buprenorphine (0.01 mg/kg) administered intravenously through a cephalic vein catheter. Anesthesia was induced with thiopental (1.5 to 5.0 mg/kg, i.v.), a cuffed tracheostomy tube (Portex, Nihon Medical Co., Japan) was inserted, and α-chloralose (50 mg/kg, i.v.) was injected slowly over 15 to 20 min. All dogs were allowed to breathe spontaneously; 100% O2 was delivered to the tracheostomy tube at a flow rate of 3 l/min, using a semi-closed circle anesthesia system (Model KA-3020, Kimura Medical Co., Japan). Anesthesia was maintained by administration of α-chloralose (5 mg/kg/hr, i.v.) with an infusion pump. Adequacy of anesthesia was assessed by determining responses to tail clamping 10 to 15 min before surgical intervention [2] and each experimental trial, and by determining responses to pinching the interdigital skin of a hind limb every 30 min [9, 11]. If any positive response (e.g., limb flinching or withdrawal or a sudden fluctuation in mean arterial blood pressure [> 5 mmHg] or heart rate [> 10%]) was observed, a supplemental dose of thiopental (0.5 mg/kg, i.v.) was given slowly.

After anesthesia was induced, dogs were positioned in dorsal recumbency. Respiratory airflow was measured with a differential pressure transducer (DD102A, Toyota...
Machine Works, Japan) through 2 sidearms connected to the tracheostomy tube, and integrated with an A-D converter (Mac Lab Scope, BRC Inc, Japan) connected to a Macintosh computer (PowerBook 5300cs, Apple Computer Inc, U.S.A.) to give tidal volume (V T). Expired volume per unit time (V E) was calculated from V T and total cycle duration. Auffed nasopharyngeal cannula (4.5 to 5.0 mm I.D.) was introduced into the nasopharynx through the tracheostomy, and a nasal cannula with a pair of cuffed tubes was inserted into the nostrils to functionally isolate the nasal cavity. A polyethylene catheter (2 mm I.D.) filled with saline solution was placed in the middle portion of the esophagus and connected to a pressure transducer (DX-300, Nihon Kohden, Japan) to record esophageal pressure. Inspiration time (T I) and expiration time (T E) were measured from tracings of esophageal pressure. Dynamic compliance (C dyn) and pulmonary resistance (R L) were calculated with breath-by-breath computer analysis using the subtraction method [1]. Arterial blood pressure was monitored with a pressure transducer (DX-300, Nihon Kohden, Japan) connected to a 20-gauge catheter inserted in the femoral artery. All signals were displayed on a thermal-array recorder (RT3100N, NEC-sanei, Japan) and stored on a magnetic tape recorder (PC 204A, Sony Co, Japan). Tidal gases were sampled by a tube connected to the tip of the tracheostomy tube, and end-tidal PCO 2 (PETCO 2) was measured with an infrared gas analyzer (Respina 1H26, NEC-sanei, Japan). Lactated Ringer’s solution was infused at a rate of 10 ml/kg/hr through the cephalic vein catheter. Rectal temperature was maintained at 37 ± 1 (mean ± SD)°C by a warming blanket.

Experimental protocol: At the commencement of each trial, PETCO 2 was adjusted to between 35 and 40 mmHg by assisted manual ventilation. At least 5 min before each trial, arterial blood was collected in heparinized syringes for measurement of blood gases (pH, PaO 2, and PaCO 2) with a blood gas analyzer (IL-1303, Instrumentation Laboratory Inc, U.S.A.). The values (mean ± SD) of pH, PaO 2, and PaCO 2 were 7.36 ± 0.07, 498 ± 22 mmHg, and 40.6 ± 2.8 mmHg, respectively. After a control period of more than 1 min, 10 ml of CAPS solution (10 µg/ml, a diluted solution of 100 µg/ml capsicain [Sigma Chemical, U.S.A.]) was instilled into the nasal passages using a Foley catheter inserted through the nasopharyngeal cannula to measure cardiopulmonary responses to the substance. Fifteen minutes later, a higher concentration of CAPS (100 µg/ml, 10 ml) was introduced into the nasal passages in order to measure the subsequent responses to CAPS (10 µg/ml, 10 ml) [13]. At the end of the experiment, 5 ml of 2% lidocaine (Xylocaine, Astra, Japan) was aerosolized with an ultrasonic nebulizer (NE-U12, Omron, Japan) driven by O 2 (5 l/min, output 2.5 ml/min), producing particles approximately 5 µm in size, and passed through the isolated nasal passages for 2 min. The nebulizer was then turned off and the procedure was repeated. The order of the trials, time interval and dose of CAPS to minimize the tachyphylaxis were determined by pilot studies. Warmed (37°C) physiological saline was used for rinsing the nasal passages between the trials. At the end of the experiments, dogs were euthanatized by administration of an overdose of pentobarbital (50 mg/kg, i.v.).

Data analysis: Respiratory variables (T I, T E, V T, V E, R L and C dyn) were analyzed on a breath-by-breath basis. Baseline values for respiratory variables were obtained by averaging values for three consecutive breaths immediately before each trial. Mean arterial blood pressure (MAP) was calculated every 5 seconds as the sum of diastolic pressure plus a third of pulse pressure. Heart rate (HR) and ECG were recorded by use of a multifunction electrocardiograph (CMO-104 Cardiovap, Datex, Finland). Baseline values for the cardiovascular variables were obtained by averaging values for 1 min before each trial. Experimental values were peak responses recorded after the onset of CAPS instilation. The onset latency (sec) of the cardiopulmonary responses were evaluated visually as the point when V E and MAP began to increase.

Statistical analysis was performed using statistical software packages (StatView, Abacus Concepts, U.S.A.). For comparisons of the differences in baseline values, one-way ANOVA followed by Tukey’s post hoc test was used. To determine whether the change from baseline to experimental values within each trial was significant, a paired Student t-test was used. Evoked changes (Δ) from baseline values in each trial were compared over treatments and dogs, using one-way ANOVA for repeated measures with Tukey’s post hoc test. Values of P<0.05 were considered significant. All data were expressed as mean ± SEM, unless otherwise indicated.

Cardiopulmonary reflexes elicited by nasal instillation of CAPS: Respiratory and cardiovascular variables associated with nasal instillation of CAPS are summarized in Table 1. A typical example of the cardiopulmonary responses to nasal instillation of CAPS (non-treatment) is shown in Fig. 1. Instillation of CAPS (10 µg/ml) into the nasal passages induced an immediate apneic response characterized by an increase in V E with an onset latency of 2.3 ± 0.3 sec (range, 0–3.0 sec) and a decrease in V T, followed by a bronchoconstrictor response characterized by a decrease in C dyn and an increase in R L. Such changes in respiratory variables resulted in a marked ventilatory depression characterized by a decrease in V T. Regarding the cardiovascular responses, nasal instillation of CAPS induced a decrease in HR and an increase in MAP with an onset latency of 3.5 ± 0.4 s (range, 1.5–5.0 s). Although the onset latency of the cardiovascular responses tended to be longer than that of the respiratory responses, there were no statistically significant differences (P=0.09).

Effects of higher dose CAPS pretreatment and local anesthesia on CAPS-evoked cardiopulmonary reflexes: Examples of the cardiopulmonary responses to nasal instillation of CAPS following higher dose CAPS pretreatment and local anesthesia with lidocaine are shown in Fig. 1. The reflex responses to CAPS were reduced significantly by pretreatment with higher dose CAPS (100 µg/ml) instillation into the nasal passages (P<0.05). All the reflex responses to nasal instillation of CAPS were no longer evoked following
Table 1. Respiratory and cardiovascular variables associated with nasal instillation of capsaicin (10 µg/ml, 10 ml) in 6 anesthetized, spontaneously breathing dogs

<table>
<thead>
<tr>
<th></th>
<th>Non-treatment</th>
<th>CAPS-pretreatment#</th>
<th>Nasal anesthesia</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>Peak response</td>
<td>Baseline</td>
</tr>
<tr>
<td>Tİ (sec)</td>
<td>1.1 ± 0.2</td>
<td>1.3 ± 0.3</td>
<td>1.2 ± 0.2</td>
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<tr>
<td>TE (sec)</td>
<td>3.0 ± 0.3</td>
<td>9.1 ± 1.6*</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td>Vİ (ml)</td>
<td>188 ± 17</td>
<td>153 ± 21*</td>
<td>182 ± 17</td>
</tr>
<tr>
<td>VE (l/min)</td>
<td>2.28 ± 0.13</td>
<td>0.93 ± 0.25*</td>
<td>2.37 ± 0.14</td>
</tr>
<tr>
<td>Cdyn (cmH2O/ml)</td>
<td>25.5 ± 2.3</td>
<td>13.7 ± 3.8*</td>
<td>24.5 ± 1.9</td>
</tr>
<tr>
<td>RL (cmH2O/l/sec)</td>
<td>1.41 ± 0.17</td>
<td>1.90 ± 0.24*</td>
<td>1.38 ± 0.14</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>112 ± 9</td>
<td>100 ± 12*</td>
<td>110 ± 10</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>106 ± 10</td>
<td>119 ± 15*</td>
<td>103 ± 11</td>
</tr>
</tbody>
</table>

TI=inspiration time; TE=expiration time; Vİ=tidal volume; VE=expired ventilation; Cdyn=dynamic compliance; RL=pulmonary resistance; HR=heart rate; MAP=mean arterial blood pressure. a) 100 µg/ml, 10 ml. * Significant difference from baseline (P<0.05). † Significant difference from non-treatment (P<0.05).

Fig. 1. Cardiopulmonary reflexes in response to nasal instillation of capsaicin (CAPS, 10 µg/ml, 10 ml) with and without pretreatment of higher dose CAPS (100 µg/ml, 10 ml) and local anesthesia with lidocaine in an anesthetized, spontaneously breathing dog. Note that cardiopulmonary reflexes elicited by CAPS (A: non-treatment) were considerably reduced by higher dose CAPS pretreatment (B) and were no longer observed following lidocaine anesthesia (C). V=respiratory airflow; PES=esophageal pressure; ABP=arterial blood pressure. □ =CAPS instillation point.
local anesthesia with lidocaine of the nasal passages.

In this study, an immediate apnea characterized by a prolongation of T_{ap} and resultant ventilatory depression were induced by nasal instillation of CAPS. Such ventilatory depressant responses to nasal CAPS coincides well with previous studies which introduced CAPS into the nasal passages, larynx or lower airways of dogs [5, 13, 14], suggesting an induction of the so-called ‘airway C-fiber reflex’ by activation of nasal capsaicin-sensitive C-fiber afferents. It is notable in this study that the nasal instillation of CAPS was accompanied by a bronchoconstriction represented by a decrease in C_{dyn} and an increase in R_{lu}. The bronchoconstrictor reaction is generally observed after administration of CAPS into the lower airways and lungs [16]. These results suggest that noxious chemical stimulus to the nasal mucosa evokes not only slowing of the breathing pattern (apnea) via the phrenic nerve efferents, but also elicits bronchoconstriction via the vagal efferent pathway, contributing to a global depression of the respiratory function.

An increase in arterial blood pressure (hypertension) and a decrease in heart rate (bradycardia) induced by nasal instillation of CAPS appears to be a characteristic of cardiovascular reflexes to CAPS in the upper airway [6, 13]. Hypotension and bradycardia are consistently elicited by CAPS in the lower airways and lungs, introduced through right arterial injection [3, 15] or intratracheal instillation in dogs [17]. It is likely that the hypertensive response to nasal CAPS, associated with sympathetic nervous system activation, was primarily mediated by stimulation of CAPS-sensitive C-fibers rather than by brief asphyxia, considering the fact that a similar effect can be provoked in animals under controlled ventilation [5, 13].

Recently, C-fiber afferents which respond to capsaicin, a potent stimulant of airway C-fibers, have been identified in the nose and larynx from the whole or single nerve unit recordings of PNN or superior laryngeal nerve afferents of the dog [18, 20]. In this study we did not record the unit activity, however, the responsiveness of the CAPS-sensitive receptors was closely associated with the elicitation of reflex responses to CAPS observed in the current study, i.e., a robust peak response followed by relative long-lasting activity.

Moreover, pretreatment with a higher concentration of CAPS (100 μg/ml, 10 ml) in the nasal passage greatly reduced the cardiopulmonary reflexes to subsequent CAPS instillation, suggesting the ‘desensitization’ of the CAPS-sensitive C-fiber endings, presumably due to a prolonged and irreversible depolarization of membrane potentials [14, 19].

It is presumed that the respiratory reflexes in response to instillation of CAPS into the nasal passages are mediated by stimulation of afferent sensory neurons in the nasal mucosa, because the effects were considerably reduced by local anesthesia of the nasal passages [10–12]. The elicitation threshold of the upper airway reflexes in response to chemical and mechanical stimuli is increased by nebulized lidocaine locally administered onto the airway mucosa without affecting the central neural reflex pathway [4, 8]. Although we did not further investigate the origin of the reflex responses here, it is known that sensory innervation of the nasal mucosa and nostril is supplied by the maxillary (PNN and infraorbital nerve) and ophthalmic (ethmoidal nerve) branches of the trigeminal nerve [18]. We have demonstrated that PNN, among these trigeminal nerve branches, is more vulnerable to irritants (e.g., capsaicin, l-menthol and cold airflow, and distilled water) applied to the nasal mucosa of dogs and the apneic reflexes induced by nasal instillation of CAPS are considerably reduced by surgical denervation of bilateral PNN [6]. Given these findings, the encoding of the CAPS-induced reflexes obtained in this study may result from stimulation of the PNN C-fiber afferents.

In conclusion, the results of this study suggest that marked cardiopulmonary reflexes are produced by nasal CAPS instillation, which may result, at least in part, from stimulation of nasal CAPS-sensitive sensory afferents.

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