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Desflurane but not sevoflurane augments laryngeal C-fiber inputs to nucleus tractus solitarii neurons by activating transient receptor potential-A1

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ABSTRACT

Aims: Volatile anesthetics have distinct odors and some are irritating to the upper airway and may cause cough and laryngospasm, which may result, in part, from stimulation of C-fiber reflex. Local exposure of such anesthetics increases the sensitivity of capsaicin-sensitive laryngeal C-fiber endings compatible with airway irritability presumably by activation of transient receptor potential (TRP) ion channels, but the physiological relevance of this sensitization transmitted to the higher-order neurons in the central reflex pathway and output is unknown.

Main methods: In anesthetized young guinea pigs, baseline and left atrial capsaicin evoked changes in the extracellular unit activity of laryngeal C-fiber-activated neurons in the nucleus tractus solitarii (NTS) and phrenic nerve activity were compared between irritant (desflurane) and non-irritant (sevoflurane) anesthetic gas exposure to the isolated larynx.

Key findings: Desflurane significantly augmented the peak and duration ($p < 0.01$) of the NTS neuronal responses and the prolongation of expiratory time ($p = 0.017$). The effect was enhanced by iontophoretic application of the TRPA1 agonist allyl-isothiocyanate ($p < 0.05$), inhibited by TRPA1 antagonist HC-030031 ($p < 0.01$), but not by TRPV1 antagonist BCTC. Sevoflurane did not affect the central pathway.

Significance: Thus, the sensitization of the laryngeal C-fiber endings by irritant volatile anesthetics is transmitted to the NTS via activation of the TRPA1 and is associated with a prolonged reflexively evoked expiratory apnea. The findings may help to explain local deleterious effects of irritant volatile general anesthetics on the airways during inhaled induction or bronchodilator therapy for status asthmatics.

Introduction

Volatile general anesthetics have distinct odors and some are irritating to the airway. Their pungent sensation may cause irritation when inhaled, and sometimes may induce a strong cough reflex, breath holding, and apnea that can precipitate laryngospasm, a potentially life-threatening complication during induction and emergence from anesthesia, particularly in children with susceptible airways (von Ungern-Sternberg et al., 2008). Although there is always no agreement with respect to the 'irritating potential' of contemporary volatile anesthetics from clinical observation, it is clear that desflurane and isoflurane are irritants and sevoflurane and halothane are non-irritant anesthetics (TerRiet et al., 2000). Unfortunately, the molecular mechanisms underlying these irritant effects remain unknown; however, the nature of the clinical responses suggests that they would involve activation of a chemosensor localized in the upper airway, particularly originating from the larynx (Canning et al., 2004; Nishino et al., 2004; Widdicombe, 1998b).

Recently, Mutoh et al. (1998b), and Mutoh and Tsubone (2003) have provided direct evidence for this proposal by showing that local exposure to isoflurane, but not sevoflurane, sensitizes laryngeal C-fibers to both chemical and mechanical stimuli. Consistent with these findings, several other studies have documented an increased excitability of primary lung sensory C-fibers (Mutoh et al., 1998a) or peripheral somatic nociceptors (Cornett et al., 2008; Eilers et al., 2010; Matta et al., 2008) after administration of irritant volatile anesthetics such as isoflurane and desflurane. It is interesting to note that volatile anesthetics appear to selectively activate the capsaicin-sensitive population of sensory neurons, suggesting that a receptor localized to these cells transduces the noxious effects of these agents (Shoudai et al., 2010; Sun et al., 2009). Transient receptor potential (TRP) V1 and TRPA1 are sensors for noxious chemical stimuli and are widely expressed in nociceptive

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sensory neurons. In particular, TRPA1, coexpressed with TRPV1 in 25–30% of capsaicin-sensitive nociceptors (Bautista et al., 2006), has emerged as a sensor for a wide variety of irritant chemicals, including volatile anesthetics.

To our knowledge, however, there are no data on whether the sensitization of primary laryngeal afferent fibers by exposure to irritant volatile anesthetics is sustained in the central circuitry of the reflex or has any physiological consequences on the reflex control of respiratory function. The nucleus tractus solitarii (NTS) is the first site in the central circuitry where afferent signals from the primary airway sensory C-fibers are transmitted and susceptible to modulation (Mazzone et al., 2005; Mutoh et al., 2000a,b; Widdicombe et al., 2011). Furthermore, TRPA1 receptors are expressed in NTS neurons and their excitatory effects via increased glutamate release at capsaicin-sensitive central terminals have been reported (Sun et al., 2009). We hypothesized that if an increased responsiveness of the laryngeal C-fibers contributes to the exaggerated respiratory symptoms produced by irritant volatile anesthetics via the central reflex pathway, then the increase should be manifested at NTS neurons in the central network through activation of TRPA1 and ultimately lead to an increase in at least some component of the reflex output. Hence, the purpose of this study was to examine the TRPA1-mediated effects of acute local exposure to two popular volatile anesthetics, desflurane and sevoflurane on the responsiveness of laryngeal C-fiber-activated NTS neurons, and associated reflex changes in phrenic nerve discharge measured in the whole animal.

Materials and methods

All experimental protocols were undertaken following the guidelines for care and use of experimental animals of the Japanese Association for Laboratory Animal Science and were reviewed and approved by the Institutional Animal Care and Use Committee.

General animal preparation

Male Dunkin–Hartley guinea pigs (age, 4–5 weeks; body weight, 250–380 g) were anesthetized with an injection of urethane (1.8 g/kg i.p.). The adequacy of anesthesia was assessed by a paw-pinching test in which the hindlimb paw was pinched and the animal was monitored for a hind limb flinch or withdrawal and/or a sudden fluctuation in arterial blood pressure or heart rate. If any positive response was detected, a supplemental dose of sodium pentobarbital (4 mg/kg i.v.) was given. Each guinea-pig was placed on a servo-controlled water blanket and body temperature was monitored via a rectal temperature probe and maintained within 37 ± 1 °C. Catheters (PE-10) were introduced into the jugular vein for administering fluids and drugs and into the femoral artery for monitoring arterial blood pressure and withdrawing samples for arterial blood gas evaluation.

The trachea was cannulated caudally just above the thoracic inlet and a catheter was connected to a side port of the endotracheal tube to monitor tracheal pressure (TP), whereas a short catheter (PE-60) was inserted cranially with its tip placed slightly below the cricoid cartilage. A latex cuffed tube (3 mm internal diameter) was introduced orally with its tip placed at the pharynx close to the epiglottis using an over-the-endoscope technique (Johnson, 2010). The oral tube and the upper tracheal catheter were connected to a semi-closed anesthesia circuit through which constant airflow could be applied locally to the larynx by minimizing the effect on the nasal cavity ('functionally isolated larynx'; Mutoh and Tsubone, 2003). Each guinea pig was prepared with bilateral pneumothoraces by incisions made in the chest wall and mechanically ventilated via the lower tracheal tube with oxygen-enriched humidified air with a tidal volume of 8 ml/kg. The ventilator rate was set initially at 35–40 breaths/min, and the positive end-expiratory pressure was set at 2 cm H_2O. Arterial blood gases and pH were maintained by adjusting the ventilator rate and by infusing sodium bicarbonate so that the pH was between 7.30 and 7.40 and the arterial PCO_2 was between 35 and 45 Torr. The pericardium was opened, and a cannula (0.58 mm internal diameter) prefilled with capsaicin (0.5 μg/ml) was inserted into the left atrium through the left atrial appendage to activate laryngeal capsaicin-sensitive primary sensory C-fibers (Mutoh and Tsubone, 2003).

The internal branches of both superior laryngeal nerves (SLNs) that convey sensory afferent information from receptors located in the laryngeal mucosa were isolated for searching the NTS cells. The external branches of the SLN that innervates all muscle fibers in the cricothyroid muscle were cut to avoid reflexes that could interfere with the activity of the receptors. Both cervical vagus nerves were separated from the carotid artery and sectioned below the diaphragm to eliminate afferent traffic from vagally innervated viscera below the diaphragm. The aortic depressor nerves were carefully separated from the vagus nerves and cut bilaterally to eliminate aortic baroreceptor input (Mifflin, 1993). The fourth cervical branch of the left phrenic nerve was isolated in the neck and cut distally to record centrally derived respiratory activity.

The guinea pigs were placed in a stereotaxic head frame. The internal branch of SLN ipsilateral to the recording site in NTS was placed on a bipolar silver hook electrode, covered with a mixture of warm petroleum jelly and mineral oil and connected to a stimulus isolation unit (SS-202J, Nihon Kohden, Tokyo, Japan) driven by a stimulator (SEN-3301, Nihon Kohden, Tokyo, Japan). This was generally the left SLN internal branch, but if a blood vessel on the surface of the left side of the brainstem interfered with proper placement of the electrode, then the right vagus nerve was isolated and the right side of the brainstem was searched. For recording neural respiratory rate and expiratory time (T_E), the central end of the phrenic nerve was placed on a bipolar silver hook electrode and covered with a mixture of warm petroleum jelly and mineral oil. A vertebral clamp was placed on the T2 spinal process and an occipital craniotomy was performed. To expose the brainstem, the caudal portion of the fourth ventricle was exposed by removing the dura mater and arachnoid membranes and then covered with warm mineral oil. At the end of the experiments, the guinea pigs were sacrificed by injection of a lethal dose of pentobarbital.

Extracellular single unit recording and iontophoresis

In vivo extracellular recordings of single unit activity were made through a glass electrode which extended ~10 μm below the remaining barrels of a four-barrelled pipette. The recording barrel was filled with 2% Pontamine Sky Blue dye (Sigma-Aldrich, St. Louis, MO, USA) in 0.5 M sodium acetate. Of the remaining barrels, one contained normal saline for balancing ejection currents. The rest of the barrels contained TRPA1 agonist allyl-isothiocyanate (AITC) (50 μM), TPRA1 antagonist HC-030031 (50 μM) or TRPV1 antagonist BCTC (50 μM). Ejection was performed using an iontophoresis circuit (DIA Medical System, Tokyo, Japan) with automatic current neutralization (500-ms current pulses at 10–15 nA for 60 s). The conditions of iontophoretic ejection were determined in pilot studies to maintain the baseline NTS neuronal or integrated phrenic nerve activities. Unit activity and phrenic nerve activity were fed via high-impedance source followers to second-stage amplifiers, filtered (0.3–3 kHz), and fed in parallel to an oscilloscope, thermal chart recorder, audio monitor, and a digital tape-recorder with a sampling rate of 11 kHz per channel for off-line analysis.

Laryngeal C-fiber-activated neurons

Based on our previous experience with airway C-fiber-activated neurons (Bonham and Joad, 1991; Mutoh et al., 2000b; Wilson et al., 1996), we focused the search in the region of the caudomedial NTS extending from 700 μm rostral to 500 μm caudal to the calamus scriptorius, from the midline to 500 μm lateral to the midline, and from 0 μm to 1800 μm ventral to the dorsal surface. The NTS was searched for potential laryngeal C-fiber-activated neurons by continually