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Isoflurane depolarizes bronchopulmonary C neurons by inhibiting transient A-type and delayed rectifier potassium channels

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ABSTRACT

Inhalation of isoflurane (ISO), a widely used volatile anesthetic, can produce clinical tachypnea. In dogs, this response is reportedly mediated by bronchopulmonary C-fibers (PCFs), but the relevant mechanisms remain unclear. Activation of transient A-type potassium current (I_A) channels and delayed rectifier potassium current (I_K) channels hyperpolarizes neurons, and inhibition of both channels by ISO increases neural firing. Due to the presence of these channels in the cell bodies of rat PCFs, we determined whether ISO could stimulate PCFs to produce tachypnea in anesthetized rats, and, if so, whether this response resulted from ISO-induced depolarization of the pulmonary C neurons via the inhibition of I_A and I_K. We recorded ventilatory responses to 5% ISO exposure in anesthetized rats before and after blocking PCF conduction and the responses of pulmonary C neurons (extracellularly recorded) to ISO exposure. ISO-induced (1 mM) changes in pulmonary C neuron membrane potential and I_A/I_K were tested using the perforated patch clamp technique. We found that: (1) ISO inhalation evoked a brief tachypnea (~7 s) and that this response disappeared after blocking PCF conduction; (2) the ISO significantly elevated (by 138%) the firing rate of most pulmonary C neurons (17 out of 21) in the nodose ganglion; and (3) ISO perfusion depolarized the pulmonary C neurons in the vitro and inhibited both I_A and I_K, and this evoked-depolarization was largely diminished after blocking both I_A and I_K. Our results suggest that ISO is able to stimulate PCFs to elicit tachypnea in rats, at least partly, via inhibiting I_A and I_K, thereby depolarizing the pulmonary C neurons.

1. Introduction

Bronchopulmonary C-fibers (PCFs) innervating the lungs and airways constitute 75–90% of the sensory fibers in the pulmonary branches of the vagal afferents (Agostoni et al., 1957; Jammes et al., 1982; Lee et al., 2003; Mei et al., 1980) and play an important role in modulating respiratory rhythm (Coleridge and Coleridge, 1986, 1994; Lee et al., 2003; Wilson and Bonham, 1997). Stimulation of the PCFs can trigger tachypnea (Coleridge and Coleridge, 1986, 1994; Kubin et al., 2006; Widdicombe, 1982) or an apnea (Lee, 2009; Xu and Frazier, 1997a; Xu et al., 2003), depending on the intensity of the stimulation. Inhalation of isoflurane (ISO), a widely used volatile anesthetic, induces tachypnea in humans (Ebert and Schmid, 2009; Hemmings and Hopkins, 2006; Sollevi and Lindahl, 1995). Mutoh et al. (1998) reported that ISO-induced tachypnea was mediated by PCFs in anesthetized dogs, based on two lines of evidence. First, the response was abolished after blocking PCF conduction using perineural capsaicin treatment of the cervical vagi, and, second, ISO markedly increased the firing rate of the PCFs. However, the cellular mechanism by which ISO stimulates the PCFs remains unexplored.

Voltage-gated K^+ channels play important roles in setting the resting membrane potential and in hyperpolarizing neurons to decrease neural excitability (Hille, 2001). Transient A-type potassium current (I_A) channels and delayed rectifier potassium current (I_K) channels are the two major voltage-gated K^+ channels in mammals (Hille, 2001; Muller et al., 1999). The former are mediated by voltage-gated K^+ channels encoded by the Kv1.4 and Kv4 family of genes, while the latter comprise currents from voltage-gated K^+ channels, including members of the Kv1, Kv2, and Kv3 gene families (Baranauskas, 2007; Gutman et al., 2005). Depolarization of neural membranes and increases in neural excitability have been induced by the inhibition of both types of currents (Birinyi-Strachan et al., 2005; Tan et al., 2006). ISO-induced depolarization has been observed in the chemosensitive neurons of the retrotrapezoid nucleus in mice (Lazarenko et al., 2010). Most importantly, ISO is able to increase dopaminergic neuron firing in the rat substantia nigra by depolarizing the membrane potential (Ishiwa et al., 2004). This depolarization results from the inhibition of I_A and I_K (Ishiwa et al., 2008). As both K^+ channels exist in pulmonary C neurons, i.e., in the cell bodies of PCFs, within the rat nodose ganglia (Gu et al., 2009), we hypothesized that ISO could stimulate PCFs to produce

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tachypnea in rats through the depolarization of pulmonary C neurons, induced by the inhibition of voltage-gated K^+ channels.

2. Methods

Male, pathogen-free Sprague–Dawley rats were purchased (Charles River Laboratories, Wilmington, MA, USA) and were housed in the animal facility at Lovelace Respiratory Research Institute (LRRI) in filter top cages. The rats were provided with water and food ad libitum. The room was constantly ventilated and the temperature was kept at 23 °C. The experimental protocols were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by LRRI's Institutional Animal Care and Use Committee, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, USA.

2.1. Animal preparation

The rats (80–150 g) were anesthetized with pentobarbital (40 mg/kg, ip). As needed, supplemental pentobarbital (10 mg/kg, ip) was administered to completely eliminate eye-blink and limb-withdrawal reflexes throughout the experiment. We used relatively young rats because our pilot study showed a higher successfulness to extracellularly record pulmonary C neurons within the nodose ganglia in young rats than in older rats. Core temperature was monitored with a rectal probe and was maintained at 36.5–37.5 °C by a water heating pad and radiant heat lamp.

In spontaneously breathing rats, the general animal preparation was the same as previously reported (Zhang et al., 2007). Briefly, the trachea below the larynx was exposed through a midline incision and the rats were tracheotomized. The trachea was cannulated and the cannula was connected to a pneumotachograph to record airflow. The pneumotachograph (Frank's Mfg. Co., Albuquerque, NM, USA) was made of stainless steel with a linear flow pressure relationship in the range of 0–10 ml s^{-1} and a flow resistance equal to 0.046 cm H_2O ml^{-1} s^{-1} with a dead space of ~0.1 ml. The other end of the pneumotachograph was placed (~5 mm deep) in a plastic tube with a diameter 5-fold greater than that of the pneumotachograph. Via this tube, 100% O_2 or 5% ISO in oxygen was given at a rate of 1 L/min through a two-way stopcock attached to the tube. The right femoral vein was cannulated for solution infusion, and the right femoral artery was cannulated for monitoring arterial blood pressure (BP) and heart rate (HR). The right jugular vein was also cannulated to allow bolus injection of PCF stimulants into the pulmonary circulation. The catheter tip was placed just above the right atrium. As in a previous study, 5% ISO was employed (Mutoh et al., 1998).

In paralyzed preparations, the animal preparation was the same as described above with the following exceptions. The animals received an intravenous infusion of pancuronium (0.1–0.3 mg/kg for induction and 0.1 mg kg^{-1} h^{-1} for maintenance) and were artificially ventilated as previously reported (Xu and Frazier, 1997b). The gas input portal of the ventilator (Model 7025, UGO Basile, Comerio, VA, Italy) was used to administer 100% O_2 or 5% ISO in oxygen (1 ml/min). To stimulate the vagal trunks, two Teflon-coated fine gold wires with bare ends were placed around the midcervical vagus nerve 1.5 cm distal to the nodose ganglion. The stimulating wires were loosely sutured to the adjacent muscle tissue to limit displacement, and the nerve-electrode interface was covered with polyvinylsiloxane dental impression material (Coltène/Whaledent Inc., Cuyahoga Falls, OH, USA), as previously reported (Mifflin, 1997).

2.2. Perineural treatment of the vagus nerves with capsaicin

The bilateral midcervical vagus nerves were exposed in the rats that were to receive perineural capsaicin treatment. Cotton strips soaked in capsaicin solution (150–250 μg/ml) or vehicle were wrapped around a 2 mm segment of the bilaterally isolated cervical vagus nerves for 20–30 min and were then removed, as reported previously (Kou et al., 1995; Lee et al., 1996). A successful perineural capsaicin treatment was confirmed by the absence of apneic responses in response to a right atrial bolus injection of capsaicin (1 μg kg^{-1}) (Lee et al., 1996).

2.3. Extracellular recording of neurons within the nodose ganglion

The nodose ganglia contain the cell bodies of the vagal sensory nerves that arise from the lungs (Springall et al., 1987). The right nodose ganglion was exposed and isolated through a side approach. The ganglion was desheathed and treated with 1% collagenase IV for 10 min. Next, a tungsten microelectrode (5 MΩ impedance, A-M Systems, Inc., Everett, WA, USA) was progressively inserted into the ganglion while the vagus nerve was electrically stimulated with 0.5 ms duration square wave pulses at an intensity of 0.5–1 mA (Liu et al., 2011). A reference electrode was placed on a skin incision near the recording electrode. The signals were amplified using a Grass P5 Series AC amplifier (Grass Instruments Co. Quincy, MA, USA) with the filter band set at 1 Hz to 5 kHz and were then digitized using a PowerLab/8sp (model ML 785; ADInstruments Inc., Colorado Springs, CO, USA) connected to a computer running PowerLab Chart 5 software. The sample frequency of digitization was 10 kHz.

2.4. Labeling of vagal pulmonary sensory neurons

Pulmonary sensory neurons in the nodose ganglia were identified by retrograde labeling with 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI), as previously reported (Gu et al., 2009). Briefly, the rats were anesthetized with pentobarbital sodium (40 mg/kg, ip). The DiI was sonicated, dissolved in ethanol, diluted in saline (1% ethanol, v/v), and then instilled into the airways and lungs (0.2 mg/ml; 2× 0.2 ml) using a polyethylene catheter. All the animals were allowed to recover for 8–10 days before the nodose ganglia were collected for cell culture.

2.5. Isolation of nodose ganglion neurons

The rats were euthanized by decapitation after being anesthetized with pentobarbital sodium (50 mg/kg, ip). Nodose ganglion neurons were isolated as previously described (Gu et al., 2009), with some modifications. Briefly, after decapitation, the head was immediately immerged in ice-cold Hanks' balanced salt solution. Ten minutes later, the nodose ganglia were extracted and incubated in ice-cold standard extracellular solution (see Section 2.6). Each nodose ganglion was desheathed, cut into 8–10 pieces under a dissecting microscope, and placed in an Eppendorf tube containing 1 ml of 0.1% type IV collagenase and 0.1% trypsin (in standard extracellular solution) at 36 °C for 45 min. The ganglion suspension was centrifuged (150 × *g*, 5 min) and the supernatant was removed before 1 ml of 0.4% trypsin inhibitor solution (in cell culture medium) was added. After 10 min, the tissue suspension was centrifuged (150 × *g*, 5 min) and the trypsin inhibitor solution was aspirated. The tube was added with cell culture medium (modified DMEM/F12 medium containing 10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 100 μM MEM nonessential amino acids). The tissue was gently triturated with three fire-polished Pasteur pipettes of progressively smaller bore