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Short communication

Airway extravasation induced by increasing airway temperature in ovalbumin-sensitized rats

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ABSTRACT

This study was carried out to determine whether hyperventilation of humidified warm air (HWA) induced airway extravasation in ovalbumin (Ova)-sensitized rats. Our results showed: (1) After isocapnic hyperventilation with HWA for 2 min, tracheal temperature (T_{tr}) was increased to 40.3 °C, and the Evans blue contents in major airways and lung tissue were elevated to 651% and 707%, respectively, of that after hyperventilation with humidified room air in Ova-sensitized rats; this striking effect of HWA was absent in control rats. (2) The HWA-induced increase in Evans blue content in sensitized rats was completely prevented by a pretreatment with either L-732138, a selective antagonist of neurokinin type 1 (NK-1) receptor, or formoterol, a selective agonist of β_2 adrenoceptor. This study demonstrated that an increase in airway temperature induced protein extravasation in the major airways and lung tissue of sensitized rats, and an activation of the NK-1 receptor by tachykinins released from bronchopulmonary C-fiber nerve endings was primarily responsible.

1. Introduction

A recent study carried out in our lab reported that hyperventilation (40% of maximal voluntary ventilation for 4 min) of humidified warm air (HWA) triggered cough and bronchoconstriction in patients with mild and stable asthma, and activation of bronchopulmonary sensory nerves by HWA was believed to be responsible (Hayes et al., 2012). A follow-up study further demonstrated that an increase in airway temperature resulting from hyperventilation of HWA evoked a pronounced increase in airway resistance in ovalbumin (Ova)-sensitized Brown–Norway rats, and that endogenous tachykinins were the primary contributing factor (Hsu et al., 2013). Furthermore, the HWA-induced airway response was not prevented by atropine and sustained for >10 min (Hsu et al., 2013), suggesting that the effect was not generated by the reflex-mediated airway smooth muscle contraction in anesthetized rats.

Tachykinins, such as substance P (SP) and neurokinin (NK) A, are pro-inflammatory neuropeptides released from pulmonary C-fiber sensory nerve endings upon intense stimulation. These neuropeptides can trigger a number of neurogenic inflammatory responses in the airways, including protein extravasation, mucosal edema and bronchoconstriction, by activating NK type 1 (NK-1) and type 2 (NK-2) receptors expressed on a number of target cells in the airways, including endothelial cells in capillaries and venules, airway and vascular smooth muscles (Steinhoff et al., 2014). However, whether airway extravasation was evoked by the HWA challenge in Ova-sensitized rats is not known (Hsu et al., 2013).

This study was carried out to answer the following questions: (1) Does hyperventilation of HWA induce airway extravasation, and if so, is the effect augmented in Ova-sensitized rats? (2) Are tachykinins responsible for the HWA-evoked airway extravasation, and if so, what are the relative roles of NK-1 and NK-2 receptors in mediating these responses?

2. Materials and methods

This study was performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health. The study protocol was approved by the University of Kentucky Institutional Animal Care and Use Committee.

2.1. Animal sensitization and preparation

Adult male pathogen-free Brown–Norway rats (267.5 ± 3.3 g) were separated into control and sensitized groups. Sensitized rats received an initial intraperitoneal (ip) injection of a suspension containing 2 mg of Ova (Sigma-Aldrich, St. Louis, MO) in 1 ml of Imject Alum (Pierce Biotechnology, Rockford, IL) as adjuvant. Three

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days later, rats were exposed to 1.25% Ova aerosol for 15 min three times per week (M/W/F) for 3 weeks, following the protocol identical to that described in our previous study (Hsu et al., 2013). Control rats received an ip injection of Imject Alum and inhalation of aerosolized vehicle (isotonic saline). One day after the last Ova or saline exposure, rats were anesthetized with α-chloralose (100 mg/kg; Sigma-Aldrich) and urethane (500 mg/kg; Sigma-Aldrich), placed in a supine position and ventilated mechanically with a respirator (model 683; Harvard, South Natick, MA) via a short tracheal cannula inserted just below the larynx. Respiratory rate (f) was set at 60 breaths/min, and tidal volume (V_T) at 6–7 ml/kg. Body temperature was maintained at 36 °C by a heating blanket. A polyethylene catheter was inserted into the left femoral vein for intravenous (iv) bolus injections of drugs.

2.2. HWA challenge

The method for the HWA challenge was described in details in a previous report (Hsu et al., 2013). Briefly, the outlet of the respirator inspiratory line was connected to an air stone that was immersed in isotonic saline contained in a bottle placed in a heated water bath. HWA was then delivered directly into the lung via the tracheal tube. Humidified room air (HRA) was delivered in the same manner except that the water bath was kept at the room temperature (~23 °C). During both HWA and HRA challenges, minute ventilation was elevated to ~375% of the baseline (by increasing V_T and f to 150% and 250% of their baselines, respectively) for 2 min; ~4.0% of CO_2 was added to the inspired air to maintain the isocapnic condition during hyperventilation. A miniature temperature probe (model IT-18; Physitemp, Clifton, NJ) was inserted into the tracheal tube and positioned near the thoracic inlet to continuously measure the air temperature in the trachea before and during HWA and HRA challenges.

2.3. Measurement of airway extravasation

Evans blue dye (30 mg/kg iv; Sigma-Aldrich) was injected 5 min before the HWA or HRA challenge. Ten min after the HWA or HRA challenge, the thorax was opened. To remove blood and intravascular Evans blue dye, pulmonary circulation and systemic circulation were perfused with isotonic saline via cannulated pulmonary artery (pressure, 30 mmHg; volume, 25 ml) and aorta (pressure, 120 mmHg; volume, 200 ml), respectively. The whole lung including trachea was then removed, separated into major airways (trachea and major bronchi) and lung tissue (lung parenchyma and intrapulmonary airways), and weighted. The intensity of airway extravasation was quantified by measuring the extravasation amount of Evans blue dye (Evans et al., 1988) extracted from tissue in formamide (Sigma-Aldrich) by incubation at 40 °C in water bath for 24 h and measured by light absorbance (SpectraMax, M2; Molecular Devices, Sunnyvale, CA) at 620 nm. The Evans blue content in tissue was determined from a standard curve of the dye in the concentration range of 0.05–20 μg/ml, and expressed as ng Evans blue dye per mg wet tissue (ng/mg).

2.4. Experimental protocols

Four series of experiments were carried out. *Series 1* aimed to determine if airway extravasation was generated by the increase of tracheal temperature (T_{tr}) induced by the HWA challenge; and to compare the responses between control and Ova-sensitized rats. Both control and sensitized rats were divided into two groups ($n = 6$ in each group) for HWA and HRA challenges. *Series 2:* To investigate the role of the endogenous tachykinins, the HWA-induced extravasation responses in Ova-sensitized rats were compared between a control group (pretreated with vehicle) and a group pretreated with a combination of L-732138 (6 mg/kg iv; Tocris, Ellisville, MO), a selective NK-1 antagonist, and SR-48968 (1 mg/kg iv; Sanofi Recherche, Montpellier, France), a selective NK-2 antagonist 20 min earlier. *Series 3*: To determine the relative contributions of NK-1 and NK-2 receptors, the HWA-induced extravasation responses were compared between two groups of Ova-sensitized rats pretreated with L-732138 alone and SR-48968 alone, respectively, 20 min earlier. *Series 4:* To study the role of β_2 adrenoceptors, the HWA-induced extravasation responses were determined in Ova-sensitized rats pretreated with formoterol (10 μg/kg iv; Sigma-Aldrich), a selective β_2 agonist, 60 min earlier.

2.5. Statistical analysis

Data were compared using one-way or two-way analysis of variance (ANOVA), followed by a post hoc Fisher's test. P values of <0.05 were considered significant. Data are reported as means ± SE.

3. Results

A total of 49 rats were used in this study. Despite that the age was matched between the two groups, the average body weight of Ova-sensitized rats (259 ± 3 g; $n = 37$) was significantly lower than that of control rats (293 ± 4 g; $n = 12$, $P < 0.01$). Hyperventilation of HWA elevated T_{tr} from 31.5 ± 0.5 °C to 40.1 ± 0.3 °C ($n = 6$, $P < 0.001$) in control rats and from 31.8 ± 0.2 °C to 40.3 ± 0.1 °C ($n = 6$, $P < 0.001$) in sensitized rats. Hyperventilation with HRA decreased T_{tr} (from 31.3 ± 0.4 °C to 24.4 ± 0.4 °C in control rats; from 31.4 ± 0.3 °C to 24.4 ± 0.2 °C in sensitized rats).

3.1. Series 1

The Evans blue content after HWA challenge was significantly higher than that after HRA in both major airways and lung tissue in Ova-sensitized rats, but not in control rats. In Ova-sensitized rats, Evans blue contents measured in major airways were 143.8 ± 34.4 ng/mg and 22.1 ± 5.3 ng/mg in the two groups receiving HWA and HRA challenges, respectively ($P < 0.01$, $n = 6$; Fig. 1A). In lung tissue, the Evans blue contents were 29.7 ± 5.7 ng/mg and 4.2 ± 0.7 ng/mg in HWA and HRA groups, respectively ($P < 0.01$, $n = 6$; Fig. 1B). In a sharp contrast, in either major airways or lung tissue, the Evans blue contents were not different between the two groups of control rats receiving HWA and HRA challenges ($P > 0.05$, $n = 6$; Fig. 1).

3.2. Series 2

Pretreatment with a combination of L-732138 and SR-48968 completely abolished the increase in Evans blue content in both major airways and lung tissue induced by the HWA challenge in Ova-sensitized rats (Fig. 2). In major airways, the Evans blue contents after HWA were 118.7 ± 11.6 in the control (vehicle) group, and 17.8 ± 1.9 ng/mg in the group pretreated with a combination of L-732138 and SR-48968 ($P < 0.01$, $n = 5$; Fig. 2A); in lung tissue, the Evans blue contents after HWA were 30.9 ± 6.3 and 5.8 ± 0.6 ng/mg in the control (vehicle) and treated (L-732138 + SR-48968) groups, respectively ($P < 0.01$, $n = 5$; Fig. 2B).

3.3. Series 3

Pretreatment with L-732138 alone significantly attenuated the HWA-induced increase in Evans blue content to 20.9 ± 1.0 ng/mg ($P < 0.01$, $n = 5$; Fig. 2A) in major airways, and to 3.8 ± 0.2 ng/mg ($P < 0.01$, $n = 5$; Fig. 2B) in lung tissue in Ova-sensitized rats. In contrast, pretreatment with SR-48968 alone did not significantly change the HWA-induced increase in Evans blue content in major