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Vagotomy reverses established allergen-induced airway hyperreactivity to methacholine in the mouse☆

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ABSTRACT

We evaluated the role of vagal reflexes in a mouse model of allergen-induced airway hyperreactivity. Mice were actively sensitized to ovalbumin then exposed to the allergen via inhalation. Prior to ovalbumin inhalation, mice also received intratracheally-instilled particulate matter in order to boost the allergic response. In control mice, methacholine (i.v.) caused a dose-dependent increase in respiratory tract resistance (RT) that only modestly decreased if the vagi were severed bilaterally just prior to the methacholine challenge. Sensitized and challenged mice, however, manifested an airway reactivity increase that was abolished by severing the vagi prior to methacholine challenge. In an innervated ex vivo mouse lung model, methacholine selectively evoked action potential discharge in a subset of distension-sensitive A-fibers. These data support the hypothesis that the major component of the increased airway reactivity in inflamed mice is due to a vagal reflex initiated by activation of afferent fibers, even in response to a direct (i.e., smooth muscle)-acting muscarinic agonist.

1. Introduction

In recent years the mouse has become the most commonly used animal model in the study of asthma and allergic airway inflammation. In these investigations, the mouse is usually actively or passively sensitized to an antigen, then the airways are exposed to the sensitizing antigen, resulting in a "T_H2 type" allergic inflammatory response. Although the strains of mice differ among studies, and there are subtle differences in the sensitization-and-challenge schemes, invariably an eosinophil-rich inflammation is evoked that is associated with "airways hyperreactivity" to methacholine. The hyperreactivity is typified by an increase in the slope of the methacholine dose–response curve, with relatively modest changes in methacholine sensitivity compared to those seen in asthmatics (Takeda et al., 1997; Park et al., 2004; Leigh et al., 2004; Williams and Galli, 2000). Despite a large and growing literature describing the essential immunologic features associated with this type of mouse airway hyperreactivity, there is comparatively little information regarding its physiological mechanisms.

Exposing mammalian airways to contractile agonists activates vagal sensory mechanosensors. Depending on the mechanosensory phenotype, this can lead to increases or decreases in parasympathetic contraction of bronchial smooth muscle (Canning, 2006). As a consequence, the bronchoconstriction evoked by contractile agonists, including methacholine, is likely to be at least partially mediated by central reflexes that result in the release of acetylcholine from postganglionic parasympathetic nerve terminals (Wagner and Jacoby, 1999; Canning, 2006). Inflammation can stimulate a subset of afferent C-fibers collectively referred to as nociceptors. One consequence of afferent C-fiber activation in airways is an amplification parasympathetic and cough reflexes. Within the context of airway inflammation, this feed-forward bronchoconstrictor reflex may be amplified through multiple mechanisms occurring at each of the sites in the sensory-CNS-autonomic reflex arc as reviewed in (Undem et al., 2000). A recent study has revealed that selective ablation of vagal C-fiber neurons in the mouse prevented allergen-induced airway hyperreactivity

Abbreviations: ROFA, residual oil fly ash; Mch, methacholine/acetyl-beta-methylcholine; OVA, ovalbumin; JNC, jugular/nodose ganglia complex; BAL, bronchoalveolar lavage.

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to acetylcholine, without apparently inhibiting the inflammatory response (Trankner et al., 2014). These data are consistent with the idea that the role inflammation plays in causing airways hyperreativitity in the mouse is dependent entirely on the effect it has in stimulating vagal C-fibers. A C-fiber mediated hyper-reflexive state in the airways would, in theory, result in greater reflex responses for any given mechanical perturbation, resulting in methacholine dose–response curves with steeper slopes.

In this study, we specifically addressed the hypothesis that the airway hyperreactivity associated with acute (1 day) allergic inflammation in mouse lungs is due, at least in part, to an exaggeration specifically in the reflex component of the methacholine response. We used a previously characterized (Gavett et al., 1999) model in which tracheal application of particulate matter (residual oil fly ash, ROFA) serves as an adjuvant that augments the allergic response, resulting in a more vigorous and consistent inflammation and airway hyperreactivity.

2. Methods

2.1. Experimental animals

Female Balb/cJ mice ($n = 7$–12 per group), 7-week old on arrival and weighing 18–22 g, were obtained from Jackson Laboratories (Bar Harbor, ME). Mice were provided with Prolab RMH-3000 meal (PMI Feeds, St. Louis, MO) and tap water ad libitum. Animals were maintained on a 12 h light/dark cycle at $22 \pm 2\,^{\circ}C$ and $50 \pm 10\%$ relative humidity in an AAALAC-approved facility, and held for a minimum of 4 days before treatment. All experiments were approved by the Institutional Care and Use Committee of the organization where they were performed and met ethical standards outlined in the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Allergen sensitization and challenge and exposure to residual oil fly ash

Exposure to ROFA, as well as allergen sensitization and challenge, were performed as previously described (Gavett et al., 1999). Mice were sensitized with 20 mg ovalbumin (OVA; Grade V, Sigma Chemical, St. Louis, MO) in 0.2 mL aluminum hydroxide gel adjuvant (Alhydrogel; Accurate, Westbury, NY) delivered via an intraperitoneal (i.p.) injection. Control mice were injected with Alhydrogel only. Two weeks later, all mice were placed in a wire rack inside a 135 L chamber and challenged for 1 h with an aerosol of 1% ovalbumin in sterile saline. The solution was nebulized using three jets of a 6-jet atomizer (Model 9306; TSI, Minneapolis, MN) at 8 L/min/jet, and house air was added to bring the total flow rate to 40 L/min (18 exchanges/h).

Residual oil fly ash (ROFA) was collected from the flue gas of a power plant burning low sulfur (1%) residual oil downstream from a 2.5-mm cutoff cyclone. One hour after ovalbumin challenge, mice were anesthetized with methoxyflurane (Mallinckrodt, Mundelein, IL) vapor in a 2.7 L Plexiglas chamber. Following anesthesia, mice were intratracheally instilled with sterile saline vehicle or ROFA in saline using a 100 μL syringe and round-tip needle (dose = 3 mg/kg, ~50 μL volume; ~60 μg ROFA instilled per mouse. Saline was used as a vehicle since the saline-soluble portion (94% by weight) has been shown to mediate ROFA's toxic effects (Gavett et al., 1997). After 1-day of recovery, airway responsiveness to intravenously administered methacholine (Mch) was assessed in each mouse. After these measurements, bronchoalveolar lavage (BAL) was performed to collect and analyze cells. Statistical differences were determined using analysis of variance (ANOVA).

2.3. Airway responsiveness measurements

Mice were anesthetized with an intraperitoneal injection of urethane (1.5 g/kg) and tracheostomized using an 18 g cannula and kept warm on a 37 °C-heated circulating water pad. Animals were ventilated with constant inspiratory flow (flexiVent, Scireq, Montreal, Quebec) using hospital grade oxygen, and spontaneous breathing was eliminated with pancuronium bromide (0.8 mg/kg i.p.). Breathing frequency ($50\,kg^{-0.25}$/min) and tidal volume (7.5 mL/kg) were determined by animal body weight. Heart rate and waveform were monitored (SRA-200, MicroMed, Louisville, KY). Animals that did not maintain a baseline heart rate of ≥400 bpm were excluded from the study.

Mice were cannulated via the jugular vein using a 27 g needle inserted into PE20 tubing which was attached to an automated syringe pump loaded with 0.2 mg/mL Mch in saline. Baseline measurements of heart rate and total respiratory resistance (RT) were taken prior to drug infusion as described in more detail here (Gavett et al., 1999). Bolus doses of Mch were delivered over two seconds every 2 min in half log doses ranging from 10.0 to 316.2 μg/kg. Following drug infusion, RT was measured and recorded at 6-s intervals for a total of one minute. The peak response was recorded and that generally was observed 12–30 s after drug infusion. To standardize lung volumes 30 s before each dose, the expiratory port was occluded until airway pressure reached 20–30 cm H_2O. Resistance of the tracheal cannula was subtracted from measured values of RT. Responses from each dose were summed to determine area under the curve for one minute. To standardize lung volumes prior to the first dose and 1 min after each dose, the expiratory port was briefly occluded until airway pressure reached ~30 cm H_2O. This maneuver eliminated artifactual differences in baseline resistance caused by focal atelectases, which could affect responses to subsequent doses of Mch. Following a series of doses, the vagi were severed bilaterally and the Mch challenge protocol was repeated. No tachyphylaxis was observed when sham vagotomy was performed between methacholine dose–response curves ($95 \pm 2.1\%$ of maximum original response, $n = 3$).

2.4. BAL fluid differential cell count

Mice were lavaged with two aliquots of Ca^{2+} and Mg^{2+}-free Hanks' balanced salt solution (35 mL/kg; HBSS). Approximately 90% of the delivered volume was consistently recovered. The lavage fluid was placed on ice and centrifuged at $360 \times g$ for 12 min at 4 °C. Cells were then resuspended in 1.0 mL HBSS and counted (Coulter, Hialeah, FL). Slides of BAL fluid cells were made (Cytospin 3; Shandon, Pittsburgh, PA) and stained with Leuko-Stat (Fisher Scientific, Fair Lawn, NJ) and at least 500 cells per sample were differentiated.

2.5. Sensory nerve recordings

Mice were killed by CO_2 inhalation and exsanguination. The blood from the pulmonary circulation was washed out by in situ perfusion with indomethacin (3 μM) containing Krebs bicarbonate buffer (KBS, composed of: NaCl, 118 mM; KCl, 5.4 mM; NaH_2PO_4, 1.0 mM; $MgSO_4$, 1.2 mM; $CaCl_2$, 1.9 mM; $NaHCO_3$, 25.0 mM; dextrose, 11.1 mM, and gassed with 95% O_2–5% CO_2, pH 7.4) through the right ventricle. The airways and lungs with intact left- and right-side extrinsic vagal innervation (including left and right jugular/nodose ganglia complex, JNC) were dissected and the tissue was pinned in a small Sylgard-lined Perspex chamber. The right and left JNC, along with the rostralmost vagi were pulled through a small hole into an adjacent chamber for extracellular recording as described in detail elsewhere (Kollarik et al., 2003).

Briefly, extracellular recordings were performed using an aluminosilicate glass microelectrode (pulled with Flaming-Brown