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House-dust mite allergen and ozone exposure decreases histamine H3 receptors in the brainstem respiratory nuclei☆

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ABSTRACT

Allergic airway diseases in children are a common and a growing health problem. Changes in the central nervous system (CNS) have been implicated in contributing to some of the symptoms. We hypothesized that airway allergic diseases are associated with altered histamine H3 receptor expression in the nucleus tractus solitarius (NTS) and caudal spinal trigeminal nucleus, where lung/airway and nasal sensory afferents terminate, respectively. Immunohistochemistry for histamine H3 receptors was performed on brainstem sections containing the NTS and the caudal spinal trigeminal nucleus from 6- and 12-month-old rhesus monkeys who had been exposed for 5 months to house dust mite allergen (HDMA) + O_3 or to filtered air (FA). While histamine H3 receptors were found exclusively in astrocytes in the caudal spinal trigeminal nucleus, they were localized to both neuronal terminals and processes in the NTS. HDMA + O_3 exposure significantly decreased histamine H3 receptor immunoreactivity in the NTS at 6 months and in the caudal spinal trigeminal nucleus at 12 months of age. In conclusion, exposing young primates to HDMA + O_3 changed histamine H3 receptor expression in CNS pathways involving lung and nasal afferent nerves in an age-related manner. Histamine H3 receptors may be a therapeutic target for allergic asthma and rhinitis in children.

Introduction

Allergic respiratory diseases such as asthma and allergic rhinitis are chronic illnesses commonly observed in children. A recent national health survey states that ~10 million U.S. children have been diagnosed with asthma, ~6.7 million (9% of total children) still have asthma, and more than 7 million suffer from allergic rhinitis (Bloom et al., 2009). These diseases cannot only disrupt the health and social activities of children and their families, but also add to health costs (Ray et al., 1999).

That repeated exposures to allergen and environmental pollutants exacerbates allergic asthma symptoms (Arshad et al., 1998; Delfino et al., 1996), and that allergen-induced release of local inflammatory mediators plays an important role in asthma exacerbations is well established (Lemanske, Jr., 2000; Undem et al., 2000). What might be underappreciated is that the inflammatory mediators released locally during allergen and ozone exposure also directly and indirectly trigger profound changes in the central nervous system (CNS) that can further exacerbate asthma-like symptoms. Increased airway resistance, mucous secretion, increased microvascular leak, and cough—all are evoked by CNS reflexes triggered by local excitation of sensory nerves innervating the airways or by manipulations of neurons in the central network (Sant'Ambrogio, 1982; Widdicombe and Lee, 2001). One CNS region that is particularly important is the nucleus tractus solitarius (NTS), located in the dorsal brainstem adjacent to the area postrema. NTS neurons temporally and spatially integrate the sensory information from lungs and airways with inputs from local networks and higher brain regions. The neurons also have direct exposure to inhaled allergens and allergen-induced blood-borne inflammatory mediators via a deficient blood-brain barrier (Gross et al., 1990).

A less studied symptom of allergic rhinitis, sneezing, is also a CNS reflex, triggered by stimulation of nasal sensory receptors innervated by the nasal trigeminal nerve, which makes synaptic contact with neurons in the caudal spinal trigeminal nucleus located in the dorsal brainstem (McCulloch et al., 2008; Rybka and McCulloch, 2006). Stimulation of the sensory nerves by irritants, mast cell products, and inflammatory mediators, including histamine, leads to sneezing in

Abbreviations: CNS, central nervous system; FA, filtered air; GFAP, glial fibrillary acidic protein; H3R-ir, H3 receptor immunoreactivity; HDMA, house-dust mite allergen; NeuN, nuclear protein; NGS, normal goat serum; NTS, nucleus tractus solitarius; PBS, phosphate buffered saline; PFA, paraformaldehyde; SYN, synaptophysin; TSA, tyrmaide signal amplification.

☆ Allergen induced neurochemical phenotype in CNS.

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addition to increases in nasal blood flow and microvascular leak. In a manner analogous to the NTS as first site of synaptic contact for vagal afferent fibers innervating the lungs and airways, the caudal spinal trigeminal nucleus is the first central site for synaptic contact of spinal afferent fibers innervating the nose, and thus is a pivotal modulatory site for rhinitis, nasal congestion and the sneeze reflex.

A number of inflammatory mediators have been implicated in contributing to the allergy symptoms; one of which, histamine, has been extensively studied because of its role in increasing vascular hyperpermeability, inducing edema, contracting smooth muscle, causing mucus secretion, and inducing leukocyte chemotaxis, largely through histamine H1 and H4 receptors. Antihistamine treatments targeting peripheral histamine H1 receptors have successfully improved symptoms in a majority of patients with seasonal allergic rhinitis. Less attention has focused on histamine receptors in the CNS, with regard to allergic asthma and rhinitis; however, the histamine H3 receptors, largely restricted to the CNS including the NTS have wide ranging effects on synaptic transmission, modulating the release of multiple neurotransmitters (Hill et al., 1997). Pharmacological activation of H3 receptors in the spinal cord of rats has been shown to attenuate formalin-induced inflammatory responses (Cannon et al., 2007). Given the potential role of histamine H3 receptors in linking the immune and neural systems, their presence in the NTS, and the capacity of NTS neurons to develop changes in their neural properties in response to extended-exposures to allergens and environmental pollutants, we hypothesized that histamine H3 receptor expression may play a role in exaggerated symptoms of allergic asthma and rhinitis. We took advantage of allergic airway disease in young rhesus monkeys using exposure to allergen + ozone which has demonstrated increased plasma histamine, hyperresponsiveness to histamine, increased airway resistance, increased number of eosinophils, increased mucus secretion, and airway remodeling (Joad et al., 2008; Plopper et al., 2007; Schelegle et al., 2003) as well as changes in the intrinsic excitability of neurons in the NTS, where lung and airway afferents terminate (Chen et al., 2001; 2003). Using this model, we studied whether exposure to house-dust mite allergen (HDMA) plus ozone (O_3) alters expression of histamine H3 receptor immunoreactivity in the NTS and caudal spinal trigeminal nucleus. Since some findings in the model have shown that critical windows of exposure exist, we studied these areas in 6-month- and 12-month-old monkeys to determine whether changes were age-related.

Methods

All protocols were approved by the Institutional Animal Care and Use Committee in compliance with the Animal Welfare Act and Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Exposure to allergen and ozone to create respiratory allergic monkey model. The 20 male rhesus monkeys used for this study were California Regional Primate Research Center colony-born rhesus macaques (*Macaca mulatta*). Care and housing of animals before, during, and after treatment complied with the provisions of the Institute of Laboratory Animal Resources and conforms to practices established by the American Association for Accreditation of Laboratory Animal Care.

Overall protocol. All infant monkeys were sensitized to house-dust mite allergen (HDMA, Dermatophygoides pteronyssinus, Greer Laboratories, Inc. Lenoir, NC). This was done by subcutaneous injection of HDMA in alum 4 times, at 3 weeks and 1 week before start of exposure and 3 weeks and 11 weeks after start of exposure. Subcutaneous injection of Infanrix (DTaP vaccine, GlaxoSmithKline, Atlanta, GA) was also performed at 3 weeks before start of exposure. Animals were randomly assigned to 4 groups, exposed to (1) filtered air (FA) from 1 to 6 months of age ($n=4$), (2) HDMA + O_3 from 1 to 6 months of age ($n=4$), (3) FA from 7 to 12 months of age ($n=6$), and (4) HDMA + O_3 from 7 to 12 months of age ($n=6$). Groups 2 and 4 received intranasal HDMA two weeks before and the week of start of exposure, as well as 4 weeks after start of exposure. HDMA was aerosolized and exposed to monkeys for 2.2 h/day, 3 days/2 weeks, and ozone (0.5 ppm) exposure was for 8 h/day, 5 days/2 weeks (Joad et al., 2008; 2006; Schelegle et al., 2003). At 6 months of age for groups (1) and (2) and at 12 months of age for groups (3) and (4), brainstem slices were obtained for immunohistochemistry for histamine H3 receptors.

Immunohistochemistry. The monkeys were anesthetized with ketamine (10 mg/kg i.m.), then euthanized with pentobarbital (Fatal-Plus, >44 mg/kg). After decapitation, the brainstem was rapidly removed and submerged into 4% paraformaldehyde (PFA) in 0.01 M phosphate-buffered saline (PBS, pH 7.4) for 24–48 h at 4 °C, and then sectioned into cryoprotectant (50 μm coronal sections) using a vibrating blade microtome VT 1000 S (Leica, Germany). Sections were stored in cryoprotectant at −20 °C until immunohistochemical labeling was performed.

Immunohistochemistry for histamine H3 receptors was performed to determine if HDMA + O_3 exposure induced a change in histamine H3 receptor expression in the NTS and caudal part of the spinal trigeminal nucleus. In addition to histamine H3 receptor labeling, sections were also simultaneously processed with antibodies to label neuronal nuclei, astroglia, or pre-synaptic terminals using neuronal specific nuclear protein (NeuN) antibody, a glial fibrillary acidic protein (GFAP) antibody, or synaptophysin (SYN) antibody, respectively. Tissue was always processed simultaneously from paired groups (a FA and HDMA + O_3 exposed animal) to minimize between-group variability.

All incubation and rinsing steps were at room temperature on a laboratory rocker unless otherwise stated. Sections were blocked in 10% normal goat serum (NGS; Vector Labs, catalog #S-1000) and 1% tyrmaide signal amplification (TSA; Invitrogen, catalog #T-20932) blocking reagent for 60 min, then incubated in a rabbit polyclonal anti-histamine H3 receptor (1:100, Thermo Scientific, catalog #OPA1-15462) primary antibody combined with either a mouse monoclonal anti-NeuN (1:100, Chemicon, catalog #MAB377), a mouse monoclonal anti-GFAP (1:500, Invitrogen, catalog #A21282), or a mouse monoclonal anti-SYN (1:200, Sigma, catalog #S5768) primary antibody in PBS containing 1% NGS and 1%TSA blocking reagent for 60–72 h at 4 °C. Sections were then rinsed and simultaneously incubated in goat anti-rabbit Alexa Fluro®-568 (1:500, Invitrogen, catalog #A11011) secondary antibody for 2 h to visualize histamine H3 receptors. Goat anti-mouse Alexa Fluro®-488 (1:500, Invitrogen, catalog #A11029) secondary antibody was also added to visualize NeuN, GFAP or SYN immunoreactivity. The brainstem sections were then rinsed, mounted on lysine coated slides, dried and coverslipped in anti-fade mounting media (Vector Labs, catalog #H-1000). Sections run without primary or secondary antibodies served as negative controls to confirm specific staining.

Up to 15 sections per monkey ranging from approximately 34.5 to 33.5 mm caudal to bregma were processed (Paxinos et al., 2000). Brainstem sections containing the area postrema were selected for analysis, since the NTS at the level of area postrema is a regulatory site for cardiorespiratory control in many animals species including rat (Bonham and Joad, 1991), mouse (Paton, 1998), cat (Silva-Carvalho et al., 1998), and rabbit (Wallach and Loewy, 1980). The ventral part of the caudal spinal trigeminal nucleus at the same level, which is responsible for nasal cardiorespiratory reflexes initiated by trigeminal (ethmoidal) sensory afferents from the nasal mucosa (McCulloch et al., 2008; Rybka and McCulloch, 2006) was also analyzed. Tissue was viewed and images were captured with a Zeiss LSM-5 inverted confocal microscope. Sections were viewed with the 488 nm (NeuN,