Please cite this article in press as: Li JN et al. Sex- and afferent-specific differences in histamine receptor expression in vagal afferents of rat: A potential mechanism for sexual dimorphism in prevalence and severity of asthma. Neuroscience (2015), http://dx.doi.org/10.1016/j.neuroscience.2015.06.049

Neuroscience xxx (2015) xxx–xxx

SEX- AND AFFERENT-SPECIFIC DIFFERENCES IN HISTAMINE RECEPTOR EXPRESSION IN VAGAL AFFERENTS OF RAT: A POTENTIAL MECHANISM FOR SEXUAL DIMORPHISM IN PREVALENCE AND SEVERITY OF ASTHMA

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Abstract—The incidence of asthma is more common in boys than in girls during the puberty, and more common in pre-menopausal female than age-matched males. Our previous study demonstrated a gender difference in histamine-mediated neuroexcitability in nodose ganglia neurons (NGNs), highlighting a possibility of histamine-mediated gender difference in asthma via visceral afferent function. In the present study, we aimed to explore the gender difference in expression profiles of histamine receptors (HRs) in nodose ganglia (NG) and individual identified NGNs to provide deeper insights into the mechanisms involved in sexual dimorphism of asthma. Western-blot and SYBR green RT-PCR showed that H_2R and H_3R were highly expressed in NG of females compared with males and downregulated in ovariectomized females. H_1R was equally expressed in NG of both sexes and not altered by ovariectomy. Furthermore, this highly expressive H_2R and H_3R were distributed in both myelinated and unmyelinated NGNs isolated from adult female rats by immunofluorescence and single-cell RT-PCR. H_3R widely distributed in all tested neuron subtypes and its expression did not show significant difference among neuron subtypes. H_2R was widely and highly expressed in low-threshold and sex-specific subpopulation of myelinated Ah-types compared with myelinated A- and unmyelinated C-type NGNs. Unexpectedly, weak expression of H_1R was detected in both myelinated and unmyelinated NGNs by immunofluorescence, which was further confirmed by single-cell RT-PCR. Our results suggest that the sexual dimorphism in the expression of H_2R and H_3R in vagal afferents very likely contributes, at least partially, to the gender difference in prevalence and severity of asthma. © **2015 Published by Elsevier Ltd. on behalf of IBRO.**

Key words: histamine receptor, vagal afferents, asthma, gender difference, electrophysiology.

INTRODUCTION

A number of studies have demonstrated that the prevalence and severity of asthma show striking difference and closely relate to gender and age. However, no single explanation has been able to fully explain the disparities in recent perspective reviews (Kynyk et al., 2011; Townsend et al., 2012). Exploring the possible mechanisms in the sexually dimorphic presentation of asthma, therefore, is an important issue to establish effective and individualized asthma management strategies for all patients. Sensory nerves have long been believed to play an important pathophysiological role in asthma (Undem and Carr, 2002). Stimulating vagal sensory fibers produce a variety of effects including bronchoconstriction (Lundberg et al., 1983; Forsberg et al., 1988), mucous secretion (Tokuyama et al., 1990), microvascular leakage (Lundberg et al., 1983) and cough (Forsberg et al., 1988), all of which are characteristic symptoms of asthma.

Histamine, a major inflammatory mediator released from mast cells, can sensitize unmyelinated C-type vagal afferents via histamine H_1 receptor (H_1R) activation (Higashi et al., 1982; Leal-Cardoso et al., 1993; Undem and Weinreich, 1993; Jafri et al., 1997; Yu et al., 2007), and then enhance the reflex response of vagal afferents to other stimuli such as mechanical or chemical stimuli (Lee and Morton, 1993; Mizumura et al., 1994; Michaelis et al., 1998). Histamine can also inhibit cholinergic neurotransmission in guinea-pig airways via histamine H_3 receptor (H_3R) activation in vagal afferents, which further inhibits the airway smooth muscle

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Abbreviations: ANOVA, analysis of variance; AP, action potential; APD_{50}, AP duration measured at 50% amplitude of AP; APFT, AP firing threshold; UV_{MAX}, the maximal upstroke velocity of depolarization; HR, histamine receptor; NG, nodose ganglion; NGNs, nodose ganglion neurons; OVX, ovariectomized; PBS, phosphate-buffered saline; SD, Sprague–Dawley.

http://dx.doi.org/10.1016/j.neuroscience.2015.06.049
0306-4522/

contraction elicited by vagal nerve stimulation (Ichinose et al., 1989). Other reports demonstrated that the activation of H_3R could inhibit neurogenic microvascular leakage in guinea-pig airways by prejunctional inhibition of neuropeptide release from airway vagal sensory nerves (Ichinose et al., 1990) and protect the lung against acetylcholine- and capsaicin-induced edema via a prejunctional modulatory effect on the C-fibers (Delaunois et al., 1995). All of these functions for histamine and histamine receptors (HRs) in vagal afferents are probably involved in the pathophysiology of asthma. Recently, a study demonstrated that lafutidine, a histamine H_2 receptor (H_2R) antagonist, could inhibit vagal afferent signaling of a gastric acid insult in adult female Sprague–Dawley (SD) rats (Edelsbrunner et al., 2009), suggesting that H_2R may also play a role in vagal afferents. Although all above evidences indicate indirectly, from a pharmacological point of view, that histamine H_1R, H_2R and H_3R may express in vagal afferents, there is no direct molecular biological evidence so far to confirm in this regard. To our knowledge, there was only one direct study (Kashiba et al., 1999) using *in situ* hybridization histochemistry showing that none of nodose ganglion neurons (NGNs) were labeled with H_1R cDNA probe in male guinea pigs, which contradict with the electrophysiological studies showing that histamine sensitizes vagal afferents by activating H_1R (Leal-Cardoso et al., 1993; Undem and Weinreich, 1993; Jafri et al., 1997).

In our previous study, we surprisingly found that in adult female SD rats, histamine could sensitize all types of NGNs including a low-threshold and sex-specific myelinated Ah-type neurons (Li et al., 2013) that exist predominantly in adult female rats and rarely in adult male rats (Li and Schild, 2007; Li et al., 2008; Qiao et al., 2009; Santa Cruz Chavez et al., 2014). In stark contrast, histamine only sensitizes unmyelinated C-type NGNs without any effects on myelinated A-type NGNs in adult male SD rats (Li et al., 2013), which is consistent with the results from male species in other reports (Higashi et al., 1982; Undem and Weinreich, 1993). These findings in our laboratory highlight a potential mechanism involved in the basic pathophysiology in the sexually dimorphic presentation of asthma; meanwhile, these data also suggest that the expression profiles of HRs in vagal afferents are probably different between adult male and age-matched female rats. Moreover, because of the important functions of H_1R, H_2R and H_3R in vagal afferents as described above, to study the gender different expression of these receptors would have important implications for better understanding the gender difference of histamine-mediated effects in vagal afferents, such as histamine-mediated vagal afferent sensitization or cholinergic neurotransmission inhibition or neurogenic microvascular leakage inhibition in airways, which probably contribute, at least partially, to the sexually dimorphic presentation of asthma.

Based on these considerations, the aim of the present study is to detect the expression of H_1R, H_2R and H_3R and reveal the sexually dimorphic expression of these receptors in nodose ganglia by using western-blot and SYBR Green RT-PCR. Furthermore, afferent-specific expression of HRs in individual identified neurons, with known afferent fiber types, isolated from adult female rats is also detected using single-cell RT-PCR. Our results will give insight into the mechanisms involved in the sexual dimorphic presentation of asthma.

EXPERIMENTAL PROCEDURES

Animals

Adult male and age-matched female SD rats (180–250 g) were provided by Weitong-Lihua Experimental Animal Tech Corp (Grade: SPF, certificate #: SCXK, 2012-0001, Beijing, China). Ovariectomy was performed as previously described (McCallister et al., 2013; Qiao et al., 2013). Briefly, the dorsal surgical area of anesthetized intact females was shaved and swabbed with surgical scrub. Bilateral flank incisions were made to provide access to the peritoneal cavity. Both ovaries and the majority of oviducts were exteriorized through the muscle wall incision. The surgical wounds were sutured closed and a topical antibiotic was applied. The animals were allowed to recover and kept under normal condition for at least 1 month before study performed. The reason we chose 1 month as the recovery time of ovariectomized (OVX) rats was based on a report, in which blood 17β-estradiol (E_2) concentration of female SD rats (180–200 g) undergone OVX surgery was markedly decreased from two weeks after the OVX operation compared with non-OVX female rats and remained at a stably low level throughout the experimental period of 12 weeks (Wang and Abdel-Rahman, 2002). Experimental protocols were pre-approved by the Institutional Animal Care and Use Committee of School of Medical Science at Harbin Medical University.

Drug and chemical agents

Primers and SuperScript™ III CellsDirect cDNA Synthesis System were obtained from Invitrogen (Carlsbad, CA, USA). Antibodies were obtained from Alomone Lab. (Jerusalem, Israel). All other experimental materials were obtained from Fisher Scientific or other regular commercial sources.

Real Time SYBR-green quantitative PCR

Total RNAs were extracted using the TRIzol® Reagent (Invitrogen) according to the manufacturer's instructions. The cDNAs were synthesized using the Reverse Transcription Kit (Applied Biosystems). The reverse transcription conditions were as follows: 25 °C for 10 min, 37 °C for 120 min, 85 °C for 5 min. RT-PCR was performed using the SYBR-Green Master Mix (Applied Biosystems) kit with a 20-μl reaction volume containing 1 μl of reverse transcription product, 10 μl of SYBR-Green, 1 μl of PCR forward primer, 1 μl of PCR reverse primer and 7 μl of double-distilled water (ddH_2O). Quantitative PCR reactions were run on an ABI 7500 fast Real-Time PCR System (Applied Biosystems) using 96-well reaction plates and began with an initial cycles of 95 °C for 10 min followed by

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