Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading

Effects of histamine H1R-H4R-agonist on the airway epithelium of rabbits

Naushad A Dilkash[1], Trivendra Tripathi[2], Aijaz Ahmed Khan[1*], Mohd Shahid[3], Haris M Khan[3], Mashiatullah Siddiqui[2], Rahat Ali Khan[4]

[1]*Department of Anatomy, Faculty of Medicine, Jawaharlal Nehru Medical College & Hospital, Aligarh Muslim University, Aligarh-202 002, India*
[2]*Department of Biochemistry, Faculty of Medicine, Jawaharlal Nehru Medical College & Hospital, Aligarh Muslim University, Aligarh-202 002, India*
[3]*Department of Microbiology, Faculty of Medicine, Jawaharlal Nehru Medical College & Hospital, Aligarh Muslim University, Aligarh-202 002, India*
[4]*Department of Pharmacology, Faculty of Medicine, Jawaharlal Nehru Medical College & Hospital, Aligarh Muslim University, Aligarh-202 002, India*

ARTICLE INFO

Article history:
Received 3 March 2010
Received in revised form 12 March 2010
Accepted 10 April 2010
Available online 20 May 2010

Keywords:
Rabbit
Airway epithelium
Histamine receptors-agonist
Hyperplasia
Hypertrophy

ABSTRACT

Objective: To explore the exact role of histamine receptors in respiratory system. **Methods:** The cohort comprised of six groups (group I control and group II-VI treated) containing five rabbits in each group. Control-group received vehicle (sterile distilled water) and treated groups received subcutaneous histamine (100 μg/kg, b.i.d.), and H1R-agonist (HTMT), H2R-agonist (amthamine), H3R-agonist (R-(-)- α -methylhistamine) and H4R-agonist (clobenpropit) each in a dose of 10 μg/kg, b.i.d. for 30 subsequent days. After completion of treatment, animals were euthanized and perfused with 10% buffered formalin. Small tissue blocks of trachea and lungs were processed for paraffin embedding. Observations were recorded in sample photomicrographs taken from 10 μm thick. Haematoxylin and eosin stained sections. **Results:** It was observed that trachea and bronchi from histamine, H1R and H4R groups had only patches of hyperplastic and hypertrophied epithelium and in general, cells in the affected region were taller heaped up. The bronchiolar epithelia from all treated groups showed hypertrophy and hyperplasia throughout with most of the cells having rounded profile and appeared to bud out from the basal cells. **Conclusions:** It is concluded that histamine receptors on induction via its specific agonist can induce hypertrophy, hyperplasia of respiratory tract epithelia suggesting its role akin to growth stimulating factor and warranting further long-term study.

1. Introduction

Histamine, a vasoactive amine, is located in most body tissues[1, 2]. It is synthesized and stored in mast cells and basophils which are effector cells involved in the immediate hypersensitivity response[1, 2]. These cells contain numerous osmophilic granules that have heparin and other proteins which support mediators including histamine, and alter cellular and vascular reactions[3]. Secretion of mediators by degranulation is provoked by certain chemical agents, which can lead to allergic reactions or anaphylactic shock in extreme cases[4]. Histamine has different pKi values of its receptors as 4.2±0.1 for H1Rs, 4.3±0.1 for H2Rs, 7.8±0.1 for H3Rs and 8.1±0.1 for H4Rs[5]. Histamine receptors (HRs) transduce extracellular signals through different G-proteins: Gq/11 for H1Rs, G α s for H2Rs, Gi/o for H3Rs and H4Rs[1]. Activation or inhibition of HRs have led to a remarkable increase in the knowledge of histamine effects in the pathophysiology of disease conditions[1,6]. Recently, we have demonstrated short-term effects of histamine on antibody generation, which were affected by the concentration of histamine[7,8]. Histamine H1Rs play principle role in modulation of total immunoglobulins and have demonstrated potent effects in IgG generation, while H2Rs play dominant role in IgM generation and have shown their principle role in B-cell differentiation and proliferation by both positively and negatively regulating the antibody production[9]. Moreover, recent immunotoxic study of HRs-agonist have revealed that HTMT, amthamine and clobenpropit (H1-, H2- and H4-agonist, respectively) play an important role in modulation of antibody generation level, among which HTMT have dominant role compared to others. Conversely, R-[-]- α -methylhistamine (H3-agonist) have dominant inhibitory role on antibody production[10]. However, several studies demonstrated that H1R was located on smooth muscle, endothelium, and central nervous system; H2R on parietal cells; H3R primarily on central and to a lesser extent on peripheral nervous system tissue; while H4R was found

*Corresponding author: Dr. Aijaz Ahmed Khan, MD, Ph.D. Professor, Department of Anatomy, Faculty of Medicine, Jawaharlal Nehru Medical College and Hospital, Aligarh Muslim University, Aligarh-202 002, UP, India
Tel: +91-2720406; Mob: 9897216343
E-mail: aijazahmedkhan7@live.com

primarily in the basophils and in bone marrow[2, 11-14]. Histamine-induced bronchiolar constriction has been implicated in the first phase of bronchial asthma[15]. In asthmatics, histamine was found to increase airway smooth muscle tone and cause mucosal edema and glandular secretion, resulting in the narrowing of the airways and limited air flow. In nonasthmatics, bronchial activity to histamine was limited, most likely due to fewer H1Rs in airway smooth muscle[16]. As histamine and its receptor's agonists act many parts of the body, but the literature on the effects of histamine and its receptor's agonists on respiratory epithelium are either not available or are very few. Therefore, the present study was undertaken to see the histological changes occurring in the respiratory epithelium and lung due to use of histamine and its receptor's agonists.

2. Materials and methods

2.1. Experimental design

To evaluate the histopathological changes, 30 New Zealand adult healthy albino rabbits of either sex weighing (1.29 ± 0.21) kg were divided into six groups. Each group contained five rabbits. Group-I was vehicle-treated (Vehicle is sterile distilled water) control group. Experimental groups of group-II, group-III, group-IV, group-V, and group-VI was treated with histamine, H1R-agonist, H2R-agonist, H3R-agonist, and H4R-agonist respectively.

They were housed in well-maintained animal facility at Central Animal House of J. N. Medical College & Hospital, Aligarh Muslim University, Aligarh, in the bioresources unit under a 12 hr light/dark cycle, at temperature of (22±2) ℃ and were fed on standard laboratory diet including fresh green vegetables and clean tap water. All studies were carried out during the light cycle and were approved by the Institutional Animal Ethical Committee.

2.2. Drugs

In the present study, following drugs were used: histamine dihydrochloride from Himedia Laboratories Pvt Limited, India; H1R-agonist (histamine trifluoro-methyl toluidide, HTMT-dimaleate), H2R-agonist (amthamine dihydrobromide), H3R-agonist (R-[-]- α -methylhistamine dihydrobromide) and H4R-agonist (clobenpropit dihydrobromide) which were kindly donated by Tocris Bioscience, Tocris Cookson Ltd., United Kingdom. All doses were referred to the weight of the salts used.

2.3. Doses

Histamine dihydrochloride (100 μg/kg) and other agonists (HTMT-dimaleate, amthamine dihydrobromide, R-[-]- α -methylhistamine dihydrobromide, clobenpropit dihydrobromide) (10 μg/kg) were administered subcutaneously (s.c.) twice a day for 30 subsequent days.

2.4. Histopathological analysis

For histopathological examination, rabbits from both experimental and control group were sacrificed by high dose ether anesthesia, after completion of drug treatments. Animals were immediately perfused with 10% freshly prepared formalin. Respiratory tract including trachea, bronchi and lungs dissected out and small blocks of tissue from different parts were processed for paraffin embedding. 10 micrometer thick sections were cut with the help of rotatory microtome and stained with haematoxyline and eosin (H&E stain). Light microscopic observations were recorded in sample photomicrographs using trinocular microscope (Olympus-B×40, Japan).

3. Results

Histological examination of trachea(Figure 1) showed patches of hyperplasia and hypertrophy (1B, 1C & 1F) which was less obvious in H2R- and H3R-agonist treated groups (1D & 1E). The lining cells in the affected region were taller and appeared more densely packed. These regions were not associated with aggregation of mucosa associated lymphoid tissues. Both extra- and intra-pulmonary bronchial epithelial lining (Figure 2) from experimental groups (2B to 2F) receiving histamine, H1R-through H4R-agonist treated showed usually localized areas of hyperplasia and hypertrophy along its epithelial lining. The longitudinal(folds) in the mucosal lining were also more prominent as compared to control (2A). These affected regions did not show any unusual aggregation mucosal associated lymphoid tissue. Cells in these regions appeared taller and heaped up closely. Lining epithelium of bronchi (Figure 3) of almost all sizes showed varying degree of hyperplasia and hypertrophy throughout its entire surface. As compared to control (3A) where lining cells were prominent and mucosa was thrown into longitudinal folds, the cell were arranged in a simple manner, those in the experimental groups (3B to 3F), lining cells appeared stratified. Most of cells appear oval or round with abundant cytoplasm. At places the supranuclear cytoplasm was very much abundant, cells were heaped over one another and places appeared to bud out from the basal cells.

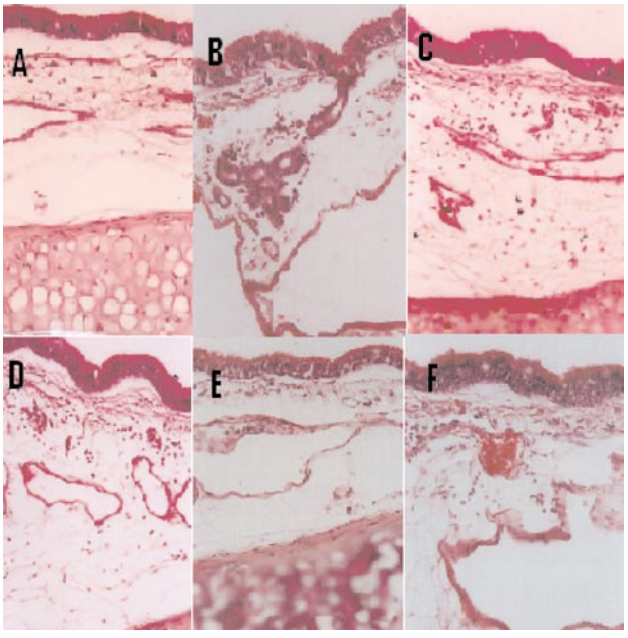

Figure 1. Photomicrographs of trachea from control (A) and experimental groups - histamine, H1R-agonist through H4R-agonist treated (B to F) respectively showing focal hypertrophy and hyperplasia of epithelial lining. The effect was less marked in H2R- and H3R-agonist treated groups. H & E stain, × 200.

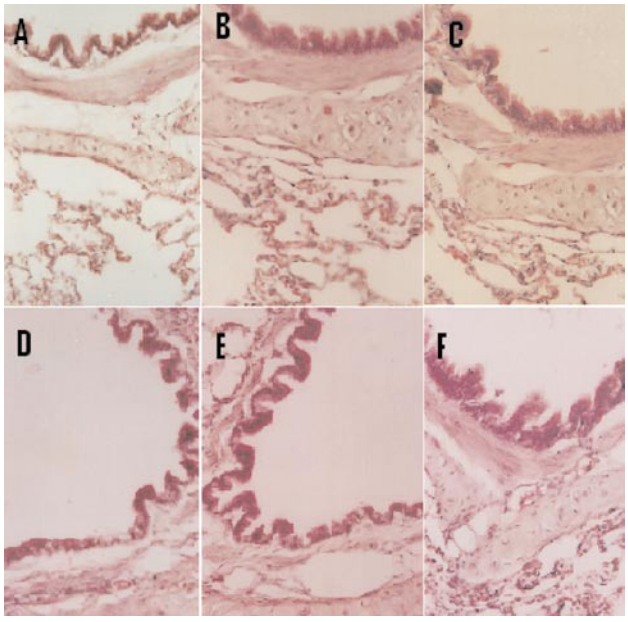

Figure 2. Photomicrographs of bronchi from control (A) and experimental groups – histamine, H1R-agonist through H4R-agonist treated (B to F) respectively showing focal hypertrophy and hyperplasia of epithelial lining. The effect was less marked in H2R- and H3R-agonist treated groups. H & E stain, × 200.

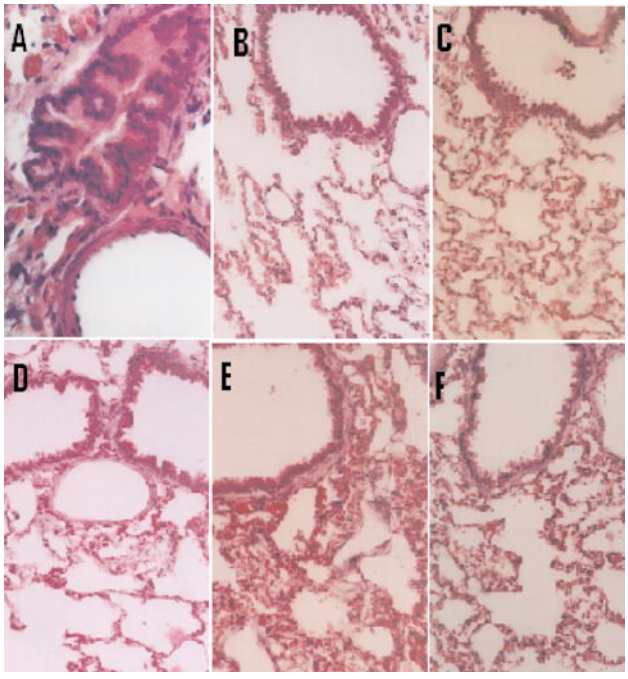

Figure 3. Photomicrographs of bronchioles from control (A) and experimental groups- histamine, H1R-agonist through H4R-agonist treated (B to F) respectively showing focal hypertrophy and hyperplasia of epithelial lining. The effect was less marked in H2R- and H3R-agonist treated groups. H & E stain, × 200.

4. Discussion

Various experimental studies (*in vivo* and *in vitro*, both normal and pathological conditions) on histamine have revealed its diverse effects on different types of tissues and in different condition. Observations in the present study suggest that histamine and its receptor's agonists in general have a differential growth promoting and mitogenic effects on the epithelium of both larger (trachea and bronchi) and small (bronchioles) respiratory tract. These observations and views can be supported by many studies which suggest that it induces endothelial cells to synthesize vascular smooth muscle relaxants including prostacycline and nitric oxide which cause vasodilation[17], increases epidermal growth factor (EGF) concentrations of salivary and gastric juice[18], act as autocrine growth factor[19], plays an important role in the development of keloid which is a progressively enlarging scar due to excessive collagen formation in the dermis[20], stimulates the growth of glioma cells *in vitro*[21], stimulates promyelocytic leukemia HL60 cell production of TPA-dependent hepatocyte growth factor[22], acts as growth factor and chemoattractant for human carcinoma and melanoma cells[23], stimulates the proliferation of human articular chondrocytes *in vitro* and is expressed by chondrocytes in osteoarthritic cartilage[24] and causes mitogenesis in intact rat tissue[25]. There are findings of other studies suggest that histamine and its receptor's agonist induce degeneration and necrosis of hepatocytes[26], cause degeneration of Purkinje cells in cerebellum[27]. These findings apparently do not correlate with findings in the present study. However, it has been shown that in addition to induction of hepatocellular degeneration of various intensities histamine and its receptor's agonist also induce multinuclearity in the hepatocytes[26] which is also believed to be a sign of regeneration.

From the above study it was concluded that histamine and its receptor's agonists have differential effects on the respiratory epithelium resulting into hypertrophy and hyperplasia warranting a further long-term study to ascertain their possible beneficial or potentially harmful effects on the respiratory epithelium.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

Trivendra Tripathi acknowledges University Grants Commission, New Delhi, India for providing UGC Fellowship [UGC letter DON F. 19-33/2006 (CU)]. The authors wish to thank Tocris Bioscience, Tocris Cookson Ltd. (United Kingdom) for kindly donating highly potent and specific histamine receptors-agonists and Prof. Abida Malik, Incharge, Central Animal House, J.N.M.C., Aligarh Muslim University, Aligarh for providing animal facilities.

References

[1]Metcalfe D, Baram D, Mekori Y. Mast cells. *Physiological Rev* 1997; **77**:1033-79.

[2]Shahid M, Tripathi T, Sobia F, Moin S, Siddiqui MU, Khan RA.

Histamine, histamine receptors, and their role in immunomodulation: an updated systematic review. *The Open Immunology J* 2009; **2**: 9-41.
[3]Jean-Francois B. *Immunology*. New York: John Wiley & Sons; 1982.
[4]Sompayrac Lauran. *How the immune system works*. Malden: Blackwell Science Ltd;1999.
[5]Huang J-F, Thurmond RL. The new biology of histamine receptors. *Curr Allergy Asthma Rep* 2008; **8**:21-27.
[6]Thurmond RL, Gelfand EW, Dunford PJ. The role of histamine H1 and H4 receptors in allergic inflammation: the search for new antihistamines. *Nature Rev Drug Dis* 2008;**7**:41-53.
[7]Tripathi T, Shahid M, Khan HM, Khan RA, Siddiqui MU, Malik A. Effect of histamine on antibody generation profile *in vivo*. *Global J Applied Physiology Allied Sciences* 2009; **1**:26-31.
[8]Tripathi T, Shahid M, Khan HM, Khan RA, Siddiqui MU. Dose-dependent effect of histamine on antibody generation *in vivo*. *Asian Pacific J Tropical Medicine* 2010;**3**:112-6.
[9]Tripathi T, Shahid M, Khan HM, Negi MPS, Siddiqui MU, Khan RA. Modulation of in vivo immunoglobulin synthesis by endogenous histamine and by H1R- and H2R- agonists/antagonists. *Pharmacological Reports* 2010; In Press.
[10]Tripathi T, Shahid M, Raza A, Khan HM, Siddiqui MU, Khan RA, et al. *In vivo* comparative immunotoxic study of histamine receptors (H1R, H2R, H3R and H4R)-agonist. *Eastern Journal of Medicine* 2010; In Press.
[11]Yamashita M, Fukui H, Sugama K, Horio Y, Ito S, Mizuguchi H, et al. Expression cloning of a cDNA encoding the bovine histamine H1 receptor. *Natl Acad Sci USA* 1991; **88**:11515-9.
[12]Gantz I, Schäffer M, DelValle J, Logsdon C, Campbell V, Uhler M, et al. Molecular cloning of a gene encoding the histamine H2 receptor. *Natl Acad Sci USA* 1991; **88**:429-33.
[13]Lovenberg TW, Roland BL, Wilson SJ, Jiang X, Pyati J, Huvar A, et al. Cloning and functional expression of the human histamine H3 receptor. *Mol Pharmacol* 1999; **55**:1101-7.
[14]Nguyen T, Shapiro DA, George SR, Setola V, Lee DK, Cheng R, et al. Discovery of a novel member of the histamine receptor family. *Molecular Pharmacol* 2001; **59**:427-33.
[15]Rang H, Dale M, Ritter J, Gardner P. *Pharmacology*. New York: Churchill Livingstone;1995,p.226-9.
[16]Goldie RG. Receptors in asthmatic airways. *Am Rev Respir Dis* 1990;**14**:S151-6.
[17]Abbas A, Lichtman A, Pober J. *Cellular and molecular immunology*. Philadelphia, PA: W. B. Saunders Company; 1994,p.287.
[18]Tunio AM, Hobsley M. Epidermal growth factor in saliva and gastric juice: response to histamine. *Gut* 1995;**37**:335-9.
[19]Rivera ES, Cricco GP, Engel NI, Fitzsimons CP, Martín GA, Bergoc RM. Histamine as an autocrine growth factor: an unusual role for a widespread mediator. *Seminars Cancer Biol* 2000; **10**:15-23.
[20]Kikuchi K, Kadono T, Takehara K. Effects of various growth factors and histamine on cultured keloid fibroblasts. *Dermatology* 1995;**190**:4-8.
[21]Van der Ven LT, Van Buul-Offers SC, Gloudemans T, Roholl PJ, Sussenbach JS, Den Otter W. Histamine-stimulated expression of insulin-like growth factors in human glioma cells. *Br J Cancer* 1997; **75**:1226-45.
[22]Suzuki M, Matsumoto K, Nakamura T, Nakano K. Stimulation by histamine of TPA-dependent hepatocyte growth factor production in promyelocytic leukemia HL-60 cells. *Biosci Biotechnol Biochem* 1998;**62**:1399-402.
[23]Tilly BC, Tertoolen LG, Remorie R, Ladoux A, Verlaan I, de Laat SW, et al. Histamine as a growth factor and chemoattractant for human carcinoma and melanoma cells: action through Ca^{2+}-mobilizing H1 receptors. *J Cell Biol* 1990;**110**:1211-5.
[24]Tetlow LC, Woolley DE. Histamine stimulates the proliferation of human articular chondrocytes in vitro and is expressed by chondrocytes in osteoarthritic cartilage. *Ann Rheum Dis* 2003;**62**:991-4.
[25]Norrby K. Evidence of mast-cell histamine being mitogenic in intact rat tissue. *Agents Actions* 1985;**16**:287-90.
[26]Tripathi T, Khan AA, Shahid M, Khan HM, Siddiqui MU, Khan RA, et al. Hepatotoxicity due to histamine trifluoro-methyl toluidide, amthamine, R-(-)- α - methyl histamine and clobenpropit (H1R-H4R-Agonists, respectively) in rabbit experimental model. *Am Med J* 2010;**1**:1-7.
[27]Rotter A, Frostholm A. Cerebellar histamine-H1 receptor distribution: an autoradiographic study of Purkinje cell degeneration, staggerer, weaver and reeler mutant mouse strains. *Brain Res Bull* 1986;**16**:205-14.