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Activation of histamine H_3 receptors in human nasal mucosa inhibits sympathetic vasoconstriction

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Abstract

The peripheral histamine H_3 receptor is a presynaptic heterologous receptor located on postganglionic sympathetic nerve fibers innervating sympathetic effector systems such as blood vessels and the heart. An extensive body of evidence shows that activation of the histamine H_3 receptor attenuates sympathetic tone by presynaptic inhibition of noradrenaline release. It is proposed that this sympathoinhibitory action, in vivo, leads to reduced vasoconstriction, thereby eliciting a vasodilatory effect.

In humans, the peripheral histamine H_3 receptor has also been shown to exert a sympathoinhibitory function on specific peripheral autonomic effector systems. For example, human saphenous vein and heart possess functional presynaptic histamine H_3 receptors on the sympathetic nerve terminals that upon activation decrease the sympathetic tone to these respective organs. The present studies were conducted to define the role of histamine H_3 receptors on neurogenic sympathetic vasoconstrictor responses in human nasal turbinate mucosa. Contractility studies were conducted to evaluate the effect of histamine H_3 receptor activation on sympathetic vasoconstriction in surgically isolated human nasal turbinate mucosa. We found that the histamine H_3 receptor agonist, (*R*)-α-methylhistamine (30 and 300 nM), inhibited electrical field stimulation-induced (neurogenic) sympathetic vasoconstriction in a concentration-dependent fashion. Pretreatment with the selective histamine H_3 receptor antagonist, clobenpropit (100 nM), blocked the sympathoinhibitory effect of (*R*)-α-methylhistamine on the neurogenic sympathetic vasoconstriction. In addition, analysis of Taqman mRNA expression studies showed a specific, high level of distribution of the histamine H_3 receptor localized in the human nasal mucosa.

Taken together, these studies indicate that histamine H_3 receptors modulate vascular contractile responses in human nasal mucosa most likely by inhibiting noradrenaline release from sympathetic nerve terminals in nasal mucosa. It is further suggested that histamine H_3 receptors may play a role in the regulation of vascular tone and nasal patency in histamine-dependent allergic nasal congestive disease.
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1. Introduction

Allergic rhinitis is an inflammation of the nasal mucosa characterized by rhinorrhea and swelling of the mucosal membranes lining the nasal cavity leading to congestion. Other symptoms of the disease include mucous secretion, sneezing and pruritis. Untreated allergic rhinitis often leads to sinusitis, aggravation of bronchial asthma, nasal polyps and otitis media (Corey et al., 2000). In the nose, histamine derived from mast cells mediates the immediate hypersensitivity reaction that occurs due to antigen exposure in allergic individuals (Kaliner, 1994; Howarth, 1995; Baraniuk, 1998). These events can be triggered by common perennial or seasonal allergens such as house dust, animal dander, mold spores, and pollen. Mast cells play a central response in this proinflammatory cascade to promote the allergic response. Upon antigen cross-linkage of immunoglobulin E (IgE), activated mast cells degranulate and release their stored mediators (Christodoulopoulos et al., 2000).

Locally released histamine exerts a myriad of effects due to the activation of pharmacologically distinct histamine receptors, namely H_1, H_2, H_3 and H_4. In the nose, histamine

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activates postjunctional histamine H_1 receptors, which elicits an increase in vascular permeability, mucus production and itch, all prominent features of acute allergic rhinitis. While H_2 and H_4 receptors do not appear to play a significant role in autonomic regulation of nasal patency, activation of prejunctional histamine H_3 receptor by either exogenous or endogenous mast-cell derived histamine has been shown to inhibit sympathetic vasoconstrictor tone in isolated porcine nasal mucosa indicating that these receptors subserve an important vasomodulatory role in this vascular bed (Varty and Hey, 2002).

There is an extensive body of evidence showing that histamine H_3 receptors modulate sympathetic and parasympathetic function broadly in the peripheral autonomic system (Coruzzi et al., 1991; Koss and Hey, 1992; Hey et al., 1992; Göthert et al., 1995). Regarding the regulation of sympathetic vascular tone, functional inhibitory prejunctional histamine H_3 receptors are located on postganglionic sympathetic nerve terminals. Specifically, functional prejunctional histamine H_3 receptors innervating the arteries, veins and heart have been identified in various species including guinea pigs, dogs and humans (Luo et al., 1994; Imamura et al., 1995; Valentine et al., 1999; Yamasaki et al., 2001). Less is known, however, about the role human histamine H_3 receptors in regulation of sympathetic effector responses in specific vascular beds such as the nasal mucosa.

The present investigations were conducted to determine whether histamine H_3 receptors are functionally expressed and contribute to the regulation of electrogenic sympathetic vasomotor responses in human nasal mucosa. Further studies to characterize the distribution and relative expression of the histamine H_3 receptor mRNA was also performed using Taqman expression analysis to provide additional information as to the specific location of the peripheral H_3 receptors. The present findings indicate that histamine H_3 receptors are abundantly expressed in human turbinate nasal mucosa. Furthermore, activation of histamine H_3 receptors also mediates a significant sympathoinhibitory action of neurogenic vasoconstriction in human nasal mucosa.

2. Materials and methods

2.1. Tissue preparation of human nasal turbinate

Turbinate strips were prepared from isolated human inferior turbinate samples. Nasal surgeries were conducted to clear nasal obstructions due to chronic nasal congestion, turbinate hypertrophy, or allergic rhinitis. Human turbinate was provided by Institut ORL de Montréal (Montréal, Canada). Tissue samples were obtained from male and female patients (14–59 years), shipped on wet ice in supplemented RPMI (Gibco, Grand Island, NY, USA) and received ≤ 48 h after removal. Turbinate strips were cut to a length of 7–12 mm and 3–5 mm wide. Turbinate mucosa strips were tied with 6-0 silk at each end and attached to tissue supports. Tissue supports were used to anchor each tissue in a 25-ml bath (Radnoti Glass Technology, Monrovia, CA, USA). Tissues were then attached to Grass FT-03 model force displacement transducers (AstroMed, West Warwick, RI, USA) for continuous recording of isometric tension. Passive tension of 1*g* was given to each tissue at the start of the 1-h equilibration. Tissue responses were recorded using an AstroMed recorder (model K2G, AstroMed).

2.2. Functional bioassay

Baths were filled with 37 °C Krebs' buffer (118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 24.9 mM $NaHCO_3$, 11.1 mM glucose, 2.55 mM $CaCl_2$), continuously aerated with 95% O_2/5% CO_2 gas. Chlorpheniramine (1 μM) and cimetidine (1 μM) were added to the buffer to block histamine H_1 and H_2 receptor mediated effects, respectively. Tissues were equilibrated and washed twice at 1.0 g initial tension for 1 h. Tissue responsiveness was then tested with noradrenaline (100 μM) after 1 h followed by three additional washings. Only tissue strips that responded to noradrenaline were used in these experiments. Studies to evaluate the effects of antagonists on (*R*)-α-methylhistamine-induced inhibition of sympathetic responses in human turbinate strips were performed as follows: (*R*)-α-methylhistamine, histamine H_3 receptor agonist, was tested against electrically induced prazosin-sensitive, sympathetic contractions. Voltage was adjusted accordingly to produce contractile responses that were approximately 40% of the maximum electrically field stimulation-induced contraction response. Antagonist equilibration time was 1 h before the start of (*R*)-α-methylhistamine challenges. Electrical field stimulation of human nasal mucosa was run for 30 min in the absence or presence of (*R*)-α-methylhistamine, followed by 30 min in the presence of prazosin (1 μM) (Varty and Hey, 2002).

2.3. Taqman gene expression

2.3.1. Human tissue panels

Human tissue autopsy samples were purchased from Zoion Diagnostics (Shrewsbury, MA). Postmortem times for tissue collection ranged from 2 to 6 h. Total RNA was isolated from the tissues using TRI-reagent (MRC, Cincinnati, OH), and tested for quality and quantity using an Agilent 2100 Bioanalyzer (Waldbroun, Germany). Tissues from three donors were used in the analysis, and included an array of 36 peripheral tissues.

2.3.2. Quantitative PCR

Taqman™ primers and probes were designed with Primer Express software (ABI), and purchased from ABI. The probes were designed away from regions of homology

with the other three histamine receptors. The fluorogenic probes were labeled with 6-carboxyfluorescein (6FAM) as the reporter and 6-carboxy-4,7,2,7′ -tetramethylrhodamine (TAMRA) as a quencher. The sequences of the primers and probes for H_3 used were as follows, with the numbers corresponding to the open reading frame of Genbank accession AF140538: forward primer 1030, TTCACC-CAGCGCTTTCG; reverse primer 1104, CCCAAA-GATGCTCACGATGA; Taqman probe 1058, ACAG-GAAAGTGGCCAAGTCGCTG. Quantitative PCR was carried out with an ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). The PCR reactions were prepared using the components from the Invitrogen Platinum Quantitative RT-PCR One-Step kit and assembled according to the manufacturer's instructions (Invitrogen). The final concentrations of the primers and probe in the PCR reactions were 200 and 100 nM, respectively. In addition, 0.25 μl of a passive reference dye (ROX, Invitrogen) was added to each reaction, and each 12.5 μl PCR reaction contained 2.5 μl (25 ng) of total RNA prepared as described above. The RT-PCR reactions were performed in a single 384-well plate according to the following protocol: one cycle for 30 min at 48 °C, followed by one 20 min cycle at 95 °C, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. Separate plates of the same RNAs were used to quantitate 18S RNA as an internal control for RNA quality, and a primer/probe set for the CD4 promoter was used to check the RNAs for genomic contamination.

The PCR data was quantitated based on a standard curve generated using serial dilutions of a plasmid DNA for the H_3 receptor. Fourfold dilutions began at 0.25 ng, and eight dilutions were used to generate the standard curve. The quantities were then calculated based on the standards and converted to fg/reaction. The data from the three donors was averaged and standard errors calculated.

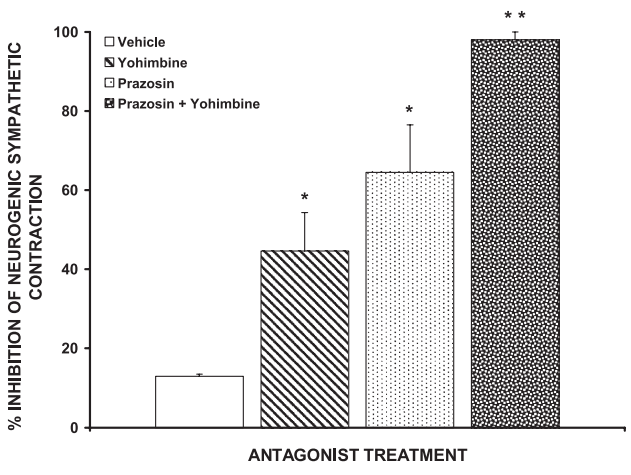

Fig. 1. Inhibitory effect of α_1 and α_2 adrenoceptor antagonists on electrical field stimulation-induced contractions of isolated human nasal mucosa. Bars represent means ± S.E.M. of yohimbine (1 μM, $n=4$), prazosin (1 μM, $n=5$), combined yohimbine + prazosin (each at 1 μM, $n=5$) or vehicle ($n=4$).

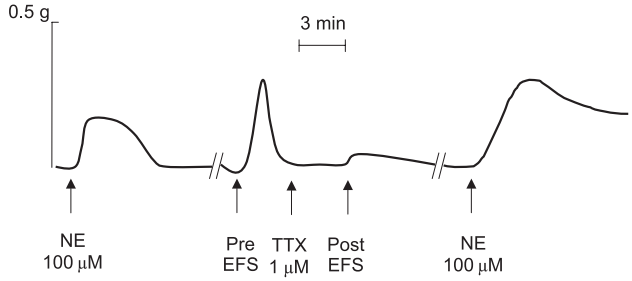

Fig. 2. Representative polygraph of electrical field stimulation-induced contractile response in isolated human nasal mucosa. Pre- and post-treatment effect tetrodotoxin on electrical field stimulation is shown. Addition of exogenous noradrenaline elicited a contractile response previous to and following the tetrodotoxin (1 μM) treatment.

2.4. Data analysis and statistics

Data was taken as percent inhibition of the noradrenergic (prazosin-sensitive) portion of electrical field stimulation. Activity in the functional bioassay was expressed as a percent of antagonist blockade of the (*R*)-α-methylhistamine-induced inhibition of the electrical-field stimulation contractile responses. Values displayed in the table and the figures represent the mean ± S.E.M. Statistical significance is achieved with $P<0.05$ using a nonparametric Kruskal–Wallis analysis of variance in conjunction with a Mann–Whitney *U*-test.

2.5. Materials

Noradrenaline, (*R*)-α-methylhistamine, clobenpropit, cimetidine, chlorpheniramine, tetrodotoxin and prazosin were

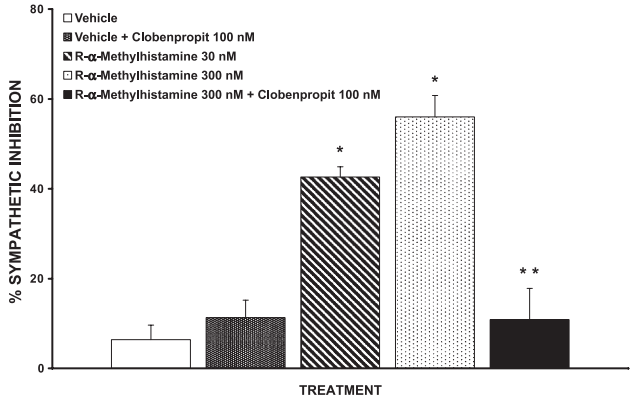

Fig. 3. Inhibition of electrical field stimulation-induced contraction in human nasal mucosa due to (*R*)-α-methylhistamine. Blockade with the histamine H_3 receptor antagonist clobenpropit on (*R*)-α-methylhistamine (300 nM) inhibition of electrical field stimulation-induced contraction in porcine mucosa. Bars represent the mean ± S.E.M. of individual tissue responses ($n=4-5$). (*) Significantly different from vehicle, (**) is significantly different from 30 and 300 nM (*R*)-α-methylhistamine; $p<0.05$.

obtained from RBI/Sigma (St. Louis, MO, USA). RPMI and L-15 were purchased from Gibco.

3. Results

We evaluated the inhibition of the electrical field stimulation-induced contraction with the α_1-antagonist prazosin and the α_2-antagonist yohimbine in human nasal mucosa. Yohimbine (1 μM), prazosin (1 μM) and the combination of both antagonists blocked the electrical field stimulation-induced contractions by 45%, 65% and 98%, respectively (Fig. 1). In addition, electrical field stimulation-induced contractions were abolished with tetrodotoxin (1 μM) a selective, neuronal Na^+ blocker, confirming that the electrically induced contractions were of neurogenic origin. Furthermore, tetrodotoxin did not affect contractility response due to exogenous noradrenaline (100 μM, Fig. 2).

In studies to characterize the effect of histamine H_3 receptor activation on sympathetic vasomotor contractions, tissues were treated with the selective histamine H_3 receptor agonist (*R*)-α-methylhistamine. (*R*)-α-methylhistamine inhibited electrical field stimulation-induced contraction at 30 and 300 nM by 43% and 56%, respectively (Fig. 3). Pretreatment with the histamine H_3-antagonist, clobenpropit (300 nM), blocked the (*R*)-α-methylhistamine inhibition (Fig. 3). Clobenpropit given alone did not affect the electrical field stimulation-induced contractions.

Studies performed using Taqman expression analysis of mRNA showed the peripheral distribution and relative expression of the histamine H_3 receptor (Fig. 4). The standard curve generated from the plasmid indicated that the primer and probe set behaved in the expected manner. In addition, the 18S and CD4 control probe sets demonstrated that the RNAs were of good quality and without genomic contamination. Among the peripheral human tissues tested, the highest level of expression was found in nasal mucosa, testes and joint tissue. In addition, low to moderate expression of the H_3 mRNA was observed in GI tract. In accordance with previously published observations, pituitary (0.874 ± 0.213 fg/25 ng cDNA) and brain tissue RNAs

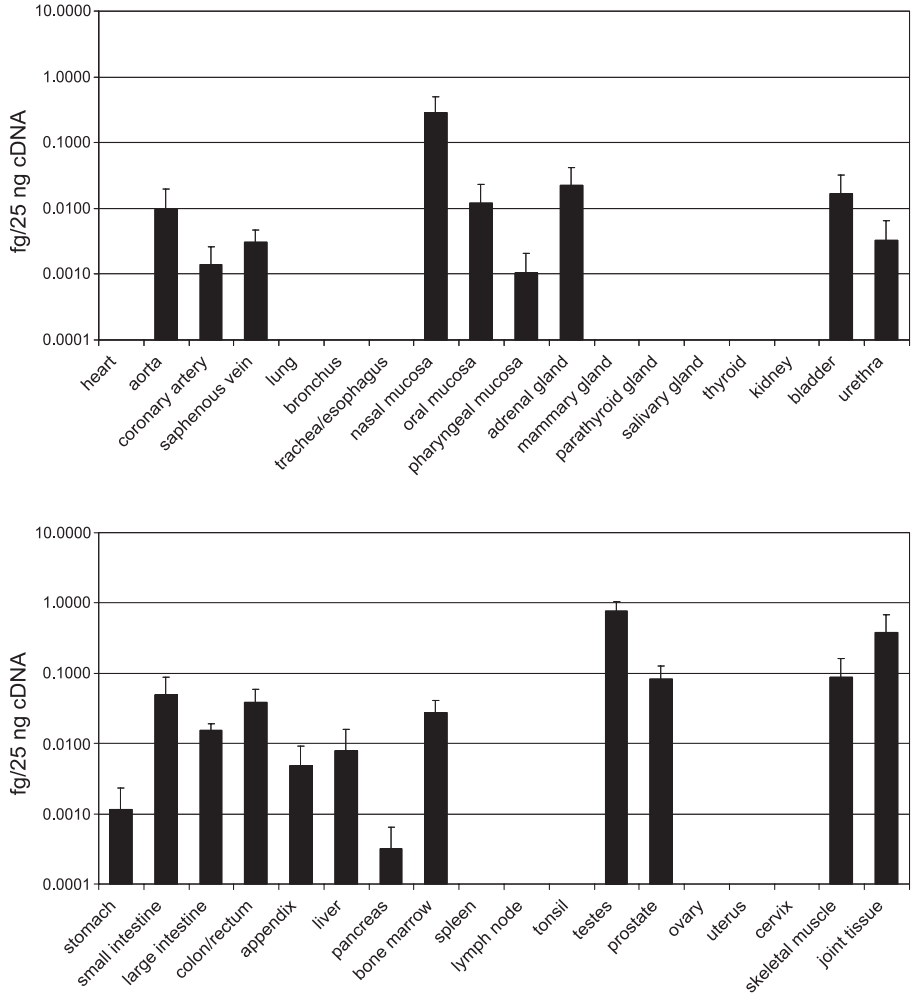

Fig. 4. Human Taqman expression analysis of mRNA showed the peripheral distribution and relative expression of the histamine H_3 receptor. Bars represent the mean ± S.E.M ($n = 3$).

(data not shown) exhibited very high levels of H_3 receptor mRNA.

4. Discussion

The present findings are the first to demonstrate the presence of functional histamine H_3-receptors in human nasal mucosa. Specifically, activation of these histamine H_3-receptors exerts a sympathoinhibitory effect on sympathetic vasoconstrictor contractions in this tissue. The findings that the selective histamine H_3-agonist, (*R*)-α-methylhistamine inhibited the electrical field-stimulation induced contractions in human nasal mucosa and the selective histamine H_3-receptor antagonist, clobenpropit, significantly blocked the (*R*)-α-methylhistamine's inhibitory action confirms that this action is mediated by histamine H_3-receptors. The present results in human nasal mucosa are also consistent with studies showing the presence of functional inhibitory prejunctional histamine H_3-receptors that modulate sympathetic vasoconstriction in pig nasal mucosa (Varty and Hey, 2002).

The presence of functionally active α_1-adrenoceptors that mediate neurogenic sympathetic vasoconstriction was also presently found. Postsynaptic α_1-adrenoceptors are activated by neurally released noradrenaline from sympathetic nerve endings. The α_1-adrenoceptor antagonist, prazosin, blocked the electrical field stimulation-induced contraction by about 60%. Prazosin, however did not produce a complete blockade of neurogenic sympathetic vasoconstriction. Addition of the α_2-adrenoceptor antagonist yohimbine (1 μM) also produced a partial blockade (approximately 40% blockade), suggesting that postjunctional α_2-adrenoceptors also contribute in the neurogenic sympathetic contractions in human nasal turbinate. Further confirmation that these neurogenic vasomotor responses are sympathetic in nature is provided by the finding that the combination of prazosin and yohimbine produced a complete inhibition of the electrical field stimulation-induced contractions. Similar to the present findings, postjunctional α_2-adrenoceptors were found to contribute to the electrical-filed stimulated neurogenic vasoconstriction with porcine nasal turbinates (Varty and Hey, 2002). Our results showed that pretreatment with yohimbine blocked the end organ neurogenic sympathetic contractions in human nasal mucosa by 45%. Interestingly, in contrast to the present human results, the magnitude of the postjunctional α_2-adrenoceptor component was smaller in the pig (approximately 10% of the neurogenic vasoconstriction), thus suggesting that postjunctional α_2-adrenoceptors may play a greater role in modulation of sympathetic vascular tone in human nasal blood vessels. The contribution of presynaptic α_2-adrenoreceptors to the neurogenic sympathetic response in nasal mucosa, which are known to mediate a feedback inhibitory effect on norepinephrine release from postganglionic sympathetic terminals, was not presently evaluated. In these studies, we used yohimbine, a nonselective α_2-adrenoreceptor antagonist that is not a suitable pharmacological tool to differentiate between presynaptic and postsynaptic α_2-adrenoreceptor subtypes. Additional pharmacological studies are thus needed to differentiate and define the relative contribution of the α_2-adrenoreceptor subtypes mediating prejunctional and postsynaptic effects in this vascular bed.

Tetrodotoxin (1 μM) (a selective sodium channel blocker that inhibits neuronal action potential propagation and neurotransmission) completely abolished the electrical field stimulation-induced contraction, but had no affect on the exogenous noradrenaline challenge. These findings support the presynaptic inhibitory site of action that takes place with peripheral histamine H_3-receptors on sympathetic nerve terminals (Göthert et al., 1984; Molderings et al., 1992; Valentine et al., 1999) and confirm that the vasoconstriction is of neural origin. These findings on the sympathoinhibitory role of human histamine H_3-receptors are also consistent with the in vitro studies in pig that show functional histamine H_3 receptors located presynaptically on the sympathetic nerve terminals of the pig nasal mucosa (Varty and Hey, 2002). Specifically, when activated the histamine H_3 receptors inhibit the electrical field stimulation-induced contraction of the pig nasal turbinate. These findings are also in agreement with previous reports of the histamine H_3 receptor-mediated inhibition of sympathetic nerve-derived noradrenaline release from sympathetic nerve terminals in human saphenous veins (Göthert et al., 1984; Molderings et al., 1992).

The human nose vasculature consists of a dense subepithelial network of capillaries, capacitance vessels or sinuses, and arteriovenous anastomoses. The capillaries supply nutrients to the epithelium and glands. When activated, prominent capacitance vessels or sinuses distend leading to swelling of the mucosal tissue. The action thereby causes blockade of the nasal cavity with resultant congestion. When empty, the capacitance vessels allow the nasal passages to be open and free breathing to occur. Arteriovenous anastomoses allow the rapid passage of blood through the mucosa and are presumed to be involved in air temperature conditioning (Widdicombe, 1997). Each of these structures is important in nasal function since they are activated by mediators such as histamine, a known cause of vasodilation (Widdicombe, 1997).

Functional histamine H_3-receptor have been reported on peripheral autonomic nerves innervating sympathetic effector systems, such as the blood vessels of human nasal mucosa. Present studies with Taqman mRNA expression confirm this, showing specific, high level of distribution of the histamine H_3-receptor message specific to the human nasal mucosa. Substructure analysis within the nasal mucosa of these data remains to be defined.

The role of the histamine H_3-receptor on the regulation of nasal patency has also been explored in experimental models of congestion. In vivo studies in an experimental feline congestion model shows that histamine H_3-receptor

antagonists, when given in combination with histamine H_1-receptor antagonists, blocks nasal congestion elicited by the histamine releasing agent compound 48/80 (McLeod et al., 1999). More recently, nasal acoustic rhinometry studies in the cat also showed that the combination of selective histamine H_3 antagonist, SCH 79687 and the histamine H_1-receptor antagonist loratadine, blocked the procongestive effects of compound 48/80 (McLeod et al., 1999, 2003). It is proposed that the activation of the histamine H_3-receptor, by endogenously released histamine, causes dilation of the blood vessels leading to congestion. These findings in combination with Taqman gene expression in human nasal tissue support the functional role of histamine H_3-receptors in human nasal mucosa.

There is a significant body of evidence showing that activation of prejunctional H_3-receptors on sympathetic nerve endings inhibits the release of noradrenaline from the nerve terminals, thus eliciting an inhibitory modulation of the tone of the vasculature. Specifically, activation of prejunctional histamine H_3-receptors on renal sympathetic nerve endings was found to inhibit noradrenergic transmission in anesthetized dogs (Yamasaki et al., 2001). A prominent role for activation of peripheral histamine H_3-receptors on sympathetic nerve terminals contributing to cardiovascular collapse has been demonstrated in a canine model of sepsis (Li et al., 1998; Cheng et al., 2002). In these studies, histamine H_3-receptor activation, most likely by circulating levels of histamine that occur in septic plasma sufficient to activate H_3-receptors (Li et al., 1998), was found to mediate a decreased in sympathetic function leading to cardiovascular collapse. A similar mechanism of histamine H_3 mediated decreases in sympathetic tone was found to depress basal vascular resistance in guinea pigs (McLeod et al., 1993) and mediate hypotension (Hey et al., 1992). Also, the histamine H_3-receptor may play an important role in the regulation of vascular tone and nasal resistance (Baraniuk, 1998). For example, H_3 receptor agonist (*R*)-α-methylhistamine inhibits sympathetic control of nasal blood flow and promotes increased nasal resistance in the cat (Hey et al., 1998; McLeod et al., 2001). Thus, the vasodilatation that occurs passively due to inhibition of sympathetic tone to nasal vasculature in turn causes the mucosal swelling, which leads to nasal congestion. Consistent with this hypothesis, combination treatment of histamine H_3 antagonists and histamine H_1 antagonist have been shown to display decongestant activity in mast cell dependent experimental feline model of nasal congestion (McLeod et al., 1999, 2003). It is thus suggested that this mechanism may contribute to the pathophysiology of allergic congestion.

In summary, the present results show that activation of human histamine H_3-receptor in nasal mucosa modulates sympathetic vasoconstriction by a prejunctional inhibitory H_3-receptor mechanism. It is further suggested that these functional histamine H_3-receptors may play an important role in the modulation of sympathetic nasal vascular tone and nasal patency.

References

Baraniuk, J.N., 1998. Mechanisms of Rhinitis. Allergy Asthma Proc. 19, 343–346.

Cheng, Z.-Q., Bose, D., Jacobs, H., Light, R.B., Mink, S.N., 2002. Sepsis causes presynaptic histamine H_3 and α_2-adrenergic dysfunction in canine myocardium. Cardiovasc. Res. 56, 225–234.

Christodoulopoulos, P., Cameron, L., Durham, S., Hamid, Q., 2000. Molecular pathology of allergic disease II: Upper airway disease. J. Allergy Clin. Immunol. Mol. Mech. Allergy Clin. Immunol. 105, 211–223.

Corey, J.P., Houser, S.M., Ng, B.A., 2000. Nasal congestion: review of its etiology, evaluation and treatment. Ear Nose Throat 79, 690–693.

Coruzzi, G., Poli, E., Bertaccini, G., 1991. Histamine receptors in isolated duodenal muscle: H_3 receptors inhibit cholinergic neurotransmission. J. Pharmacol. Exp. Ther. 258, 325–331.

Göthert, M., Schicker, E., Hentrich, F., Rohm, N., Zerkowski, H.-R., 1984. Modulation of noradrenaline release in human saphenous vein via presynaptic α_2-adrenoceptors. Eur. J. Pharmacol. 102, 261–267.

Göthert, M., Garbag, M., Hey, J.A., Schicker, E., Schwatrz, E., Levi, R., 1995. New aspects of the role of histamine in cardiovascular function: identification, characterization and potential pathophysiological importance of H_3 receptors. Can. J. Physiol. Pharm. 73, 558–564.

Hey, J.A., del Prado, M., Egan, R.W., Kreutner, W., Chapman, R.W., 1992. Inhibition of sympathetic hypertensive responses in the guinea-pig by prejunctional histamine H_3-receptors. Br. J. Pharmacol. 107, 347–351.

Hey, J.A., Aslanian, R., Bolser, D.C., Chapman, R.W., Egan, R.W., Rizzo, C.A., Shih, N.Y., Fernandez, X., McLeod, R.L., West, R., Kreutner, W., 1998. Studies on the pharmacology of the novel histamine H_3 receptor agonist Sch 50971. Arzneim.-Forsch. 48, 881–888.

Howarth, P.H., 1995. The cellular basis for allergic rhinitis. Allergy 50, 6–10.

Imamura, M., Seyedi, N., Lander, H.M., Levi, R., 1995. Functional identification of histamine H_3-receptors in the human heart. Circ. Res. 77, 206–210.

Kaliner, M.A., 1994. The mediators of the early and late phases of allergic rhinitis. Adv. Prostaglandin Thromboxane Leukotriene Res. 22, 271–278.

Koss, M.C., Hey, J.A., 1992. Activation of histamine H_3 receptors produces presynaptic inhibition of neurally evoked cat nictitating membrane responses in vivo. Naunyn-Schmiedeberg's Arch. Pharmacol. 346, 208–212.

Li, X., Eschun, G., Bose, D., Jacobs, H., Yang, J.J., Light, R.B., Mink, S.N., 1998. Histamine H_3 activation depresses cardiac function in experimental sepsis. J. Appl. Physiol. 85 (5), 1693–1701.

Luo, X.X., Song, L., Jiang, Y.P., Tan, Y.H., 1994. Inhibition of sympathetic neurotransmission via NEM-sensitive H_3-receptors in the guinea pig vas deferens. Inhibition of sympathetic neurotransmission via NEM-sensitive H_3-receptors in the guinea pig vas deferens. Methods Find. Exp. Clin. Pharmacol. 16, 185–189.

McLeod, R.L., Gertner, S.B., Hey, J.A., 1993. Production by (*R*)-methylhistamine of a histamine H_3 receptor mediated decrease in basal vascular resistance in guinea pigs. Br. J. Pharmacol. 110, 553–558.

McLeod, R.L., Egan, R.W., Cuss, F.M., Bolser, D.C., Hey, J.A., 2001. Histamine H_3-antagonists. Prog. Respir. Res. 31, 133–136.

McLeod, R.L., Mingo, G.G., Herczku, C., DeGennaro-Culver, F., Shih, N.-Y., Egan, R.W., Hey, J.A., 1999. Combined histamine H_1 and H_3 blockade produces nasal decongestion in an experimental model of nasal congestion. Am. J. Rhinol. 13, 391–399.

McLeod, R.L., Rizzo, C.A., West Jr., R.E., Aslanian, R., McCormick, K., Bryant, M., Hsieh, Y., Korfmacher, W., Mingo, G.G., Varty, L., Williams, S.M., Shih, N.-Y., Egan, R.W., Hey, J.A., 2003. Pharmacological characterization of the novel histamine H_3-receptor antagonist *N*-(3,5-

Dichlorophenyl)-[[4-(1*H*-imidazol-4-ylmethyl]-urea (SCH 79687). J. Pharmacol. Exp. Ther. 305, 1037–1044.

Molderings, G.J., Weiβenborn, G., Schlicker, E., Likungu, J., Göthert, M., 1992. Inhibition of noradrenaline release from the sympathetic nerves of the human saphenous vein by presynaptic histamine H_3 receptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 346, 46–50.

Valentine, A.F., Rizzo, C.A., Rivelli, M.A., Hey, J.A., 1999. Pharmacological characterization of histamine H_3 receptors in human saphenous vein and guinea pig ileum. Eur. J. Pharmacol. 366, 73–78.

Varty, L.M., Hey, J.A., 2002. Histamine H_3 receptor activation inhibits neurogenic sympathetic vasoconstriction in porcine nasal mucosa. Eur. J. Pharmacol. 452, 339–345.

Widdicombe, J., 1997. Microvascular anatomy of the nose. Allergy 52 (40), 7–11.

Yamasaki, T., Tamai, I., Matsumura, Y., 2001. Activation of histamine H_3 receptors inhibits renal noradrenergic neurotransmission in anesthetized dogs. Am. J. Physiol., Regul. Integr. Comp. Physiol. 280, R1450–R1456.