Acta Oto-Laryngologica, 2011; 131: 1155–1159

informa
healthcare

ORIGINAL ARTICLE

Topical application of betahistine improves eustachian tube function in an animal model

BURKHARD FRANZ, ANTHONY D. SHAFTON & COLIN R. ANDERSON

Department of Anatomy and Cell Biology, University of Melbourne, Victoria, Australia

Abstract

Conclusion: Betahistine dihydrochloride, a drug used widely in the systemic treatment of balance disorders such as Ménière's disease, was found to improve eustachian tube function when applied topically in the nasopharynx of rats. *Objectives:* The study tested the effect of betahistine, a histamine receptor agonist, on eustachian tube function and tested the involvement of H1 and H3 histamine receptors. *Methods:* Eustachian tube function was measured in anaesthetized rats while middle ear pressure was increased and then monitored during induced swallowing. Betahistine and other drugs were applied topically in the nasopharynx, bulla and epipharynx, and administered intraperitoneally. *Results:* Systemic application of betahistine hardly changed eustachian tube function, but topical application significantly improved it. The action of topical betahistine was unaffected by the HI receptor antagonist mepyramine and was mimicked by the H3 agonist, ciproxifan.

Keywords: *Middle ear, barotrauma, otitis media, Ménière's disease, H3 receptor, cyproxifan*

Introduction

Betahistine is a popular drug used in the management of balance disorders such as Ménière's disease. The exact mechanism of its action is still unclear. While it is often cited that the effect of betahistine is on histamine H1 receptors and is therefore similar to histamine itself (e.g. Taylor-Clark et al. [1]), it is only a weak H1 agonist and its strongest actions are as an antagonist at H3 receptors [2]. Betahistine appears to improve blood flow and hence oxygenation of the ear and to dampen central vestibular activity [3], both of which would be expected to relieve the symptoms of balance disorders. Histamine has an adverse effect on nasal airway [1] and eustachian tube patency [4], but it is unclear whether betahistine will have the same action. However, clinical experience does not reveal these effects in patients treated with betahistine. It suggests that betahistine may have a different action on the nasal airways or on the eustachian tubes, perhaps even a positive action that assists with the management of balance disorders. In this study we were interested in the effect of betahistine on active eustachian tube function in the rat, following application of the drug into the epipharynx, bulla or systemically.

Material and methods

Animal experiments were conducted on female and male adult Sprague Dawley rats weighing 250–450 g as described previously [5]. To monitor middle ear pressure, a polyethylene tube was inserted and sealed in the bulla with superglue. The polyethylene tube was connected to a pressure transducer connected to an AD Instruments data recording system to monitor pressure changes within the middle ear. For each experiment, the passive opening pressure was initially determined by increasing pressure in the bulla by a continuous air flow (30 ml/min) until the eustachian tube opened spontaneously. This value was recorded as the passive opening pressure of the eustachian tube. To measure the active behaviour of the eustachian tube, middle ear pressure was increased to 75% of the

Correspondence: Dr Colin Anderson, Department of Anatomy and Cell Biology, University of Melbourne, Parkville 3010, Australia.
E-mail: c.anderson@unimelb.edu.au

(Received 11 June 2011; accepted 7 July 2011)

ISSN 0001-6489 print/ISSN 1651-2251 online © 2011 Informa Healthcare
DOI: 10.3109/00016489.2011.607847

passive opening pressure and the superior laryngeal nerve was electrically stimulated with a bipolar hook electrode (1–3 V, 10 Hz, 500 ms) to induce swallowing. The drop in pressure brought about by a predetermined number of swallows was recorded and compared before and after drug treatment. An experiment consisted of three control runs followed by drug or vehicle administration and then repeated runs in the presence of the drug. Before each active run, 5 µl betahistine were injected into the bulla and then flushed through the eustachian tube (five animals), applied to the nasopharynx (five animals) or three injections of 1, 5 and 10 µl Vasomotal were cumulatively injected peritoneally (five animals). Five animals were treated with 5 µl normal saline applied to the nasopharynx and in five animals 5 µl normal saline were injected into the bulla. The latter served as a control. From previous experiments it was known that normal saline applied to the bulla had the effect of improving eustachian tube function [6]. Here we were interested to compare the effect with betahistine. In each animal femoral artery blood pressure was monitored using a pressure transducer. For each group of five animals, the raw data were first normalized with respect to the last control run before experimental manipulations and subjected to an arcsine transformation. The control and experimental results for each of the six measurements were compared using unpaired t tests, with $\alpha = 0.05$.

Results

The model of eustachian tube function used in this study relies on the generation of positive pressure in the middle ear of an anaesthetized rat and the measurement of the pressure drop when the animal swallowed after stimulation of the superior laryngeal nerve on one side [7]. This model has been described previously [5]. The salient feature of this model is that an improvement in eustachian tube function is represented by an increase in the amount of air shifted by a standard swallow, which is presented below as a percentage normalized to the control levels.

The initial experiments used a commercial liquid preparation of 8 mg/ml betahistine dihydrochloride designed for oral administration (Vasomotal). When this compound was applied within the tympanic bulla and allowed to penetrate into the eustachian tube, it significantly improved eustachian tube function by a mean of 29% (Figure 1), 2 min after application (paired t test, $t = 3.67$, $p = 0.010$, $n = 5$). The same dose of Vasomotal applied within the nasopharynx to the medial openings of the eustachian tubes had a similar action (increase 23.5%, paired t test, $t = 3.34$, $p = 0.02$, $n = 5$). To ensure that the effect observed was

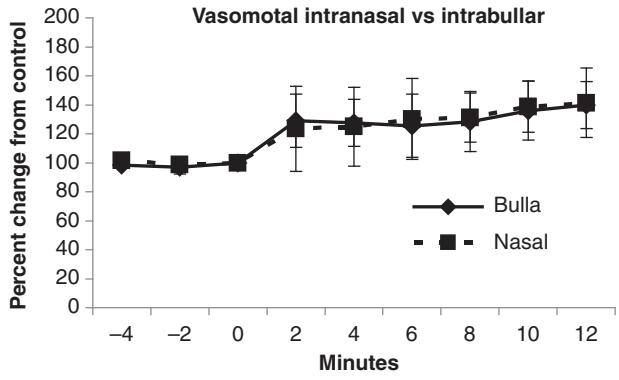

Figure 1. Effect of applying Vasomotal (5 µl of 8 mg/ml betahistine hydrochloride) into the nasopharynx (nasal) or within the tympanic bulla (bulla) of an anaesthetized rat. Both modes of delivery resulted in improved eustachian tube performance, with more total air passed during induced swallowing. Error bars are SEM, $n = 5$ in each case.

caused by a local effect of the topically applied Vasomotal, we injected increasing doses of Vasomotal intraperitoneally to test for systemic effects of the drug. Eustachian tube function was not significantly affected by systemic treatment with Vasomotal (maximum 4.5% increase after 18 min, t test, $t = 0.21$, $p = 0.423$, see Figure 2).

The initial experiments demonstrating a positive effect of Vasomotal on eustachian tube function were carried out in experiments over 30 min. To study the effects of betahistine over a longer timespan and to eliminate any potential confounding actions of the flavouring, stabilizing and antibacterial agents present in Vasomotal, we modified our model to use pure betahistine dihydrochloride in experiments lasting up to 2 h.

Like Vasomotal, pure betahistine dihydrochloride (5 µl of 4 mg/ml dissolved in phosphate-buffered

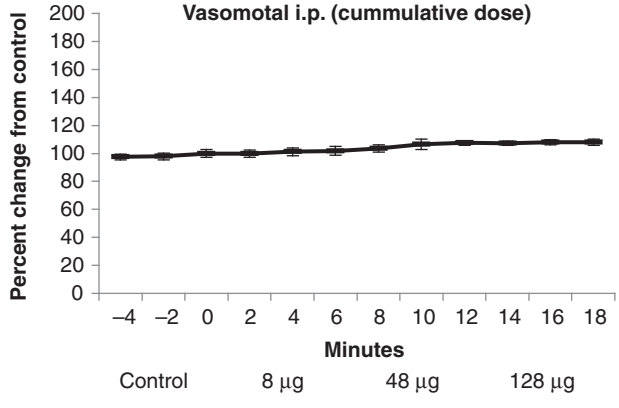

Figure 2. The effect of cumulative treatment with Vasomotal injected intraperitoneally (8 mg/ml betahistine hydrochloride) on eustachian tube function. No significant change in the amount of air transferred during swallowing occurred. The horizontal axis records the cumulative dose. Error bars are SEM, $n = 5$.

saline) caused a significant increase in eustachian tube function when applied to the nasopharynx of anaesthetized rats (63% increase at 3 min, paired *t* test, $p = 0.01$, $n = 7$) that was sustained for nearly 2 h (Figure 3). Doses of 2 and 8 mg/ml also improved eustachian tube function to a similar extent, but a dose of 1 mg/ml had no significant action.

To ascertain which histamine receptors betahistine, a weak H1 agonist and strong H3 antagonist [2] was working through, we pretreated animals with the HI antagonist, mepyramine (5 mg/kg i.v.) 10 min before betahistine application. In animals treated with mepyramine, betahistine still had a significant effect in improving eustachian tube function (54%, $p = 0.012$, paired *t* test, $n = 5$, Figure 4). Betahistine acts to inhibit H3 receptors and so is likely to be acting as an inverse agonist at these constitutively active receptors [8]. We tested the action of a known H3 inverse agonist, Ciproxifan, on eustachian tube function. A dose of 5 µl of 16 mg/ml cyproxifan applied into the nasopharynx in place of betahistine had a similar effect (77% increase, $p = 0.035$, paired *t* test, $n = 5$) to 4 mg/ml betahistine in improving eustachian tube function (Figure 5), suggesting that the effect of betahistine was mediated through the inhibition of constitutive H3 receptor activity.

Discussion

While betahistine is often described as a histamine agonist, its actions on the eustachian tube are quite different to histamine. Histamine has been reported to cause eustachian tube dysfunction [4] and increases nasal airway resistance [9]. These effects are presumed to occur by actions on H1 receptors, although

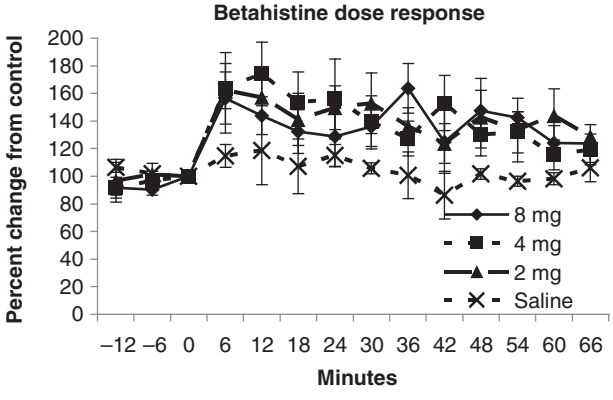

Figure 3. The effect of three doses of betahistine hydrochloride (volume10 µl) applied into the nasopharynx of an anaesthetized rat and compared to vehicle (saline). All three doses produced a similar effect with no sign of a dose response; 1 mg/ml betahistine was without significant effect (not shown). Error bars are SEM, n = minimum of 4 in each case.

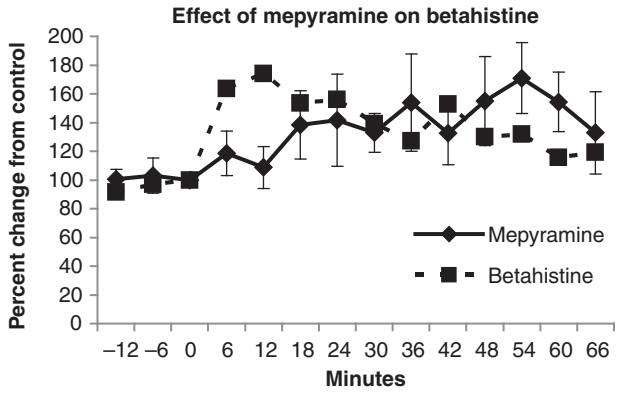

Figure 4. The effect of pretreating anaesthetized rats with the H1 antagonist mepyramine on the action of betahistine (10 µl of 4 mg/ml in nasopharynx), compared to the action of betahistine (10 µl of 4 mg/ml) alone (trace from Figure 3). Mepyramine blunts the intitial effect of betahistine but has no effect on the prolonged actionof betahistine. Error bars are SEM, $n = 5$.

HI receptor antagonists do not always reverse these effects [1]. In contrast, our results suggest that betahistine improves eustachian tube function. Betahistine is in fact only a weak agonist at H1 receptors, but a strong antagonist at H3 receptors [2] and we suggest that the actions we see on the eustachian tube are due the effect of antagonism of H3 receptors, rather than the weak agonist effect at H1 receptors. This conclusion is supported by the results of our experiments. Ciproxifan, an H3 antagonist like betahistine, but without H1 agonist actions [10], has an identical action to betahistine. Conversely, blockade of H1 receptors with mepyramine had no significant effect on the action of betahistine in our experiments.

The H3 histamine receptor is a relatively recently discovered receptor [11] and has a complicated pharmacological profile with around 20 isoforms of the receptor [12]. The H3 receptor also shows constitutive

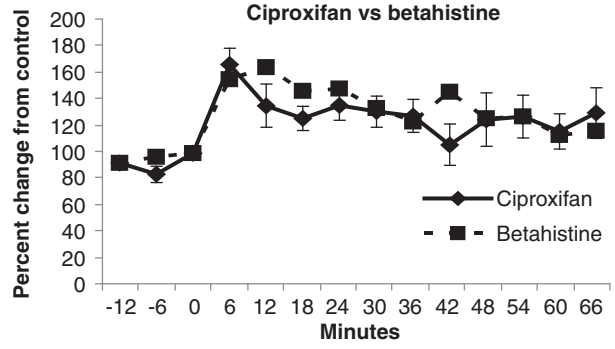

Figure 5. The ability of the H3 antagonist, Ciproxifan (10 µl of 16 mg/ml) to mimic the action of betahistine (10 µl of 4 mg/ml, trace from Figure 3) applied to the nasopharynx of an anaesthetized rat. The actions of ciproxifan are indistinguishable from that of betahistine. Error bars are SEM, $n = 7$.

activity. That is, it is active in the absence of ligand binding. When it does bind a ligand, the activity of the receptor can be increased (an agonist action) or decreased (an antagonist or inverse agonist action). Ciproxifan is an inverse agonist at H3 receptors and we assume, because of the identity of action, that betahistine is as well.

H3 receptors have been localized to the nasal mucosa of humans and rodents [13,14], where they are associated with sympathetic nerve endings [1] and submucosal glands [14] in the nasal mucosa. We have previously postulated that eustachian tube function can be altered by changes in the properties of secretions in the lumen of the eustachian tube [6]. Changes in surface tension or adhesion can alter the ease with which the eustachian tube opens [15].

In the nasal mucosa H3 receptors have been associated with allergic rhinitis. In mice, the effects of allergic rhinitis are reduced when H3 receptors are stimulated with an agonist [16]. In contrast, in humans, H3 antagonists alleviate the symptoms of allergic rhinitis [1].

Our hypothesis is that the action of betahistine is through H3 receptors and that it improves eustachian tube function by making the eustachian tube easier to open. This action could involve an inhibition of noradrenaline release from sympathetic nerve terminals in the nasal mucosa, although we have been unable to implicate the sympathetic system directly in controlling the eustachian tube [5]. Alternatively, H3 receptors have been found associated with submucosal glands of the nasal mucosa [14] and so the action of betahistine may be directly onto the source of secretions within the eustachian tube. H3 receptors are also reported to mediate direct vasodilation of blood vessels [17] and so a third possibility is that betahistine affects the vasculature supplying the nasal mucosa to bring about a change in the quality of the secretion and perhaps shrinkage of the nasal mucosa, both of which may favour easier opening of the eustachian tubes.

Betahistine applied to the nasopharyngeal openings of the eustachian tube presumably penetrates far enough into the closed section of the eustachian tube to alter adhesion and affect opening dynamics of the eustachian tube on swallowing. The same level of effect was observed when betahistine was placed in the middle ear and forced through the entire length of the eustachian tube by increasing the middle ear pressure until the eustachian tube opened passively. This suggests that the key area controlling eustachian tube opening is the nasopharyngeal end of the collapsed segment of the tube.

Topical applications of betahistine were not mimicked by systemic treatment with the same or higher doses of betahistine, indicating that betahistine's action when topically applied is local, rather than systemic after absorption from nasal mucosa or stomach. The highest betahistine dose tested systemically in the rat (0.77 mg/kg) is still ineffective and is twice a typical dose used systemically in humans to treat balance disorders (16 mg per dose or 0.23 mg/kg), suggesting that it is unlikely that betahistine could be used systemically to affect eustachian tube function in humans.

In this study we have not examined the action of H2 or H4 histamine receptors. Betahistine does not have an action at H2 receptors. H4 receptors are the most recently discovered of the histamine receptors and relatively little is known about them. They have been associated with immune tissues [18] and have been recently reported on neurons in the central nervous system (CNS) [19]. They have also been found on nerve fibres in the nasal mucosa, where they are co-localized with H3 receptors [20]. No information is available on the action of betahistine on H4 receptors.

The action of betahistine in improving eustachian tube function is novel and has clinical potential. No compounds exist that are used routinely to improve eustachian tube function. Betahistine has potential to treat disorders such as otitis media or barotrauma.

Declaration of interest: The University of Melbourne hold a patent for the use of betahistine as a topical treatment for eustachian tube disorder. B.F. and C.R.A. act as consultants to Otifex Pty Ltd who are developing the drug for clinical use.

References

[1] Taylor-Clark T, Sodha R, Warner B, Foreman J. Histamine receptors that influence blockage of the normal human nasal airway. Br J Pharmacol 2005;144:867–74.

[2] Arrang JM, Garbarg M, Quach TT, Dam Trung Tuong M, Yeramian E, Schwartz JC. Actions of betahistine at histamine receptors in the brain. Eur J Pharmacol 1985; 111:73–84.

[3] Chavez H, Vega R, Valli P, Mira E, Benvenuti C, Guth PS, et al. Action mechanism of betahistine in the vestibular end organs. Acta Otorhinolaryngol Ital 2001;21 (3 Suppl 66):8–15.

[4] Ebert CS Jr, Pollock HW, Dubin MG, Scharer SS, Prazma J, McQueen CT, et al. Effect of intranasal histamine challenge on Eustachian tube function. Int J Pediatr Otorhinolaryngol 2002;63:189–98.

[5] Franz B, Anderson C. The effect of the sympathetic and sensory nervous system on active Eustachian tube function in the rat. Acta Otolaryngol 2007;127:265–72.

[6] Franz B, Anderson C. A model of Eustachian tube function in the rat: effect of modulating the parasympathetic innervation. Acta Otolaryngol 2002;122:374–81.

[7] Kessler JP, Jean A. Inhibition of the swallowing reflex by local application of serotonergic agents into the nucleus of the solitary tract. Eur J Pharmacol 1985;118:77–85.
[8] Hancock AA. The challenge of drug discovery of a GPCR target: analysis of preclinical pharmacology of histamine H3 antagonists/inverse agonists. Biochem Pharmacol 2006; 71:1103–13.
[9] Shotts RF, Jackson RT. Changes in the patency of the dog's eustachian tube induced by histamine and antihistamines. Arch Otolaryngol 1972;96:57–61.
[10] Kathmann M, Schlicker E, Marr I, Werthwein S, Stark H, Schunack W. Ciproxifan and chemically related compounds are highly potent and selective histamine H3-receptor antagonists. Naunyn Schmiedebergs Arch Pharmacol 1998;358:623–7.
[11] Leurs R, Bakker RA, Timmerman H, de Esch IJ. The histamine H3 receptor: from gene cloning to H3 receptor drugs. Nat Rev Drug Discov 2005;4:107–20.
[12] Hancock AA, Esbenshade TA, Krueger KM, Yao BB. Genetic and pharmacological aspects of histamine H3 receptor heterogeneity. Life Sci 2003;73:3043–72.
[13] Nakaya M, Fukushima Y, Takeuchi N, Kaga K. Nasal allergic response mediated by histamine H3 receptors in murine allergic rhinitis. Laryngoscope 2005;115:1778–84.
[14] Suzuki S, Takeuchi K, Majima Y. Localization and function of histamine H3 receptor in the nasal mucosa. Clin Exp Allergy 2008;38:1476–82.
[15] Hill BA. Surface tension versus straight adhesion in the Eustachian tube. Otol Neurotol 2002;23:620–1.
[16] Yokota E, Kuyama S, Sugimoto Y, Ogawa M, Kamei C. Participation of histamine H3 receptors in experimental allergic rhinitis of mice. J Pharmacol Sci 2008;108:206–11.
[17] Sun P, Jin X, Koyama T, Li S, Kitamura Y, Kawasaki H. R-(-)-alpha-methylhistamine, a histamine H3 receptor agonist, induces endothelium-dependent vasodilation in rat mesenteric resistance arteries. Biol Pharm Bull 2010; 33:58–63.
[18] Leurs R, Chazot PL, Shenton FC, Lim HD, de Esch IJ. Molecular and biochemical pharmacology of the histamine H4 receptor. Br J Pharmacol 2009;157:14–23.
[19] Connelly WM, Shenton FC, Lethbridge N, Leurs R, Waldvogel HJ, Faull RL, et al. The histamine H4 receptor is functionally expressed on neurons in the mammalian CNS. Br J Pharmacol 2009;157:55–63.
[20] Nakaya M, Takeuchi N, Kondo K. Immunohistochemical localization of histamine receptor subtypes in human inferior turbinates. Ann Otol Rhinol Laryngol 2004;113: 552–7.