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ORIGINAL ARTICLE

Effect of Betahistine on Isolated Rats' Tracheal Smooth Muscles

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Background: Betahistine is used as an H3 antagonist. It has been used to treat balance disorders. During the administration of the drug, the trachea may be affected through oral intake. **Aim:** This study aimed to determine the effects of betahistine on the tracheal smooth muscle of rats *in vitro*. **Methods:** On a rat trachea that had been isolated and immersed in Krebs solution in a muscle bath, we evaluated the efficacy of betahistine. We examined how the application of parasympathetic mimetic agents altered tracheal contractility. The betahistine was evaluated using the following criteria: the drug's effects on tracheal smooth muscle contractions triggered by parasympathetic mimetic 10^{-6} M methacholine, electrically induced tracheal smooth muscle contractions, and resting tracheal smooth muscle tension were listed below. **Results:** At preparation concentrations as high as 10^{-4} M, betahistine produced a substantial relaxing response. The medication also prevented spike contraction brought by electrical field stimulation. However, betahistine alone had a negligible effect on the basal tension of the trachea at increasing concentrations. **Conclusion:** According to this study, excessive levels of betahistine might actually oppose cholinergic receptors and prevented the tracheal smooth muscles parasympathetic activity.

Key words: Betahistine, trachea, smooth muscle, *in vitro* study

INTRODUCTION

Histamine, or 2-(4-imidazole)-ethylamine, is a known mediator of inflammation that works by activating histamine receptors (HRs), of which four varieties (H1R, H2R, H3R, and H4R) are now known to exist.[1] A H3 antagonist, betahistine works similarly to a histamine H1 agonist. Despite being widely used, its pharmacological route of action is still unclear. The systemic therapy of balance disorders such as Ménière's illness has made extensive use of it.[2] It has been assumed that betahistine's influence on the bigger feeder vessels or lateral wall capillary bed in the cochlea vascular system is what causes betahistine-evoked increases in cochlear blood flow. As such, the mechanism of action could be due to the inhibition of H3R.[3] In general, asthma is accompanied by allergies or other immune dysfunctions, and some patients with asthma experience dizziness and hearing problems. High-frequency hearing loss was reported to be more common in asthma patients than in the control group.[4,5] In addition, asthma patients having a higher prevalence of balance problems were reported.[6] Association between asthma and Meniere's disease is also noted.[7]

Because betahistine may impact the trachea through oral ingestion during drug delivery, this effect on the trachea is a matter that warrants more research. Clinically, asthma or bronchospasm may be affected by this drug. The aim of this study was to evaluate the effects of betahistine on isolated tracheal smooth muscle. This study used a rat tracheal model to examine the effects of betahistine on isolated tracheal contraction *in vitro*. This model shows how the tension of an intact tracheal ring changes in response to the addition of parasympathetic mimetic agents and potential tracheal

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contraction agents and identifies substances that directly affect tracheal smooth muscle.

MATERIALS AND METHODS

Tissue preparation

The purest chemicals on the market were used. Every chemical reagent was purchased from Sigma (St. Louis, MO, USA). This study, which was approved by an animal experiment review board, used a total of 18 rats (LAC-2017-0036). Eighteen healthy male Sprague Dawley rats were humanely killed by CO_2 gas asphyxiation, and two portions of each rat's trachea (each measuring about 5 mm in length) were taken. As in earlier research,[8,9] the tracheal specimens were mounted using two steel hooks and immersed in a 30-mL muscle bath at 37°C. Thirty mL of Krebs solution, which included (in mmol/L) NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$ $7H_2O$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; and glucose, 10.0, was added to the bath [Figure 1]. A steel hook and a 3-O silk ligature were used to affix the top ends of the tracheal strips to a Grass FT-03 force displacement transducer (AstroMed, West Warwick, RI, USA). The strips' opposite ends were fastened to a steel hook that was affixed to the bathtub. The strips were passively tensioned to 0.3 g, and variations in tension were then constantly recorded using Chart ver. 4.2 (PowerLab, ADInstruments, Colorado Springs, CO, USA).

Methacholine and electrical field stimulation

Methacholine was put to the test as a tracheal contraction aid. A tracheal strip submerged in the bath solution utilized for further trials did not shrink when basal tension was applied, according to preliminary tests. Isolated trachea samples were equilibrated in the bath solution for 15–30 min before drug testing, during which time continuous aeration with a solution of 95% O_2 and 5% CO_2 was used. To investigate the contraction or relaxation responses of the tracheal strips, the dosages of the medicines were gradually increased. By adding a specific volume of stock solution to the tissue bath solution, all medications were given.

Two wire electrodes linked to a direct-current stimulator were positioned parallel to the trachea strip to apply electrical field stimulation (EFS) to the trachea strips at a frequency of 5 Hz, a pulse width of 5 ms, and a voltage of 50 V (Grass S44, Quincy, MA, USA). An interval of 2 min was imposed between each stimulation period to allow recovery from the response. The trachea was continuously stimulated at a temperature of 37°C.

We evaluated the following effects of betahistine: (1) impact on tracheal smooth muscle resting tension: this test looked at how the medication affected a model of a resting trachea condition; (2) the impact of 10^{-6} M methacholine on contraction: this experiment looked at postsynaptic events such as muscle receptor blockage, enhancement, and second messengers; and (3) effect on electrically induced tracheal smooth muscle contractions: the trachea releases a parasympathetic transmitter in response to electrical stimulation of this tissue (acetylcholine). Electrical stimulation does not elicit contraction if the test substance prevents transmitter release. This approach made it easier to see presynaptic events. Six tracheal testing strips were included in each treatment group.

The amount of each medication in the 30-mL bath solution is used to express the drug concentrations. Each experiment tested the effect of betahistine on tracheal resting tension or on electrically induced contraction using an untreated strip as the

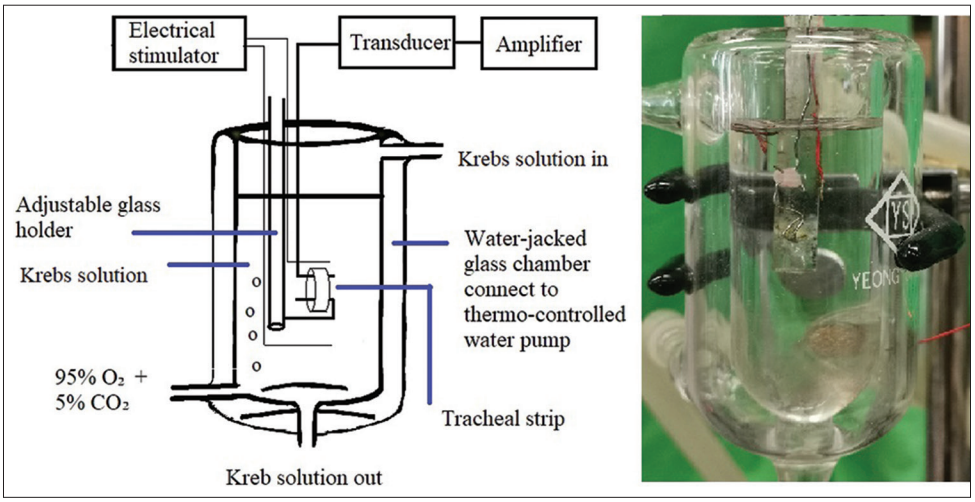

Figure 1: Schematic diagram and actual photo describing the measurement of tension in isolated rat tracheal smooth muscle

control group. The strip's degree of contraction following the addition of methacholine served as the control value in the other studies using 10^{-6} M methacholine. The percentage results were reported as the mean value and standard deviations (SDs) normalized to the control value in comparison to the control value.

Statistical analysis

Data of the basal tension and methacholine experiment were collected by the mean of tension between the two different concentration agents that were added. The mean of the EFS peak between the two varied concentration agents that were added served as the data in the EFS experiment. A one-way ANOVA was used to examine the statistical significance of the data, with $P < 0.01$ being regarded as significant. The data were given as mean ± SD.

RESULTS

The strain placed on the transducer was used to estimate the degree of tracheal strip contraction or relaxation. Methacholine, a parasympathetic agent, quickly elicited the contractile responses of the trachea, and the tissue remained in a contracted state until the substance was washed from the tissue. The results indicated that each test had the same tendency, despite the tiny differences in the graph variations between each test.

The addition of betahistine had a negligible effect on basal tension [Figure 2] but resulted in a relaxation of the trachea when introduced after the addition of a constricting agent such as 10^{-6} M methacholine [Figures 3 and 4]. Low doses of betahistine resulted in a slight decrease in tracheal contraction and higher doses relaxed the trachea much more quickly. The tension was 98.2% ± 2.6%, 94.8% ± 2.6%, 92.8% ± 4.5%, and 88.2% ± 5.8% of the control values at 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} M betahistine, respectively, while at 10^{-4} M betahistine, the tension was decreased to 56.7% ± 10.3% [Figure 4]. The inhibition of contraction was statistically significant at 10^{-4} M as compared with that of the control ($P < 0.01$). Betahistine also influenced the spike contraction induced by EFS. Although low doses of this drug resulted in little change in the spike contraction, a higher dose of up to 10^{-4} M prominently lowered the peak tension of the control value. The peak tensions of the tracheal strip evoked by EFS upon the addition of 10^{-6}, 10^{-5}, and 10^{-4} M betahistine were 97.1% ± 2.7%, 93.8% ± 3.7%, and 27.2% ± 10.6% of the control value, respectively. Following the addition of 10^{-4} M betahistine, the peak tension value of the tracheal strip was significantly lower than that of the control group ($P < 0.01$) [Figures 5 and 6].

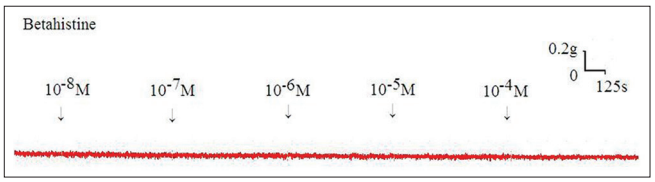

Figure 2: Tension changes in the rat trachea after the application of various betahistine concentrations. The basal tension was 0.3 g (n = 6)

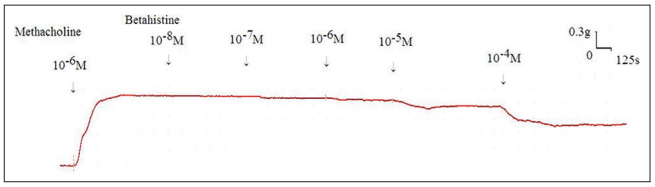

Figure 3: Effects of betahistine on 10^{-6} M methacholine-induced contraction of the rat trachea ($n = 6$)

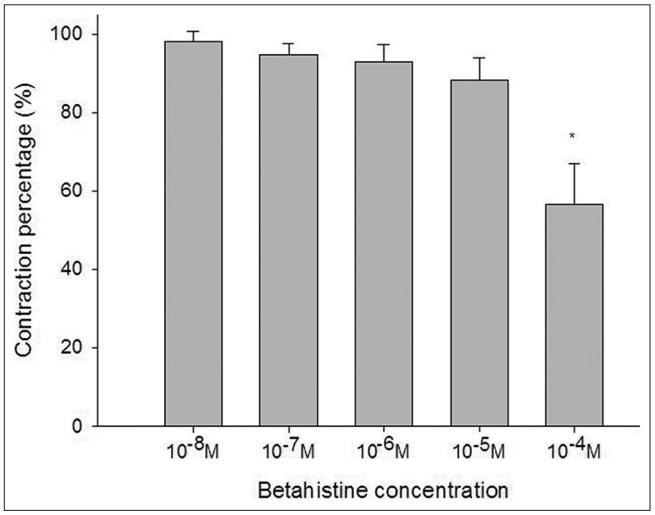

Figure 4: Effects of betahistine on 10^{-6} M methacholine-induced contraction (contraction area was calculated at 100% with no addition of betahistine) of rat trachea. The inhibition of contraction was statistically significant at 10^{-4} M as compared with that of the control ($P < 0.01$). The results are represented as mean ± SD ($n = 6$). SD: Standard deviation

DISCUSSION

The context of the test items used should be considered when interpreting the outcomes of our investigations. In our earlier study, [8,9] we explored the device's mechanics. The characteristics of the particular tissue and its reaction to particular medications give some hint as to which tissue component of the trachea was in charge of the drug-induced contraction, despite the difficulty of making this determination. First of all, the tracheal strips utilized in our investigation were undeveloped preparations that included tracheal smooth muscle and cartilage. The other tissues (epithelium, glands, connective tissue, nerves, and cartilage) did not contract to a substantial

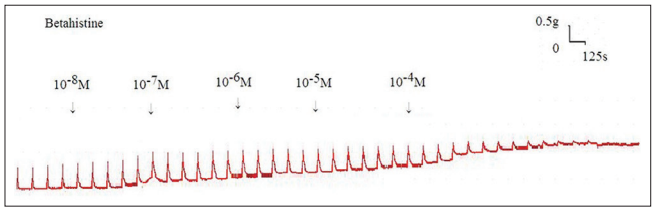

Figure 5: Effects of betahistine on electrically induced tracheal smooth muscle contractions ($n = 6$)

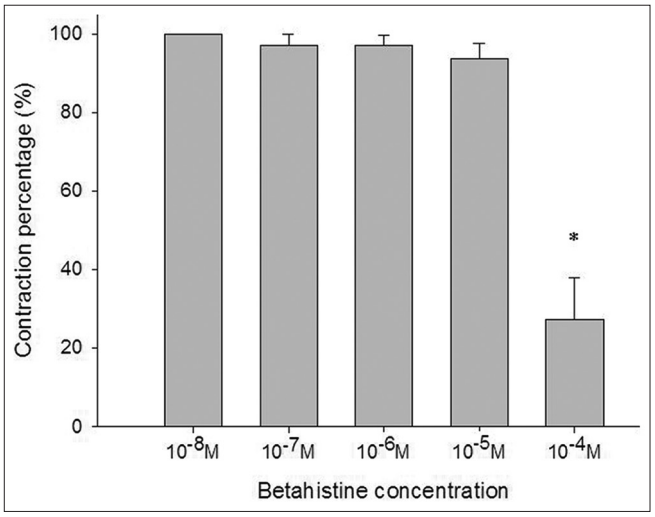

Figure 6: Effects of betahistine on electrically induced tracheal smooth muscle contractions (contraction area was calculated at 100% with no addition of betahistine). The inhibition of spike contraction was statistically significant at 10^{-4} M as compared with that of the control ($P < 0.01$). The results are represented as mean ± SD ($n = 6$). SD: Standard deviation

degree, suggesting that the trachea's smooth muscle is the primary tissue component involved in contraction. Due to the method's utilization of cross contraction, the tracheal ring's radial contraction was what generated tension fluctuations. Similar formulations have demonstrated responses to medication and electrical stimulation. [10,11] The observed contractile response, however, was likely a combination of reactions from several kinds of muscle tissue. Second, a rat's isolated trachea was surgically removed for our investigations without causing any harm to the endothelium or smooth muscle. Therefore, it is plausible to believe that the tracheal reactions to the test agents in our investigation were similar to those seen after administering medication to the trachea during an asthma episode. The effect of this medication on isolated human tracheal smooth muscle still needs more research because it is challenging to get human tissue for comparable experiments. The response could be significantly more complicated in an *in vivo* scenario than it would be in an *in vitro* scenario.

Histidine decarboxylase converts the amino acid histidine into histamine. Mast cells, basophils, gastric enterochromaffin-like cells, and histaminergic neurons in the brain are a few of the sources of histamine in humans. It is now known that histamine activates HRs, of which four types have been identified, to produce its pleiotropic effects. These receptors are all members of the family of G protein-coupled receptors.[1,12] While H2R mediates intracellular events primarily characterized by elevations in cyclic adenosine monophosphate, H1R mediates a series of intracellular events primarily characterized by changes in free cytosolic calcium levels (cAMP). A different combination of cytosolic free calcium and cAMP is used to activate the H3R and H4R.[1] Ash and Schild[12] separated HRs for the first time in 1966. The class of HR that is sensitive to inhibition by promethazine and mepyramine, currently recognized as H1R antagonists, was first described by these researchers using the term H1R. The guinea pig trachea, uterus, and longitudinal smooth muscle of the ileum are just a few examples of the many visceral smooth muscles that contract as a result of the basic H1R-mediated reactions. However, H1R antagonists such as mepyramine cannot stop the gastric mucosa's acid release. Burimamide, a specific antagonist of the acid-secretion response (also known as an H2R), was created in 1972 by Black *et al*. [13] To explain the unusual pharmacological characteristics of the histamine auto receptor in charge of regulating the release of histamine from rat cerebral cortical slices, Arrang *et al*. [14] proposed the H3R in 1983. According to many studies, [1,15] H3R is found in both central and peripheral organs and serves a variety of purposes. H4R is primarily expressed on cells that participate in inflammatory and immunological reactions. It is highly concentrated on peripheral immune cells such as lymphocytes, eosinophils, monocytes, mast cells, and dendritic cells as well as hematopoietic cells in the bone marrow. [16] H3R may control the neurotransmitter release of postganglionic cholinergic neurons and cholinergic ganglia in the airway. [1,15] Additionally found in the tracheal smooth muscle and bronchial epithelium, H3R causes the trachea's precontracted muscles to relax. The suppression of noradrenaline release from sympathetic nerve terminals in the nasal mucosa by H3R is also thought to influence vascular contractile responses, which would increase mucus output in allergic rhinitis. [1,17]

Methacholine, the contracting agent in this test, is a synthetic choline ester that functions as a nonselective cholinergic agonist and is frequently employed in research. It is important to note that the tissue relaxation brought on by the medication required that the smooth muscle has previously partially contracted in reaction to methacholine. As a result, it should be possible to evaluate the effects of popular medications and other substances that are thought to be responsible for reducing asthma symptoms.

According to the results of methacholine challenge tests, betahistine was proved to possess the ability to

prominently relax the isolated trachea at 10^{-4} M. This phenomenon indicated a direct anticholinergic effect of betahistine. Despite the distribution of H3R on the trachea, contraction effects on the trachea were not activated due to the absence of histamine in methacholine tests. Hence, it appeared an unknown mechanism for betahistine to relax the trachea which had been treated with an exogenous nonselective cholinergic agonist, methacholine. Previous studies have suggested that H3R could relax the airway. [18,19] The interactions with cholinergic or other uncertain receptors were presumably responsible for the anticholinergic effect of betahistine. Further biomolecular investigation is necessary to clarify this experimental result. Although the mechanism by which the H3R antagonist affects the trachea smooth muscle remains unclear, the drug may represent another potential therapy for asthma attacks.

EFS is a frequently used experimental technique that causes endogenous neurotransmitters to be released from the tissue's nerve terminals, which then causes the smooth muscle to contract. After ipsilateral cervical sympathetic ganglionectomy, EFS-induced canine nasal mucosal contraction vanishes[20] and stimulation of sympathetic innervation were used to demonstrate EFS-induced nasal mucosal contraction spikes. In this investigation, activation of parasympathetic innervation caused the tracheal smooth muscle to contract with an EFS-induced increase. Therefore, the EFS-induced contraction of the trachea decreased as the concentration of betahistine increased. This suggests that betahistine could antagonize not only the effect of H3R but also parasympathetic innervation in tracheal smooth muscle contraction. In the isolated ileum of the guinea pig, activation of H3R decreased the electrical stimulation-induced contraction. [21] The mechanisms by which H3R modulates the cholinergic neurotransmitter have been proposed, [21] but the physiological influence of H3R on the airway is still uncertain. Our observations in this study were very interesting, and further study is required to clarify these phenomena.

CONCLUSION

To explore the effects of betahistine on tracheal contraction, we developed a test system that included a short, undamaged ring of the trachea. According to the study, large levels of betahistine might both impede the trachea's parasympathetic function and counteract the effects of cholinergic receptors.

Acknowledgments

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Data availability statement

The data that support the findings of this study are available from the corresponding author, HW Wang, upon reasonable request.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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