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EFFECTS OF 3-METHYLHISTAMINE AND PHENYLETHYLAMINE ON HISTAMINE ACTION ON ISOLATED GUINEA-PIG TRACHEA RINGS

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It is well known that histamine produces constriction via H_1 receptors and decreases tracheal smooth muscle tone via H_2 and H_3 receptors. In addition, it has already been reported that 3-methyl-histamine and phenylethylamine are competitive antagonists of histamine N-methyl-transferase (HMT), the enzyme responsible for rapid inactivation of histamine. Our results suggest the possibility that 3-methyl-histamine and phenylethylamine as competitive antagonists of histamine N-methyl-transferase lead to potentiation of histamine induced constriction of isiolated guinea-pig trachea, probably due to the decrease of histamine methylation and consequent inactivation. In as much, 3-methyl-histamine and phenylethylamine had no effect on the basal tone of isolated trachea smooth muscle, as well as on other mechanisms leading to increased responsiveness of guinea-pig tracheal smooth muscle (acetylcholine, KCl, electro stimulation).

Key words: 3-methyl-histamine, airways, histamine N-methyl-transferase, phenylethylamine

INTRODUCTION

Histamine plays one of the mayor roles in the control of airway responsiveness. The action of histamine on tracheal smooth muscles is very complex. It can be shortly described as "a subtle balance of contraction and relaxation" (Jolly and Desmecth, 2003). Histamine produces constrictions of tracheal smooth muscles via H_1 receptors (Ash and Schild, 1966; Barnes *et al.* 1998), but at the same time histamine decreases tracheal smooth muscle tone via H_2 (Chand, 1980; Eyre, 1973) and H_3 receptors (Burgaud J-L and Oudart, 1993; Ichinose and Barnes, 1989).

Histamine N-methyl-transferase (HMT) is the enzyme responsible for rapid inactivation of histamine by methylatation of ring tele-nitrogen in histamine (Fogel *et al.,* 2007; Fram and Green, 1968). HMT represents in the airways the primary enzyme witch degrades histamine and the epithelium of the airways is a rich

sourse of HMT mRNA (Ohrui *et al.,* 1992). It has been already confirmed that some products of transmethylation reactions regulate the activity of histamine N-methyl-transferase (Barth *et al.*, 1973). 3-methyl-histamine, a major inactive metabolite of histamine (Herman *et al.,* 1985) and phenylethylamine are reported to be competitive antagonist of histamine N-methyl-transferase (Tachibana *et al.,* 1986). Phenylethylamine is an endogenous amine related structurally and pharmacologically to amphetamine (Rambali *et al*., 2002; Marc *et al*., 2010).

In the airways phenylethylamine caused an initial relaxation at a lower concentration, followed by contraction at a higher concentration (Rambali *et al.,* 2002). The relaxation effect of phenylethylamine seems to be mediated by β-adrenoreceptors, since contraction effect does not seem to be mediated by α-adrenergic, muscarinic histaminergic, seratonergic or dopaminergic receptor simulation. It is not clear which receptors are involved in contraction effects of phenylethylamine (Hawthorn *et al.,* 1985), but a family og G protein-coupled receptors have shown to specifically bind and/or be activated by phenylethylamine in different tissues (brain, stomach, kidney, lung, blood vessel, pituitary, skeletal muscle) (Borowsky *et al.,* 2010; Broadley, 2010; Bunzow *et al.,* 2001; Fehler *et al*., 2010; Zucchi *et al.,* 2006).

The aim of the this study was to investigate whether the presence of competitive HMT antagonists, 3-methyl-histamine and phenylethylamine affects histamine action on isolated guinea-pig trachea rings. The second aim was to find out whether 3-methyl-histamine and phenylethylamine influence some of the well known mechanisms leading to increased tracheal smooth muscle tone, underlying the effects of acetylcholine, KCl and electro stimulation.

MATERIALS AND METHODS

Preparation of guinea-pig trachea rings

Twenty-five guinea-pigs of both sexes, weighing between 250 g and 300 g, were used in this study. Guinea-pigs were killed by cervical dislocation (according to Schedule 1 of the Animals, Scientific producers, Act 1986, UK) and exsanguinated. Each experiment was conducted on isolated preparations from five different animals. Rings (2 mm of length) were excised by scissors from the lower third of the trachea and put in an organ bath.

Experimenntal design

Each isolated preparation was mounted in the 10 mL organ bath with constant flow (5 mL/min) of Krebs solution (NaCl-94.7 mM, $MgSO_4$ x 7 H_2O-2.4 mM, $CaCl_2$-2.52 mM, KH_2 PO_4-1.18 mM, $NaHCO_3$- 24.88 mM and glucose 11.7 mM). The bath was aerated continously with 95% O_2 and maintained at 37°C. One end of the isolated tracheal ring was fixed to the bath, and the other was fixed to a force-displacement transducer (IT-1 sensor, EMKA Technologies) coupled with a tension amlifier and chart recorder.

All rings were loaded with 0.5 g weight and allowed to equilibrate 90 minutes. A first set of experiments consisted of recording the trachea rings contractile responses to histamine (5, 10, 25, 50, 75, 100 and 150 X 10^{-6} M for 1

minute), acetylcholine (1, 13, 26, 39, 53 and 66 X 10^{-6} M for 2 minutes), and electro stimulatation (5, 10, 20, 50 H_z, 40 V, 5 ms for 15 seconds). Second set of experiments consisted of recording the trachea rings contractile responses to same agonists in the presence of 3-methyl-histamine (permanent perfusion for 5 minutes before agonists use and during agonist action, with final concentrations of 28, 84, 170 and 300 X 10^{-6} M). Third set of experiments consisted of recording the trachea rings contractile responses to same agonists in the presence of phenylethylamine (permanent perfusion for 5 minutes before agonists use and during agonist's action, with final concentrations of 0.23, 2.3, 23 and 230 x 10^{-6} M). The next agonist was tried on the same preparation only after a period of 15 min. All drugs were applied into the organ bath using a micro infusion pump with constant flow of 125 μL/min.

Contractile responses were measured as changes in isometric tension and converted into a percentage of the reference maximum for each group of experiments. Total duration of contractile response was measured, as well.

Chemicals

Drugs used in these experiments were histamine, 3-methyl-histamine, acetylcholine (Sigma-Aldrich, USA), phenylethylamine (Calbiochem, GB) and KCl (Zorka Sabac, Serbia). The drugs were prepared on the day of the experiment in NaCl 0.9% (Zorka Sabac, Serbia). Concentrations reported are expressed as final concentration within the organ bath.

Statistical analysis

Each concentration was assayed on isolated preparations from five different animals. Concentration-response curves were constructed using linear regression according to least-squares analysis (Kenakin RT, 1984; Tallarida JR, Murray RB, 1986). Effective concentration of agonists that produced 50% of maximal response and response duration (EC_{50}) was calculated for each agonist together with its confidence limits (1.96 x standard error). The results were considered statistically significant when $p \leq 0.05$.

RESULTS

The effects of acetylcholine, KCl and electro stimulation on the isolated guinea-pig trachea rings

Acetylcholine (1 x 10^{-6} M to 66 x 10^{-6} M) produced concentration-dependent tonic contractions of isolated guinea-pig trachea rings ($EC_{50} = 16.87 \pm 1.1 \times 10^{-6}$ M, $p < 0.001$). KCl (2x10^{-3} M to 100x10^{-3} M) produces concentration-dependent tonic contractions of isolated guinea-pig trachea rings ($EC_{50} = 20.24 \pm 1.1 \times 10^{-3}$ M, $p < 0.001$). Electro stimulation (5 to 50 Hz, 40 V, 5 ms) produced tonic contractions of isolatid guinea-pig trachea rings, reaching 50% of maximal response at aprroximately 7.5 Hz.

The effects of histamine on the isolated guinea-pig trachea rings

Histamine (5 x10^{-6} M to 66 x 10^{-6} M) produced concentration-dependent tonic contractions of isolated guinea-pig trachea rings (EC_{50}=20.79±1.1x10^{-6} M, p<0.001). Furthermore, duration of tonic concentration of isolated guinea-pig trachea rings to the same histamine doses is also concentration-dependent (EC_{50}=18.76±1.1x10^{-6} M, p<0.001).

The effects of 3-methyl-histamine on the isolated guinea-pig trachea rings

3-methyl-histamine did not affect the basal tone of isolated guinea-pig trachea rings in all applied concentrations In addition, 3-methyl-histamine in all applied doses had no influence effects of acetylcholine, KCl, and electro stimulation on isolated preparations. On the other hand, 3-methyl-histamine (28, 84, 170 and 300x10^{-6} M) caused concentration-dependent potentiation of tonic contractions of isolated guinea-pig trachea rings produced by histamine (EC_{50} = 16.14 ± 1.1 x 10^{-6} M, p<0.001, EC_{50} = 14.71 ± 1.2 x 10^{-6} M, p<0.001, EC_{50} = 10.95 ± 1.2 x 10^{-6} M, p<0.001 and EC_{50} = 8.78 ± 1.2 x 10^{-6} M, p<0.001 respectively) (Figure 1).

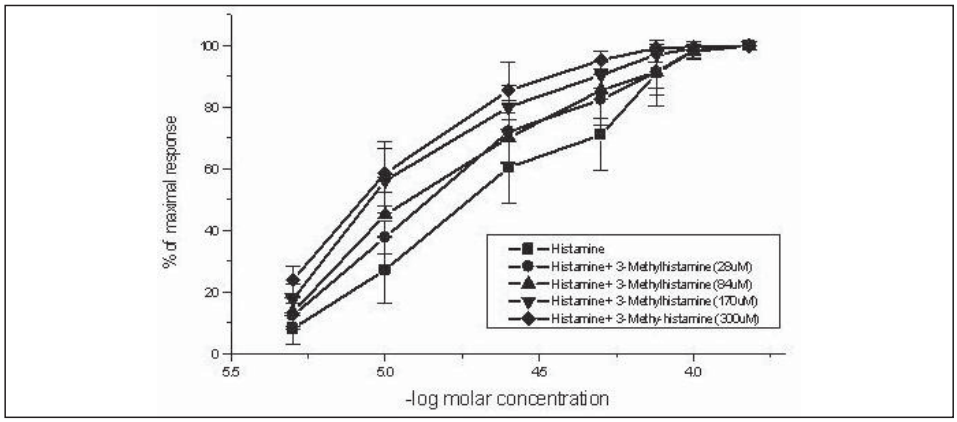

Figure 1. Effects of 3-methyl-histamine (28, 84, 170 and 300 x 10^{-6} M) on histamine action on isolated guinea-pig trachea rings

At the same time, 3-methyl-histamine (28, 84, 170 and 300 x 10^{-6} M) caused concentration-dependent enhancing of tonic contractions duration of isolated guinea-pig trachea rings produced by histamine (EC_{50}=12.51±1.1x10^{-6} M, p<0.001, EC_{50}=9.16±1.2x10^{-6} M, p<0.001, EC_{50}=6.27±1.2x10^{-6} M, p<0.001 and EC_{50}=4.02±1.3x10^{-6} M, p<0.001 respectively) (Figure 2).

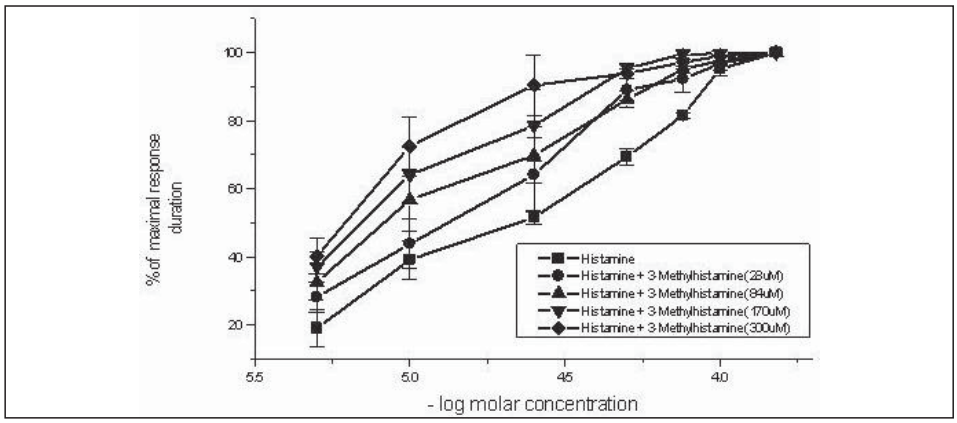

Figure 2. 3-methyl-histamine (28, 84, 170 and 300 x 10^{-6} M) caused concentration-dependent enhancing of tonic concentration duration of isolated guinea-pig trachea rings produced by histamine

The effects of phenylethylamine on the isolated guinea-pig trachea rings

Phenylethylamine did not affect the basal tone of isolated guinea-pig trachea rings in all applied concentrations. In addition, phenylethylamine in all applied doses had no effects on acetylcholine, KCl and electro stimulatin on isolated preparations. On the other hand, phenylethylamine (0.23, 2.3 and 230 x 10^{-6} M) caused concentration-dependent potentiation of tonic contractions of isolated guinea-pig trachea rings produced by histamine ($EC_{50}=16.42\pm1.1x10^{-6}$ M, $p<0.001$, $EC_{50}=12.94\pm1.2x10^{-6}$ M, $p<0.001$, $EC_{50}=9.21\pm1.2x10^{-6}$ M, $p<0.001$ and $EC_{50}=6.87\pm1.2x10^{-6}$ M, $p<0.001$ respectively) (Figure 3).

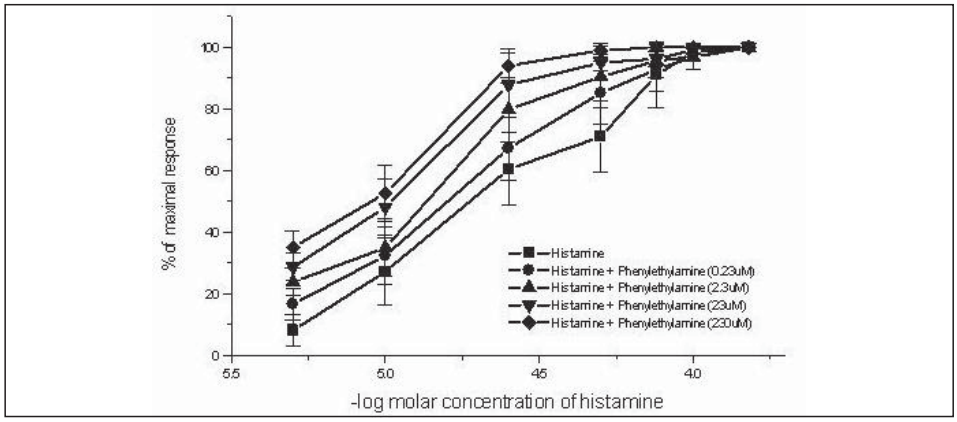

Figure 3. Effects of phenylethylamine (0.23, 2.3, 23 and 230 x 10^{-6} M) on histamine action on isolated guinea-pig trachea rings

At the same time phenylethylamine (0.23, 2.3, 23 and 230 x 10^{-6} M) caused concentration-dependent potentiation of tonic contractions duration of isolated guinea-pig trachea rings produced by histamine (EC_{50}=13.70±1.1x10^{-6} M, P.001, EC_{50}=8.54±1.2x10^{-6} M, p<0.001, EC_{50}=4.26±1.23x10^{-6} M, p<0.001 and EC_{50}=2.89±1.23x10^{-6} M, p<0.001 respectively) (Figure 4).

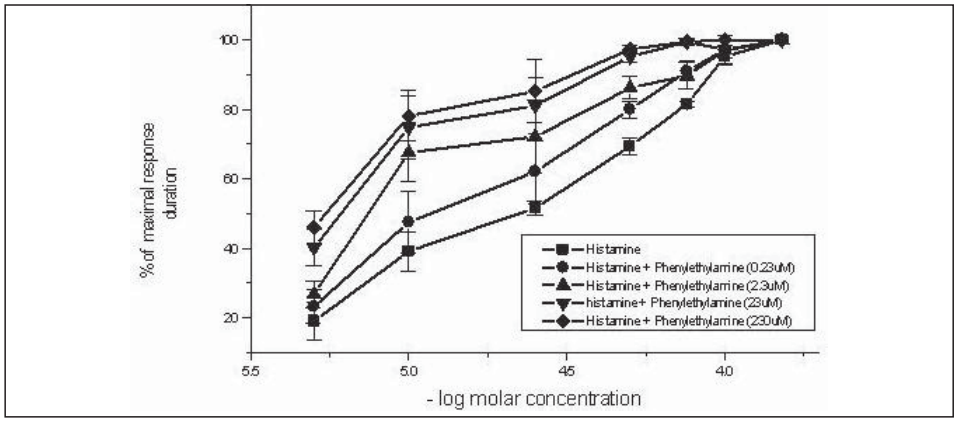

Figure 4. Phenylethylamine (0.23, 2.3, 23 and 230 x 10^{-6} M) caused concentration-dependent enhancing on tonic contraction duration of isolated guinea-pig trachea rings produced by histamine

Phenylethylamine did not affect the effects of acetylcholine, KCl and electro stumulation on isolated preparations.

DISCUSSION

Effects of acetylcholine, KCl and electro stimulation on tone of airway smooth muscle are already well known, as well as their mechanisms of action. Still, we performed such a trial in order to check our experimental setting (sensitivity and reproducibility) comparing to previously reported data.

However, the main purpose of this study was to evaluate 3-methyl-histamine and phenylethylamine effects on different mechanisms leading to the same final consequence i.e. contraction of trachea smooth muscle. 3-methyl-histamine and phenylethylamine had no effects on basal tone of isolated guinea-pig trachea. Also, they had no influence on the effects of acetylcholine, KCl and electro stimulation action to the musculature of guinea-pig trachea.

3-methyl-histamine is the major inactive metabolite of histamine and is formed by histamine N-methyl-transferase (Herman *et al.,* 1985). Phenylmethylamine is an endogenous amine. It is know that it causes relaxation of the guinea-pig isolated lung parenchymal strip at lower concentrations (10^{-7} - 10^{-5} M). This effect seems to be mediated by β-adrenoreceptors (Rambali *et al.,* 2002). In our experimental conditions, phenylethylamine did not affect the basal

tone of isolated guinea-pig trachea rings at all applied concentrations (0.23, 2.3, 23 and 230 x 10^{-6} M). Comparing to previous reports, our results suggest that applied doses of phenylethylamine were too small to produce any effects on isolated trachea rings.

Absence of effects of 3-methyl-histamine and phenylethylamine on basal tone suggests that they did not activate any of the mechanisms responsible for the change in contractile response of tracheal rings smooth muscles. This includes the absence of action on any kind of receptors in our experimental conditions, as on histamine receptors in guinea-pig trachea, because histamine itself produces constriction via H_1 receptors (Barnes *et al*., 1973) and decreases tracheal smooth muscle tone via H_2 (Chand, 1980) and H_3 receptors (Burgaud and Oudart, 1993). However, both 3-methyl-histamine and phenylethylamine strongly potentate histamine induced constriction of guinea-pig trachea smooth muscle and the duration of response, as well. Taking in consideretion all facts mentioned above with previous reports that 3-methyl-histamine and phenylethylamine are competitive antagonist of histamine N-methyl-transferase, i.e. the enzyme responsible for rapid inactivation of histamine, we suggest a possibility that decrease in histamine has effects on guinea-pig trachea.

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REFERENCES

1. *Ash ASF, Schild HO*, 1966, Receptors mediating some actions of histamine. *Br J Pharmacol*, 14, 427-39.
2. *Barnes PJ, Chung KF, Page CP,* 1998, Inflammatory mediators of asthma: an update. *Pharmacol Rev*, 50, 515-96.
3. *Barth H, Lorenz W, Niemeyer I,* 1973, Inhibition and activation of histamine methyltransferase by methylated histamines, *Hoppe Seylers J Phyisol Chem*, 354, 1024-5.
4. *Borowsky B, Adham W, Jones KA, Raddatz R, Artymishyn R, Ogozalek KL et al.,* 2001, Trace amines: Identification of a family of mammalian G protein-coupled receptors, *Proc Natl Acad Sci USA*, 98, 16, 8966-71.
5. *Broadley KJ,* 2010, The vascular effects of trace amines and amphetamines, *Pharmacol Therap,* 125, 363-75.
6. *Bunzow JR, Sonders MS, Arttamangkul S, Harrison LM, Zhang G, Quigley DI et al.*, 2001, Amphetamine, 3,4-methylenedioxymethamphetamine, lysergic acid diethylamide, and metabolites of the catecholamine neurotransmitters are agonists of a rat trace amine receptor, *Mol Pharmacol,* 60, 6, 1165-7.
7. *Burgaud J-L, Oudart N*, 1993, Bronchodilatation of guinea-pig perfused bronchioles induced by the H3-receptor for histamine: role of epithelium, *Br J Pharmacol*, 109, 960-9.
8. *Chand N*, 1980, Distribution and classification of airway histamine receptors: the physiological significance of histamine H2-receptors, *Adv Pharmacol Chemother*, 17, 103-31.
9. *Eyre P*, 1973, Histamine H2-receptors in the sheep bronchus and cat trachea: the action of burinamide, *Br J Pharmacol*, 48, 321-3.

10. *Fehler M, Broadley KJ, Ford WR, Kidd EJ*, 2010, Identification of trace-amine-associated receptors (TAAR) in the rat aorta and their role in vasoconstriction by β-phenylethylamine, Naunyn Schmiedebergs *Arch Pharmacol*, 382, 4, 385-98.
11. *Fogel WA, Lewinski A, Jochem J,* 2007, Histamine in food: is there anything to worry about? *Biochemical Society Transactions*, 35, 2, 349-52.
12. *Fram DH, Green JP,* 1968, Methylhistamine in guinea pig brain, *J Neurochem*, 15, 597-602.
13. *Hawthorn MH, Broadley KJ, Gibbon CJ*, 1985, Examination of the bronchoconstrictor responce of guinea-pig isolated lung to beta-phenylethylamine, *Gen Pharmacol*, 16, 4, 371-8.
14. *Hawthorn MH, Broadley KJ, Gibbon CJ*, 1985, Examination of the bronchoconstrictor responce of guinea-pig isolated lung to beta-phenylethylamine, *Gen Pharmacol*, 16, 4, 371-8.
15. *Ichinose M, Barnes PJ*, 1989, Inhibitory histamine H3-receptors on cholinergic nerves in human airways, *Eur J Pharmacol*, 163, 383-6.
16. *Jolly S, Desmecth D, 2003,* Functional identification of epithelial and smooth muscle histamine-dependent relaxing mechanisms in the bovine trachea, but not in bronchi. Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology, 134, 1, 91-100.
17. *Kenakin RT*, 1984, The classification of drugs and drug receptors in isolated tissues, *Pharmacol Rev*, 36, 165-222.
18. *Marc DT, Ailts JW, Ailts Campeau DC, Bull MJ, Olson KL,* 2010, Neurotransmiters excreted in the urine as biomarkers of nervous system activity: Validity and clinical applicability, *Neurosc and Biobehav Rew*. doi:10.1016/j.neubiorev.2010.07.007
19. *Ohrui T, Yamauchi K, Sekizawa K, Ohkawara Y, Maeyama K, Sasaki M et al.*, 1992, Histamine N-methyl-transferase controls the contractile response of guinea pig trachea to histamine, *J Pharmacol Exp Ther*, 261, 1268-72.
20. *Rambali B, Van Andel I, Schenk E, Wolterink G, Van de Werken G, Stevenson H et al.*, 2002, The contribution of cocoa additive to cigarette smoking addiction. RIVM report 650270002, 142-59.
21. *Tachibana T, Taniguchi S, Fujiwara M, Imamura S*, 1986, Regulation of the activity of histamine N-methyltransferase from guinea pig skin by biogenic amines, *Exp Mol Path*, 45, 257-69.
22. *Tallarida JR, Murray RB*, 1987, Manual of pharmacological calculations with computer programs. 2nd ed. New York: Springer-Verlag, 297.
23. *Zucchi R, Chiellini G, Scanlan TS, Grandy DK*, 2006, Trace amine-associated receptors and their ligands, *British J Pharmacol*, 149, 967-78.

UTICAJ 3-METIL-HISTAMINA I FENILETILAMINA NA REAKTIVNOST IZOLOVANE TRAHEJE ZAMORČIĆA

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SADRŽAJ

Poznato je da histamin izaziva kontrakciju glatkih mišića traheje preko H_1 receptora a smanjuje njihov tonus posredstvom H_2 i H_3 receptora. Takođe je objavljeno da su 3-metil-histamin i feniletilamin kompetitivni antagonisti histamin metil transferaze (HMT), enzima odgovornog za brzu inaktivaciju histamina.

Naši rezultati sugerišu mogućnost da 3-metil-histamin i feniletilamin kao kompetitivni antagonisti histamin N-metil-transferaze mogu potencirati histaminom izazvanu kontrakciju izolovane traheje zamorčića, verovatno zbog smanjenja metilacije histamina i posledične inaktivacije. Fenil-etil-amin i 3-metil-histamin nemaju efekta na bazalni tonus glatkih mišića traheje, kao acetilholin, KCl i elektrostimulacija koji dovode do povećanja odgovora glatkih mišića traheje zamorčića.