

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 H₁ receptors and decreases tracheal smooth muscle tone via H₂ and H₃ 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 isolated 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 H₁ receptors (Ash and Schild, 1966; Barnes *et al.* 1998), but at the same time histamine decreases tracheal smooth muscle tone via H₂ (Chand, 1980; Eyre, 1973) and H₃ 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 methylation 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

source of HMT mRNA (Ohrui *et al.*, 1992). It has been already confirmed that some products of transmethylation reactions regulate the activity of histamine N-methyltransferase (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-methyltransferase (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, serotonergic or dopaminergic receptor stimulation. It is not clear which receptors are involved in contraction effects of phenylethylamine (Hawthorn *et al.*, 1985), but a family of 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 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 procedures, 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.

Experimental 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, MgSO₄ x 7 H₂O-2.4 mM, CaCl₂-2.52 mM, KH₂ PO₄-1.18 mM, NaHCO₃- 24.88 mM and glucose 11.7 mM). The bath was aerated continuously with 95% O₂ 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 amplifier 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 stimulation (5, 10, 20, 50 Hz, 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 (EC₅₀) was calculated for each agonist together with its confidence limits (1.96 x standard error). The results were considered statistically significant when p≤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 (EC₅₀ = 16.87 ± 1.1 x 10⁻⁶ M, p < 0.001). KCl (2 x 10⁻³ M to 100 x 10⁻³ M) produces concentration-dependent tonic contractions of isolated guinea-pig trachea rings (EC₅₀ = 20.24 ± 1.1 x 10⁻³ M, p < 0.001). Electro stimulation (5 to 50 Hz, 40 V, 5 ms) produced tonic contractions of isolated guinea-pig trachea rings, reaching 50% of maximal response at approximately 7.5 Hz.

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

Histamine (5×10^{-6} M to 66×10^{-6} M) produced concentration-dependent tonic contractions of isolated guinea-pig trachea rings ($EC_{50} = 20.79 \pm 1.1 \times 10^{-6}$ M, $p < 0.001$). Furthermore, duration of tonic concentration of isolated guinea-pig trachea rings to the same histamine doses is also concentration-dependent ($EC_{50} = 18.76 \pm 1.1 \times 10^{-6}$ 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 300×10^{-6} M) caused concentration-dependent potentiation of tonic contractions of isolated guinea-pig trachea rings produced by histamine ($EC_{50} = 16.14 \pm 1.1 \times 10^{-6}$ M, $p < 0.001$, $EC_{50} = 14.71 \pm 1.2 \times 10^{-6}$ M, $p < 0.001$, $EC_{50} = 10.95 \pm 1.2 \times 10^{-6}$ M, $p < 0.001$ and $EC_{50} = 8.78 \pm 1.2 \times 10^{-6}$ M, $p < 0.001$ respectively) (Figure 1).

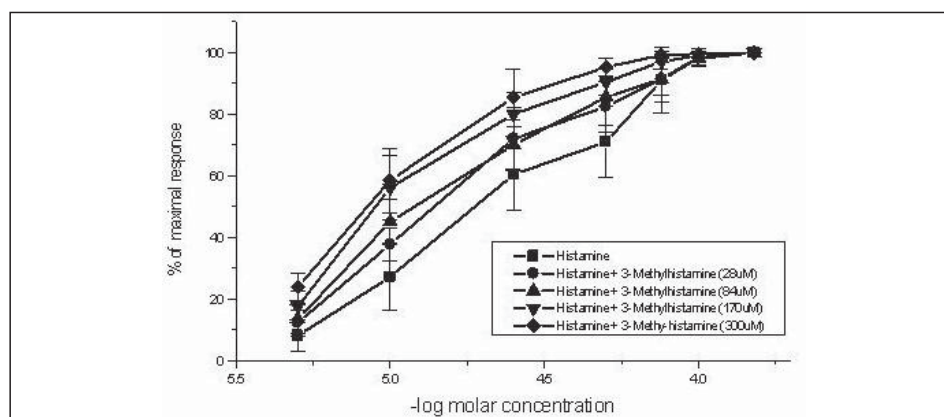


Figure 1. Effects of 3-methyl-histamine (28, 84, 170 and 300×10^{-6} M) on histamine action on isolated guinea-pig trachea rings

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

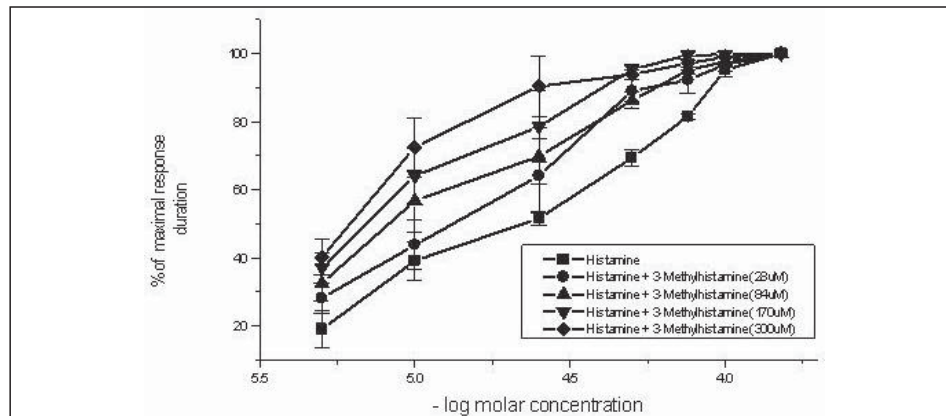


Figure 2. 3-methyl-histamine (28, 84, 170 and 300 $\times 10^{-6}$ M) caused concentration-dependent enhancing of tonic concentration duration of isolated guinea-pig trachea rings produced by histamine

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 stimulin on isolated preparations. On the other hand, phenylethylamine (0.23, 2.3 and 230 $\times 10^{-6}$ M) caused concentration-dependent potentiation of tonic contractions of isolated guinea-pig trachea rings produced by histamine ($EC_{50} = 16.42 \pm 1.1 \times 10^{-6}$ M, $p < 0.001$, $EC_{50} = 12.94 \pm 1.2 \times 10^{-6}$ M, $p < 0.001$, $EC_{50} = 9.21 \pm 1.2 \times 10^{-6}$ M, $p < 0.001$ and $EC_{50} = 6.87 \pm 1.2 \times 10^{-6}$ M, $p < 0.001$ respectively) (Figure 3).

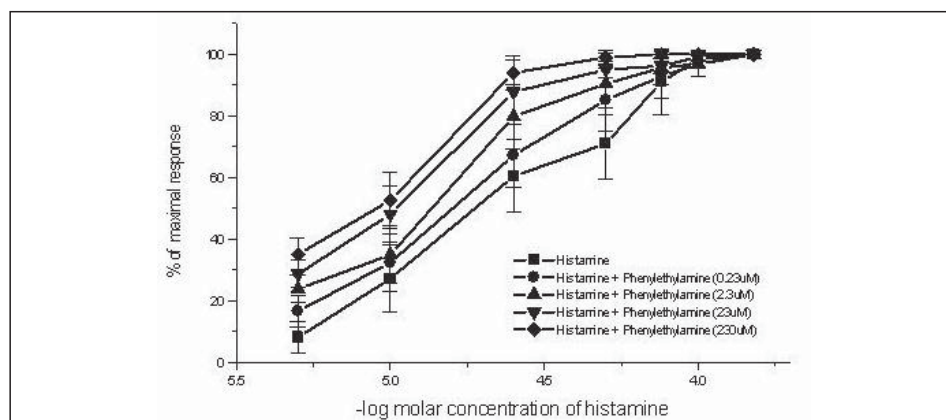


Figure 3. Effects of phenylethylamine (0.23, 2.3, 23 and 230 $\times 10^{-6}$ M) on histamine action on isolated guinea-pig trachea rings

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 (EC₅₀ = 13.70 ± 1.1 × 10⁻⁶ M, P.001, EC₅₀ = 8.54 ± 1.2 × 10⁻⁶ M, p < 0.001, EC₅₀ = 4.26 ± 1.23 × 10⁻⁶ M, p < 0.001 and EC₅₀ = 2.89 ± 1.23 × 10⁻⁶ M, p < 0.001 respectively) (Figure 4).

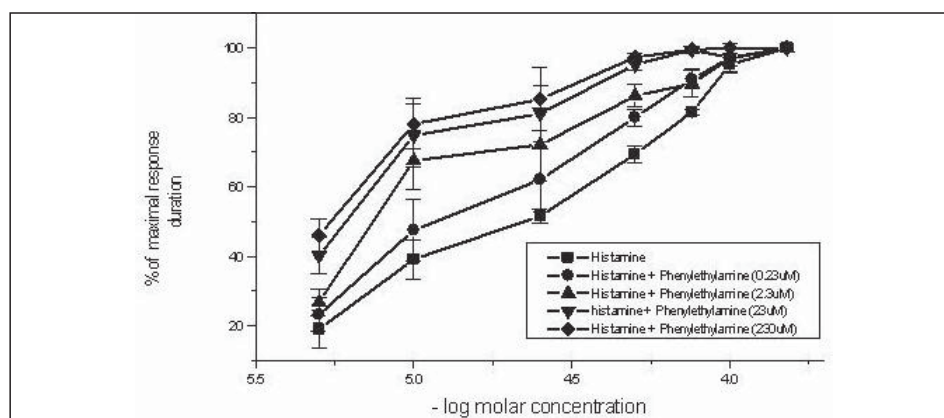


Figure 4. Phenylethylamine (0.23, 2.3, 23 and 230 x 10⁻⁶ M) caused concentration-dependent enhancing on tonic contraction duration of isolated guinea-pig trachea rings produced by histamine

Phenylethylamine did not affect the effects of acetylcholine, KCl and electro stimulation 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). Phenylmethylaniline is an endogenous amine. It is known 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 H₁ receptors (Barnes *et al.*, 1973) and decreases tracheal smooth muscle tone via H₂ (Chand, 1980) and H₃ receptors (Burgaud and Oudart, 1993). However, both 3-methyl-histamine and phenylethylamine strongly potentiate histamine induced constriction of guinea-pig trachea smooth muscle and the duration of response, as well. Taking in consideration 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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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 H₁ receptora a smanjuje njihov tonus posredstvom H₂ i H₃ 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.