

## PHENYLETHYLAMINE EFFECTS ON HISTAMINE-INDUCED CONTRACTION OF ISOLATED GUINEA-PIG TRACHEA RINGS

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**Summary:** Histamine produces constriction of tracheal smooth muscle via H1 receptors, but it also decreases tracheal smooth muscle tone via H2 and H3 receptors. In addition, it has already been reported that phenylethylamine is competitive antagonist of histamine N-methyl-transferase (HMT), enzyme responsible for rapid inactivation of histamine. Our results suggest possibility that phenylethylamine as competitive antagonist of histamine N-methyl-transferase leads to potentiation of histamine induced constriction of isolated guinea-pig trachea, which could be consequence of decreased histamine methylation and subsequent histamine inactivation. At the same time, phenylethylamine had no direct effect on basal tone of intact isolated trachea rings, as well as on other mechanisms leading to increased responsiveness of guinea-pig tracheal smooth muscle (acetylcholine, KCl, electro stimulation).

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

### Introduction

Histamine, already considered as a multifunctional hormone, has a significant influence in control of airway smooth muscle tone. Histamine exhibits various actions on tracheal smooth musculature. Namely, histamine produces constriction of tracheal smooth muscle via H1 receptors (1), but at the same time histamine decreases tracheal smooth muscle tone via H2 (2) and H3 receptors (3).

Histamine N-methyl-transferase (HMT) is enzyme responsible for rapid inactivation of histamine by methylation of ring tele-nitrogen in histamine (4). In the airways, HMT represents the primary enzyme which degrades histamine and airways epithelium is a rich source of HMT mRNA (5). Some products of transmethylation reactions regulate the activity of histamine N-methyl-transferase (6). Phenylethylamine is reported to be competitive antagonist of histamine N-methyl-transferase (7, 8), but still its action on tracheal smooth muscle tone was not evaluated.

The primary aim of this study was to evaluate whether or not the phenylethylamine, competitive

HMT antagonist, affects histamine action on isolated guinea-pig trachea rings. The second aim was to find out whether phenylethylamine influences some of the well known mechanisms leading to increase of tracheal smooth muscle tone, such as effects of acetylcholine, KCl and electro stimulation.

### Materials and Methods

#### Preparation of guinea-pig trachea rings

Fifteen 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 trachea and put in 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 mmol/L, KCl 4.7 mmol/L, MgSO<sub>4</sub> × 7H<sub>2</sub>O 2.4 mmol/L, CaCl<sub>2</sub> 2.52 mmol/L, KH<sub>2</sub>PO<sub>4</sub> 1.18 mmol/L, NaHCO<sub>3</sub> 24.88 mmol/L and glucose 11.7 mmol/L). The bath was aerated conti-

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nuously with 95% O<sub>2</sub> and 5% CO<sub>2</sub>, and maintained at 37 °C. One end of the isolated trachea ring was fixed to the bath, and the other was fixed to a force-displacement transducer (IT-1 sensor, EMKA Technologies) coupled with 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 of trachea rings contractile responses to histamine (5, 10, 25, 50, 75, 100 and 150 × 10<sup>-6</sup> mol/L for 1 minute), acetylcholine (1, 13, 26, 39, 53 and 66 × 10<sup>-6</sup> mol/L for 1 minute), KCl (2, 10, 20, 40, 60, 80 and 100 × 10<sup>-3</sup> mol/L for 2 minutes) and electro stimulation (5, 10, 20, 50 Hz, 40 V, 5 ms for 15 seconds). Electro stimulation was performed by means of gold-plated electrodes fixed on opposite walls of organ bath, using electro stimulator (ECM-Kragujevac, Serbia). Second set of experiments consisted of recording of trachea rings contractile responses to same agonists in the presence of phenyl ethylamine (permanent perfusion for 5 minutes before agonists use and during agonist's action, with final concentrations of 0.23, 2.3, 23 and 230 × 10<sup>-6</sup> mol/L). Next concentration of agonist on the same preparation was tried only after a period of 15 min. All drugs were applied to organ bath using 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 contractions for each group of experiments.

### Chemicals

Drugs used in these experiments were histamine, acetylcholine (Sigma-Aldrich, USA), phenylethylamine (Calbiochem, GB) and KCl (Zorka Šabac, Serbia). The drugs were prepared on the day of experiment in 154 mmol/L NaCl (Zorka Šabac, Serbia). Concentrations reported are expressed as final concentrations within the organ bath.

### Statistical analysis

Each concentration was assayed on isolated preparatus from five different animals. Concentration-response curves were constructed using linear regression according to least-squares analysis (13, 14). Effective concentration of agonists that produced 50% of maximal response was calculated for each agonist together with its confidence limits (1.96 × standard error). The results were considered statistically significant when  $p \leq 0.05$ .

## Results

Acetylcholine (1 × 10<sup>-6</sup> mol/L to 66 × 10<sup>-6</sup> mol/L) produced concentration-dependent tonic contractions of isolated guinea-pig trachea rings (EC<sub>50</sub> = 16.87 ± 1.1 × 10<sup>-6</sup> mol/L,  $p < 0.001$ ). KCl (2 × 10<sup>-3</sup> to 100 × 10<sup>-3</sup> mol/L) produced concentration-dependent tonic contractions of isolated guinea-pig trachea rings (EC<sub>50</sub> = 20.24 ± 1.1 × 10<sup>-3</sup> mol/L,  $p < 0.001$ ). 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.

Histamine (5 × 10<sup>-6</sup> mol/L to 150 × 10<sup>-6</sup> mol/L) produced concentration-dependent tonic contractions of isolated guinea-pig trachea rings (EC<sub>50</sub> = 20.79 ± 1.1 × 10<sup>-6</sup> mol/L,  $p < 0.001$ ).

Phenylethylamine did not affect the basal tone of isolated guinea-pig trachea rings in all applied concentrations. In addition, phenyl ethylamine in all applied doses had no influence effects of acetylcholine, KCl and electro stimulation on isolated preparations. On the other hand, phenylethylamine (0.23, 2.3, 23 and 230 × 10<sup>-6</sup> mol/L) caused concentration-dependent potentiation of tonic contractions of isolated guinea-pig trachea rings produced by histamine (EC<sub>50</sub> = 16.42 ± 1.1 × 10<sup>-6</sup> mol/L,  $p < 0.001$ , EC<sub>50</sub> = 12.94 ± 1.2 × 10<sup>-6</sup> mol/L,  $p < 0.001$ , EC<sub>50</sub> = 9.21 ± 1.2 × 10<sup>-6</sup> mol/L,  $p < 0.001$  and EC<sub>50</sub> = 6.87 ± 1.2 × 10<sup>-6</sup> mol/L,  $p < 0.001$ , respectively) (Figure 1).

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

## Discussion

Even effects of acetylcholine, KCl, electro stimulation and histamine on tone of airway smooth muscle are already well known, as well as their mechanisms of action, we performed such a trial in order to check our experimental setting (sensitivity and reproducibility) comparing to previous reports.

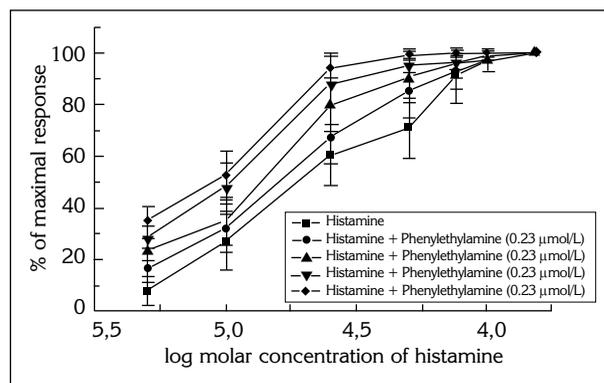


Figure 1 Effects of phenylethylamine (0.23, 2.3, 23 and 230 × 10<sup>-6</sup> mol/L) on tonic contractions of isolated guinea-pig trachea rings produced by histamine

The basic aim of this study was evaluation of phenylethylamine effects on different mechanisms leading to same final consequence i.e. contraction of trachea smooth muscle. Phenylethylamine had no effect on basal tone of isolated guinea-pig trachea meaning that it has no direct action on histamine receptors in guinea-pig trachea, because histamine itself produces constriction via H1 receptors (1), and decreases tracheal smooth muscle tone via H2 (2) and H3 receptors (3). It, also, had no influence on effects of acetylcholine, KCl and electro stimulation action to the musculature of guinea-pig trachea.

On the other hand, phenylethylamine strongly potentiated histamine induced constriction of guinea-pig trachea smooth muscle. Taking in consideration all facts mentioned above with previous reports that phenylethylamine is competitive antagonist of histamine N-methyl-transferase (4), enzyme responsible for rapid inactivation of histamine (6), we suggest a possibility that potentiation of histamine effects on guinea-pig trachea may be due to decrease of histamine methylation in presence of this competitive antagonist.

## FENILETILAMIN UTIČE NA HISTAMINOM INDUKOVANU KONTRAKCIJU IZOLOVANE TRAHEJE ZAMORČIĆA

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*Kratak sadržaj:* Histamin dovodi do konstrikcije glatke muskulature traheje preko H1 receptora i smanjuje njen tonus preko H2 i H3 receptora. Poznato je, takođe, da je feniletilamin kompetitivni antagonist histamin N-metil-transferaze (HMT), enzima koji je odgovoran za brzu inaktivaciju histamina. Dobijeni rezultati sugerišu mogućnost da feniletilamin kao kompetitivni antagonist histamin N-metil-transferaze, dovodi do potenciranja histaminom indukovane konstrikcije izolovane traheje zamorčića, verovatno usled smanjenja metilacije histamina, odnosno njegove inaktivacije. U isto vreme, feniletilamin ne utiče na bazalni tonus intaktnih izolovanih prstenova traheje, kao i na druge mehanizme koji dovode do povećanja odgovora glatke muskulature traheje zamorčića (acetilholin, KCl, elektro stimulacija).

*Ključne reči:* feniletilamin, histamin N-metil-transferaza, disajni putevi

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