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# PARTICIPATION OF $H_1$- AND $H_3$-RECEPTORS IN THE REGULATION OF GUINEA-PIG TRACHEAL TONE - INFLUENCE OF INDOMETHACIN

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## ABSTRACT

**The tracheal tone is influenced by histaminergic receptors and by the epithelium derived relaxing factor (EpDRF) which contains metabolites of arachidonic acid. The aims of the present study were the following: 1) to investigate the role of $H_1$- and $H_3$-receptors in the tracheal tone regulation using as test substances histamine (H), the $H_1$-receptor antagonist mepyramine and the $H_3$-receptor agonist (R)α-methylhistamine [(R)α -MHA]; 2) to study the influence of the cyclooxygenase (COX) inhibitor indomethacin on the tracheal responsiveness to the examined substances. The experiments were carried out on isolated guinea-pig tracheal rings. Histamine at cumulative concentrations contracts the trachea. In the presence of mepyramine the concentration-response curves to H are shifted to the right that proves the concept of the $H_1$-receptor mediated tracheal contraction. In the presence of indomethacin the concentration-response curves to H are shifted to the left and the maximal contraction is significantly increased due to the inhibition of the COX pathway. (R)α-MHA at concentrations up to $1x10^{-4}$ M leads to a slight tracheal contraction, which is probably $H_1$-receptor mediated as it is reduced in the presence of mepyramine. The $3x10^{-4}$ M concentration of (R)α-MHA relaxes the trachea probably due to its $H_3$-receptor agonistic activity. In the presence of indomethacin this relaxation is turned to a further contraction. After tracheal precontraction by H, (R)α-MHA concentration dependently relaxes the trachea. This spasmolytic effect is insignificantly influenced by mepyramine and is almost completely abolished in the presence of indomethacin, which shows that it is probably due to an increased production of metabolites of arachidonic acid.**

**Key words**: tracheal tone, indomethacin, $H_1$-receptor, $H_3$-receptor, guinea pig

## INTRODUCTION

The histaminergic $H_1$- and $H_3$-receptors as well as the epithelium derived relaxing factor (EpDRF) take part in the tracheal tone modulation. The EpDRF contains metabolites of arachidonic acid, in particular prostaglandins $E_1$ and $E_2$ (PGE1 and PGE2), and nonprostanoid substances as well (4,5,6,8). The histamine-induced bronchoconstriction is due to the stimulation of $H_1$-receptors. Mepyramine is an $H_1$-receptor antagonist, while (R)α-methylhistamine [(R)α-MHA] is a potent and selective $H_3$-receptor agonist and possesses only 1% of the activity of histamine on $H_1$- and $H_2$-receptors (3).

Indomethacin, a non-selective cyclooxygenase (COX) inhibitor, leads to an airway hyperresponsiveness *in vitro* as a result of the decreased synthesis of the inhibitory prostanoids (PGE2 and PGI1). These prostanoids are normally synthesized by the bronchial epithelium and inhibit the bronchial contraction by LTC4 and LTD4 (1,2).

The aims of the present study were:

1. To study the role of $H_1$- and $H_3$-receptors in the regulation of guinea-pig tracheal tone *in vitro* using as test substances histamine, mepyramine and (R)α-MHA.
2. To investigate the influence of indomethacin on the tracheal responsiveness to $H_1$- and $H_3$-receptor agonists.

## MATERIAL AND METHODS

Rings, two adjacent cartilages wide, were isolated from male guinea pigs (450-600g) and tied to force-displacement transducers for the measurement of isometric tension responses.

They were placed in organ baths (37°C) containing a solution with the following composition (millimolar): NaCl 119, KCl 4.6, $CaCl_2$ 1.5, $MgCl_2$ 1.2, $NaHCO_3$ 15, $NaH_2PO_4$ 1.2 and glucose 11. The solution was gassed with

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95% $O_2$ and 5% $CO_2$ for maintenance of pH 7,4. Before each experiment the tissues were equilibrated for 60min under a resting load of 2g and washed every 15min with fresh solution. The number of organs (taken from different animals) was six in all the experiments. The drugs used were the following: histamine dihydrochloride (Fluka Chemie, Switzerland), mepyramine (Sigma Chemical Co, USA), (R)α-MHA (Free University, Berlin, Germany) and indomethacin (Fluka Chemie, Switzerland).

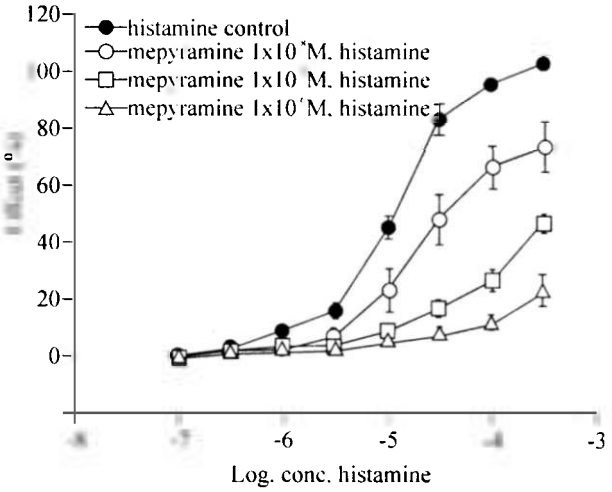


*Fig. 1A. Effect of histamine alone and in the presense of mepyramine on guinea-pig trachea*

The experiments included:

1. Registration of tissue isometric tension responses to cumulative concentrations of histamine (from $1x10^{-7}$M to $3x10^{-4}$M) alone and in the presence of three concentrations of mepyramine ($1x10^{-8}$M, $1x10^{-7}$M and $1x10^{-6}$M).
2. Registration of tissue responses to the same cumulative concentrations of histamine after 20min. equilibration of the organs in the presence of indomethacin ($1x10^{-5}$M).
3. Registration of organ responses to cumulative concentations of (R)α-MHA (from $1x10^{-7}$M to $3x10^{-4}$M) alone and in the presence of two concentrations of mepyramine ($1x10^{-7}$M and $1x10^{-6}$M).
4. Registration of the responses to the same cumulative concentrations of (R)α-MHA after 20min. equilibration of the organs in the presence of indomethacin ($1x10^{-5}$M).
5. Precontraction of the trachea by histamine ($3x10^{-4}$M) and registration of the organ responses to cumulative concentrations of (R)α-MHA (from $1x10^{-7}$M to $1x10^{-4}$M) applied alone and in the presence of mepyramine ($1x10^{-7}$M).
6. Precontraction of the trachea by histamine ($3x10^{-4}$M) in the presence of indomethacin ($1x10^{-5}$M) and registration of the responses to cumulative concentrations of (R)α-MHA (from $1x10^{-7}$M to $3x10^{-4}$M).

*Calculation of organ responses*

The effect induced by $3x10^{-4}$M histamine was taken as

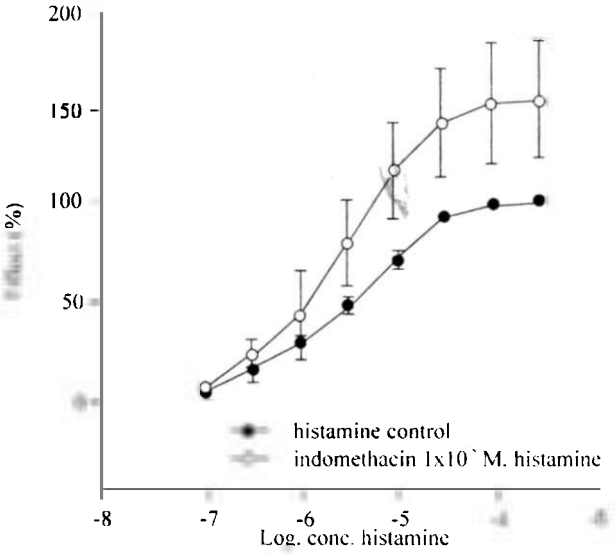


*Fig. 1B. Responses of guinea-pig trachea to cumulative concentrations of histamine alone and in the presence of $1x10^{-5}$M of indomethacin, n = 6 in all the experiments*

100% contraction. The tissue responses to each concentration of the examined substances were calculated as a percentage of the maximal contraction. The relaxation was calculated as a percentage of histamine precontraction.

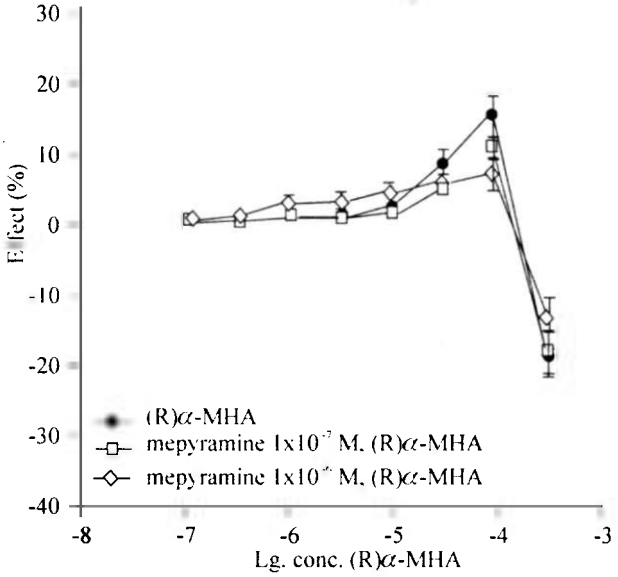


*Fig. 2A. Responses of guinea-pig trachea to cumulative concentrations of (R)-MHA alone and in the presence of $1x10^{-7}$M and $1x10^{-6}$M of mepyramine, n = 6 in all the experiments*

*Statistical analysis*

Results are given as mean ± SEM. Differences between means were considered significant at $p<0,05$. The $IC_{50}$ (the concentration causing 50% inhibition of the maximal tra-

cheal contraction) of mepyramine was determined by the method of Litchfield-Wilcoxon.

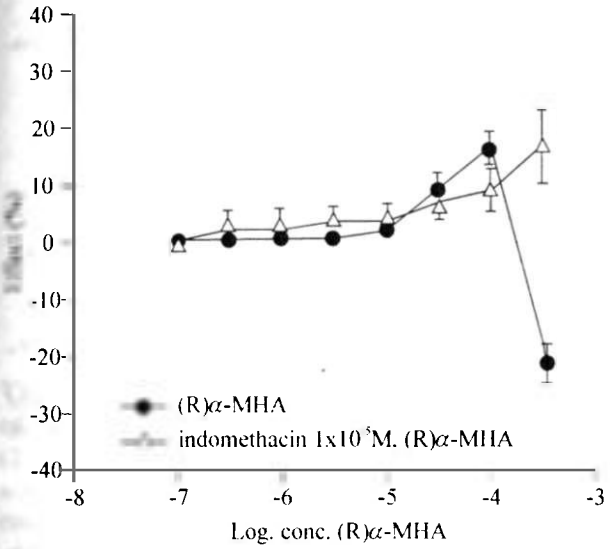


*Fig. 2B. Responses of guinea-pig trachea to cumulative concentrations of (R)α-MHA alone and in the presence of 1x10⁻⁵M of indomethacin, n = 6 in all the experiments*

## RESULTS AND DISCUSSION

*Experiments with histamine*

Histamine at cumulative concentrations from 1x10⁻⁷M to 3x10⁻⁴M leads to a concentration-dependent contraction of guinea-pig tracheal rings (Fig. 1A); $pD_2$ for histamine is 5,4±0,05.

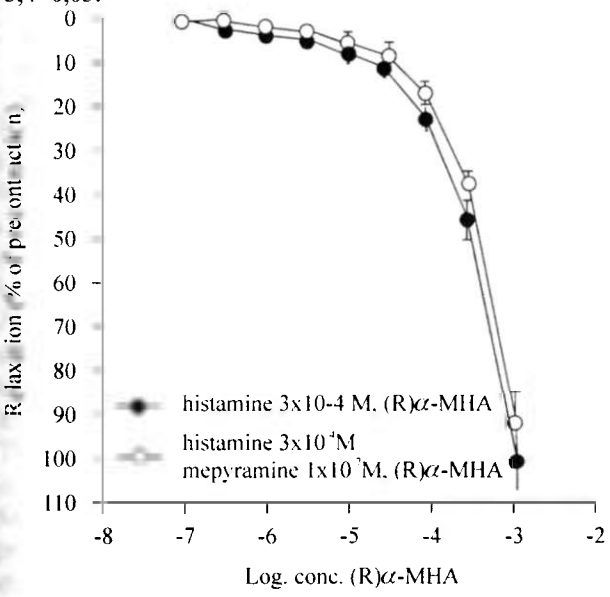


*Fig.3A. Responses of guinea-pig trachea precontracted by histamine (3x10⁻⁴M) to cumulative concentrations of (R)α-MHA alone and in the presence of 1x10⁻⁷M of mepyramine, n = 6 in all the experiments*

In the presence of mepyramine (1x10⁻⁸M, 1x10⁻⁷M and 1x10⁻⁶M) the concentration-response curves to histamine are dose-dependently shifted to the right (Fig. 1A); $pD_2$ for mepyramine is 7,02±0,89. In the presence of indomethacin (1x10⁻⁵M) the dose-dependent curve to histamine is shifted to the left and the maximal contraction is increased up to 152,7±12,4% (Fig. 1B); $pD_2$ for histamine in this case is 5,78±0,4.

*Experiments with (R)α-MHA*

(R)α-MHA (from 1x10⁻⁷M to 1x10⁻⁴M) leads to a slight dose-dependent contraction of guinea-pig tracheal smooth muscle ($E_{max}$ =14,22±2,58%) while the 3x10⁻⁴M concentration of (R)α-MHA relaxes the trachea (E =-19,28±3,06%) (Fig. 2A).

The presence of 1x10⁻⁷M mepyramine leads to an insignificant reduction of the contractile responses to (R)α-MHA. In the presence of 1x10⁻⁶M mepyramine the contractile response to 1x10⁻⁴M (R)α-MHA is significantly ($p < 0,05$) reduced ($E_{max}$ =6,1±2,27%) (Fig. 2A). Mepyramine at both concentrations does not influence the relaxing effect of the 3x10⁻⁴M concentration of (R)α-MHA (Fig. 2A).

In the presence of indomethacin (1x10⁻⁵M) the responses to (R)α-MHA from 1x10⁻⁷M to 1x10⁻⁴M are insignificantly changed while the 3x10⁻⁴M concentration of (R)α-MHA does not relax but further contracts the trachea (E=14,44±5,67%) (Fig. 2B).

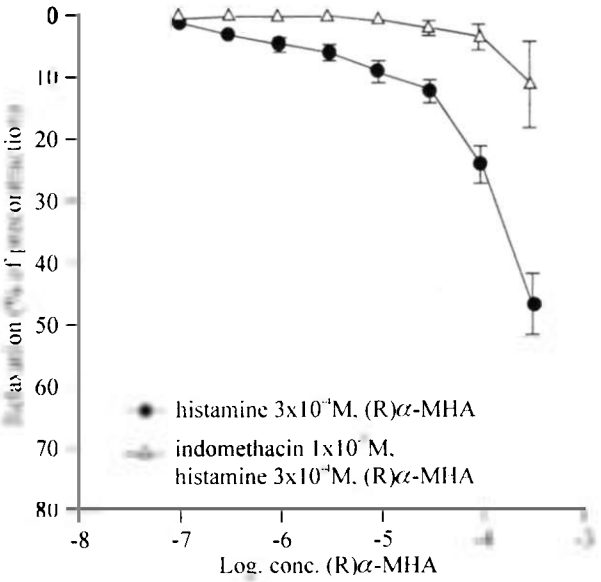


*Fig.3B. Responses of guinea-pig trachea precontracted by histamine (3x10⁻⁴M) in the presence of indomethacin (1x10⁻⁵M) to cumulative concentrations of (R)α-MHA, n=6 in all the experiments*

After precontraction of the trachea by 3x10⁻⁴M of histamine (R)α-MHA (from 1x10⁻⁷M to 1x10⁻³M) leads to a concentration-dependent relaxation of the tracheal rings and the tracheal tone is almost turned to the pretreatment base level; $pD_2$ for (R)α-MHA is 3,29±0,048 (Fig. 3A).

The spasmolytic effect of (R)α-MHA on the trachea precontracted by histamine is not significantly changed in the presence 1x10⁻⁷M concentration of mepyramine (Fig. 3A). (R)α-MHA produces a slight concentration-dependnt relaxation of the tracheal rings precontracted by histamine in the presence of indomethacin. The relaxant effect of

$3x10^{-4}$M (R)α-MHA in this case is 10,97 ±7,02% while the relaxant effect of the same concentration of (R)α-MHA in the absence of indomethacin is 46,39 ±4,88% (Fig. 3B).

## CONCLUSIONS

1. The tracheal contraction by histamine is a result of its agonistic action on the $H_1$- receptors, which are coupled to inositol phosphate hydrolysis (6). The concept of the $H_1$- receptor-mediated tracheal contraction is supported by the mepyramine-induced rightward shift of the concentration-response curves to histamine. Tracheal responsiveness to histamine is increased in the presence of the COX inhibitor indomethacin. This may be a result of the actions of LTC4 and LTD4 the effects of which are normally antagonized by the inhibitory prostaglandins (PGE2 and PGI1) synthesized in the COX pathway (1,2).
2. The contractile effect of the lower concentrations of (R)α-MHA might be due to an $H_1$- receptor agonistic activity as it is reduced in the presence of mepyramine.
3. The relaxant effect of the $3x10^{-4}$M concentration of (R)α-MHA applied alone as well as the relaxation of histamine-precontracted guinea-pig trachea exerted through cumulative (R)α-MHA concentrations is, probably, due to its agonistic action on the $H_3$-receptors.

Several mechanisms may be involved in this tracheal relaxation. There is evidence that $H_3$-receptor stimulation inhibits vagal cholinergic transmission in the airways and non-adrenergic non-cholinergic bronchoconstriction as well (5). $H_3$-receptor stimulation also mediates the relaxation of guinea-pig trachea by increasing the release of EpDRF containing metabolites of arachidonic acid as this relaxation is abolished in the presence of the COX inhibitor indomethacin (4,7.8).

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