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## INFLUENCE OF XANTHINE DERIVATIVES ON COUGH AND AIRWAY REACTIVITY IN GUINEA PIGS

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Xanthine derivatives may inhibit phosphodiesterases without selective action on their single isoforms. In this study, effects of theophylline and theobromine on cough and airway reactivity were evaluated in awake guinea pigs using double-chamber whole body plethysmograph. Pre-treatment with theophylline and theobromine (10 mg/kg, i.p.) decreased the number of cough efforts evoked by inhalation of citric acid aerosol (0.6 mol/l) in both healthy and ovalbumin-sensitized animals. Theophylline and theobromine decreased *in vivo* airway reactivity, *i.e.*, specific airway resistance measured after nebulization of citric acid and histamine aerosol ( $10^{-6}$  mol/l), only in ovalbumin-sensitized animals, whereas *in vitro* reactivity to cumulative doses of histamine and acetylcholine ( $10^8$ - $10^{-3}$  mol/l) measured in organ chambers significantly decreased in both healthy and ovalbumin-sensitized animals, with more pronounced effect in the latter group. In conclusion, administration of theophylline and theobromine influenced the cough and airway reactivity in ovalbumin-sensitized guinea pigs, indicating the anti-inflammatory potential of xanthine derivatives.

Key words: *airway reactivity, cough, ovalbumin, phosphodiesterase, xanthine derivatives*

### INTRODUCTION

Cough is a frequent sign of many respiratory diseases, persecuting the patients. As many currently available antitussive drugs show insufficient effectivity, or are associated with serious adverse effects (1), searching for new approaches in the therapy of cough is still a hot topic.

In the recent experiments, antitussive effects of naturally occurring agents (plant extracts) from *Embllica officinalis* (2), *Paederia foetida* (3) or *Gnaphalium liebmannii* (4) were demonstrated. Suppression of cough by plant extracts may be mediated by various mechanisms. However, Sanchez-Mendoza *et al.* (4) found that relaxing effect of crude flowers extract of *Gnaphalium liebmannii* on tracheal smooth muscle was caused by phosphodiesterase (PDE) inhibition. Beside the above mentioned substances, agents influencing different types of ion channels and receptor structures are nowadays increasingly used for their antitussive effects (5).

Nevertheless, in the therapy of airway diseases associated with cough and inflammation, such as bronchial asthma and chronic obstructive pulmonary disease, several agents from a group of xanthine derivatives are historically used. Although they are generally considered to be non-selective inhibitors of PDE without selective action on its single isoforms, in therapeutically relevant plasma concentrations several other mechanisms are involved in their effects, *e.g.*, antagonism with adenosine receptors, activation of histone-deacetylases, and others (6-8). Although bronchodilating and anti-inflammatory actions of PDE inhibitors have been elucidated, little is known

about antitussive effects of xanthine derivatives (9, 10) or selective PDE inhibitors (PDE3, PDE4, PDE5) (11-13).

To better understand the underlying mechanisms and relations between the cough and airway reactivity, in the present study the effects of theophylline and theobromine, representatives of xanthine derivatives, on cough and *in vivo* and *in vitro* airway reactivity were assessed.

### MATERIAL AND METHODS

The study protocol was approved by local Ethics Committee at Jessenius Faculty of Medicine, Comenius University in Martin, Slovakia. Forty eight healthy male guinea pigs (Trik, 250-350 g) were used for the study. They were kept in an animal house and had food and water *ad libitum*. Animals were divided into six groups, n=8 in each group. In three groups, airway hyperresponsiveness was induced by exposure to ovalbumin antigen. Other three groups served as non-sensitized controls. In both ovalbumin-sensitized and non-sensitized animals, the first group was left without treatment, the second one was treated with theophylline (Sigma Aldrich, Germany) and the third one was treated with theobromine (Sigma Aldrich, Germany), both at a dose of 10 mg/kg.

#### *Antigen-induced airway hyperresponsiveness*

Sensitization of animals by antigen ovalbumin, which causes changes in airway reactivity on immunological basis, was

performed within 14 days (14, 15). The allergen (1% ovalbumin) was administered i.p. on the 1<sup>st</sup> day of sensitization (0.5 ml) and subcutaneously (0.5 ml), on the 3<sup>rd</sup> day intraperitoneally (1 ml) and on the 14<sup>th</sup> day only by inhalation (3 min). The measurement of *in vivo* airway reactivity to mediators of bronchospasm followed immediately the inhalation of ovalbumin. The measurement of *in vitro* reactivity was done after sacrificing the animal. In the treated groups, theophylline or theobromine was administered 30 min before the nebulization.

#### Cough reflex assessment

To assess the cough reflex, the method of chemically-induced cough was used (5, 14). The animal was placed in a double chamber whole body plethysmograph and aerosol of citric acid at a concentration of 0.6 M in saline was used for cough provocation. During 2 min of inhalation of citric acid and the following 2 min, a well trained observer evaluated visually and acoustically the number of cough efforts. To distinguish cough from sneezing or movement artifacts, subsequent evaluation of the computer records of air-flow in the nasal chamber was performed.

#### Evaluation of *in vivo* airway reactivity

*In vivo* airway reactivity was evaluated using a double chamber plethysmograph immediately after administration of bronchoconstrictors (14). Specific airway resistance and its

changes after a short-term inhalation (2 min) of bronchoconstricting agents (citric acid and histamine at a concentration of 10  $\mu$ M in saline) were considered as an indicator of the *in vivo* reactivity changes. For comparison, reactivity after nebulization of saline was used. Between two exposures, there was an interval of minimum 5 min. During intervals, fresh air was insufflated into the nasal chamber.

#### Evaluation of *in vitro* airway reactivity

After sacrificing the animals, trachea, and lungs were immediately excised. Tracheal strips (approximately 15 mm) were cut on the opposite side of a smooth muscle. Lung tissue strips (2 x 2 x 15 mm) were cut from the margin of upper lobe of right and left lungs. The strips were mounted between two hooks and placed into the 30 ml organ chambers containing Krebs-Henseleit's buffer (NaCl 110.00 mmol/l, KCl 4.80 mmol/l, CaCl<sub>2</sub> 2.35 mmol/l, MgSO<sub>4</sub> 1.20 mmol/l, K<sub>2</sub>HPO<sub>4</sub> 1.20 mmol/l, NaHCO<sub>3</sub> 25.00 mmol/l, and glucose 10.00 mmol/l in glass-distilled water). The chambers were maintained at 36.5 $\pm$ 0.5°C and aerated continuously with a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub> to maintain pH 7.5 $\pm$ 0.1. One of the hooks was connected to a force transducer (TENSIL 10, RES Martin, Slovakia) and an amplifier (TEMES 1052, RES Martin, Slovakia), and tension changes were recorded online using special computer software (TEMES 1, RES Martin, Slovakia). The tissue strips were initially set to 4 g of tension for 30 min (loading phase). Then, in each strip the tension was readjusted to a baseline value of 2 g for

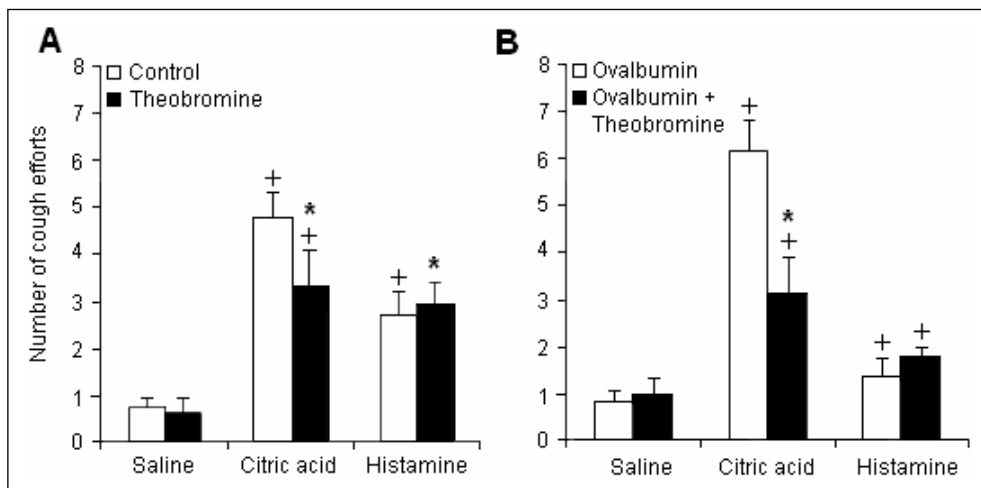


Fig. 2. Number of cough efforts before and 30 min after administration of theobromine at a dose of 10 mg/kg i.p. in healthy (A) and ovalbumin-sensitized guinea pigs.  $^+P<0.05$  vs. saline,  $^*P<0.05$  vs. control or ovalbumin.

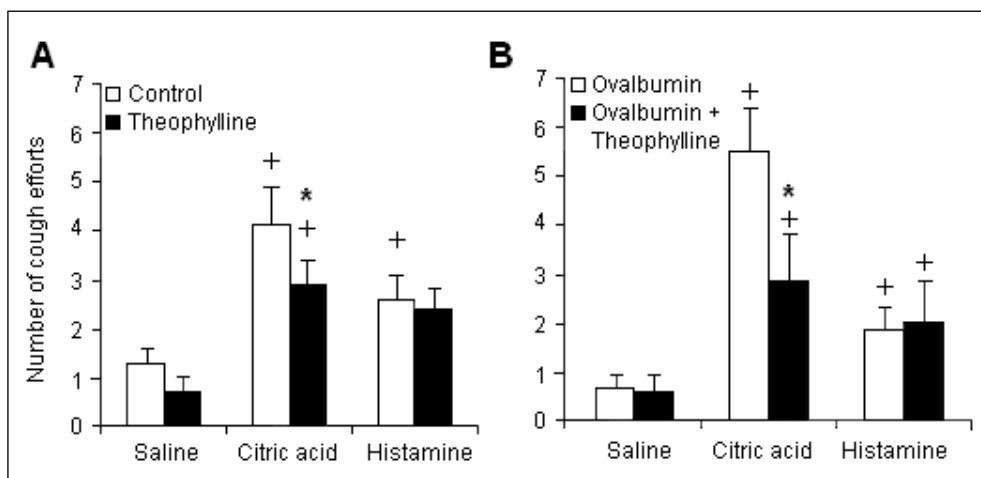


Fig. 1. Number of cough efforts before and 30 min after administration of theophylline at a dose of 10 mg/kg i.p. in healthy (A) and ovalbumin-sensitized guinea pigs.  $^+P<0.05$  vs. saline,  $^*P<0.05$  vs. control or ovalbumin.

another 30 min (adaptation phase). During both periods, the tissue strips were washed at 10 min intervals. Cumulative doses of histamine and acetylcholine ( $10^{-8}$  to  $10^{-3}$  mol/l, Sigma-Aldrich, Germany) were added after the adaptation phase had been finished and a continuous recording of contractions was made (16, 17). Data of the tracheal and lung tissue reactivity are shown in grams of the smooth muscle tension.

All data are shown as means $\pm$ SE. For statistical analysis, one-way ANOVA was used. A  $P < 0.05$  was considered statistically significant.

## RESULTS

Sensitization of guinea pigs with ovalbumin significantly increased the number of cough efforts as well as the specific airway resistance. *In vitro* studies confirmed significantly increased tracheal and lung tissue reactivity to histamine and acetylcholine.

In the healthy non-sensitized animals, pre-treatment with theophylline and theobromine, respectively, decreased the number of cough efforts evoked by inhalation of citric acid (Fig. 1A and Fig. 2A). The number of cough efforts evaluated by inhalation of saline and histamine aerosols was lower compared to citric acid, but without any significant effect of either theophylline or theobromine.

A 14-day long sensitization with ovalbumin did not influence the response to administration of theophylline and

theobromine with respect to the number of cough efforts (Fig. 1B and Fig. 2B).

By assessing specific airway resistance, as a marker of *in vivo* airway reactivity, we confirmed the bronchodilating effect of both theophylline and theobromine. However, both drugs significantly decreased this parameter only in the ovalbumin-sensitized guinea pigs, with no activity observed in healthy animals (Fig. 3).

Beneficial effects of theophylline and theobromine on ovalbumin-induced airway hyperreactivity were confirmed also in *in vitro* measurements. Both xanthine derivatives significantly decreased the lung smooth muscle contractile responses to cumulative doses of histamine in the ovalbumin-sensitized guinea pigs, confirming their anti-inflammatory potential (Fig. 4). Similar results were observed also in tracheal tissue, as well as after cumulative doses of acetylcholine (data not shown).

## DISCUSSION

The phosphodiesterases represent 11 superfamilies of metallophosphohydrolases, hydrolyzing cAMP and cGMP to their inactive metabolites (18, 19). PDE isoenzymes play an important role in the regulation of diameter of the airways and the functions of smooth muscle. PDE3 and PDE4, both hydrolyzing cAMP, were confirmed as major PDE isoforms active in the airways. However, airway smooth muscle contains more PDE isoenzymes, e.g., PDE 1, 3, 4, 5, and 7.

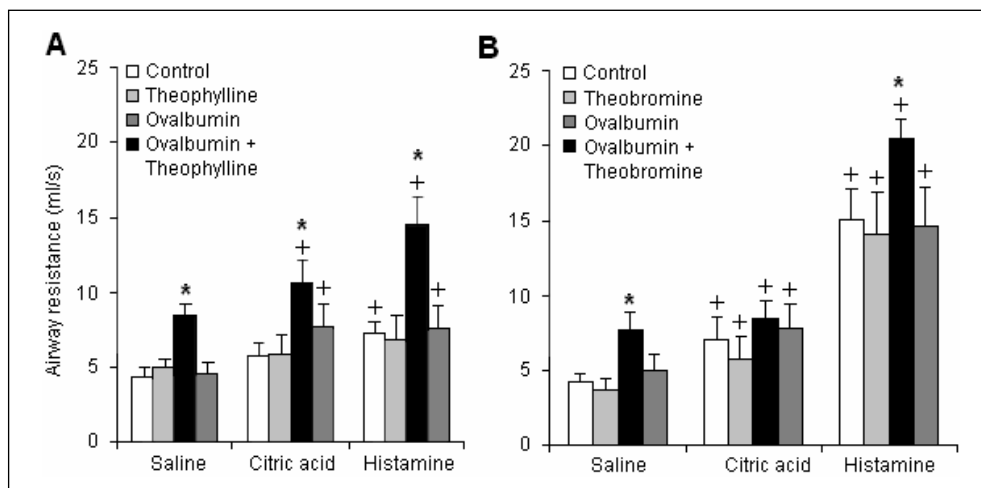


Fig. 3. Specific airway resistance in healthy and ovalbumin-sensitized guinea pigs before and after administration of theophylline (A) and theobromine (B) at a dose of 10 mg/kg i.p. after inhalation of aerosol with saline, citric acid - 0.6 mol/l, and histamine  $10^{-6}$  mol/l. \* $P < 0.05$  vs. saline, \* $P < 0.05$  vs. control, theophylline/theobromine + ovalbumin, and theophylline/theobromine.

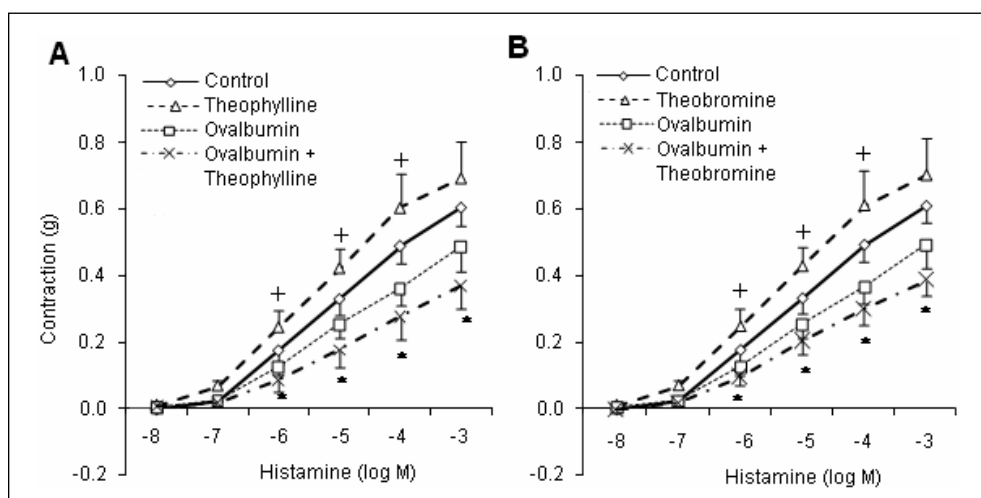


Fig. 4. *In vitro* airway reactivity (lungs) to cumulative doses of histamine ( $10^{-8}$ - $10^{-3}$  mol/l) in healthy and ovalbumin-sensitized guinea pigs before and after theophylline (A) or theobromine (B) administration at a dose of 10 mg/kg i.p. \* $P < 0.05$  ovalbumin vs. control, \* $P < 0.05$  ovalbumin + theophylline/theobromine vs. ovalbumin.

Inhibition of the phosphodiesterase PDE3, PDE4, and PDE7 may be used to assess airway inflammation and contractility (20). PDE3 seems the most suitable target to affect airway reactivity and cough. PDE3 is expressed in airway smooth muscle, myocardium, vessels, and the gastrointestinal tract. However, some authors consider inhibitors of PDE4 as the most important therapeutic tools. Although a first generation inhibitor of PDE4, rolipram, has not been introduced into clinical practice, due to adverse effects (nausea, vomiting), new perspectives occurred after testing the second generation of PDE4 inhibitors (roflumilast, cilomilast), as they maintain anti-inflammatory and immunomodulating effects at a lower incidence of adverse effects (21, 22). The antitussive effect of citalopram, another selective PDE4 inhibitor, has recently been confirmed in an experimental setting (10). Nevertheless, because of lack of clinical data on their antitussive effects, the selective inhibitors (including the second generation agents) have not yet been approved for respiratory therapy.

Low specificity of the mechanism of action, interactions with other drugs, and a narrow therapeutic range limit the use of xanthine derivatives as antitussives (21, 22). However, both xanthine derivatives tested in the present study effectively suppressed cough and made significant bronchodilation. Our findings confirm also the previous observations that theophylline and theobromine show more pronounced effects in ovalbumin-sensitized animals (with airway hyperresponsiveness) (23, 24).

Theophylline and theobromine-induced decrease in the number of cough efforts triggered by inhalation of citric acid aerosol confirms our previous results in cats (9) as well as the data published by Usmani *et al.* (11). Both effects - suppressed intensity of cough by theophylline after mechanical stimulation of cough receptors with a nylon fiber in laryngopharyngeal and tracheobronchial areas (9) and decreased number of chemically-induced cough with inhalation of citric acid aerosol - suggest a potential use of xanthine derivatives as antitussives. As demonstrated in our previous study, intraperitoneal administration of theophylline in conscious cats has a stronger antitussive effect than the non-narcotic antitussive drug dextrometorphan (9).

As selective inhibition of PDE3 and PDE4 leads to similar results, inhibition of phosphodiesterases seems a major mechanism responsible for antitussive effects of xanthine derivatives. However, there are still other mechanisms, which could participate in the suppression of cough - especially, an indirect anti-inflammatory action in animals with airway hyperresponsiveness based on the antagonism with adenosine receptors, or activation of histone acetylases (in lower therapeutic concentrations) (6). Participation of anti-inflammatory effects in the action of xanthine derivatives is supported by the *in vitro* results of the present study where stronger suppressive effects on airway reactivity (contractile responses of tracheal and lung tissue strips to cumulative doses of histamine and acetylcholine) were found in ovalbumin-sensitized guinea pigs compared with the healthy group.

In conclusion, suppression of cough and airway reactivity by xanthine derivatives (theophylline and theobromine) indicates the anti-inflammatory potential of these agents in the model of ovalbumin-sensitized guinea pigs, which suggests their possible usefulness in therapy of cough associated with chronic inflammatory airway conditions.

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Conflict of interests: None declared.

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