

EXPERIMENTAL STUDY

The influence of the PDE inhibitors on cough reflex in Guinea pigs

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Abstract: In this study the effects of non-selective PDE inhibitors (theophylline and theobromine) and selective inhibitors of PDE 1, 3, 4 and 5 on cough, induced by citric acid, were evaluated.

Inhalation of citric acid aerosol was used for cough provocation in healthy and ovalbumin-sensitized guinea pigs and the number of cough efforts was registered after visual and acoustic control by a skilled observer, with subsequent evaluation of airflow changes in a double chamber whole body plethysmograph.

The pre-treatment with theophylline and theobromine (10 mg/kg b.w. intraperitoneally) decreased the number of cough efforts evoked by inhalation of citric acid aerosol (0.6 mol/l) in both healthy and ovalbumin-sensitized animals. The selective inhibitors (all 1 mg/kg b.w. intraperitoneally) of PDE1 (vinpocetin), PDE3 (cilostazol), and PDE4 (citalopram) showed antitussive effects in healthy guinea pigs. Conversely, the antitussive potential of PDE1 (vinpocetin), PDE4 (citalopram), and PDE5 (zaprinas) was observed in ovalbumin-sensitized animals. We conclude that the administration of non-selective PDE inhibitors influenced the citric acid-induced cough both in healthy and ovalbumin-sensitized guinea pigs, indicating the participation of a bronchodilating action and suppression of airway hyperreactivity in the cough suppression. With selective inhibitors, PDE4 inhibition seems to be the most effective in cough suppression, confirming its positive effects tested in chronic airway inflammatory diseases associated with bronchoconstriction and cough (Fig. 6, Ref. 27). Full Text in free PDF www.bmj.sk. Key words: phosphodiesterase, cough reflex, PDE inhibitors, xanthine derivatives, antitussive drugs.

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

In the therapy of airway diseases associated with cough and inflammation, such as bronchial asthma and chronic obstructive pulmonary disease (COPD), several historical agents from a group of xanthine derivatives are still used. They are generally considered to be non-selective inhibitors of phosphodiesterase (PDE) without selective actions on its single isoforms. Nevertheless, 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 (2, 3, 4). Furthermore, low specificity of their mechanism of action, interactions with other drugs, and a narrow thera-

peutic range can often lead to an occurrence of adverse effects, which can limit (especially in some groups of patients) their use (5, 6). Thus, the use of selective (PDE3, PDE4) or dual (PDE3/4, PDE4/7) PDE inhibitors in the therapy of these diseases and influencing cough could be beneficial.

Since bronchodilation and the anti-inflammatory action of PDE inhibitors is at least partially elucidated, little is known about the antitussive effects of xanthine derivatives (7, 8) or selective PDE inhibitors (PDE3, PDE4, PDE5) (9, 10, 11). Selective inhibitors of PDE have attracted increasing attention in the therapy of respiratory diseases (12). PDE isoenzymes play an important role in the regulation of airways diameter and smooth muscle functions. PDE3 and PDE4, both hydrolyzing cAMP, were confirmed as major PDE isoforms in the airways. However, airway smooth muscle contains more PDE isoenzymes, e.g. PDE1, 3, 4, 5 and 7.

To evaluate the antitussive properties of xanthine derivatives as non-selective PDE inhibitors, the effects of theophylline and theobromine on citric acid-induced cough were studied. Furthermore, to elucidate the participation of PDE1, PDE3, PDE4, and PDE5 isoenzymes in cough, antitussive effects of their selective inhibitors were assessed.

Materials and methods

The study protocol was approved by the local Ethics Committee at the Jessenius Faculty of Medicine, Comenius University in Martin, Slovakia. Healthy male guinea pigs (Triuk, 250–

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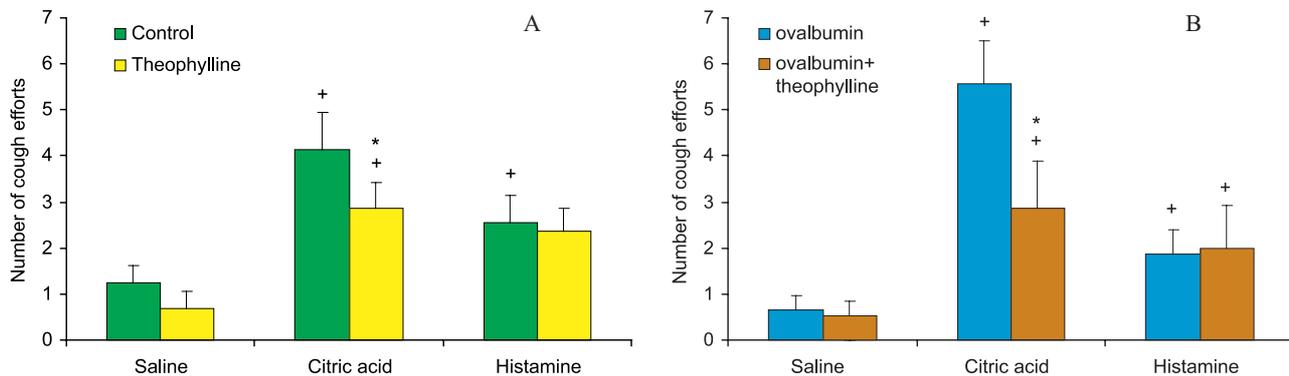


Fig. 1. Number of cough efforts before and 30 min after administration of theophylline at a dose of 10 mg/kg intraperitoneally in healthy (A) and ovalbumin-sensitized (B) Guinea pigs. (+ $p < 0.05$ vs. saline, * $p < 0.05$ vs control, or ovalbumin)

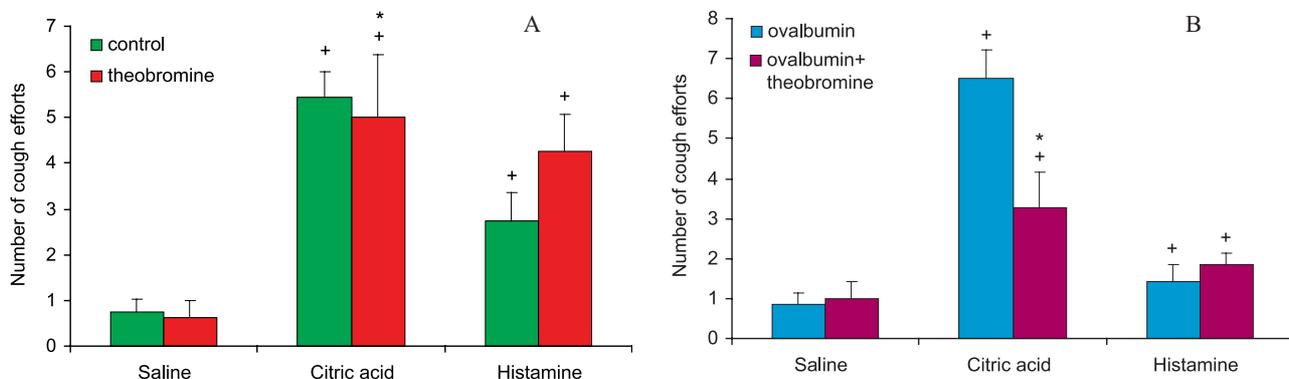


Fig. 2. Number of cough efforts before and 30 min after administration of theobromine at a dose of 10 mg/kg intraperitoneally in healthy (A) and ovalbumin-sensitized (B) Guinea pigs. (+ $p < 0.05$ vs. saline, * $p < 0.05$ vs control, or ovalbumin)

350 g) were used. They were kept in an animal house and had food and water *ad libitum*. Animals were divided into groups, each consisting of 6 guinea pigs. In seven groups, airway hyperresponsiveness was induced by exposure to ovalbumin antigen. The other seven groups served as non-sensitized controls. In both ovalbumin-sensitized and non-sensitized animals, the one group was left without treatment, and the others were treated with theophylline, theobromine (both at 10 mg/kg b.w.) or vinpocetin (PDE1 inhibitor), cilostazol (PDE3 inhibitor), citalopram (PDE4 inhibitor), and zaprinast (PDE5 inhibitor) (all at a dose of 1 mg/kg b.w.) 30 min before cough measurement. All PDE inhibitors were purchased from Sigma-Aldrich, Germany.

Antigen-induced airway hyperresponsiveness

Sensitization of animals by antigen ovalbumin, which causes changes in airway reactivity on an immunological basis, was performed within 14 days (13,14). The allergen (1 % ovalbumin) was administered on the 1st day of sensitization intraperitoneally (0.5 ml) and subcutaneously (0.5 ml), on the 3rd day intraperitoneally (1 ml) and on the 14th day only by inhalation (3 min). The number of cough efforts evoked by citric acid was

measured immediately after the inhalation of ovalbumin (1 %). In the treated groups, respective PDE inhibitors were administered 30 minutes before the nebulization.

Cough reflex assessment

To assess the cough reflex, the method of chemically-induced cough was used (13,15, 16). The animal was placed in double chamber whole body plethysmograph and an aerosol of citric acid in a concentration of 0.6 M in saline was used for cough provocation. As a control, inhalation with saline or histamine at a concentration of 10^{-6} M in saline was used. During 2 min of inhalation of citric acid and during following 2 min, a well trained observer evaluated visually and acoustically the number of cough efforts. To distinguish cough from sneezing or movement artefacts, subsequent evaluation of the computer records of air-flow in the nasal chamber was performed.

Statistical analysis

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

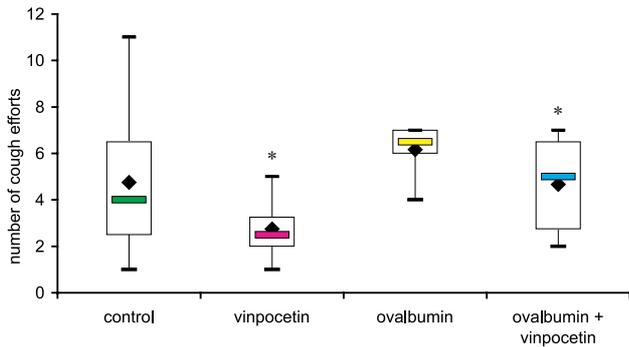


Fig. 3. Number of cough efforts during (2 min) and after (2 min) inhalation of citric acid aerosol in healthy, ovalbumin-sensitized and vinpocetin pre-treated Guinea pigs. Diamond represents mean, thick middle line median, lower and upper side of box plot 25th and 75th percentile and error bars minimum and maximum. (* $p < 0.05$ vs control)

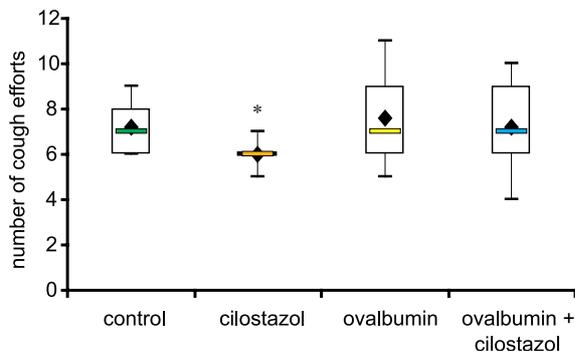


Fig. 4. Number of cough efforts during (2 min) and after (2 min) inhalation of citric acid aerosol in healthy, ovalbumin-sensitized and cilostazol pre-treated Guinea pigs. For explanations see Figure 3.

Results

The sensitization of guinea pigs with ovalbumin significantly increased the number of cough efforts evoked by inhalation of citric acid. In healthy non-sensitized animals, pre-treatment with theophylline and theobromine, respectively, decreased the number of cough efforts evoked by citric acid (Figs 1A and 2A). The number of cough efforts caused by inhalation of saline and histamine aerosols (0.6 M) was lower compared to citric acid, but without any significant effect of neither theophylline nor theobromine. Fourteen days lasting sensitization with ovalbumin did not influence the response to administration of theophylline and theobromine on the number of cough efforts (Figs 1B and 2B).

Intraperitoneal administration of vinpocetin, a PDE1 inhibitor, at a dose of 1 mg/kg b.w. 30 min before the experiment led to a significant suppression of cough (decreased number of cough efforts due to citric acid) both in healthy and ovalbumin-sensitized guinea pigs (Fig. 3). The PDE3 inhibitor cilostazol at the same dose significantly decreased the number of cough efforts in healthy guinea pigs. In ovalbumin-sensitized animals,

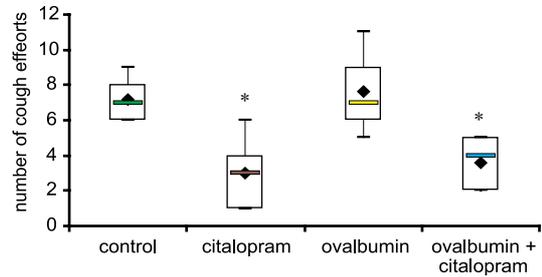


Fig. 5. Number of cough efforts during (2 min) and after (2 min) inhalation of citric acid aerosol in healthy, ovalbumin-sensitized and citalopram pre-treated Guinea pigs. For explanations see Figure 3.

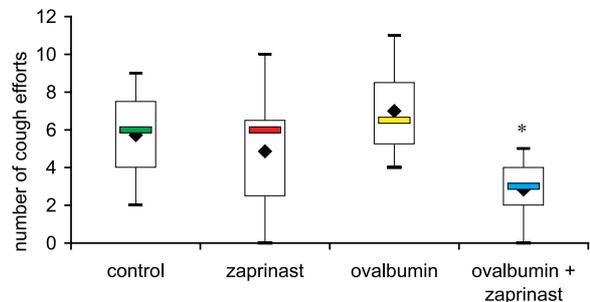


Fig. 6. Number of cough efforts during (2 min) and after (2 min) inhalation of citric acid aerosol in healthy, ovalbumin-sensitized and zaprinast pre-treated Guinea pigs. For explanations see Figure 3.

cilostazol did not influence cough significantly, suggesting its weaker effect in inflammation (Fig. 4). PDE4 inhibition by citalopram at the same dose led to a significant suppression of cough both in healthy and ovalbumin-sensitized guinea pigs (Fig. 5). On the other hand, selective PDE5 inhibition by zaprinast was accompanied by a significant decrease in number of cough efforts only in ovalbumin-sensitized animals (Fig. 6).

Discussion

Phosphodiesterases represent 11 superfamilies of metallophosphohydrolases, hydrolyzing cAMP and cGMP to their inactive metabolites (17,18). PDE isoenzymes play an important role in the regulation of diameter of the airways and the functions of smooth muscle.

Inhibition of PDE, especially PDE3 and PDE4, was previously confirmed as a suitable target for influencing the airway inflammation as well as the contractility of airway smooth muscle (12). In affecting airway reactivity and cough, the inhibition of PDE3 seems to be the most suitable target. PDE3 is expressed in airway smooth muscle, myocardium, vessels, and gastrointestinal tract. However, some authors consider inhibitors of PDE4 as the most important therapeutic tool. Although the inhibitor of PDE4 of the first generation (rolipram) was not introduced into

clinical practice for this indication due to its adverse effects (nausea, vomiting), new perspectives occurred after testing the second generation of PDE4 inhibitors (roflumilast, cilomilast), as they maintained anti-inflammatory and immunomodulating effects with a lower incidence of adverse effects (5, 6, 19). However, these selective inhibitors (including the second generation agents) were still not approved for the therapy of respiratory diseases due to remaining adverse effects.

In our experiments, cilostazol as a selective inhibitor of PDE3 showed the antitussive effect only in healthy animals (8). Similar results were observed by Matsuda et al (20), who considered inhibition of PDE3 as the most suitable way to influence cough and airway reactivity. Our results are in accordance with another study, where cilostazol in clinical conditions decreased the cough reflex sensitivity to capsaicin in patients with bronchial asthma (10). In a recent paper Fujimura and Liu (11) showed that the antitussive effect of olprinon (PDE3 inhibitor) to capsaicin was present in normal and sensitized guinea pigs with negligible effect on eosinophilia in bronchoalveolar lavage fluid. However, this effect was not observed after PDE4 inhibition with SB207499. Similarly, previously published data described the antitussive effects of PDE3 inhibitors (20, 21, 22). These data were complemented by our results, where the PDE4 inhibitor citalopram suppressed the cough both in healthy and ovalbumin-sensitized guinea pigs, compared to PDE3 inhibitor cilostazol suppressing the cough only in healthy guinea pigs.

There are no relevant data about the antitussive effects of PDE4 inhibitors, but our results indicate their efficiency in influencing cough. Furthermore, the administration of dual inhibitors (PDE3/4) should be considered, as previous findings demonstrated a more significant airway relaxing effect of siguazodan (PDE3 inhibitor) compared to rolipram (PDE4 inhibitor). On the other hand, their simultaneous administration led to an additive relaxation, suggesting an interaction or even synergism between the inhibition of PDE3 and PDE4 (23). Furthermore, zardaverine (dual inhibitor of PDE3/4) led after its inhalational administration to significant bronchodilation (12).

As the selective inhibition of PDE3 and PDE4 leads to similar results, the inhibition of phosphodiesterases seems to be the major mechanisms responsible for antitussive effect of the xanthine derivatives. However, there are also other mechanisms, which could participate in the suppression of cough – especially an indirect anti-inflammatory action in animals with airway hyperresponsiveness based on antagonism with adenosine receptors, or activation of histone de-acetylases (in lower therapeutic concentrations) (2). The participation of anti-inflammatory effects in the antitussive action of xanthine derivatives is supported also by the results of in vitro airway reactivity (contractile responses of tracheal and lung tissue strips to cumulative doses of histamine and acetylcholine), where stronger effects were found in ovalbumin-sensitized guinea pigs compared to the healthy group (24). A low specificity of the mechanism of action, interactions with other drugs, and a narrow therapeutic range often leading to an occurrence of adverse effects limits the use of xanthine derivatives as antitussives (5,6). However, both xanthine deriva-

tives tested in this study suppressed the cough effectively. Our findings confirm also the previous observations that theophylline and theobromine show more pronounced effects in ovalbumin-sensitized animals (with airway hyperresponsiveness) (21, 22, 24).

The decrease in the number of cough efforts evoked by inhalation of citric acid aerosol by theophylline and theobromine observed in this study confirms our previous results in cats (7) as well as the data published by Usmani et al. (9). It means that both effects – suppressed intensity of cough attack by theophylline after a mechanical stimulation of cough receptors by nylon fibre in the laryngopharyngeal and tracheobronchial areas (7), and decreasing the number of chemically-induced coughs by 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 had a stronger antitussive effect than the commercially used non-narcotic antitussive drug dextrometorphan (7).

Besides the selective PDE3 and PDE4 inhibitors, we have tested vincerin – PDE1 inhibitor and zaprinast – PDE5 inhibitor. Administration of both agents led to significant suppression of cough, with a smaller effect on bronchodilation. Thus, their antitussive effect is not associated with their weak bronchodilating effect and other mechanisms must be involved in their action, probably of anti-inflammatory origin. This is in concordance with results of Sebki et al (25), who did not show any effect of zaprinast on bronchodilation and eosinophil accumulation. Conversely, sildenafil as a newer PDE5 inhibitor led to a significant inhibition of bronchial hyperresponsiveness, leukocyte infiltration and levels of exhaled nitric oxide (26). This suggests a possible usefulness of PDE4/5 combination in asthma or COPD therapy. Any effects of PDE5 inhibitors on cough do not seem to have been described.

Furthermore, it is important to mention that some of the changes in cough could have been due to changes of nasal mucosa and stimulation of afferent nerves in upper airways during inflammation and its modulation by PDE inhibitors (27). However, the confirmation of this statement was beyond the study protocol and thus can be neither confirmed nor excluded based on our results.

In conclusion, the administration of non-selective PDE inhibitors influenced the cough due to citric acid both in healthy and ovalbumin-sensitized, indicating the participation of bronchodilation and suppression of airway hyperreactivity in cough suppression. From the selective inhibitors, PDE4 inhibition seems to be the most effective in cough suppression, confirming its positive effect found in chronic airway inflammatory diseases associated with bronchoconstriction and cough.

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