ELSEVIER

European Journal of Pharmacology 278 (1995) 253–256

ejp

Short communication

Furosemide inhibits bradykinin-induced contraction of human bronchi: role of thromboxane A_2 receptor antagonism

Mathieu Molimard [a,b,*], Emmanuel Naline [a], Albert Hirsch [b], Charles Advenier [a]

[a] *Laboratoire de Pharmacologie, Faculté de Médecine Paris Ouest, 15, rue de l'École de Médecine, 75270 ParisCedex 06, France*
[b] *Service de Pneumologie, Hôpital Saint Louis, 1, avenue Claude Vellefaux, 75010 Paris, France*

Received 14 February 1995; revised 7 March 1995; accepted 10 March 1995

Abstract

Bradykinin contracts human isolated small bronchi through prostanoid release and subsequent TP receptor stimulation. Furosemide 10^{-4} to 10^{-3} M concentration dependently inhibited bradykinin- and the stable TP receptor agonist U-46619-induced contraction of human isolated small airways. The inhibitory effect of furosemide on U-46619-induced contraction involves competitive antagonism at TP receptors. Such an inhibition of TP receptors could a least partly explain the inhibitory effect of furosemide on bradykinin-induced contraction, and could be one of the mechanisms of the protective effect of furosemide in asthma.

Keywords: Bradykinin; Furosemide; Thromboxane A_2 receptor; Smooth muscle; Airway

1. Introduction

Inhalation of the loop diuretic, furosemide, has been shown to protect against various bronchocontrictor challenges in asthma (Chung and Barnes, 1992; Lockhart and Slutsky, 1994). The mechanism of this action remains uncertain. Inhibition of Na^+/Cl^- co-transport processes in the membrane of epithelial cells, release of bronchodilatator prostaglandins from airway epithelium and inhibition of C-fiber stimulation have been put forward to explain this effect. We have demonstrated earlier that furosemide inhibits the bradykinin-induced contraction of the guinea pig isolated trachea (Molimard and Advenier, 1993). Recently, Rajakulasingam et al. (1994) have demonstrated that aerosolized furosemide inhibits bradykinin-induced bronchoconstriction in asthmatics.

Since we and others have demonstrated that bradykinin induces contraction of human isolated small airways through prostanoid release (Molimard et al., 1994), and thromboxane A_2 receptor (TP receptor) stimulation (Hulsmann et al., 1994; Molimard et al., 1995), we devised this study to investigate the possible inhibitory effect of furosemide on contractions of human isolated small bronchi induced by bradykinin and the stable thromboxane A_2 mimetic, U-46619.

2. Materials and methods

Bronchial tissues were removed at the time of surgery from patients (mean age 58 years; range 40–71 years) with lung cancer. All were male and previous smokers. None was asthmatic. Just after resection, segments of bronchi with an inner diameter 0.5–1 mm (small bronchi) were taken from an area as far as possible from the malignancy. They were placed in oxygenated Krebs-Henseleit solution (NaCl, 119; KCl, 5.4; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25; glucose 11.7 mM) and stored overnight at 4° C. After removal of adhering fat and connective tissues, 4–8 rings of the same bronchus were prepared. Each set of bronchial rings was suspended under an initial tension of 1.5 g in Krebs-Henseleit solution, bubbled with 95% O_2-5% CO_2 and maintained at 37° C.

* Corresponding author. Laboratoire de Pharmacologie Respiratoire, 15, rue de l'École de Médecine, 75006 Paris, France. Tel. 33-1-43 29 28 71, fax 33-1-44 07 13 52.

SSDI 0014-2999(95)00159-X

Changes in force of contraction were measured isometrically with strain gauge amplifiers and displayed on an I.O.S.-Moise 3 recorder (EMKA, Mitry Mory, France).

Each experiment began by contracting the bronchial strips to maximal tension with acetylcholine (1 mM), and then maximal relaxation was induced with theophylline (3 mM). During the next 90 min, the tissues were washed every 15 min. Experiments were conducted on parallel groups of 4–8 rings, one ring serving as control.

Concentration-response curves for bradykinin (10^{-9} to 10^{-5} M) or U-46619 (10^{-9} to 10^{-5} M) were recorded by applying increasing concentrations of drugs at 3- to 6-min and 10- to 20-min intervals respectively, in logarithmic increments. Contractile responses were expressed as percentages of the maximal effect induced by acetylcholine 1 mM.

Pretreatments with furosemide (10^{-4} to 10^{-3} M) or indomethacin (10^{-6} M) were made 1 h before the cumulative concentration-response curves for bradykinin or U-46619.

Concentrations and pretreatment duration were chosen according to results of our previous studies (Molimard and Advenier, 1993; Molimard et al., 1994, 1995).

pA_2 values were determined according to Arunlakshana and Schild (1959). All values were expressed as means ± standard error of the mean (S.E.M). Statistical analysis of the results was performed using variance analysis and Student's t-test. P values lower than 0.05 were considered to be significant.

Drugs used were: bradykinin, U-46619 (9,11-dideoxy-11α,9α-epoxymethano-prostaglandin $F_{2\alpha}$), indomethacin (Sigma, St. Louis, MO, USA), furosemide (Hoechst, Puteaux, France), acetylcholine (PCH, Paris, France); theophylline sodium anisate was used as the proprietary injectable solution (Delalande, Paris, France). All drugs, except indomethacin which was dissolved in ethanol, were dissolved in distilled water and then diluted in Krebs solution. The final amount of ethanol (0.03%) did not alter acetylcholine and U-46619 reactivity.

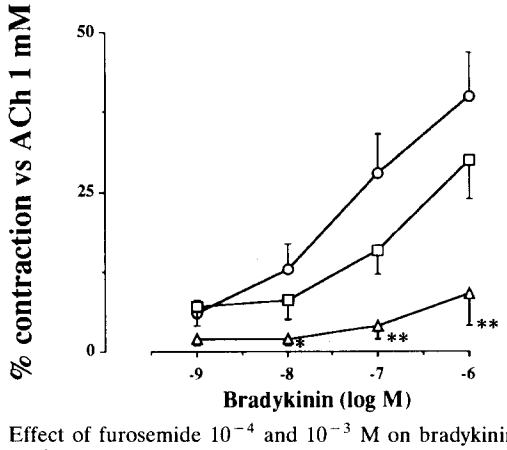

Fig. 1. Effect of furosemide 10^{-4} and 10^{-3} M on bradykinin (10^{-9} M to 10^{-6} M)-induced contraction of the human isolated small bronchus. Control (○), furosemide 10^{-4} M (□), furosemide 10^{-3} M (△). Significant differences versus control are expressed by: $^{*}P < 0.05$; $^{**}P < 0.01$ ($n = 7$).

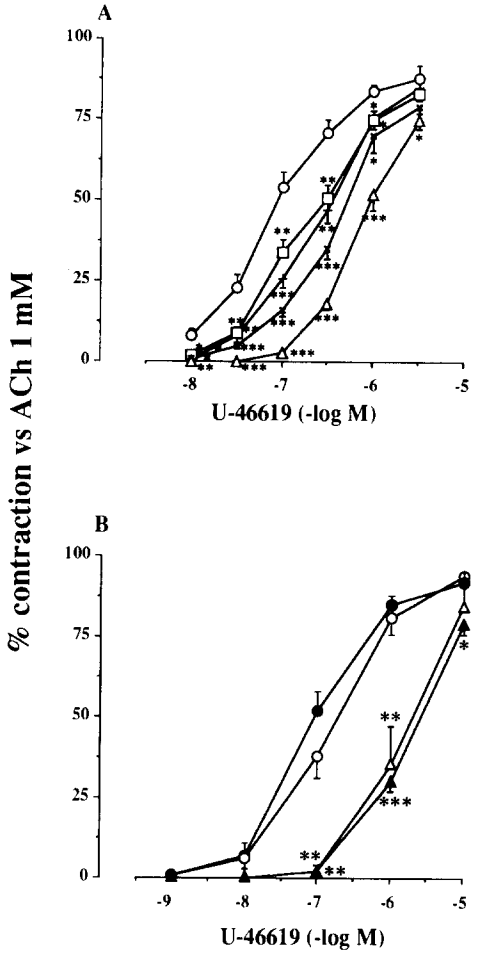

Fig. 2. Effect of furosemide 10^{-4} to 10^{-3} M on the action of the thromboxane A_2 mimetic, U-46619 (10^{-9} M to 10^{-5} M), in the absence (A) or the presence (B) of indomethacin 10^{-6} M on the human isolated small bronchus. Control in the absence (○) or presence of indomethacin (●), furosemide 10^{-4} M (□), 2.5×10^{-4} M (+), 5×10^{-4} M (×) and 10^{-3} M in the absence (△) or presence (▲) of indomethacin. Significant differences versus control are expressed by: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ (A: $n = 8$; B: $n = 4$).

3. Results

Furosemide 10^{-4} and 10^{-3} M induced a basal tone decrease of 54 ± 9% and 87 ± 6% of the maximal relaxation induced by theophylline ($n = 7$) respectively, but did not reduce acetylcholine-induced contraction.

Furosemide 10^{-4} to 10^{-3} M dose dependently inhibited bradykinin-induced concentration-response curves in a non-competitive manner ($n = 7$) (Fig. 1).

Furosemide 10^{-4} to 10^{-3} M dose dependently inhibited U-46619-induced concentration-response curves in a competitive manner with a pA_2 of 4.01 ± 0.09 (slope of the Schild plot regression curve: 0.98 ± 0.08, $n = 8$) (Fig. 2A). The cyclooxygenase inhibitor, indomethacin 10^{-6} M, had no effect on U-46619-induced contraction and on the inhibitory effect of furosemide on U-46619-induced contraction ($n = 4$) (Fig. 2B).

4. Discussion

Our results showed that high concentrations of furosemide inhibit bradykinin-induced contraction of human isolated small bronchi. These results are consistent with our previous report of the inhibitory effect of furosemide on bradykinin-induced contraction of guinea-pig trachea (Molimard and Advenier, 1993). In guinea pig trachea, we had moreover demonstrated that the inhibitory effect of furosemide is reversed by indomethacin. We have been unable to study the effect of indomethacin on the inhibition by furosemide of bradykinin-induced contraction in human bronchi since bradykinin-induced contraction of the human small isolated bronchus is abolished by indomethacin itself (Molimard et al., 1994).

We and others have recently demonstrated that the bradykinin-induced contraction of human isolated small bronchi is entirely mediated by TP receptor stimulation by prostanoids (mainly thromboxane A_2) and that, after TP receptor blockade, bradykinin induces relaxation due to relaxant prostanoid release (Hulsmann et al., 1994; Molimard et al., 1995). Thus, furosemide could inhibit bradykinin-induced contraction of human small isolated bronchi through modulation of the balance of the bradykinin-induced relaxant/contractile prostanoid effect, either by an increased bradykinin-induced relaxant prostanoid release or by a decrease in bradykinin-induced contractile prostanoid release or antagonism of the contractile prostanoid receptor step of bradykinin action. By studying the effects of furosemide on prostaglandin synthesis by human airway epithelial cells in culture, we have demontrated otherwise that furosemide 10^{-3} M was unable to increase relaxant prostanoid release even after arachidonic acid stimulation and, moreover, inhibited the synthesis of the major relaxant prostaglandin induced by bradykinin prostaglandin E_2 (Levasseur-Acker et al., 1994). Thus, an increase of relaxant prostanoid release is unlikely to be involved in the inhibitory effect of furosemide on bradykinin-induced contraction. To determine the step at which the inhibitory effect of furosemide on bradykinin-induced contraction occurs, we then studied the effect of furosemide on the contraction of human isolated small bronchi induced by the TP receptor-specific agonist, U-46619. Our results show that furosemide inhibits the thromboxane A_2 mimetic U-46619-induced contraction in a competitive manner since the slope of the Schild plot regression curve is not significantly different from 1. This effect is independent of prostaglandin synthesis since indomethacin is unable to reverse the inhibitory effect of furosemide on U-46619-induced contraction. Since the inhibitory effect of furosemide on bradykinin- and on U-46619-induced contraction is observed at a similar range of furosemide concentrations, we suggest that antagonism by furosemide of the TP receptor step of bradykinin action could at least partly explain the mechanism by which furosemide inhibits bradykinin-induced contraction.

Our results could be consistent with the reversal by indomethacin of the inhibitory effect of furosemide on bradykinin-induced contraction of guinea pig isolated trachea we observed earlier. Indeed, since bradykinin-induced contraction of guinea pig isolated trachea involves prostaglandin and tachykinin release and is therefore not abolished by indomethacin (Saria et al., 1988; Molimard and Advenier, 1993), conversely to what is observed in human bronchi, we suggest that TP receptor blockade by furosemide could reveal the bradykinin-induced relaxant prostanoid effect and that this effect can be reversed by indomethacin in guinea pig isolated trachea.

Since such high concentrations of furosemide are likely to be reached in human airways only on local treatment with an aerosol, we suggest that the antagonism by furosemide of the TP receptor could participate in the protective effects of inhaled furosemide in asthma. Furthermore, that such high concentrations are needed to observe any effect of furosemide in vitro could explain the lack of protective effect of oral furosemide in asthma.

To the best of our knowledge, this report is the first evidence of an antagonism by furosemide of the TP receptor and of the inhibitory effect of furosemide on bradykinin-induced contraction of human isolated bronchi.

References

Arunlakshana, O. and H.O.J. Schild, 1959, Some quantitative uses of drug antagonists, Br. J. Pharmacol. Chemother. 14, 48.

Chung, K.F. and P.J. Barnes, 1992, Loop diuretics and asthma, Pulm. Pharmacol. 5, 1.

Hulsmann, A.R., H.R. Raatgeep, P.R. Saxena, K.F. Kerrebijn and J.C. DeJonste, 1994, Bradykinin-induced contraction of human peripheral airways mediated by both bradykinin β_2 and thromboxane prostanoid receptors, Am. J. Respir. Crit. Care Med. 150, 1012.

Levasseur-Acker, G.M., M. Molimard, J. Regnard, E. Naline, C. Freche, J. Marsac and A. Lockhart, 1994, Effects of furosemide on prostaglandin synthesis by human nasal and bronchial cells in culture, Am. J. Respir. Cell Mol. Biol. 10, 378.

Lockhart, A. and A.S. Slutsky, 1994, Furosemide and loop diuretics in human asthma, Chest 106, 244.

Molimard, M. and C. Advenier, 1993, Effect of frusemide on bradykinin- and capsaicin-induced contraction of the guinea-pig trachea, Eur. Respir. J. 6, 434.

Molimard, M., C.A.E. Martin, E. Naline, A. Hirsch and C. Advenier, 1994, Contractile effects of bradykinin on the isolated human small bronchus, Am. J. Respir. Crit. Care Med. 149, 123.

Molimard, M., C.A.E. Martin, E. Naline, A. Hirsch and C. Advenier, 1995, Role of thromboxane A_2 in bradykinin-induced human isolated small bronchi contraction, Eur. J. Pharmacol. 278, 49.

Rajakulasingam, K., R. Polosa, M.K. Church, P.H. Howarth and S.T. Holgate, 1994, Effect of inhaled frusemide on responses of airways to bradykinin and adenosine 5′-monophosphate in asthma, Thorax 49, 485.

Saria, A., C.-R. Martling, Z. Yan, E.E. Theodorsson-Norheim, R. Gamse and J.M. Lundberg, 1988, Release of multiple tachykinins from capsaicin-sensitive sensory nerves in the lung by bradykinin, histamine, dimethylphenyl piperazinium, and vagal nerve stimulation, Am. Rev. Respir. Dis 137, 1330.