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Effect of frusemide on bradykinin- and capsaicin-induced contraction of the guinea-pig trachea

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Effect of frusemide on bradykinin- and capsaicin-induced contraction of the guinea-pig trachea. M. Molimard, C. Advenier. ©ERS Journals Ltd 1993.

ABSTRACT: Frusemide, a loop diuretic, inhibits the bronchial response to various bronchoconstrictor stimuli in asthmatic subjects. The underlying mechanisms remain unclear.

In order to determine whether frusemide inhibits pharmacologically induced C-fibre stimulation, we investigated the effect of frusemide on bradykinin-, capsaicin-, neurokinin A-, and substance P-induced contraction of the guinea-pig isolated trachea.

Frusemide 10^{-5} and 10^{-4} M produced a significant inhibition of concentration-response curves to bradykinin, which was markedly reduced by indomethacin 10^{-6} M. Frusemide significantly reduced capsaicin-induced contraction only in the presence of indomethacin 10^{-6} M. Neurokinin A- and substance P-induced contractions were not affected by frusemide and/or indomethacin.

Our data suggest that a cyclo-oxygenase pathway is involved in the inhibition by frusemide of the bradykinin-induced contraction, but not in the inhibition of the capsaicin-induced contraction.

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Inhalation of the loop diuretic frusemide has been shown to inhibit bronchoconstrictor response to exercise [1], ultrasonically nebulized distilled water [2], antigen [3–5], metabisulphite [6, 7] or adenosine [8]. The mechanism of this action remains uncertain. Frusemide is unlikely to inhibit smooth muscle contraction directly, since it does not influence the bronchoconstriction induced by histamine, KCl, and hypertonic NaCl *in vitro* [9]. The effect of frusemide could be due to inhibition of the Na^+/Cl^- cotransport process, as suggested by BIANCO *et al.* [1]. Frusemide might also act by releasing bronchodilatator prostaglandins from airway epithelium, as has been shown on vascular endothelium and kidney tubular epithelium [10, 11]. Recently, frusemide has been shown to inhibit, in the presence of indomethacin, the contraction induced by electrical field stimulation of guinea-pig bronchial smooth muscle [12].

Afferent vagal C-fibres are stimulated by bradykinin [13, 14], and capsaicin [15], and induce the release of multiple tachykinins, such as substance P (SP) and neurokinin A (NKA) [14–17]. The aim of the present study was to investigate the possible inhibitory effect of frusemide on neurally evoked bronchoconstrictor pathways. We studied the effect of frusemide on bradykinin-, capsaicin-, SP- and NKA-induced bronchoconstriction in guinea-pig.

Material and methods

Guinea-pig isolated trachea

Male guinea-pigs weighing 250–350 g were killed by cervical dislocation. The trachea was removed and cut into rings. In some experiments, the epithelium was removed by gently rubbing the luminal surface with a cotton-tipped applicator soaked in Kreb's solution, according to the method described by DEVILLIER *et al.* [18]. The preparations were mounted isometrically and subjected to an initial tension of 1.50 g, in a Kreb's solution at 37°C bubbled with a 95% O_2 and 5% CO_2 mixture. Kreb's solution was composed of (mM): NaCl 113; KCl 4.7; $CaCl_2$ 1.9; $MgSO_4$ 1.2; KH_2PO_4 1.2; $NaHCO_3$ 25; glucose 11.5. Following equilibration for 1.25 h, the resting tension was between 0.8–1.5 g.

Tensions were measured with Celaster UF-1 gauges connected to Celaster AC-261 amplifiers. Analogical and digital signal acquisition, enabling treatment of pharmacological data, was performed using the Moise 3 program (Dei Lierre, 77290 Mitry-Mory, France).

Tracheal rings were first contracted to maximal tension with acetylcholine 1 mM, then relaxed with theophylline 3 mM to sensitize and stabilize the preparations.

Experiments were separated by intervals of a least 1 h. Experiments were conducted on parallel groups of 4–8 rings, one ring serving as control. Each of the other rings was incubated for 1 h with a given concentration of the reagents or substances tested (frusemide (10^{-5} and 10^{-4} M) and/or indomethacin (10^{-6} M)). Following this incubation, and in the absence (control) or presence of reagents or substances tested, a range of one of the following contraction-inducing agents (bradykinin (10^{-10}–10^{-6} M), capsaicin (10^{-9}–10^{-6} M), SP (10^{-9}–10^{-6} M), NKA (10^{-9}–10^{-6} M)) was established. Thereafter, acetylcholine 1 mM was added to the bath to obtain 100% maximal contraction.

The effects of the contractile agents were expressed as a percentage of the effects of acetylcholine 1 mM.

Statistical analysis of results

All values in the text and figures are mean±SEM. Statistical analysis was performed using variance analysis and Student's t-test for paired or unpaired data.

Drugs and chemicals

The substances used were: frusemide (Hoechst), acetylcholine (Pharmacie centrale des hôpitaux, Paris, France); indomethacin (Merck); substance P, neurokinin A, bradykinin, capsaicin (Sigma, St. Louis, USA); theophylline sodium anisate (Bruneau, Paris, France). All drugs were dissolved in distilled water and then diluted in saline for the *in vivo* studies, or in Kreb's solution for the *in vitro* studies, except for indomethacin which was dissolved in ethanol, and then diluted in such a way that the amounts of ethanol did not alter acetylcholine reactivity.

Results

Effect of frusemide on concentration-response curves to bradykinin on the guinea-pig trachea

Bradykinin induced a transient contraction within 5 min after injection, followed by a relaxation. We studied the cumulative contractile effect of bradykinin by adding the next concentration of bradykinin when the induced contraction reached its maximum.

Figure 1a shows that epithelium removal significantly enhanced the concentration response curve to bradykinin on guinea-pig trachea. On epithelium-denuded trachea (fig. 1b and c), frusemide 10^{-5} and 10^{-4} M induced a significant decrease of bradykinin (BK) concentration-response curves (fig. 1b); indomethacin 10^{-6} M did not significantly influence bradykinin control concentration-response curves, but significantly inhibited the decrease of bradykinin concentration-response curves induced by frusemide (fig. 1c)

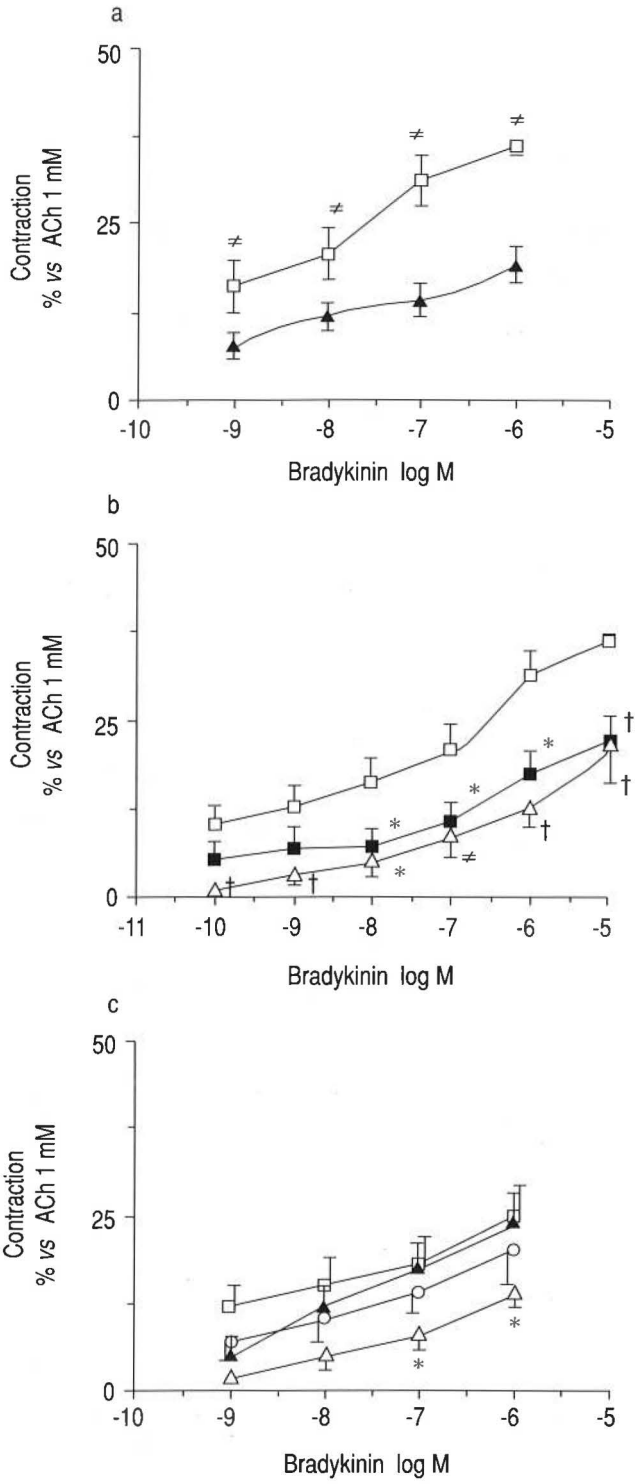

Fig. 1. – a) Concentration response-curves to bradykinin on intact (▲) and denuded (□) guinea-pig isolated trachea.
b) Influence of frusemide (10^{-5} (■) and 10^{-4} (Δ) M) on bradykinin concentration-response curves on guinea-pig isolated trachea without epithelium. □: control. c) Influence of indomethacin 10^{-6} M on bradykinin concentration-response curves on guinea-pig denuded trachea pretreated by frusemide 10^{-4} M (▲). □: control (no treatment); Δ: frusemide 10^{-4} M alone; ○: indomethacin 10^{-6} M alone. Values are mean±SEM (n=6). Significant differences with control are indicated by: *: $p<0.05$; ≠ : $p<0.02$; †: $p<0.01$ except for figure c where the comparison was made with indomethacin 10^{-6} M. ACh: acetylcholine.

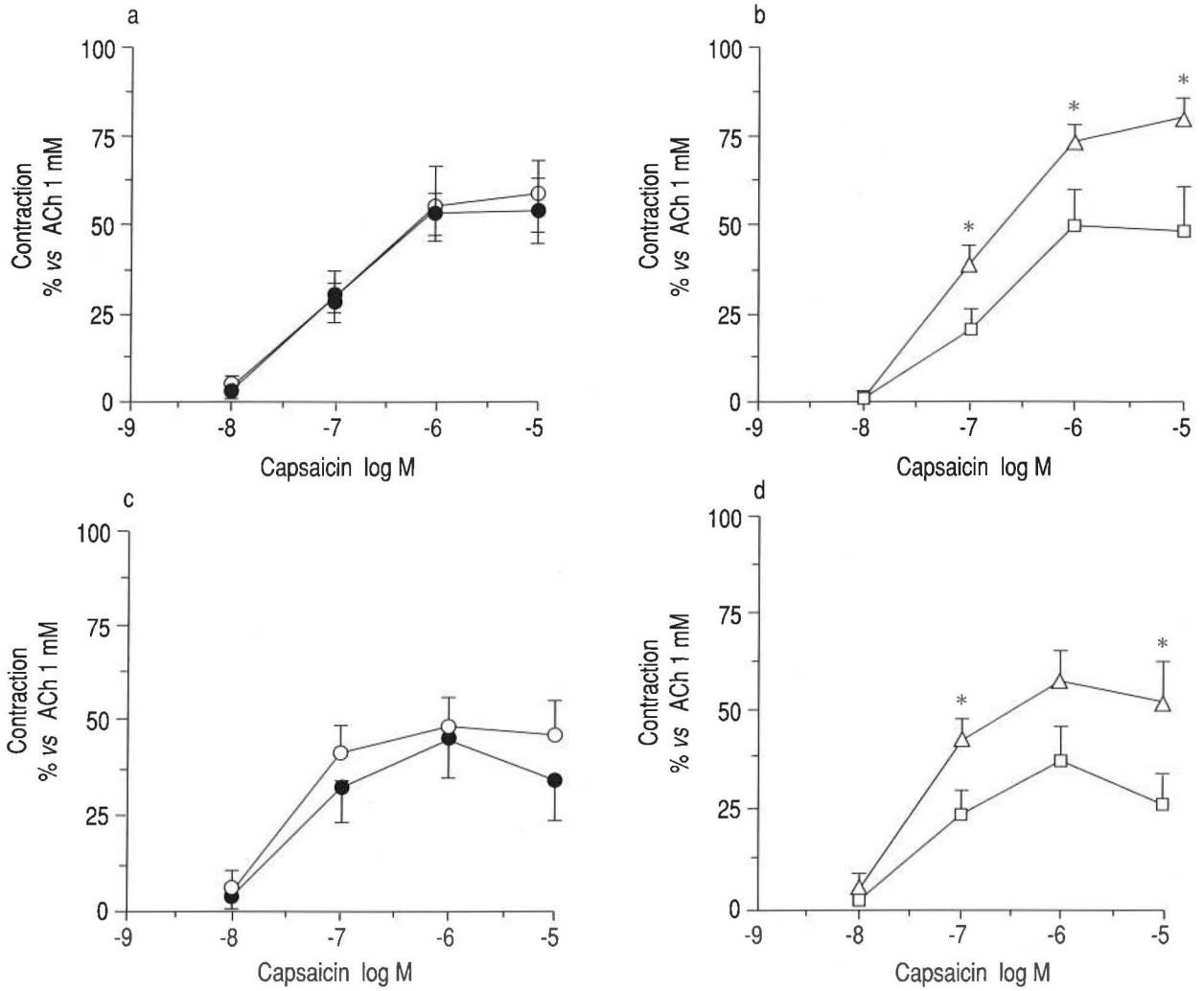

Fig. 2. – Effect of frusemide (10^{-3} M) on capsaicin concentration-response curves without (a, c, ●) or with indomethacin 10^{-6} M pretreatment (b, d, □) on intact (a, b) and denuded (c, d) guinea-pig isolated trachea. Δ: indomethacin 10^{-6} M alone. ○: control (no treatment). Values are mean±SEM (n=6). Significant differences with capsaicin response curves in the presence of indomethacin (Δ) indicated by *: $p<0.05$.

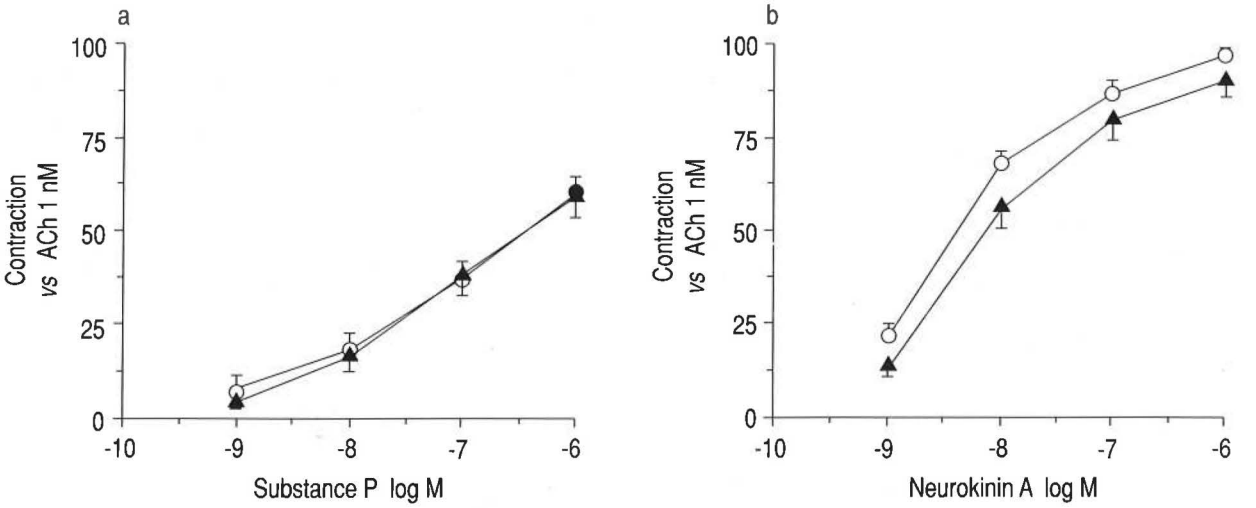

Fig. 3. – a) Influence of frusemide 10^{-4} M (▲) on substance-P concentration-response curves on guinea-pig isolated trachea without epithelium pretreated by indomethacin. b) Influence of frusemide 10^{-4} M (▲) on neurokinin A concentration-response curves on guinea-pig denuded isolated trachea pretreated by indomethacin. Values are mean±SEM (n=6 and n=5, respectively). There was no significant difference with substance-P or neurokinin A control response curves (○).

Effect of frusemide on concentration-response curves to capsaicin on the guinea-pig trachea

Administration of capsaicin (10^{-8} to 10^{-5} M) produced a concentration-related transient contraction, which reached a maximal value within 3–6 min. A cumulative concentration-response curve to capsaicin is shown in figure 2a. Neither epithelium removal (fig. 2c), nor indomethacin 10^{-6} M (fig. 2d), significantly modified concentration-response curves to capsaicin (n=6).

Frusemide 10^{-3} M did not influence capsaicin concentration-response curves with or without epithelium (fig. 2a and c).

Figure 2b shows that on indomethacin 10^{-6} M pretreated tracheal spirals, frusemide significantly inhibited the concentration response curve to capsaicin. This inhibition remained after epithelium removal (fig. 2d).

Effect of frusemide on concentration-response curve to substance P and neurokinin A on the guinea-pig denuded trachea

Figure 3 shows that frusemide in the presence of indomethacin had no significant effect on the contractile response induced by exogenous addition of substance P 10^{-9} to 10^{-6} M (fig. 3a) and neurokinin A 10^{-9} to 10^{-6} M (fig. 3b) (n=6).

Discussion

We have shown that the loop diuretic frusemide inhibits the airway muscle contraction induced by bradykinin. The mechanism of this action appears to be complex, because the effects of bradykinin involve several components. Bradykinin acts firstly by stimulating B_2 bradykinin-receptors, since its effects on airways are inhibited by B_2 bradykinin-receptors antagonists and not by B_1 bradykinin-receptors antagonists [19–21].

It has been demonstrated, by recording vagal impulses in anaesthesized dogs, that bradykinin induces stimulation of afferent vagal C-fibres in intrapulmonary airways [13]. On the perfused guinea-pig isolated lung, Saria *et al.* [14] have shown, by radio-immunoassay, that bradykinin releases SP and NKA. On the guinea-pig isolated trachea, Inoue *et al.* [22] have shown that bradykinin-induced contraction is inhibited by tetrodotoxin. These results confirm the neurally-mediated effect of bradykinin. Furthermore, in the latter study [22], atropine does not modify the bradykinin concentration-response curve, thereby excluding a cholinergic response to bradykinin *in vitro*.

Bradykinin also acts on arachidonic acid metabolism. Thus, Bramley *et al.* [23] have demonstrated that bradykinin stimulates and increases prostaglandins E_2 and I_2 (PGE_2 and PGI_2) release (about 5 and 2 times, respectively) from intact guinea-pig isolated trachea. Epithelium removal significantly inhibits PGE_2 release (but not PGI_2) and enhances bradykinin-induced contraction of smooth muscle. Bramley *et al.* [23] concluded that PGE_2 is important in the secondary relaxation induced by bradykinin on intact guinea-pig trachea, and considered PGI_2 to be less important in the response to bradykinin. This is in agreement with Mizrahi *et al.* [24], who suggested that bradykinin exerts its relaxant effect on the guinea-pig trachea *via* an indirect mechanism which is entirely dependent on PGE_2 generation. Frusemide increases the release of arachidonic acid from phospholipids, probably by stimulating phospholipase A_2 [25], and it decreases PGE_2 degradation by inhibiting renal PGE_2 9-ketoreductase [26], and 15-hydroxyprostaglandin dehydrogenase [27]. Frusemide releases PGE_2 from the renal tubules, and this action is involved in its diuretic effect [11].

We studied bradykinin-induced contraction only on denuded trachea, because the bradykinin contraction on intact trachea is too weak and interferes with prostaglandin release from the epithelium. Our results show that the effects of bradykinin are inhibited or abolished by frusemide, and we suggest that this effect of frusemide might be mediated by cyclo-oxygenase products, such as PGE_2, because it is abolished by indomethacin. As the epithelium was removed, it could not be involved in this release. The cyclo-oxygenase product released by frusemide could have a relaxant effect on smooth muscle directly and/or indirectly by inhibiting the cholinergic and non-cholinergic neurotransmission, as has been shown for PGE_2 on the isolated ferret trachea [28].

On the other hand, we have shown that frusemide inhibits capsaicin-induced tracheal contraction in the presence of indomethacin alone. From these experiments we cannot rule out the possibility that frusemide could inhibit a nonsignificant enhancement of the capsaicin response due to indomethacin. It would be surprising, however, if indomethacin at 10^{-6} M induces a contractile prostaglandin synthesis that nonsignificantly enhances the effect of capsaicin, and that the inhibition of this hypothetical effect by frusemide could be significant. It seems more likely that frusemide may inhibit C-fibre stimulation without involving prostaglandins. Elwood *et al.* [12] demonstrated that frusemide inhibits the non-adrenergic noncholinergic bronchial contraction induced by electrical field stimulation in the presence of indomethacin. In agreement with their conclusion, we suggest that the site of action of frusemide is presynaptic, because frusemide does not affect substance P- and neurokinin A-induced contraction. Neither indomethacin nor epithelium removal affect capsaicin-induced contraction, indicating that prostanoids are involved only little or not at all in this contraction under basal conditions. This confirms the direct stimulation of C-fibres without release of prostanoids [15, 17]. The lack of inhibitory effect of frusemide on capsaicin-induced contraction in the absence of indomethacin suggests that frusemide plus capsaicin induces the synthesis of prostanoids, which enhance the action of capsaicin. The epithelium is unlikely to be involved in this synthesis, since even after epithelium removal frusemide inhibited capsaicin-induced contraction only after indomethacin pretreatment. To support the hypothesis of a released prostanoid product masking the direct inhibitory effect of frusemide on C-fibres, we may

argue that frusemide increases arachidonic acid release [25], and may also induce release of PGI_2 [10] which can increase the response of unmyelinated sensory neurons [29]. Indeed, MAPP *et al.* [29] have shown that prostacyclin activates tachykinin release from capsaicin-sensitive afferents in guinea-pig bronchi *via* a ruthenium red (functional antagonist of capsaicin) sensitive pathway.

Thus, the "paradoxical" effects of frusemide on the bradykinin- and capsaicin-induced contraction suggest that more than one mechanism is involved, including inhibition of C-fibres and an increased output of cyclo-oxygenase products. The type and/or concentration of prostaglandin produced differs with the nature of the bronchoconstrictor agent tested. The site of action of this prostanoid is probably on C-fibres rather than on smooth muscle, because neurokinin A- as well as substance P-induced contraction are not affected by frusemide, and also because frusemide has no effect *in vitro* on histamine-, KCl- or hypertonic NaCl-induced contraction [9].

The high doses of frusemide (10^{-5} to 10^{-3} M) tested are similar to those studied by ELWOOD *et al.* [12], and are compatible with the concentration obtained after a local treatment by aerosol.

To conclude, frusemide acts through release of cyclo-oxygenase products and possibly through C-fibre inhibition. We suggest that experiments on frusemide should be made, with and without prostaglandin synthesis inhibitors, to assess the involvement of prostaglandins. Finally, our results may, in part, explain the potentiation of the protective effect of inhaled frusemide on allergen-induced bronchoconstriction by inhaled lysine acetylsalicylate, described by BIANCO *et al.* [30].

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