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Effect of frusemide on the induction and potentiation of cough induced by prostaglandin $F_{2\alpha}$

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We examined whether inhaled frusemide could reduce the potentiation of capsaicin-induced cough by prostaglandin (PG) $F_{2\alpha}$. Eight non-smoking normal subjects, after a baseline capsaicin challenge were given inhaled frusemide or saline followed by capsaicin challenge, then $PGF_{2\alpha}$ and finally capsaicin challenge again. $PGF_{2\alpha}$-induced coughs were reduced after frusemide to 3.6 ± 1.0 compared with 5.7 ± 1.2 after saline ($P < 0.05$). $PGF_{2\alpha}$ increased capsaicin-induced coughs by 11.1 ± 3.7 and 7.9 ± 3.4 after placebo and frusemide, respectively ($P < 0.05$). Frusemide had no effect on capsaicin-induced cough alone. Changes in local ionic concentrations by frusemide, particularly chloride ions within the vicinity of epithelial cough receptors, may determine the cough response to low chloride solutions and to $PGF_{2\alpha}$, but not to capsaicin which acts directly on the cough receptors, and alter the sensitivity of the receptors to capsaicin.

Keywords cough frusemide capsaicin $PGF_{2\alpha}$

Introduction

Inhaled frusemide inhibits bronchoconstriction induced by a number of agents including allergen (Bianco *et al.*, 1989) and exercise (Bianco *et al.*, 1988). In addition, it reduces the cough induced by low-chloride and chloride-free solutions but it is ineffective against capsaicin-induced cough (Ventresca *et al.*, 1990), suggesting that frusemide may act indirectly on cough receptors in airway epithelium, either by preventing local ionic changes or by inhibiting some aspect of neural function. Because $PGF_{2\alpha}$ released in the airways can enhance the cough response in normal subjects (Nichol *et al.*, 1990), we have now examined whether it could also reduce the cough responses induced by $PGF_{2\alpha}$, and the potentiation of capsaicin-induced cough by $PGF_{2\alpha}$.

Methods

Eight healthy, non-smoking subjects on no medication and who have been free of upper respiratory tract infections for at least 4 weeks gave informed consent for the study approved by our Ethics Committee. Each subject attended on two different occasions, at least 2 days apart, at the same time of the day were challenged with capsaicin and then were given frusemide or placebo in a double-blind, balanced cross-over fashion. Immediately after treatment they were challenged again with capsaicin, followed by a $PGF_{2\alpha}$ challenge and then another capsaicin challenge. Frusemide 10 mg ml^{-1} (Frusemide Injection BP, Antigen Ltd, Roscrea, Ireland) was diluted with 0.9% saline to 3.75 mg ml^{-1} which was inhaled for 8 min using the De Vilbiss 65 ultrasonic nebuliser; 0.9% saline was used as placebo. The total amount of frusemide delivered at the mouthpiece was 30 mg.

One breath of capsaicin (Sigma Chemical Company, Dorset, UK; stock solution of 3.6 mM in ethanol diluted with 0.9% saline to 2.4 nM) was inhaled 4 times every 1 min. One breath of $PGF_{2\alpha}$ 5 mg ml^{-1} (Prostin F2 alpha, Upjohn, Crawley, UK) was inhaled 1 min after the second capsaicin challenge and was followed after 1 min by the last capsaicin challenge. Aerosols were delivered from a jet nebuliser (output = 7.7 μl per breath; mass median diameter = 3.5 μm) (MEFAR MB3, Brescia, Italy) which was set to trigger for 1 s at a pressure of 22 psi. Each breath was inhaled over a 5 s period from functional residual capacity to total lung capacity. The number of coughs was counted by the same independent blinded observer for 30 s after each inhalation. Total respiratory resistance (Rrs; mean of three consecutive measurements) was measured by the forced oscillation technique (Oscillaire; Jones Instruments Co, Chicago, USA) (Landser *et al.*, 1976) before the baseline capsaicin challenge, before and after treatment and after the last capsaicin challenge.

Two-way ANOVA and Tukey's test were used to compare the number of coughs of the capsaicin challenges and the values of Rrs before and after each treatment. Three-way ANOVA (treatments, subjects, periods) was

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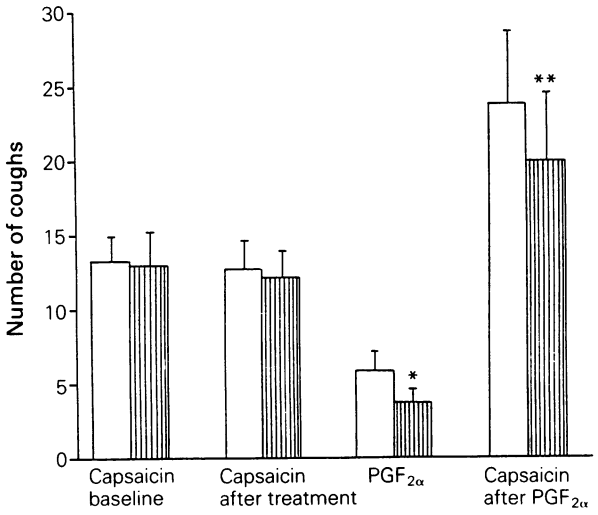

Figure 1 Mean cough numbers (s.e. mean) in eight normal subjects after capsaicin challenges performed at baseline, after treatment with either placebo or frusemide and after inhaling $PGF_{2\alpha}$. Cough numbers after $PGF_{2\alpha}$ challenge is also shown. Open bars represent results with placebo and lined bars with frusemide * = $P < 0.05$ and ** = $P < 0.01$ as compared with placebo.

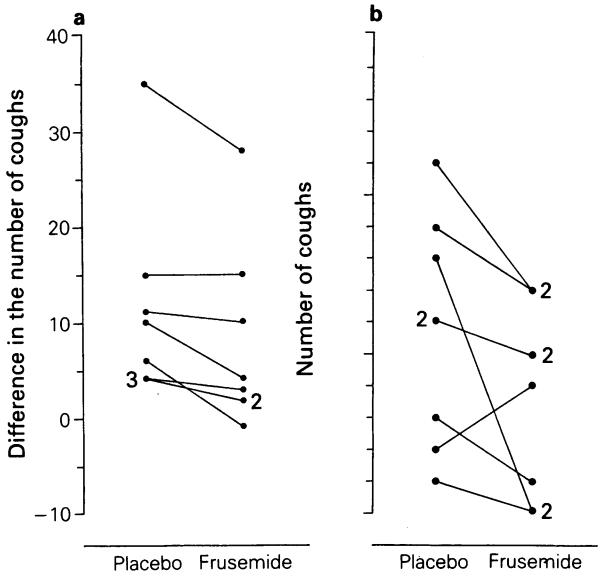

Figure 2 a) Individual values of the difference in cough numbers induced by capsaicin after and before $PGF_{2\alpha}$ challenge in eight subjects treated with either placebo or frusemide. The points indicated by 2 and 3 represent two and three superimposed data-points, respectively. b) Individual cough numbers induced by $PGF_{2\alpha}$ after placebo or frusemide pretreatment in eight normal subjects. The points indicated by 2 represent two superimposed data points.

used to assess the effect of treatments on the number of coughs with each challenge, on the increase in the number of capsaicin-induced coughs after $PGF_{2\alpha}$ and on the values of Rrs. A P value < 0.05 was considered significant.

Results

Baseline capsaicin-induced coughs were not different with the two treatments. After both frusemide and placebo a small but non-significant decrease in the number of capsaicin-induced coughs was observed. $PGF_{2\alpha}$ significantly increased capsaicin-induced cough when compared with post-treatment responses after placebo ($P < 0.05$).

The increase after frusemide was at the limits of statistical significance ($0.05 < P < 0.1$) but significantly less than after placebo (Figure 1). $PGF_{2\alpha}$ increased capsaicin-induced coughs by 11.1 ± 3.7 and 7.9 ± 3.4 after placebo and frusemide, respectively ($P < 0.05$); the increase in the number of coughs was less in all six of the seven subjects after frusemide than after placebo (Figure 2a). Frusemide significantly reduced $PGF_{2\alpha}$-induced cough ($P < 0.05$; Figure 1) in all six of the seven subjects (Figure 2b). There were no significant differences in Rrs after either treatments or cough challenges.

Discussion

Inhaled frusemide partially inhibited the cough induced by $PGF_{2\alpha}$ and the potentiation of capsaicin-induced cough by $PGF_{2\alpha}$. We have confirmed that frusemide is ineffective against capsaicin-induced cough (Ventresca *et al.*, 1990). Therefore, it is unlikely that the inhibitory effect of frusemide against the $PGF_{2\alpha}$-induced potentiation is secondary to an effect against capsaicin. Our data suggest that frusemide inhibits both the direct and sensitising effects of $PGF_{2\alpha}$ on the cough receptor in normal subjects.

We can only speculate on the mechanism by which $PGF_{2\alpha}$ elicits cough and augments capsaicin-induced cough. $PGF_{2\alpha}$ and PGE_2 have direct effects in stimulating cough receptors, in addition to enhancing the response of cough receptors to subsequent capsaicin stimulation. The capacity of prostaglandins to increase pain sensitivity to other chemical mediators such as bradykinin and histamine in the skin is a useful analogy to our observations on cough (Collier & Schneider, 1972; Zurier, 1974). $PGF_{2\alpha}$ and PGE_2 also stimulate the activity of airway unmyelinated afferent C-fibres and also rapidly-adapting receptors (Coleridge *et al.*, 1978). Capsaicin may also activate similar afferent nerves (Coleridge & Coleridge, 1977; Lundberg & Saria, 1983). However, frusemide inhibited the potentiation of capsaicin-induced cough and the cough induced by low chloride-content solutions, without affecting capsaicin-induced cough. These observations suggest that frusemide does not have a primary effect on the unmyelinated C-fibre activity. The induction of cough by low-chloride content solutions is inhibited by frusemide, an event which is probably due to changes in ionic environment such as chloride concentrations within the vicinity of cough receptors. A similar effect may be involved in the mechanism of $PGF_{2\alpha}$-induced cough and potentiation of cough as $PGF_{2\alpha}$ and PGE_1 are known to stimulate chloride secretion across dog tracheal epithelium (Al-Bazzaz *et al.*, 1981).

We did not find any significant changes in Rrs either after frusemide or cough challenges making it unlikely

that changes in airway calibre were related to inhibition of the cough responses observed.

Inhibition of the Na-K-2Cl cotransporter in the luminal cell membrane of the thick ascending limb of the loop of Henle in the kidney by frusemide is the postulated mechanism of its diuretic action (Burg, 1976). A similar action may operate at the level of airway afferent nerves. Frusemide and bumetanide inhibit neurally-mediated airway smooth muscle contraction induced by stimulation of cholinergic and non-cholinergic (presumably peptidergic) nerves in guinea-pig airways (Elwood *et al.*, 1991). Frusemide may interfere with the co-transporter known to be present on airway epithelium (Knowles *et al.*, 1984) to induce ionic changes within the neighbourhood of airway sensory nerves.

References

Al-Bazzaz, F., Yadava, V. P. & Westenfelder, C. (1981). Modification of Na and Cl transport in canine tracheal mucosa by prostaglandins. *Am. J. Pysiol.*, **240**, F101–F105.

Bianco, S., Pieroni, M. G., Refini, R. M., Mattoli, L. & Sestini, P. (1989). Protective effect of inhaled furosemide on allergen-induced early and late asthmatic reactions. *New Engl. J. Med.*, **321**, 1069–1073.

Bianco, S., Vaghi, A., Robuschi, M. & Pasargiklian, M. (1988). Prevention of exercise-induced bronchoconstriction by inhaled frusemide. *Lancet*, **ii**, 252–255.

Burg, M. B. (1976). Mechanisms of action of diuretic drugs. In *The Kidney*, eds Brenner, M. B. & Rector, F. C., pp 737–762. Vol 1. Philadelphia: Saunders.

Coleridge, H. M. & Coleridge, J. C. G. (1977). Impulse activity in afferent vagal C-fibres with endings in the intrapulmonary airways of dogs. *Resp. Physiol.*, **29**, 125–142.

Coleridge, H. M., Coleridge, J. C. G., Baker, D. G., Ginzel, K. H. & Morrison, M. A. (1978). Comparison of the effects of histamine and prostaglandin on afferent C-fiber endings and irritant receptors in the intrapulmonary airways. *Adv. exp. Med. Biol.*, **99**, 291–305.

Collier, H. O. J. & Schneider, C. (1972). Nociceptive response to prostaglandins and analgesic actions of aspirin and morphine. *Nature, New Biol.*, **236**, 141–143.

Elwood, W., Lotvall, J. O., Barnes, P. J. & Chung, K. F. (1991). Loop diuretics inhibit cholinergic and non-cholinergic nerves in guinea-pig airways. *Am. Rev. resp. Dis.*, **143**, 1340–1344.

Knowles, M., Murray, G., Shallal, J. *et al.* (1984). Bioelectric properties and ion flow across excised human bronchi. *J. appl. Physiol.*, **56**, 868–877.

Landser, F. J., Nagels, J., Demedts, M., Billiet, L. & van de Woestijne, K. P. (1976). A new method to determine frequency characteristics of the respiratory system. *J. appl. Physiol.*, **41**, 101–106.

Lundberg, J. M. & Saria, A. (1983). Capsaicin-induced desensitisation of airway mucosa to cigarette smoke, mechanical and chemical irritants. *Nature*, **302**, 251–253.

Nichol, G. M., Alton, E. W. F. W., Nix, A., Geddes, D. M., Chung, K. F. & Barnes, P. J. (1990). Effect of inhaled furosemide on metabisulfite and methacholine-induced bronchoconstriction and nasal potential difference in asthmatic subjects. *Am. Rev. resp. Dis.*, **142**, 576–580.

Nichol, G. M., Nix, A., Barnes, P. J. & Chung, K. F. (1990). Prostaglandin $F_{2\alpha}$ enhancement of capsaicin induced cough in man: modulation by beta$_2$-adrenergic and anticholinergic drugs. *Thorax*, **45**, 694–695.

Ventresca, P., Nichol, G. M., Barnes, P. J. & Chung, K. F. (1990). Inhaled furosemide inhibits cough induced by low-chloride content solutions and not by capsaicin. *Am. Rev. resp. Dis.*, **142**, 143–146.

Zurier, R. B. (1974). Prostaglandins, inflammation and asthma. *Arch. intern. Med.*, **133**, 101–110.

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