3. Dolovich M, Vanzielegham M, Hidinger K-G, Newhouse MT. – Influence of inspiratory flow rate on the response to terbutaline sulphate inhaled *via* the Turbuhaler. *Am Rev Respir Dis*, 1988, 137, A433.
4. Engel T, Heinig JH, Madsen F, Weeke B, Nikander K. – Peak inspiratory flow rate and inspiratory vital capacity of patients with asthma, measured with and without Turbuhaler. Proceedings of European Academy of Allergology and Clinical Immunology, Copenhagen, 1988.
5. Pedersen S, Hansen OR, Fuglsang G. – Influence of inspiratory flow rate upon the effect of a Turbuhaler. *Arch Dis Childhood*, 1990, 65, 308–310.

LETTER TO THE EDITOR

Effects of frusemide on human airway epithelium

In the June 1990 issue of the Journal, POLOSA *et al.* [1] show that inhalation of 28±2.5 mg of frusemide reduces the bronchial effects of inhaled methacholine and, to a greater degree, of adenosine 5'-monophosphate (AMP) in asthma. Although increase in the group-average geometric mean of the provocation concentration causing a 20% fall in FEV_1 from baseline (PC_{20}) for methacholine is small, and probably due to a large decrease in 4 of 12 subjects (No. 1, 2, 6 and 7), this is an interesting observation that confirms previous findings with 40 mg of inhaled frusemide by FUJIMARA *et al.* [2].

The authors speculate that the small protective effect against methacholine, and some of the inhibitory effects against AMP are best explained by interference with ion transport by airway epithelium. This is supported by our finding that frusemide deposited on the nasal mucosa causes a dose-dependent decrease in transepithelial nasal potential difference (PD) in man [3], a finding which we have subsequently reproduced (MIALON, REGNARD and LOCKHART, unpublished observation). The reduction in nasal PD strongly suggests either reduced electrical resistance or diminished ionic current across this epithelium; the latter being the most likely because of the known effects of frusemide and other loop diuretics on epithelial cells [4-8]. Therefore, we agree with POLOSA *et al.* that a direct effect of frusemide on airway epithelium is very likely, and may account for some of the protective effects of this drug against several bronchial provocants [9], the more so since osmotic stimuli, which certainly interfere with ion transport, induce epithelial-dependent relaxation of isolated guinea pig trachea [10].

However, we disagree with POLOSA *et al.*''s suggestion that frusemide acts *via* inhibition of the Na^+-K^+-ATPase. There is no doubt that Na-K-Cl co-transport of airway, and other, epithelial cells necessitates both establishment and maintenance of a low intracellular Na^+ activity through operation of the Na^+-K^+-ATPase located at the basolateral cell membrane [4-8], and phosphorylation of the co-transporter protein [8, 11]. However, experimental evidence suggests that there is no direct inhibition by loop diuretics of Na^+-K^+-ATPase in animal cells [11], including airway epithelial cells [6]. Rather, loop diuretics oppose Na-K-Cl co-transport through a direct effect on the Na-K-Cl co-transporter.

In conclusion, we do agree that frusemide has very likely a direct effect on airway epithelium that may account, at least in part, for its protective effect against bronchial obstruction induced by several bronchial provocants in asthma [3, 9], but we question the primary effect of frusemide on Na^+-K^+-ATPase suggested by POLOSA *et al.* [1]. We also agree with POLOSA et *al.* that frusemide certainly modifies ion transport and, henceforth, biological activity of other cell types, *e.g.* mast cells, since Na-K-Cl co-transport is a ubiquitary mechanism [11].

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References

1. Polosa R, Lau LCK, Holgate ST.- Inhibition of adenosine 5'-monophosphate- and methacholine-induced bronchoconstriction in asthma by inhaled frusemide. *Eur Respir J*, 1990, 3, 665-672.
2. Fujimara M, Sakamoto S, Kamio Y. - Effect of inhaled furosemide on bronchial responsiveness to methacholine [Letter to the Editor]. *N Engl J Med*, 1990, 322, 935-936.
3. Wood AM, Dinh Xuan AT, Higenbottam TW, Cremona G, Lockhart A. - Methoxamine and frusemide inhibit nasal transepithelial potential difference: a possible protective mechanism in the prevention of exercise-induced asthma [Abstract]. *Am Rev Respir Dis*, 1989, 139 (4), A478.
4. Al Bazzaz FJ. - Regulation of salt and water transport across airway mucosa. *In: Clin Chest Med*, 1986, 7, 259-272.
5. Welsh MJ. - Inhibition of chloride secretion by furosemide in canine tracheal epithelium. *J Membr Biol*, I983, 71, 219-226.
6. Widdicombe JH, Nathanson IT, Highland E. - Effects of "loop" diuretics on ion transport by dog tracheal epithelium. *Am J Physiol*, 1983, 245, C388-C396.
7. Welsh MJ. - Electrolyte transport by airway epithelial. *Physiol Rev*,1987, 67, 1143-1184.
8. Nadel JA, Widdicombe JH, Peatfield AC. - Regulation of airway secretions, ion transport and water movement. *In*: Handbook of Physiology. Section 3, The Respiratory System. Eds, Fishman AP, Fisher AB. American Physiological Society, 1985, Bethesda, Vol 1, pp 419-446.
9. Anonymous. - Inhaled frusemide and asthma. *Lancet*, 1990, 335, 944-946.
10. Munakata M, Mitzner W, Menkes H. - Osmotic stimuli induce epithelial dependent relaxation in the guinea pig trachea. *J Appl Physiol*, 1988, 64, 466-471.
11. Saier MH Jr, Boyden DA. - Mechanism, regulation and physiological significance of the loop diuretic-sensitive NaCl/KCl symport system in animal cells. *Mol Cell Biochem*, 1984, 59, 11-32.

REPLY TO LETTER

Effects of frusemide on human airway epithelium

I read with interest the letter from WOOD *et al.* and was glad to learn that our observations, that frusemide aministered by inhalation reduces the bronchial effects of inhaled methacholine in human asthma [1], are in agreement with the findings by FUJIMARA *et al.* [2].

In their letter, WOOD *et al.* propose that frusemide acts *via* inhibition of Na^+/K^+ adenosine triphosphatase (ATPase) at the epithelial level. I agree with them entirely as already mentioned in the discussion of our paper [1], in which we clearly state: "...since the drug inhibits Cl-flux only when added to the basolateral side of the epithelium [3–5] whereas in these experiments the drug reached the epithelium from the luminal face of the bronchial mucosa, it is unlikely that the inhibitory effect of the drug on the epithelial cell Na^+/K^+ ATPase may account for its effect in reducing provoked bronchoconstriction".

In contrast to the data presented by WOOD *et al.* [6], a recently published paper has clearly shown that frusemide up to 30 mg has no effect on epithelial ion transport as reflected by nasal potential difference in man [7], thus suggesting that this drug does not affect electrogenic epithelial ion transport. Therefore, whether frusemide has some direct electrogenic effect upon epithelium lining of the respiratory tract remains highly controversial.

Drugs such as frusemide may modulate the bronchoconstrictor responses to inhaled methacholine by a variety of other mechanisms. Frusemide's capacity to generate protective prostaglandins [8] with functional effects [9] could account for some of the inhibition of the bronchospasm provoked by methacholine. Frusemide affects neural function in the inner ear [10] so that it might inhibit both cholinergic and non-cholinergic nervous activity in the airways [11]. Finally, frusemide, in being an effective venodilator agent [12], could also enhance bronchial blood flow and, therefore, affect the kinetics of the clearance of the inhaled mediator.

The capacity of frusemide to produce a greater protection against the bronchospastic response to inhaled adenosine monophosphate (AMP) rather than methacholine [1] suggested the phenomenon of an additional pharmacological action, possibly at the level of mucosal mast cells. Prompted by the findings in rat mast cells a loop diuretic sensitive cell Ca^{++}/Mg^{++} ATPase has been shown to modulate mast cell degranulation to various stimuli [13], we proposed that an additional action on mast cell functions was a likely possibility. However, to date there is no published data in support of the effect of frusemide on human mast cell function and clearly further studies are needed to clarify this hypothesis.

As science advances and new technologies emerge, our hypotheses will require re-examination and modification, but surely up to now the mechanisms whereby inhaled frusemide protect against various bronchoconstrictor stimuli remains uncertain.

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References

1. Polosa R, Lau LCK, Holgate ST. – Inhibition of adenosine 5'-monophosphate and methacholine induced bronchoconstriction in asthma by inhaled frusemide. *Eur Respir J*, 1990, 3, 665–672.
2. Fujimara M, Sakamoto S, Kamio Y. – Effect of inhaled furosemide on bronchial responsiveness to methacholine. *N Engl J Med*, 1990, 332, 935–936.
3. Welsh MJ. – Intracellular chloride activities in canine tracheal epithelium: direct evidence for sodium coupled intracellular chloride accumulation in chloride secreting epithelium. *J Clin Invest*, 1983, 71, 1392–1401.
4. Knowles M, Murray G, Shallal J, Askin F, Ranga V, Gatzy J, Boucher R. – Bioelectric properties and ion flow across excised human bronchi. *J Appl Physiol: Respirat Environ Exercise Physiol*, 1984, 56, 868–877.
5. Widdicombe JH. – Ion transport by tracheal epithelial cells in culture. *Clin Chest Med*, 1986, 7, 299–305.
6. Wood AM, Dinh Xuan AT, Higenbottam TW, Cremona G, Lockhart A. – Methoxamine and frusemide inhibit nasal transepithelial potential difference: a possible protective mechanism in the prevention of exercise-induced asthma. *Am Rev Respir Dis*, 1989, 139(4), A478 (abstract).
7. Nichol GM, Alton EWFW, Nix A, Geddes DM, Chung KF, Barnes PJ. – Effect of inhaled frusemide on metabisulfite and methacholine induced bronchoconstriction and nasal potential difference in asthmatic subjects. *Am Rev Respir Dis*, 1990, 142, 576–580.
8. Weber PC, Scherer B, Larsson C. – Increase of free arachidonic acid by furosemide in man as the cause of prostaglandin and renin release. *Eur J Pharmacol*, 1977, 41, 329–332.
9. Hardy CC, Bradding P, Robinson C, Holgate ST. – Bronchoconstrictor and antibronchoconstrictor properties of inhaled prostacyclin in asthma. *J Appl Physiol*, 1988, 64(4), 1567–1574.
10. Rybak LP. – Pathophysiology of furosemide ototoxicity. *J Otolaryngol*, 1982, 11, 127–133.
11. Barnes PJ. – Neural control of human airways in health and disease. *Am Rev Respir Dis*, 1973, 288, 1087–1090.
12. Dikshit K, Vyden J, Forrester JS, Chatterjee K, Prakash R, Swan HJC. – Renal and extrarenal hemodynamic effects of furosemide in congestive heart failure after acute myocardial infarction. *N Engl J Med*, 1973, 288, 1087–1090.
13. Chakravarty N. – Role of Ca^{++}/Mg^{++} ATPase on the mast cell surface in calcium transport and histamine secretion. *Agents Actions*, 1987, 20, 185–187.