Rhinology, 33, 22-25, 1995

Effects of inflammatory mediators on ciliary function in vitro*

Rauf Khan[1], Jan Dolata[2], Sven Lindberg[2]

[1] Department of Clinical Research, Draco AB, Lund, Sweden
[2] Department of Otorhinolaryngology, University Hospital, Lund, Sweden

SUMMARY

Prostaglandins and histamine released during inflammatory and allergic reactions can affect the mucociliary system in different ways. By studying the effect of these mediators on ciliary beat frequency (CBF) with a photo-electrical technique in airway explants from different species, i.e. guinea-pig trachea, rabbit maxillary sinus, and human adenoid, the mechanisms underlying the effects of prostaglandin and histamine were further elucidated. Prostaglandin E_1 (PGE_1) produced a modest increase in CBF in preparations from guinea-pig trachea. The maximum response was 12.9±3.4% for the dose of 0.1 μg/ml, corresponding with 0.28 μM. Prostaglandin E_1 produced a dose-dependent increase in explants from rabbit maxillary sinus, the maximum effect was 35.9±14.1% at a dose of 1.0 μg/ml. PGE_1 produced a lesser increase in CBF in explants from human adenoids. A maximum increase of 4.1±1.6% was observed at a dose of 0.1 mg/ml. Histamine produced a moderate increase in CBF in explants from human adenoid at concentrations of 0.01-0.1 mM, corresponding with 1.84-18.4 μg/ml. In contrast, histamine did not significantly alter CBF in explants from the rabbit maxillary sinus or guinea-pig trachea. These results indicate that there are interspecies differences in the responsiveness to prostaglandins, and that PGE_1 seems to have more powerful effects on CBF in the upper than in the lower airways. The weak effects of histamine on CBF in vitro as compared to the relatively strong in vivo effects on mucociliary activity might be due to that histamine lacks direct effects on the ciliated epithelium, but rather exerts an indirect effect via other mediators or is dependent on neurogenic mechanisms which is demonstrable only in intact animals.

Key words: inflammatory mediators, in vitro ciliary, prostaglandins, histamine

INTRODUCTION

The influence of pharmaceuticals on ciliary activity has been the subject for extensive research, and present knowledge originates mainly from investigations performed on explants from the lower airways (Wanner, 1983), whereas the ciliated epithelium in the upper airways has not been extensively studied in this respect. During inflammatory and allergic reactions in the airways there is a release of mediators, such as prostaglandins and histamine (Okazaki et al., 1977; Naclerio et al., 1983; Shaw et al., 1985), which could affect the function of the mucociliary system. During such reactions, a stimulation of ciliary activity seems beneficial since an increased load on the mucociliary system can be expected, due to the increased production of mucus. Prostaglandins stimulate ciliary beat frequency (CBF) in explants from the lower airways in different species (Iravani and Melville, 1975; Melville et al., 1980; Wanner et al., 1983, 1986). In contrast, histamine failed to affect ciliary activity in previous *in vitro* studies (Ballenger, 1949; Scudi, 1951) or produced at relatively high concentrations (10^{-5}–10^{-3} M) only a modest stimulation of CBF in isolated tracheal cells from ewes (Wanner et al., 1983). Prostaglandin E_1 stimulates mucociliary activity in the rabbit maxillary sinus *in vivo*, an effect which is partially mediated through capsaicin-sensitive sensory C-fibres (Dolata et al., 1989a). Histamine produces a more intense stimulation of mucociliary activity, and this effect involves both capsaicin-sensitive sensory C-fibres and release of the neuropeptide substance P (Dolata et al., 1989b; 1990). Although the ciliated epithelia in the lower and upper airways display no major morphological differences, the responsiveness to inflammatory mediators might differ since prostaglandins

* Received for publication October 6, 1992; accepted June 6, 1994

produce a more powerful stimulation of ciliary activity in the lower airways than in the upper airways (Melville et al., 1980; Dolata et al., 1989a), whereas histamine shows an opposite potency (Wanner et al., 1983; Dolata et al., 1990). The aim of the present study was therefore to elucidate the mechanisms underlying the effects of prostaglandin and histamine on mucociliary activity, and to investigate if mediators known to stimulate CBF in the lower airways have the same effect on explants from the upper airways. This was done by studying the effect of these mediators on CBF with a photo-electrical technique in airway explants from different species including man.

MATERIAL AND METHODS

Human adenoids were obtained from children (aged between 3 and 8 years) undergoing adenoidectomy at the ENT Department of the University Hospital in Lund, Sweden. After removal, the tissues were placed in Krebs' solution and transported to the laboratory where they were thoroughly washed with aerated (95% O_2 and 5% CO_2) Krebs' solution. Small areas (about 0.5 mm^2) of ciliated epithelium were dissected under the microscope and transferred to a microscopic coverslip in 30 µl HEPES buffer. The coverslip was then placed upside down on a glass slide, 2 mm thick with a 1.2-mm deep cavity in the centre, giving a preparation hanging freely in a drop of Krebs' solution. To obtain guinea-pig and rabbit preparations the animals were killed by a blow on the head. The trachea was removed immediately and placed in aerated Krebs' solution. Connective tissue was carefully removed and the trachea was cut into small rings of about 0.5–0.8 mm in width. Adhering mucus was washed off with Krebs' solution. The tracheal preparation in a drop of HEPES buffer was transferred to a coverslip as described above. Rabbit maxillary epithelium was obtained by cutting through the bone, and prepared in a similar way to that for human adenoids. The hanging drop preparation was placed on a microscope stage maintained at room temperature (22–23°C; see Figure 1). The beating cilia were viewed at a magnification of ×400 in a Nikon-Optiphoto CF (chromatic aberration-free) microscope. The cilia were oriented in such a way to interrupt the passage of light through a slit-diaphragm (0.2 mm) into the photometer (Nikon photometer P1), which transduced the light energy into an electrical signal. The electrical signal generated was converted into a reading of ciliary beat frequency (CBF) displayed on the screen of a Nicolet 3091 oscilloscope. The signals displayed on the oscilloscope were also recorded on paper by means of an X-Y BD 90 recorder. The preparation was kept under the microscope for at least 15 min before the start of the experiments. CBF in each preparation was recorded from six different sites and a mean frequency was calculated. The results were expressed as means±SEM. Statistical evaluations were performed by means of the Student's t-test for paired observations.

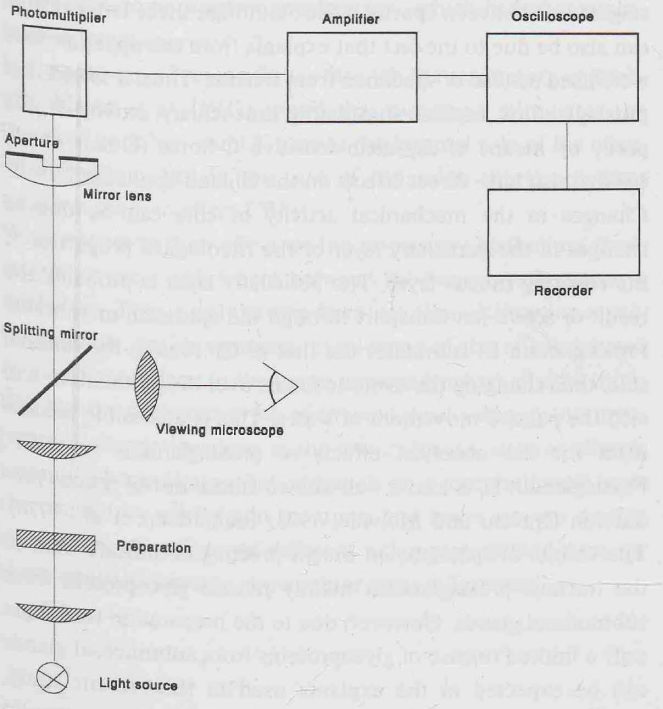

Figure 1. Schematic drawing of the experimental set-up (for details, see material and methods).

Solutions

Krebs' solution had the following composition: 120 mM NaCl, 4.0 mM KCl, 20 mM $NaHCO_3$, 1.5 mM NaH_2PO_4, 1.5 mM $MgSO_4$, 20 mM sodium acetate, 1.5 mM $CaCl_2$, 10 mM glucose (pH 7.4). HEPES buffer had the following composition: 135 mM NaCl, 4.6 mM KCl, 1.2 mM $MgSO_4$, 1.5 mM $CaCl_2$, 11 mM glucose, 10 mM HEPES. The pH was adjusted to 7.4 with tris(hydroxymethyl)aminomethane and aerated with 100% O_2. The following mediators were used: PGE_1 (Prostivas; Upjohn, USA) and histamine dihydrochloride (Sigma, USA). Prostivas is a solution of PGE_1 in ethanol at a concentration of 0.5 mg/ml. Immediately before the experiments a small volume of the stock solution was diluted in Krebs' solution and the effect on CBF was compared with controls containing Krebs' solution only. Histamine was diluted in Krebs' solution at the appropriate concentrations. Doses are expressed as concentrations of the respective salts. CBF was measured 2 min after the administration of the mediators.

RESULTS

Prostaglandin E_1 produced a modest increase in CBF in guinea-pig trachea, which was significant for doses of 0.01 µg/ml and above. The maximum response was 12.9±3.4% at a dose of 0.1 µg/ml, corresponding to 0.28 µM. Figure 2 shows the log-dose response curve for PGE_1 on CBF in preparations from guinea-pig trachea. However, the dose-response curve was shaped like an inverted U, and the highest dose tested (20.0 µg/ml) produced a significant decrease in CBF (-23.6±4.6%).

In human adenoids, PGE_1 produced a lesser increase in CBF than in explants from guinea pig trachea. After exposure to this compound there was only a slight increase (4.1±1.6%) which was significant only at a dose of 0.1 µg/ml.

In explants from rabbit maxillary sinus, PGE_1 produced a rather strong, dose-dependent increase in CBF, significant for the doses 0.1 and 1.0 µg/ml (0.28 and 2.8 µM, respectively). The maximum increase was 35.9±14.1% for the dose of 1.0 µg/ml.

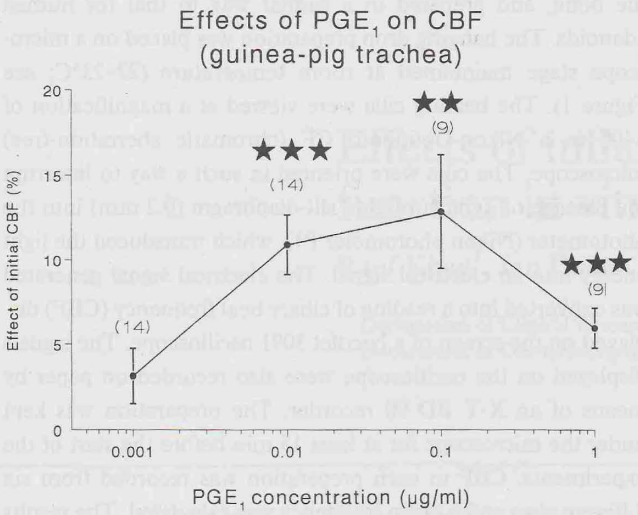

Figure 2. The log-dose response of various doses of prostaglandin E_1 on CBF in guinea-pig tracheal rings. The results are expressed as means±SEM (n=9-14 for the various concentrations). The initial CBF prior to challenges was 10.12±0.16 Hz. Note that the effect of the highest concentration investigated (20.0 µg/ml) is omitted for the sake of clarity (20.0 µg/ml produced a decrease of -23.6 ±4.6% instead of an increase in CBF; n=10). **: $p < 0.01$; ***: $p < 0.001$.

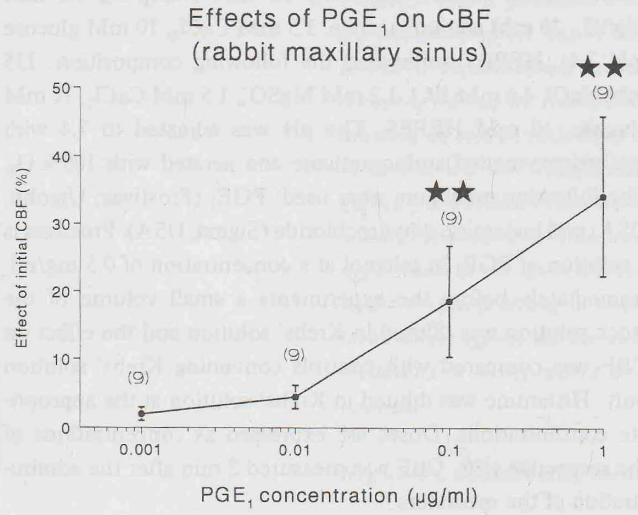

Figure 3. The log-dose response of various doses of prostaglandin E_1 on CBF in rabbit maxillary sinus. The results are expressed as means±SEM (n=9 for the various concentrations). The initial CBF prior to challenges was 11.62±0.83 Hz. **: $p < 0.01$.

Figure 3 shows the log-dose response curve for PGE_1 on CBF in preparations from rabbit maxillary sinus.

Histamine produced a slight increase in CBF in explants from human adenoid at concentrations of 0.01–0.1 mM, corresponding to 1.84-18.4 µg/ml. The maximum increase was 8.6±2.5% at the dose of 0.01 mM. In contrast, histamine did not significantly alter the CBF in explants from the rabbit maxillary sinus, nor did histamine in the doses of 0.01, 0.1, and 1.0 mM affect the CBF in explants from guinea pig trachea (Figure 4).

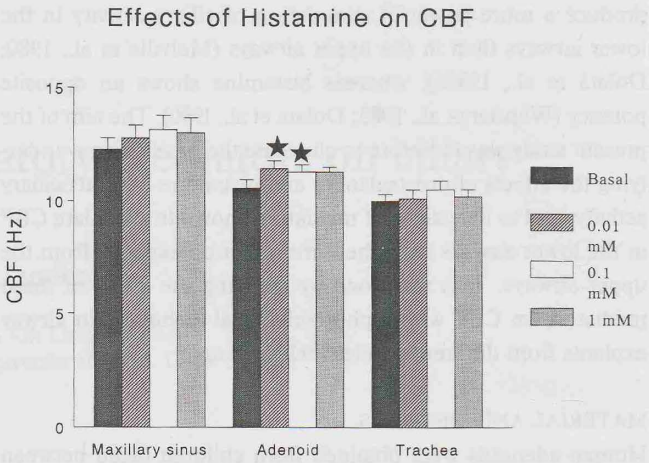

Figure 4. The effect of various doses of histamine on CBF in specimens from the rabbit maxillary sinus, human adenoids, and guinea-pig trachea. The results are expressed as means±SEM (n=8–15 for the various concentrations). The baseline CBF were 12.29±0.49 Hz (maxillary sinus), 10.58±0.38 Hz (adenoids), and 10.0±0.3 Hz (guinea-pig trachea). *: $p < 0.05$.

DISCUSSION

In the present study PGE_1 produced a mild stimulation of CBF in the trachea of guinea pigs in the dose range 0.01–1.0 µg/ml, corresponding to 2.8×10^{-8}–2.8×10^{-6} M). In explants from sheep trachea PGE_1 and PGE_2 produced a more powerful stimulation of CBF in the same dose range (Wanner et al., 1983; 1986). However, in the latter experiments PGE_1 stimulated CBF also in lower doses (10^{-10}–10^{-8} M; Wanner et al., 1986), whereas no effects were observed in our study. Furthermore, PGE_1 was more potent in stimulating CBF in the trachea of sheep as compared to that in guinea pigs, which indicates that there is a different sensitivity to prostaglandins between species. In the present study PGE had a rather strong stimulating effect on CBF in explants from rabbit maxillary sinus. In contrast, PGE_1 had only a slightly stimulatory effect on human adenoid, which further stresses that a variation in the responsiveness to prostaglandins between species exists, although these latter results can also be due to the fact that explants from the upper airways were used instead of specimen from trachea. Thus, it seems that prostaglandins, besides stimulating mucociliary activity *in vivo* partly by means of capsaicin-sensitive C-fibres (Dolata et al., 1989b), also have direct effects on the ciliated epithelium itself. Changes in the mechanical activity of cilia can be due to changes in the periciliary layer or the rheological properties of the covering mucus layer. The periciliary layer is probably the result of active ion transport through the epithelial membrane. Prostaglandin E_1 stimulates the flux of Cl^- ions to the luminal side, thus changing the osmotic forces over the epithelium, and also the passive movement of water. This is a possible mechanism for the observed effects of prostaglandins on CBF. Prostaglandin E_1 is also a well-known stimulator of mucus production (Iravani and Melville, 1975; Richardson et al., 1978). The results of radiolabeled mucin precursors indicate that, in the trachea, prostaglandins mainly release glycoprotein from submucosal glands. However, due to the preparation technique only a limited release of glycoproteins from submucosal glands can be expected in the explants used in the present study, making this mechanism as an explanation for the prostaglandin effect less probable. PGE_1 has been found to have a rather

strong excitatory effect on CBF in the lower airways, i.e. PGE_1 caused a maximum stimulation of about 40% in the tracheal-bronchial tree from bronchitic rats (Melville at al., 1980), and a maximum stimulation of 26% in preparations from the trachea of ewes (Wanner et al., 1983). In the present investigation the stimulation of CBF in explants from the upper airways was more modest, at least with regard to the effect on human adenoids (maximum response being 4.1±1.6%), while the effect on rabbit maxillary sinus was more powerful (maximum response being 35.9±14.1%).

In several *in vitro* investigations no effect of histamine on mucociliary function has been demonstrated (Ballenger, 1949; Scudi 1951), which is in accordance with the results obtained in the present study where histamine had no significant effect on CBF, neither in explants from the rabbit maxillary sinus nor in explants from guinea-pig trachea. In contrast, a small but significant increase in CBF was produced by histamine in explants from human adenoids at concentrations of 0.01-0.1 mM, corresponding to 1.84-18.4 µg/ml. Earlier experiments have shown that inhalation of histamine aerosol increases tracheal mucous transport rate in dogs (Wanner et al., 1975). Moreover, in the rabbit maxillary sinus histamine produces a powerful *in vivo* dose-dependent stimulation of mucociliary activity via H_1-receptors, the effect being mediated through capsaicin-sensitive C-fibres and involving the release of substance P (Dolata et al., 1990). An opposite finding, a depression of ciliary function, was reported by Van de Donk et al. (1982) who found ciliostasis after topical application of anti-histamines in chicken trachea embryos.

A possible explanation for the discrepancy between histamine's strong effects *in vivo* and its weak effects *in vitro* might be that histamine lacks direct effects on the ciliated epithelium, but rather exerts an indirect effect via other mediators or may be dependent on neurogenic mechanisms, which is demonstrable only in intact animals.

Histamine can affect ion fluxes through the respiratory epithelium. Marin et al. (1977) found that histamine stimulates the fluxes of both Na^+ and Cl^- ions to the luminal side of the ciliated epithelium, but in the case of histamine this mechanism does not seem to affect CBF.

With respect to their effect on the respiratory epithelium there are rather great differences between the various inflammatory mediators. This is well known from the clinical situation since, for example, anti-histamines reveal some of the effects caused by allergic reactions in the upper airways (such as allergic rhinitis), but do not seem to be of therapeutical value in the treatment of allergic reactions in the lower airways, such as allergic asthma. This indicates that, although on a morphological basis the respiratory epithelia in the upper and lower airways display similarities, the effects of different inflammatory mediators on these epithelia seem to show rather great differences.

REFERENCES

1. Ballenger JJ (1949) A study of ciliary activity in the respiratory tract of animals. Ann Otol Rhinol Laryngol 58: 351-69.
2. Dolata J, Lindberg S, Mercke U (1989a) The effects of prostaglandins E_1, E_2 and $F_{2\alpha}$ on mucociliary activity in the rabbit maxillary sinus. Acta Otolaryngol (Stockh) 108: 290-297.
3. Dolata J, Lindberg S, Mercke U (1989b) Cholinergic and C-fibre mediated mechanisms in the stimulation of mucociliary activity induced by prostaglandins and histamine. Acta Otolaryngol (Stockh) 108: 456-463.
4. Dolata J, Lindberg S, Mercke U (1990) Histamine stimulates mucociliary activity in the rabbit maxillary sinus. Ann Otol Rhinol Laryngol 99: 666-671.
5. Iravani J, Melville GN (1975) Mucociliary activity in the respiratory tract as influenced by prostaglandin E_1. Respiration 32: 305-315.
6. Marin MG, Davis B, Nadel JA (1977) Effect of histamine on electrical and ion transport properties of tracheal epithelium. J Appl Physiol 42: 735-738.
7. Melville GN, Ismail S, Sealy C (1980) Tracheobronchial function in health and disease. Respiration 40: 329-336.
8. Naclerio RM, Meier HL, Adkinson Jr NF, Kagey-Sobotka A, Meyers DA, Norman PS, Lichtenstein LM (1983) In vivo demonstration of inflammatory mediator release following nasal challenge with antigen. Eur J Respir Dis 64 Suppl 128: 26-32.
9. Okazaki T, Reisman RE, Arbesman CE (1977) Prostaglandin E in the secretions of allergic rhinitis. Prostaglandins 13: 681-690.
10. Richardson PS, Phipps RJ, Balfre K, Hall RL (1978) The roles of mediators, irritants and allergens in causing mucin secretion from the trachea. In: R Porter (Ed.) Respiratory Tract Mucus (Ciba Foundation Symposium, New Series, 54). Elsevier/Excerpta Medica, Amsterdam, pp. 111-131.
11. Scudi JV, Kimura ET, Reinhard JF (1951) Study of drug action on mammalian ciliated epithelium. J Pharmacol Exp Ther 102: 132-137.
12. Shaw RJ, Fitzharris P, Cromwell O, Wardlaw AJ, Kay AB (1985) Allergen-induced release of sulphidopeptide leukotrienes (SRS-A) and LTB4 in allergic rhinitis. Allergy 40: 1-6.
13. Van de Donk HJM, Van Egmond ALM, Van den Heuvel AGM, Zuidema J, Merkus FWHM (1982) The effect of drugs on ciliary motility. III. Local anaesthetics and anti-allergic drugs. Int J Pharmaceut 12: 77-85.
14. Wanner A, Zarzecki S, Hirsch J, Epstein S (1975) Tracheal mucous transport in experimental canine asthma. J Appl Physiol 39: 950-957.
15. Wanner A (1983) Allergic mucociliary dysfunction. Laryngoscope 93: 68-70.
16. Wanner A, Maurer D, Abraham WM, Szepfalusi Z, Sielczak M (1983) Effects of chemical mediators of anaphylaxis on ciliary function. J Allergy Clin Immunol 72: 663-667.
17. Wanner A, Sielczak M, Mella JF, Abraham WM (1986) Ciliary responsiveness in allergic and non-allergic airways. J Appl Physiol 60: 1967-1971.

Dr. Jan Dolata
Dept. of Otorhinolaryngology
Helsingborg Hospital
S-25187 Helsingborg
Sweden