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Production of PGE_2 by bovine cultured airway smooth muscle cells and its inhibition by cyclo-oxygenase inhibitors

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1 Prostaglandin E_2 (PGE_2) is thought to be an important inhibitory modulator of inflammatory processes in the airway. It inhibits inflammatory cell function and cholinergic neurotransmission *in vitro* and roles have been postulated *in vivo* in refractoriness and in the mechanism of action of the diuretic agent, frusemide.

2 The production of PGE_2 by bovine cultured airway smooth muscle cells has been studied under a range of conditions. The effects of cyclo-oxygenase inhibitors (flurbiprofen, indomethacin, acetyl salicylic acid) on serum-induced production of PGE_2 were assessed over a range of concentrations (10^{-7}–10^{-4} M).

3 Serum-stimulated production of PGE_2 in control wells ranged from 350 to 800 ng PGE_2 ml^{-1} in cells from different animals. All three cyclo-oxygenase inhibitors inhibited PGE_2 production with an order of potency, flurbiprofen > indomethacin > acetyl salicylic acid. Log IC_{50} values were − 6.24 for flurbiprofen, − 5.23 for indomethacin and − 3.50 for acetyl salicylic acid.

4 PGE_2 production was stimulated by arachidonic acid (10^{-5} M) or addition of the proinflammatory mediator, bradykinin (10^{-8}–10^{-5} M).

5 Incubation of cells for 24 h with 5 bromo deoxyuridine (BRDU) (10^{-4} M) to prevent DNA synthesis did not alter PGE_2 production in response to serum, suggesting that it was not a function of proliferation *per se*.

6 Our study suggests that airway smooth muscle may be an important source of PGE_2. Production of PGE_2 may be a novel feedback mechanism whereby airway smooth muscle cells can negatively modulate airways inflammation. The differing potencies of the cyclo-oxygenase inhibitors may explain the contrasting effect of these drugs in recent studies in asthma.

Keywords: PGE_2; airway smooth muscle; indomethacin; flurbiprofen; acetyl salicylic acid; bradykinin

Introduction

Prostaglandin E_2 (PGE_2) is thought to be an important inhibitory prostaglandin in airway tissue which has potent effects both *in vitro* and *in vivo*. *In vitro*, PGE_2 inhibits acetylcholine release from parasympathetic nerve endings in human airway smooth muscle (Ito *et al.*, 1990). It inhibits release of mediators from human lung mast cells (Peters *et al.*, 1982) and inhibits cellular responses in other inflammatory cells such as eosinophils (Giembycz *et al.*, 1990), macrophages (Christman & Christman, 1990) and T-lymphocytes (Minakuchi *et al.*, 1990). In asthmatic subjects *in vivo*, PGE_2 is a potent inhibitor of bronchoconstriction induced by metabisulphite (Pavord *et al.*, 1991), allergen (Pavord *et al.*, 1992a), exercise and ultrasonically nebulised distilled water (Pasargiklian *et al.*, 1976; 1977). A role for PGE_2 has also been postulated in the refractoriness to bronchoconstrictor challenges which occurs in asthma (Pavord *et al.*, 1992a) and in the effect of the diuretic drug, frusemide, which protects against several bronchoconstrictor challenges in asthma (Pavord *et al.*, 1992a).

It is not known which of the many types of airway cells are the most important in PGE_2 production as this has not been studied in detail with the exception of the epithelial cell. Airway smooth muscle has been relatively ignored as a possible site of production. A few studies in lung homogenates (Masumoto & Masuda, 1976) or whole airway preparations (Haye-Legrand *et al.*, 1986) have documented production of PGE_2, suggesting that airway smooth muscle is capable of PGE_2 synthesis but these preparations contain heterogenous populations of airway cells. As smooth muscle is an important source of prostaglandins in other organs (Chamley-Campbell *et al.*, 1979) it would seem likely that airway smooth muscle cells may also be an important source of PGE_2. In one preliminary report on dog trachealis smooth muscle strips, PGE_2 was produced in response to histamine, suggesting that airway smooth muscle is capable of PGE_2 synthesis (Anderson *et al.*, 1980). One method of studying PGE_2 production by airway smooth muscle more closely is to study cultured cells.

Cyclo-oxygenase inhibitors have been recently shown to have conflicting effects in asthma (Robuschi *et al.*, 1992; Bianco *et al.*, 1992; Pavord *et al.*, 1992b; Polosa *et al.*, 1992; O'Connor *et al.*, 1993). In view of this and the potential importance of PGE_2 in the airway, we studied the production of PGE_2 by cultures of bovine airway smooth muscle cells and its inhibition by flurbiprofen, indomethacin and acetyl salicylic acid.

Methods

Tissue preparation

Bovine trachea was obtained from the local abattoir and bathed immediately in ice cold Krebs-Henseleit solution. The trachealis muscle was then dissected free of epithelium and connective tissue. The muscle was minced with a McIlwain tissue chopper (Brinkmann Instruments, Westbury, New York, U.S.A.) and subjected to an enzymatic digestion procedure (Felbel *et al.*, 1988; Kamm *et al.*, 1989). The chopped tissue was incubated in 5 ml Krebs-Ringer-HEPES solution (KRH) without enzymes and shaken in a waterbath at 37°C for 30 min. The tissue was then incubated in 5 ml Krebs-Ringer-HEPES solution (KRH), containing 3.5 mg type IV collagenase, 5 mg soya bean trypsin inhibitor and 5 mM $CaCl_2$ at 37°C in a shaking water bath for 30 min. This

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procedure was then repeated twice more. The slurry was filtered, the cells were centrifuged, resuspended in Dulbecco's modified eagles medium (DMEM), counted and then plated out in several 175 cm^2 flasks. Cells were grown to confluence in DMEM + 10% fetal calf serum (FCS) containing penicillin/streptomycin/amphotericin B and L-glutamine (concentrations given below). Once confluent, cells were trypsinized with 0.25% trypsin, 0.02% EDTA and either passaged immediately or resuspended in 90% FCS + 10% dimethyl sulphoxide, frozen in liquid nitrogen and stored until required. Cells were thawed prior to use and plated at a density of 0.2×10^5 cells/well in 12 well plates containing DMEM + 10% fetal calf serum + penicillin/streptomycin/amphotericin B + L-glutamine.

Cell counting

Cells were removed from plates by incubation with 0.25% trypsin, 0.02% EDTA for 8–10 min. Cells were counted in a haemocytometer and viability assessed by trypan blue exclusion.

Radioimmunoassay for PGE_2

PGE_2 was measured by radioimmunoassay (Hawthorne *et al.*, 1991). Assays were performed in triplicate and expressed as pg ml^{-1}. Samples and standards were incubated overnight with assay buffer, [^{3}H]-PGE_2 (Amersham TRK 431) and rabbit PGE_2 antiserum (Sigma P5164). Free and bound PGE_2 were separated by incubation for 12 min with dextran-coated charcoal and centrifuged for 15 min at 3,500 r.p.m. (4°C). Supernatants were diluted in scintillation fluid and counted for 5 min. Doubling dilutions of PGE_2 standard (Sigma P5640) over the concentration-range 1000 to 7.5 pg 100 μl^{-1} were made up in ethanol and used to construct the standard curve. The antiserum has a low degree of cross reactivity with $PGF_{2\alpha}$ (3.4%) and $PGF_{1\alpha}$ (3.2%) but does not discriminate PGE_2 from PGE_1. The sensitivity of the assay, or the 90% intercept of the B/B_0 standard curve was 15 pg 100 μl^{-1}. Intra-assay variability was +/−6.8 pg 100 μl^{-1} ($n = 14$) and inter-assay variability +/−7.5 pg 100 μl^{-1} ($n = 45$). The assay results were comparable with those obtained with a sensitive ELISA assay (Cascade, UK).

Staining

Cells were grown on coverslips, washed with ice cold PBS (with 0.1% sodium azide) and fixed in methanol at − 20°C. Coverslips of cells were incubated in 1% goat serum in PBS for 45 min at room temperature. Cells were then incubated for 1 h at room temperature in monoclonal anti smooth muscle actin (Sigma A2547) antisera diluted in PBS. The optimum dilution was determined for each batch (between 1 in 100 and 1 in 400 dilution). The cells were then washed in PBS/azide and incubated for 1 h at room temperature in the dark with fluorescein isothiocyanate (FITC) conjugated anti-mouse antibody (Dako F313) (up to 1 in 8 dilution) before being washed and viewed by fluorescent microscopy.

Drugs

Indomethacin, acetyl salicylic acid and flurbiprofen were obtained from Sigma Chemicals, Poole, Dorset, UK. Stock solutions (10^{-2} M) were made up of each inhibitor. Indomethacin and acetyl salicylic acid were dissolved in the minimum amount of 1 M sodium hydroxide required and diluted in DMEM. Flurbiprofen was dissolved in water and 1 M NaOH in a 6:1 ratio and then diluted with DMEM.

[^{3}H]-PGE_2 was obtained from Amersham Interational, Amersham, U.K. Rabbit PGE_2 antiserum, PGE_2 standard, type IV collagenase, protease, DMEM, L-glutamine, penicillin/streptomycin, arachidonic acid, bradykinin, 5 bromo deoxyuridine, ascorbic acid, insulin, transferrin and soya bean trypsin inhibitor were obtained from Sigma Chemicals, Poole, U.K. Amphotericin was obtained from ICN Flow, High Wycombe, U.K. Bovine foetal calf serum was obtained from Seralab, Crawley Down, U.K.

Buffers: Krebs-Henseleit solution had the following composition (mmol l^{-1}): Na^+Cl^- 118, K^+Cl^- 4.7, $Mg^{2+}SO_4^{2-}$ 1.2, $Na^+H_2PO_4^-$ 1.2, $Ca^{2+}Cl_2^-$ 2.5, $Na^+HCO_3^-$ 25, glucose 11.1, pH 7.4 when gassed with 95% O_2, 5% CO_2.

Krebs-Ringer-HEPES was of the following composition (mmol l^{-1}):Na^+Cl^- 105, K^+Cl^- 5, $Ca^{2+}Cl_2^-$ 2, $K^+H_2PO_4^-$ 1, $Mg^{2+}SO_4^{2-}$ 1, glucose 14, HEPES 25 and phenol red 10 μg ml^{-1}, pH 7.4.

Culture medium

Penicillin/streptomycin solution (5000 u penicillin + 5 mg streptomycin ml^{-1}) 1 ml + 0.5 ml amphotericin (250 μg ml^{-1}) + 1 ml L-glutamine (200 mM) was added to 50 ml of DMEM before use.

Protocols

Cells were used on the first passage and plated at a concentration of 0.2×10^5 cells per well in 12 well culture plates containing DMEM + 10% foetal calf serum (FCS). Initial experiments were performed to determine the day in culture that PGE_2 production was maximal by counting cells and measuring PGE_2 production on days 4, 5, 6 and 7 of culture. PGE_2 production had plateaued by days 6 and 7 and subsequent experiments were performed to look at the effects of cyclo-oxygenase at this time. The time course of PGE_2 production in 10% FCS over 24 h was studied. For these experiments medium was changed on Day 6 and the fresh medium subsequently removed for measurement of PGE_2 at 2, 6 and 24 h from separate wells.

Experiments were performed to look at the effects of different cyclo-oxygenase inhibitors in tissue from four different animals, assays being performed in triplicate for each animal. Indomethacin, acetyl salicylic acid or flurbiprofen were added over a concentration range 10^{-7}–10^{-4} M on day 5 to separate wells of cells. On day 6, after 24 h incubation in each inhibitor, the supernatant from each well was collected, PGE_2 was measured and cells were trypsinized and counted.

Additional experiments were performed to determine the effects of bradykinin, arachidonic acid and foetal calf serum in quiescent cells. In these experiments cells were grown to confluence and growth arrested for 24 h by incubation in serum-free DMEM + insulin 6 μg ml^{-1} + transferrin 5 μg ml^{-1} + ascorbic acid 35 μg ml^{-1} prior to the addition of each agent. PGE_2 production was measured over 6 h.

In order to determine if production of PGE_2 was a function of cell proliferation we performed experiments where cells were incubated in 10^{-4} M 5-bromo deoxyuridine (BRDU) for 24 h prior to the addition of 10% FCS and measurement of PGE_2.

Results

Cells grown in the manner described reached confluence after 5–6 days, showed the characteristic appearance of smooth muscle cells in culture with a hill and valley appearance when confluent and stained positively for smooth muscle alpha actin in contrast to fibroblasts grown from connective tissue implants (Figure 1).

The initial experiments showed that airway smooth muscle cells cultured in 10% FCS produce large quantities of PGE_2 and that this was maximal after 6–7 days of culture (Table 1). PGE_2 production increased progressively over a 24 h period (Figure 2). Serum-stimulated PGE_2 production in control wells ranged from 350 to 800 ng per well. PGE_2 production was inhibited by all three cyclo-oxygenase inhibitors in a concentration-dependent manner. The concentrations used in

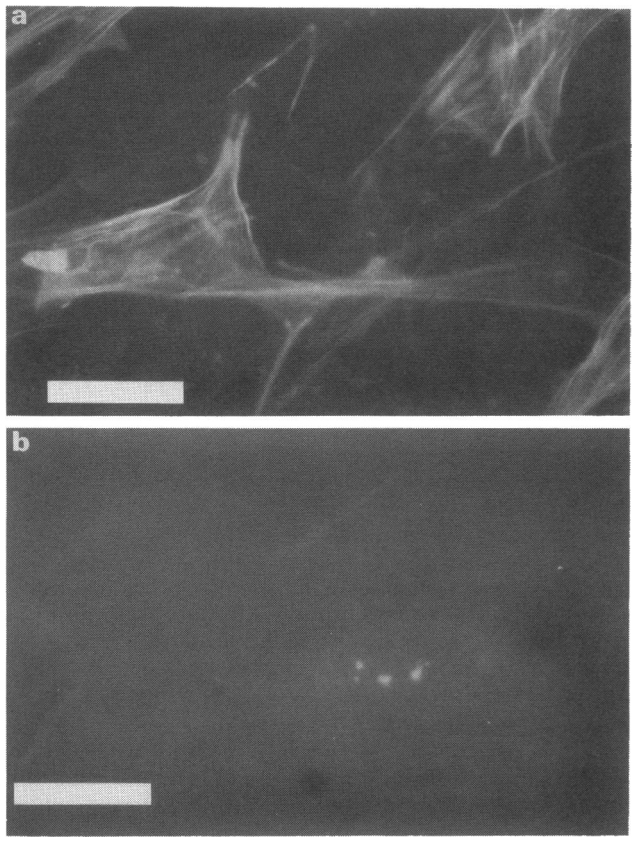

Figure 1 (a) Bovine airway smooth muscle cells in culture showing staining for smooth muscle actin in contrast to (b) fibroblasts grown from connective tissue explants. Cells were grown on coverslips and stained with monoclonal antismooth muscle actin antisera, labelled with fluorescein isothiocyanate antibody and veiwed by fluorescent microscopy. Scale bar = 50 μm.

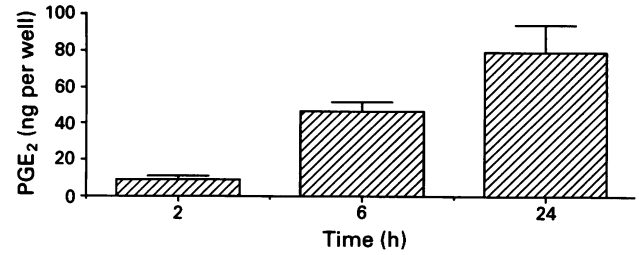

Figure 2 Time course of prostaglandin E_2 (PGE_2) production in cells grown to confluence in serum, medium changed at time 0 and PGE_2 measured over 24 h. Values shown are the mean ± s.e.mean of 4 experiments.

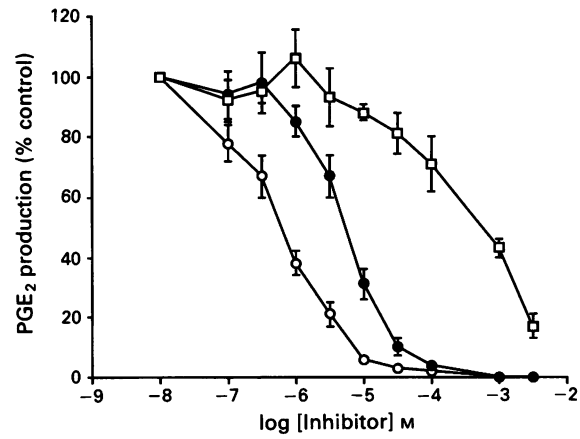

Figure 3 The effects of flurbiprofen (○), indomethacin (●) and acetyl salicylic acid (□) on serum induced production of prostaglandin E_2 (PGE_2) from confluent airway smooth muscle cells. Results are the mean ± s.e.mean values of 4 experiments performed in triplicate.

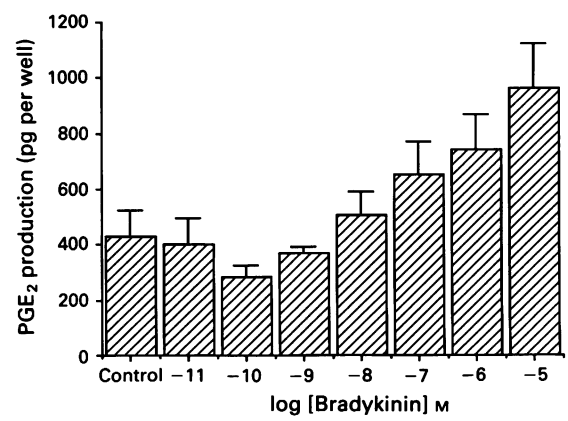

Figure 4 Effect of a range of concentrations of bradykinin on prostaglandin E_2 (PGE_2) production by growth arrested airway smooth muscle cells. Results are mean ± s.e.mean, $n = 4$.

the first experiment for acetyl salicylic acid (up to 10^{-4} M) did not inhibit PGE_2 production enough to obtain an IC_{50} value. Thus for the remaining experiments, acetyl salicylic acid was studied in concentrations up to 3×10^{-3} M. Figure 3 illustrates the mean (s.e.) production of PGE_2 in the presence of the three inhibitors shown as concentration-response curves. Flurbiprofen was ten times more potent than indomethacin and 500 times more potent than acetyl salicylic acid. Flurbiprofen at 3×10^{-5} M inhibited PGE_2 production by 97%, indomethacin at 10^{-4} M inhibited PGE_2 production by 96% and acetyl salicylic acid at 3×10^{-3} M inhibited PGE_2 production by 93%. Log IC_{50} values were -6.24 ± 0.1 for flurbiprofen, -5.23 ± 0.1 for indomethacin and -3.50 ± 0.3 for acetyl salicylic acid. None of the cyclo-oxygenase inhibitors altered cell counts significantly at any concentration.

Incubation of cells in serum-free medium for 24 h produced a lower basal level of PGE_2 production. This was stimulated over 6 h by 10^{-5} M arachidonic acid to 148 ± 17% of control wells ($P < 0.05$, $n = 4$ experiments each performed in triplicate). Addition of 10% FCS produced a marked increase in PGE_2 production over 6 h compared to serum-free alone ($P < 0.05$). PGE_2 production over 6 h was 10.4 ± 4 ng per well in serum-free medium and 190 ± 78 ng per well in 10% FCS ($n = 4$ experiments each performed in triplicate).

Arachidonic acid (10^{-5} M) increased PGE_2 production to 148 ± 17% of control values ($P < 0.05$, $n = 4$ experiments each performed in triplicate). Bradykinin caused a concentration-related increase in PGE_2 production. Bradykinin (10^{-6} M) increased PGE_2 production to 239 ± 80% of control values ($n = 4$ experiments each performed in triplicate) ($P <$ 0.05, Figure 4). Incubation of cells with 10^{-4} M BRDU for

Table 1 Cell counts and prostaglandin E_2 (PGE_2) production from bovine airway smooth muscle cells in culture, day 4–7

Day	*Mean cell count* ($\times 10^5$ cells ml^{-1})	*Mean PGE_2 prod^n* (ng ml^{-1})	*PGE_2 prod^n* (ng per 10^5 cells)
4	6.2 ± 0.5	174 ± 5.2	28.1
5	24.3 ± 2.7	327 ± 33.7	13.5
6	16.3 ± 0.9	747 ± 24.0	45.8
7	17.0 ± 0.4	837 ± 14.1	49.2

Values shown are mean ± s.e.mean, $n = 3$.

24 h prior to the addition of 10% FCS did not significantly alter PGE_2 production. PGE_2 produced was 313 ± 78 ng per well in control wells and 391 ± 71 ng per well in cells treated with 10^{-4} M BRDU ($P = 0.5$, $n = 4$).

Discussion

The aim of the study was to measure PGE_2 production by airway smooth muscle cells under different conditions and to study its inhibition by three cyclo-oxygenase inhibitors. We performed experiments on bovine cultured airway smooth muscle cells *in vitro*. The advantage of using cell culture for these studies is that lipid metabolism can be readily studied and it is easier to manipulate the system experimentally than in studies in tissue slices or whole animals. It also allows the study of individual cell types such as airway smooth muscle without confounding effects due to epithelial and inflammatory cells. We have been able to culture bovine airway smooth muscle cells readily from enzyme digested tissue strips and have confirmed that these cells stain positively for the smooth muscle contractile protein alpha actin unlike confluent cultures of fibroblasts grown from connective tissue explants.

We measured PGE_2 by radioimmunoassay. This is a convenient and reliable technique. The monoclonal antibody used, however, does not discriminate between PGE_1 and PGE_2. However, PGE_1 is produced in minute amounts relative to PGE_2 in airway tissue (Coleman *et al.*, 1990) and in preliminary experiments we showed that we were measuring PGE_2 rather than PGE_1 in our assay by comparing results from the radioimmunoassay with a more sensitive enzyme-linked immunosorbent assay (ELISA) which distinguishes PGE_1 from PGE_2. We did not measure other arachidonic acid metabolites as h.p.l.c. studies have shown that PGE_2 is by far the dominant arachidonic acid metabolite of airway smooth muscle (Tanaka *et al.*, 1993).

Ours is the first study to examine PGE_2 production in detail in cultured airway smooth muscle cells. There have, however, been studies measuring PGE_2 production in whole airway tissue preparations which have contained airway smooth muscle in addition to other cell types (Masumoto & Masuda, 1976; Haye-Legrand *et al.*, 1986) and one previous study measuring PGE_2 in canine trachealis strips (Anderson, 1980). However, studies in whole tissue preparations cannot identify the cell type responsible for prostaglandin production.

PGE_2 was stimulated over basal levels in our experiments by addition of arachidonic acid, the substrate for phospholipase A_2. As bradykinin has been shown to increase PGE_2 production in other cell types (White *et al.*, 1992) and is thought to be an important proinflammatory mediator in asthma, we studied the effect of bradykinin on PGE_2 production. Bradykinin caused a concentration-related increase in PGE_2. The finding that proinflammatory mediators such as bradykinin increase PGE_2 is important for several reasons. As PGE_2 is an inhibitory prostaglandin, the PGE_2 released may be involved in a negative feedback mechanism protecting the airway from excessive bronchoconstriction. Proinflammatory mediators such as histamine and bradykinin have also been shown to stimulate PGE_2 production from canine tracheal strips (Anderson *et al.*, 1980) and in human cultured tracheal epithelial cells (Churchill *et al.*, 1989) respectively. Any PGE_2 release by these mediators might feed back to inhibit inflammatory cell function (Giembycz *et al.*, 1990; Christman & Christman, 1990; Minakuchi *et al.*, 1990) and acetylcholine release from cholinergic nerve terminals (Ito *et al.*, 1990), thus suggesting a novel role for airway smooth muscle and airway epithelium in producing an anti-inflammatory mediator which may exert a braking effect on the inflammatory process. The protective effects of PGE_2 are likely to be due to its anti-inflammatory effects rather than effects on smooth muscle as its effects on airway smooth muscle are weak and variable *in vitro* (Sweatman & Collier, 1968). When PGE_2 is administered by nebuliser to normal and asthmatic subjects *in vivo* it has also been shown to cause variable effects on airway calibre with an initial transient bronchoconstriction being followed by bronchodilatation (Walters & Davies, 1982).

We were surprised by the large quantities of PGE_2 produced by bovine airway smooth muscle cells in our experiments where serum stimulated PGE_2 production was as high as 490 ng/10^6 cells in a 24 h period. This is large compared to PGE_2 production in other tissues. For example, a study in human tracheal epithelial cells grown in 5% fetal calf serum showed PGE_2 production of 1.5 ng/10^6 cells over a 24 h period. We considered whether differences in the concentration of fetal calf serum used might be responsible. The cells in our study were cultured in medium containing 10% fetal calf serum. Serum contains many growth factors including epidermal growth and platelet-derived growth factor and these have both been shown to stimulate PGE_2 production from other cell types by inducing enzymes in the cyclo-oxygenase pathway (Habenicht *et al.*, 1985; Casey *et al.*, 1988). The differences in PGE_2 production (300 fold) between our study and the study in tracheal epithelial cells (Churchill *et al.*, 1989) would seem to be too large, however, to be due solely to the differences in the concentration of fetal calf serum used (10% vs 5%). Even in serum-free conditions, production of PGE_2 by our airway smooth muscle cells was in excess of that reported in airway epithelium. This suggests that airway smooth muscle may be a more important site of PGE_2 production than epithelial cells. There was some variability in the amount of PGE_2 produced by different cultures in our studies. This probably reflected differences in the degree of confluence when PGE_2 production was studied.

As smooth muscle cells can change their phenotypic properties in culture we performed experiments where cells were incubated with BRDU to prevent DNA synthesis prior to stimulating PGE_2 production with serum. We found no inhibitory effect of BRDU on PGE_2 production suggesting that PGE_2 production is not a function of proliferation *per se*.

In addition to quantifying PGE_2 production by airway smooth muscle cells, we were interested in looking at the contrasting effects of three inhibitors of cyclo-oxygenase. PGE_2 production was inhibited by the three different cyclo-oxygenase inhibitors that we used in a concentration-dependent manner. We studied the effects of these inhibitors on PGE_2 production after 6 days in culture as initial standardization experiments showed that production per cell was large at that time. All three inhibitors, flurbiprofen, indomethacin and acetyl salicylic acid inhibited PGE_2 production. Flurbiprofen was ten times more potent than indomethacin and 500 times more potent than acetyl salicylic acid. Several previous studies have examined the effect of these three inhibitors in other tissues and shown a similar order of potency. In a study of guinea-pig lung homogenates *in vitro* (Masumoto & Masuda, 1976), flurbiprofen was found to be five times more potent than indomethacin and 100 times more potent than acetyl salicylic acid. In other tissues, and species however, these agents have been shown to have different potencies. In a cell-free homogenate of rabbit renal medulla, flurbiprofen was equipotent with indomethacin and 300 times more potent than aspirin (Crook *et al.*, 1976). In platelets the three agents were almost equipotent at inhibiting prostaglandin synthesis (Cockbill *et al.*, 1979). The varying results of the above studies indicate that the three inhibitors can have differential effects in different tissues with the IC_{50} values varying according to tissue type as well as the species studied. This may be due to the existence of different isoenzymes of cyclo-oxygenase in different tissues (Crook *et al.*, 1976). There are two cyclo-oxygenase (Cox) isoenzymes, Cox 1 and Cox 2 (Meade *et al.*, 1993). We were probably looking at Cox 1 in our studies.

We have considered whether the differences in potency of

the three prostaglandin synthesis inhibitors might explain their contrasting effects in asthma. It has been known for years that some asthmatic subjects respond to orally administered acetyl salicylic acid and similar drugs by bronchoconstriction (Szczeklik, 1976). However, recently Bianco and colleagues have shown that inhaled acetyl salicylic acid has protective effects in asthma and that its effects are additive with the diuretic agent frusemide. (Robuschi *et al.*, 1992; Bianco *et al.*, 1992). In contrast, oral indomethacin inhibits the protective effect of frusemide on exercise-induced bronchoconstriction (Pavord *et al.*, 1992a) giving rise to the hypothesis that frusemide may be acting by producing PGE_2. Oral flurbiprofen did not inhibit the protective effect of frusemide on exercise-induced bronchoconstriction in asthma (O'Connor *et al.*, 1993) but did inhibit the protective effect of frusemide on methacholine-induced bronchoconstriction in normal subjects (Polosa *et al.*, 1992). In our study acetyl salicylic acid was a relatively poor inhibitor of prostaglandin synthesis and it is therefore unlikely that the beneficial reported effects of inhaled aspirin in asthma are due to inhibition of prostaglandin synthesis. However, the potent effects of indomethacin on prostaglandin synthesis in airway smooth muscle cells *in vitro* would be consistent with its inhibitory effects on the action of frusemide *in vivo* if frusemide were acting through prostanoid mediated pathways.

The other implications of our study may be in aspirin-induced asthma which occurs in a small proportion of asthma patients. Several theories have been put forward to explain this, including an imbalance of constrictor and dilator prostaglandins or shunting of arachidonic acid metabolism or leukotriene synthesis (Szczeklik, 1976). The low potency of acetyl salicylic acid at inhibiting PGE_2 production would be consistent with the lack of effect of oral aspirin in the majority of patients. It may be that aspirin-sensitive asthmatic subjects are more sensitive to the effects of aspirin on prostaglandin synthesis.

In conclusion we have demonstrated that bovine airway smooth muscle cells produce large quantities of PGE_2 and that this can be inhibited by cyclo-oxygenase inhibitors. PGE_2 produced by airway smooth muscle may be an important negative modulator of inflammation in the airways.

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