

Effects of topical corticosteroids on inflammatory mediator-induced eicosanoid release by human airway epithelial cells

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Background: Airway epithelial cells are among the first cells to come in contact with aerosolized corticosteroids. However, the relative potencies and time course of action of the several commonly used aerosolized corticosteroids on eicosanoid production by airway epithelial cells are unknown.

Objectives: This study compared the effects of fluticasone, budesonide, and triamcinolone on eicosanoid output by human airway epithelial cells in vitro. We also determined the spectrum of eicosanoids affected and the mechanism for corticosteroid action.

Methods: Cultured BEAS-2B airway epithelial cells (a transformed cell line) were exposed to corticosteroids (1 nmol/L to 1 μ mol/L) for 2 to 48 hours and then assayed for basal- and bradykinin (BK)-stimulated eicosanoid output. The eicosanoid profile was identified by HPLC in tritiated arachidonic acid prelabelled cells, and PGE₂, the major eicosanoid product, was quantitated by RIA. The effect of corticosteroids on the immunoreactivity of key proteins involved in eicosanoid metabolism (ie, cyclooxygenase [COX], phospholipase A2 [PLA₂], and Clara cell protein, a PLA₂ inhibitor) was determined by Western blotting.

Results: Eicosanoid output was largely confined to prostaglandins with values of 5 ± 2 and 82 ± 35 ng PGE₂/10⁶ cells for basal- and BK stimulation, respectively (n = 8). All 3 corticosteroids inhibited basal- and BK-induced PGE₂ output in a dose- and time-dependent manner. Fluticasone and budesonide completely eliminated PGE₂ output in nanomolar concentrations in contrast to triamcinolone, which required micromolar concentration. The rank order of potency was: fluticasone = budesonide > triamcinolone. The time course of action for PGE₂ inhibition also differed, with budesonide acting more slowly than the other 2 corticosteroids (P = .04). All 3 corticosteroids markedly reduced COX2 with little effect on COX1, cPLA₂ (Type IV), or iPLA₂ (Type VI) immunoreactivi-

ty or their relative distribution in cytosol versus membrane fractions. Clara cell protein immunoreactivity was undetectable in control and corticosteroid-treated cell lysates.

Conclusion: These results show that in a human airway epithelial cell line, the 3 inhaled corticosteroids commonly used to treat asthma differ in onsets of action as inhibitors of prostaglandin synthesis and vary considerably in potency. All 3 corticosteroids act mechanistically in similar fashion by inhibiting COX2 synthesis. (*J Allergy Clin Immunol* 1999;103:1081-91.)

Key words: Airway epithelium, corticosteroids, fluticasone, budesonide, triamcinolone, eicosanoids, prostaglandins, phospholipase A2, cyclooxygenase

Eicosanoid products of arachidonate metabolism appear to contribute significantly to the pathogenesis of asthma.^{1,2} Eicosanoids released into the airway affect the recruitment and activation of inflammatory cells, mucous secretion, bronchoconstriction, parasympathetic neurotransmission, and mucosal edema.^{1,2} Prostaglandin and leukotriene levels in bronchoalveolar lavage³⁻⁸ and prostaglandins released by epithelial cells⁹ from asthmatic subjects are increased when compared with normal subjects.

In the airways, epithelial cells are the first cells to encounter inhaled corticosteroids, mainstays in the treatment of asthma. However, little or nothing is known about the relative potencies and time courses of action of the several corticosteroid drugs currently in use to treat asthma on eicosanoid synthesis by human epithelial cells. Accordingly, this study compared the magnitude and time course of effects of 3 widely used corticosteroids, fluticasone, budesonide, and triamcinolone, on eicosanoid production by cultured human airway epithelial cells. The profile of eicosanoids was assessed by HPLC.

In the airways, corticosteroids produce complex, cell-type dependent effects on eicosanoid metabolism. For example, corticosteroids reduce production of thromboxane B₂ (TxB₂) in isolated macrophages^{8,10} but have no effect on TxB₂ production by isolated mast cells.¹¹ Yet few studies on airway epithelial cells have examined the effects of corticosteroids on the expression of phospholipase A2 (PLA₂) and cyclooxygenase (COX), the principal enzymes catalyzing eicosanoid synthesis.^{12,13} There-

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Supported by National Institutes of Health grant HL40295 and a CFF/NIH award from the Cystic Fibrosis Foundation.

Received for publication Nov 19, 1998; revised Jan 5, 1999; accepted for publication Jan 5, 1999.

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0091-6749/99 \$8.00 + 0 1/1/96952

Abbreviations used

AA:	Arachidonic acid
BK:	Bradykinin
CC10:	Clara cell 10-kd protein
COX:	Cyclooxygenase
DMSO:	Dimethyl sulfoxide
KHS:	Krebs-Henseleit solution
PLA ₂ :	Phospholipase A ₂
TBST:	Tris/saline/Tween-20 solution
TxB ₂ :	Thromboxane B ₂

fore corticosteroid effects on epithelial cell PLA₂ and COX protein expression were also examined in this study by using Western blotting.

METHODS

Cell culture

In these studies we used the R1 subtype of the BEAS-2B epithelial cell line (a gift of Dr Curtis Harris, National Cancer Institute), which is derived from SV40-transformed human bronchial epithelial cells.¹⁴ This BEAS-2B cell line has been used by us¹⁵ and others^{16,17} to study the regulation of eicosanoid metabolism in human airway epithelial cells. Cells were cultured in 100-mm flasks or in 6-well plates with RPMI-1640 medium plus 10% FBS, which was changed at 48-hour intervals. Cultures were grown at 37°C in 5% CO₂/95% air until confluent (10 to 14 days). Confluent cell monolayers were treated with corticosteroids and assayed for eicosanoid release by HPLC or RIA (see below). Cells were then harvested by exposure to trypsin/EDTA at 37°C for 7 minutes. Cell number was determined by Coulter counter or hemocytometer, and viability was determined by trypan blue exclusion. Cell numbers ranged from 5 to 10 × 10⁶/flask and 1 to 5 × 10⁶/well.

Protocols

Fluticasone (Glaxo, Research Triangle Park, NC), budesonide, and triamcinolone (Sigma, St Louis, Mo) were diluted in medium to final concentrations of 0.1 nmol/L to 1 μmol/L. In each experiment, cell cultures were incubated with medium plus corticosteroid or vehicle (dimethyl sulfoxide [DMSO]) control.

HPLC. The profile of eicosanoids released in response to bradykinin (BK) was measured in confluent cell cultures radiolabelled with tritiated arachidonic acid (AA) (Du Pont-NEN, Boston, Mass) as previously described.^{18,19} Over the last 24 hours of corticosteroid treatment, cell monolayers were immersed in medium containing 10% FBS, 0.1 mg/mL BSA, and tritiated AA (2 μCi/10⁶ cells). Under these conditions, approximately 90% of the radiolabelled AA is absorbed by the cells (Table I). Cells were then washed twice with 5 mL of Krebs-Henseleit solution (KHS) and incubated in 5 mL of KHS containing the corresponding corticosteroid (KHS-C) for 60 minutes. The cells were then sequentially exposed for 60 minutes to fresh KHS-C to measure basal eicosanoid output and then exposed to KHS-C plus 10 to 30 μmol/L BK. These steroid-containing and vehicle-control solutions were processed for HPLC, as previously described.^{18,19}

Reverse-phase HPLC was performed on a 1090M chromatograph (Hewlett Packard, Palo Alto, Calif) equipped with a diode array detector and a Dynamax 3-μm ODS column (4.6 mm × 100 mm) with guard cartridge (Rainin Instruments, Inc, Woburn, Mass). Eicosanoids were eluted by using a double gradient solvent system according to the method of Aksoy et al.¹⁸ This double gradient effectively resolves prostaglandins, mono-acids, dihydroxy-acids, and epoxy-acids, as well as leukotriene B₄ and isomers (Fig 1).

TABLE I. Effect of corticosteroid pretreatment on tritiated AA uptake in BEAS-2B cells

Treatment*	DMSO	FLU	BUD	TRI
Percent AA uptake over 24 hours	89.3 ± 1.3	90.1 ± 0.3	91.3 ± 0.6	87.6 ± 2.3

FLU, Fluticasone; BUD, budesonide; TRI, triamcinolone.

*BEAS-2B cell monolayers were pretreated with solvent vehicle DMSO or 1 μmol/L corticosteroid for 48 hours. Tritiated AA was present during the final 24-hour period. Data are means ± SE for 4 experiments.

Eicosanoid samples were dissolved in 35 μL of acetonitrile, and eluate from a 25-μL injection was collected as 30-second (0.5 mL) fractions over a 55-minute period and β-counted for tritium activity. The eicosanoid standards (Cayman Chemical Co, Ann Arbor, Mich) shown in Fig 1 underwent chromatography in series with each sample and monitored by UV light (Fig 1).

RIA. PGE₂ release was also measured by RIA in cells not labelled with tritiated AA for potency and time course studies. After corticosteroid exposure over a time range of 2 to 48 hours, BEAS-2B cells were treated with BK as described above. Sample solutions were then collected, centrifuged at 2500g for 15 minutes, and analyzed for PGE₂ by using a commercially available RIA kit (Du Pont-NEN, Boston, Mass) as described previously.¹⁸

Western blotting for immunoreactivity

Expression of PLA₂ isoforms cPLA₂ (Type IV) and iPLA₂ (Type VI), COX isoforms COX1 and COX2, and the PLA₂ inhibitor Clara cell 10-kd protein (CC10) were determined by Western blotting. Confluent BEAS-2B cells (5 to 10 × 10⁶ cells/dish) were dispersed in 100 to 300 μL of ice-cold cell lysis buffer (50 mmol/L HEPES [pH 8.0], 1 mmol/L EDTA, 1 mmol/L EGTA, 1 mmol/L dithiothreitol, 0.5 mmol/L phenylmethylsulfonyl fluoride, 50 μg/mL leupeptin, 10 μmol/L phosphoramidon, 10 μg/mL soybean trypsin inhibitor, and 100 μg/mL aprotinin). Lysates were centrifuged at 1000g for 5 minutes, and the resultant low-speed supernatant was then ultracentrifuged at 100,000g for 1 hour to yield cytosolic (supernatant) and membrane (pellet) fractions, which were frozen at -80°C until needed. Total cell fraction protein was determined by the Biorad DC microtiter plate assay (Biorad). Electrophoresis and transfer were then performed as previously described.²⁰

Specific, non-cross-reactive COX1 and COX2 antibodies were obtained from Oxford Biomedical Research Co. Antibodies for cPLA₂ (Type IV) and iPLA₂ (Type VI) were obtained from Cayman Chemical Co and used in conjunction with a second cPLA₂ antibody from Santa Cruz Biotechnology Corp. CC10 antibody and standard were gifts of Dr Gurmukh Singh (University of Pittsburgh) and Dr A. L. Pilon (Claragen, Inc, Bethesda, Md), respectively. All antibodies were incubated with the membrane for 1 hour in TBST (20 mmol/L Tris [pH 7.5], 500 mmol/L NaCl, 0.05% Tween 20) plus 5% nonfat dry milk at 25°C. After reaction with the primary antibody, membranes were washed 6 times with TBST and incubated at room temperature for 1 hour with a 1:25,000 dilution of goat anti-rabbit IgG conjugated to horseradish peroxidase (Pierce). Membranes were washed 6 times in TBST and visualized by enhanced chemiluminescence (SuperSignal kit, Pierce) on conventional x-ray film. Several different exposure times were used to ensure that images obtained were in the linear range of detection.

Data analysis

Group data are reported as the mean ± 1 SE and represent data from at least 3 experiments. Statistical significance of differences in

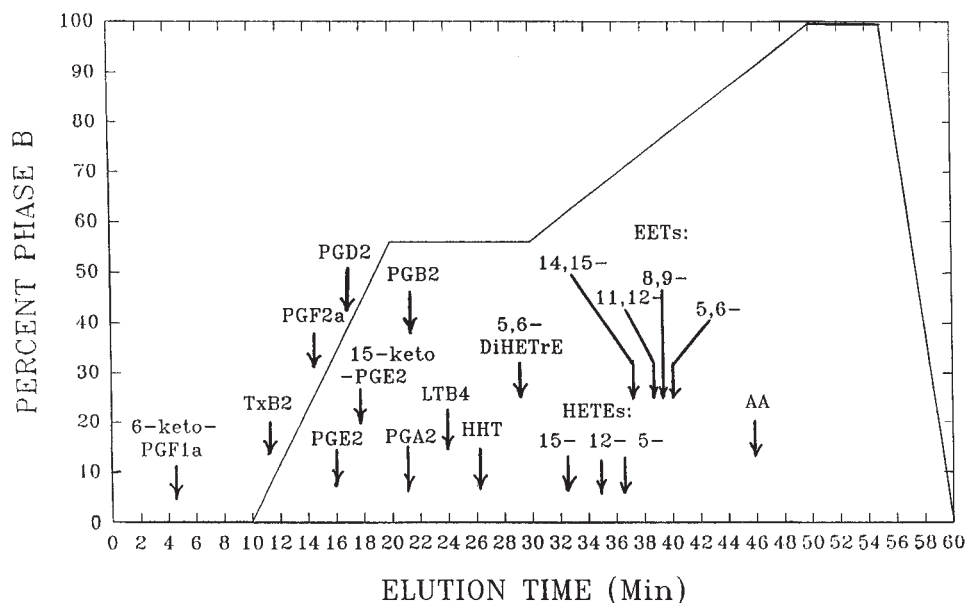


FIG 1. Reverse phase HPLC of eicosanoids. Elution times of eicosanoid standards along with the gradient time course plotted as percent phase B versus time are shown. *EET*, Epoxyeicosatrienoic acid; *HETE*, hydroxyeicosatetraenoic acid; *DiHETRe*, dihydroxyeicosatetraenoic acid.

sample means was analyzed by the 2-tailed Student's *t* test. Time-course data were assessed by repeated measures ANOVA, with the Tukey test as a post hoc multiple comparison procedure. In all cases statistical significance was accepted at *P* values of less than .05. Curve fitting of dose-response and time-course data was performed by linear regression with second- or third-order polynomial fits.

RESULTS

Effect of BK stimulation on eicosanoid release by BEAS-2B epithelial cells

BEAS-2B cells treated with DMSO solvent vehicle released PGE₂ basally in addition to arachidonate and trace amounts of the non-COX product, 5, 6-epoxyeicosatrienoic acid (EET) (Fig 2, *A*, dotted line). A 1-hour stimulation by 30 μmol/L BK markedly increased prostanoid output, yielding PGE₂ as the major product (Fig 2, *A*, solid line). PGE₂ output (as determined by RIA) increased from 5 ± 2 ng/10⁶ cells to 82 ± 35 ng/10⁶ cells (*n* = 8) as a result of BK stimulation. Smaller amounts of PGF_{2α}, PGA₂ (a metabolite of PGE₂), 12-hydroxyheptadecatrienoic acid, and 3 unidentified peaks that eluted at 22, 24, and 28 minutes were also released. Leukotriene B₄, TxB₂, PGD₂, and 6-keto-PGF_{1α} (derived from PGI₂) were not detected.

Effect of corticosteroid treatment on the profile of prostanoid release by BEAS-2B cells

Corticosteroid treatment (1 μmol/L for 48 hours) had no effect on tritiated AA uptake by BEAS-2B cells (Table I).

All 3 corticosteroids (fluticasone, budesonide, and triamcinolone) inhibited basal- and BK-stimulated prostanoid release in a dose-dependent fashion. In representative radiochromatograms (with total PGE₂ peak areas listed in

TABLE II. Effect of corticosteroid exposure (1 μmol/L) on total eluted radioactivity released by BK in cultured BEAS-2B cells

Treatment*	FLU	BUD	TRI
Total released cpm (% control)†	27 ± 5	22 ± 7	33 ± 12

FLU, Fluticasone; BUD, budesonide; TRI, triamcinolone.

*BEAS-2B cell monolayers were preincubated with tritiated AA plus DMSO vehicle or 1 μmol/L corticosteroid and then equilibrated in KHS and exposed to BK.

†For each radiochromatogram, cpm for all eluted fractions were summed to obtain the total eluted radioactivity released in the presence of BK. Total released cpm represents a combination of free tritiated AA and labelled eicosanoid products and is normalized to the DMSO control. Data are means ± SE for 5 experiments. Corticosteroid treatment versus control was significant (*P* < .05) for all 3 steroids.

the legend), 1 μmol/L corticosteroid inhibited basal- and BK-stimulated release of the identified COX metabolites (eg, >95% reduction of PGE₂) (Fig 2, *B* to *D*). At a lower concentration (10 nmol/L), inhibition of prostanoid release was less but still apparent (Fig 3, *A* to *C*).

All 3 corticosteroids also inhibited the total counts per minute (ie, the sum of radiolabelled arachidonate plus eicosanoid products) released by BK stimulation (Table II). Inhibition by all 3 corticosteroids at 1 μmol/L was significant (*P* < .05) and ranged from 67% for triamcinolone to 78% for budesonide.

Dose dependence of corticosteroid inhibition of PGE₂ release by BEAS-2B cells

At 1 and 0.1 μmol/L, all 3 corticosteroids significantly inhibited PGE₂ release (analyzed by RIA) to less than 10% of the control level (*P* < .05) (Fig 4). At 10 and 1

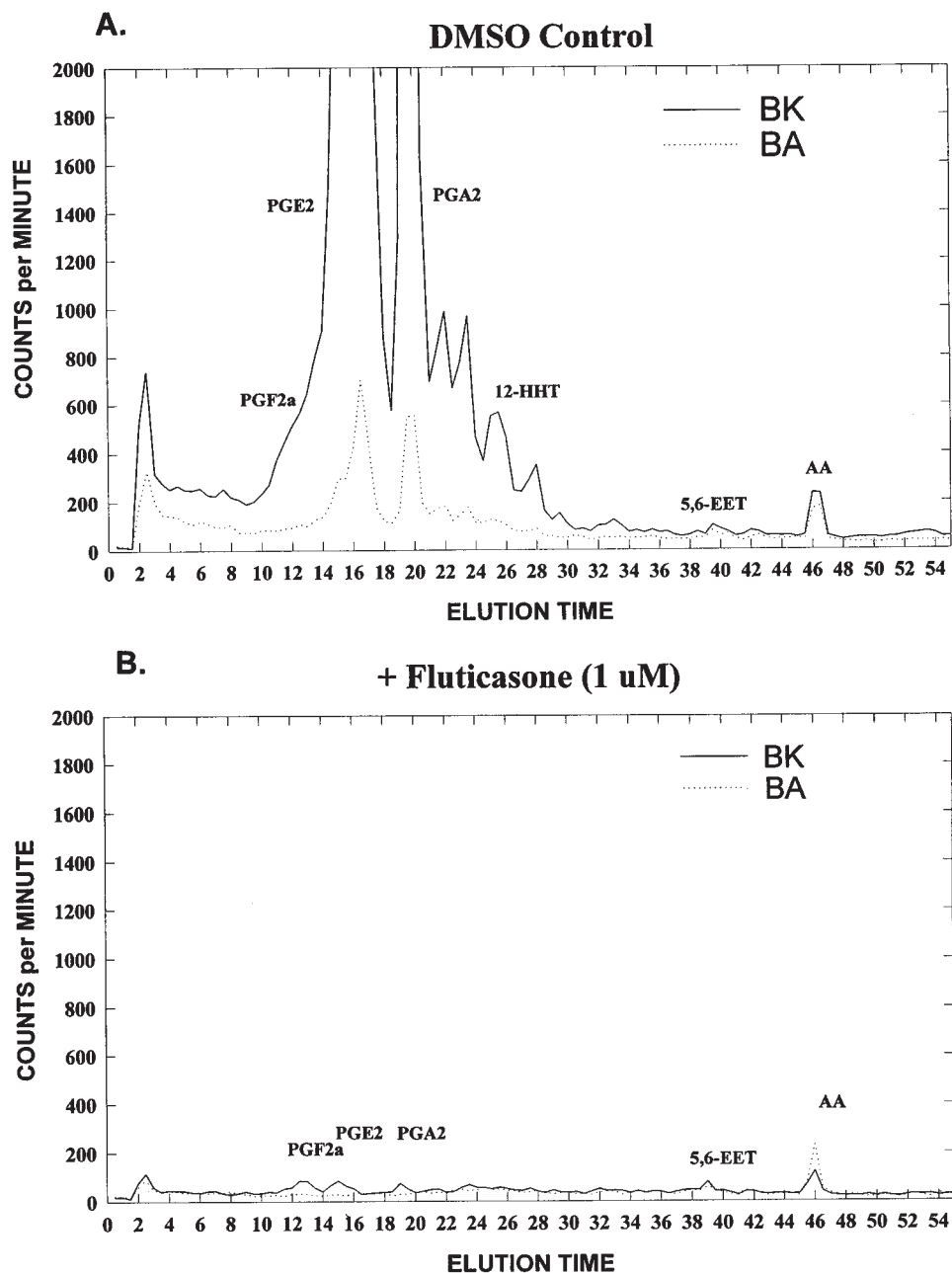


FIG 2. Effect of 1 μ mol/L corticosteroid exposure on eicosanoid release by BK in cultured BEAS-2B cells. Radiochromatograms show basal- (dotted line) and BK-evoked (solid line) eicosanoid profile for cultures pre-treated with DMSO vehicle (**A**); fluticasone (**B**); budesonide (**C**); and triamcinolone (**D**). Labels show elution times of eicosanoid standards. The total peak areas for PGE₂ in cpm are as follows: DMSO 32546 (**A**); fluticasone 223 (**B**); budesonide 125 (**C**); and triamcinolone 483 (**D**). Data of 1 experiment representative of 4, 5, 6-EET, 5,6-epoxyeicosatrienoic acid; 12-HHT, 12-hydroxyheptadecatrienoic acid.

nmol/L, fluticasone and budesonide inhibited PGE₂ release to less than 15% of the control level. In contrast, at 10 nmol/L triamcinolone inhibited PGE₂ release to only 54% $\pm$ 9% of the control level and had no effect at 1 nmol/L ($P > .1$). Fluticasone and budesonide continued to inhibit PGE₂ release at 0.1 nmol/L (to 64% and 74%

of the control level, respectively). These data yield the following rank order of potency for corticosteroid-induced inhibition of PGE₂ release: fluticasone = budesonide > triamcinolone. Median effective concentration values were 0.5 nmol/L for fluticasone and budesonide and 10 nmol/L for triamcinolone.

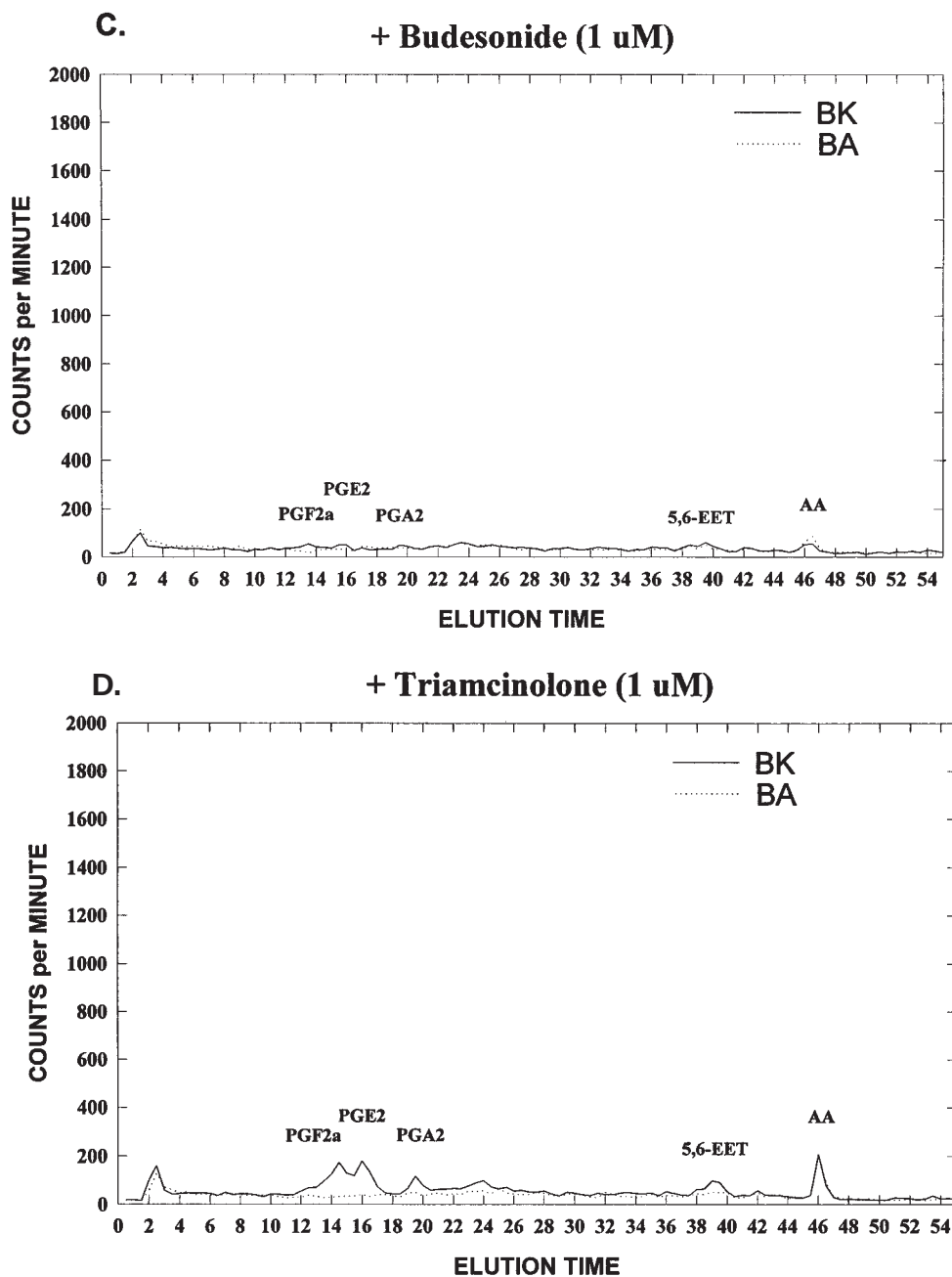


FIG 2. CONT.

Time course of corticosteroid inhibition of PGE₂ release by BEAS-2B cells

The time course of PGE₂ inhibition was examined for all 3 corticosteroids ($n = 9$ experiments). Over the 26-hour interval shown in Fig 5, inhibition was observed by 2 hours and was maximal at 14 hours. However, the time course of fluticasone (10 nmol/L) was significantly more rapid than that for budesonide (10 nmol/L) ($P = .043$). Triamcinolone given in a 10-fold greater concentration (100 nmol/L) to achieve a maximal effect similar to that

of fluticasone and budesonide showed a time course similar to that of fluticasone (data not shown).

Effect of corticosteroid treatment on COX, PLA₂, and CC10 protein synthesis by BEAS-2B cells

Control cells expressed primarily COX2 with little COX1 (Fig 6, A). COX2 immunoreactivity was concentrated in the membrane fraction. Fluticasone (10 nmol/L), budesonide (10 nmol/L), or triamcinolone (100

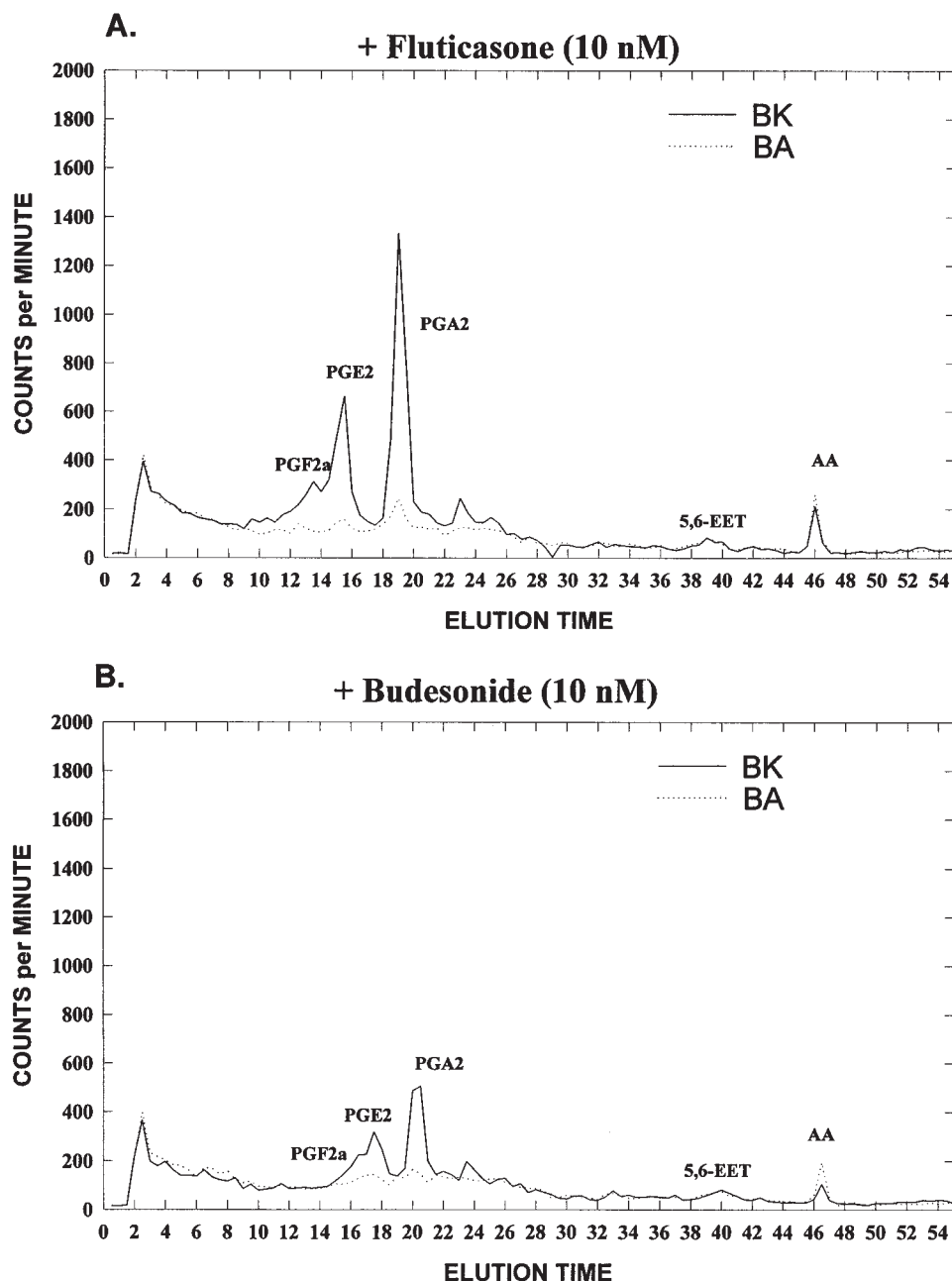


FIG 3. Effect of 10 nmol/L corticosteroid exposure on eicosanoid release by BK in cultured BEAS-2B cells. Radiochromatograms show basal- (dotted line) and BK-evoked (solid line) eicosanoid profile for cultures pre-treated with fluticasone (**A**); budesonide (**B**); and triamcinolone (**C**). The peak areas for PGE₂ in cpm are as follows: fluticasone 2321 (**A**); budesonide 1005 (**B**); and triamcinolone 14646 (**C**). Data of 1 experiment representative of 4. 5, 6-EET, 5,6-epoxyeicosatrienoic acid; 12-HHT, 12-hydroxyheptadecatrienoic acid.

nmol/L) administered for 48 hours completely eliminated COX2 immunoreactivity (Fig 6, C). In contrast, COX1 immunoreactivity was not decreased (Fig 6, B).

cPLA₂ immunoreactivity was concentrated in the cytosol. In 3 experiments on separate cultures, fluticasone (10 nmol/L) had no consistent effect on cPLA₂ immunoreactivity or its partition between cytosolic and

membrane fractions (Fig 7, A). Immunoreactivity for iPLA₂ was also detected, but in contrast to cPLA₂ and COX2 it was equally distributed between the membrane and cytoplasm. Like cPLA₂, iPLA₂ showed no consistent change with corticosteroids (Fig 7, B).

No CC10 immunoreactivity was detected in cytosolic or membrane fractions from control or fluticasone-treat-

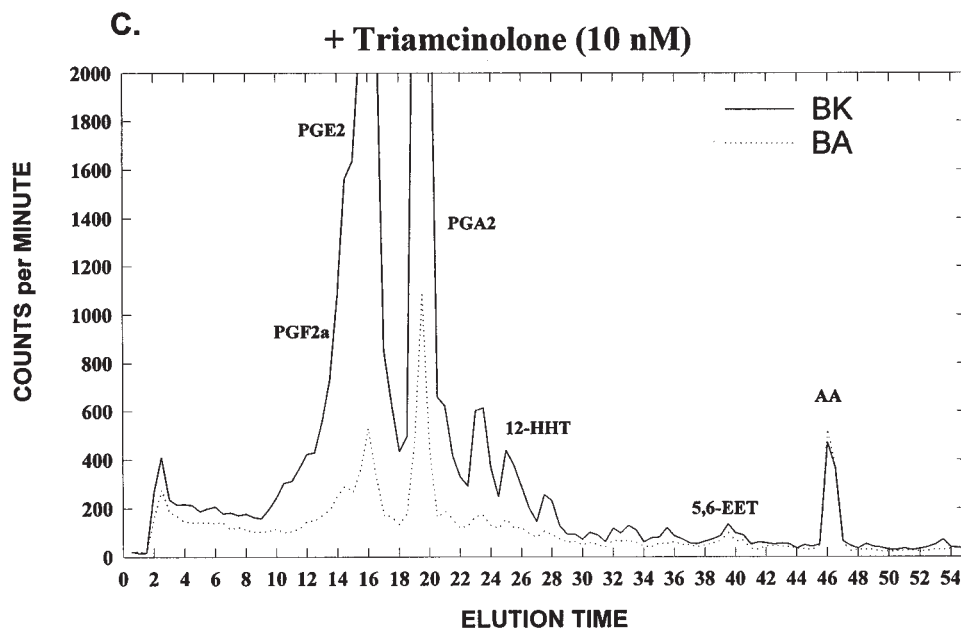


FIG 3. CONT.

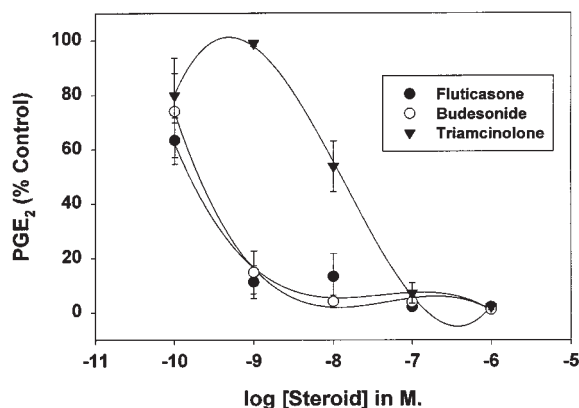


FIG 4. Concentration response relationship for corticosteroid inhibition of PGE_2 release from BK-stimulated BEAS-2B cells. Data shown are means $\pm$ SE of released PGE_2 concentration measured by RIA and are expressed as a percent of the DMSO vehicle control ($n = 8$ experiments). Lines represent linear regression best fit with a third-order polynomial.

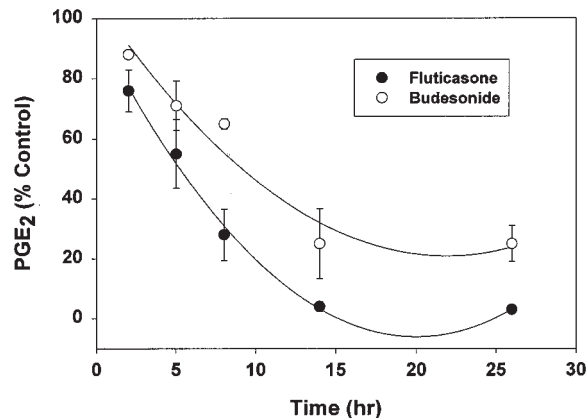


FIG 5. Time course for corticosteroid inhibition of PGE_2 release from BK-stimulated BEAS-2B cells. Confluent BEAS-2B cells were pretreated with fluticasone (10 nmol/L) or budesonide (10 nmol/L) over a time range of 2 to 26 hours and then assayed for PGE_2 release in response to BK as described in the Methods section. Data are expressed as a percent of the DMSO vehicle control ($n = 9$ experiments) and are calculated as means $\pm$ SE. Lines represent linear regression best fit with a second order polynomial.

ed BEAS-2B cells with up to 70 μ g of protein loaded in each lane.

DISCUSSION

In this study BK stimulation elicited a pronounced increase in the COX products PGE_2 and PGA_2 (a breakdown product of PGE_2) and a smaller release of 12-hydroxyheptadecatrienoic acid and $PGF_{2\alpha}$, as well as free arachidonate. Accordingly, under the conditions of our experiment, eicosanoid production depended on the activity of both PLA_2 , which releases AA from membrane phospholipids,²¹ and COX, which converts AA to the PGG_2/PGH_2 intermediates necessary for PG synthe-

sis.²² In fact, the BEAS-2B system is a suitable model to study corticosteroid effects on airway prostanoid metabolism because the prostanoid profile in response to BK is similar to that elicited in primary cultured human airway epithelial cells.^{23,24} However, the applicability of our findings to eicosanoid metabolism of human airway epithelial cells in vivo requires further study.

To our knowledge, this study is the first to compare the relative potency of several corticosteroids commonly used in the treatment of asthma on eicosanoid production in human airways epithelial cells. All 3 corticosteroids

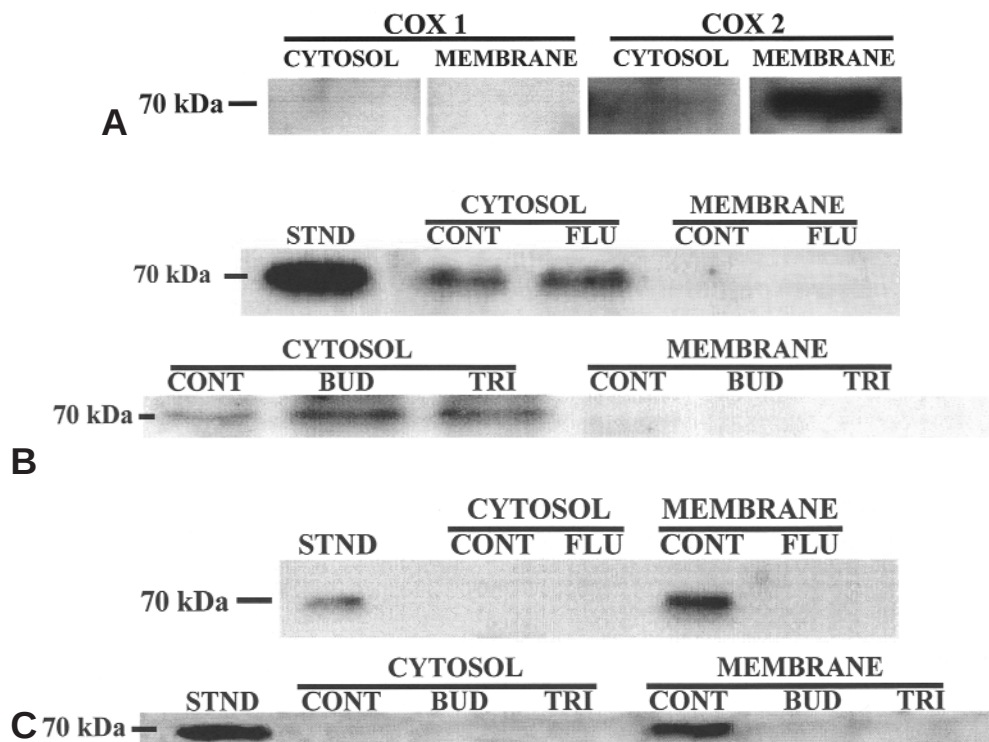


FIG 6. Effect of corticosteroid treatment on COX expression in BEAS-2B cells. **A** shows COX1 and COX2 immunoblots from the same lysate processed under identical conditions except that the anti-COX1 concentration was 10 times greater than that for anti-COX2. COX2 immunoreactivity markedly exceeded that for COX1. **B** and **C** are immunoblots showing COX1 (**B**) and COX2 (**C**) immunoreactivity in control- and corticosteroid-treated lysates. COX1 immunoblots are enlarged, and exposure time is increased relative to COX2 to facilitate visualization of COX1 levels. The predominant isoform, COX2, was concentrated in the membrane fraction and was markedly reduced by all 3 steroids. *STND*, Standard; *CONT*, control; *FLU*, fluticasone; *BUD*, budesonide; *TRI*, triamcinolone.

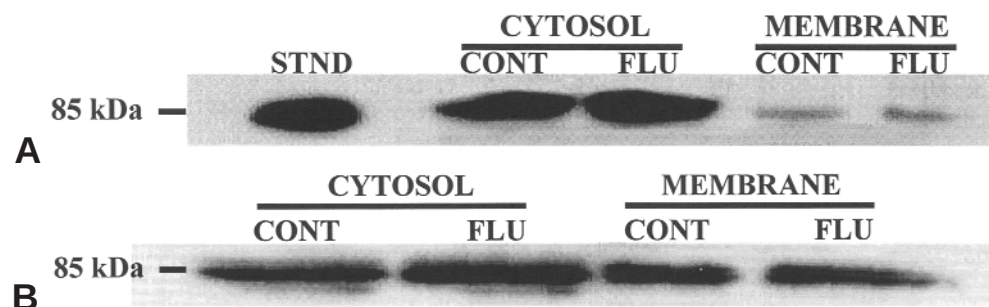


FIG 7. Effect of corticosteroid treatment on PLA₂ expression in BEAS-2B cells. Immunoblots showing cPLA2 (**A**) and iPLA2 (**B**) immunoreactivity in control- and fluticasone-treated BEAS-2B cell lysates. Immunoreactivity of cPLA2 and iPLA2 isoforms was not significantly reduced by 10 nmol/L fluticasone. *STND*, Standard; *CONT*, control; *FLU*, fluticasone.

inhibited basal- and BK-evoked release of PGE₂ and other prostanoids (PGA₂ and PGF_{2α}). Inhibition of prostaglandin production was dose dependent. At the highest concentration used (1 μmol/L), all 3 corticosteroids almost completely eliminated basal- and BK-stimulated prostaglandin output. At lower concentrations, the effectiveness of the 3 corticosteroids differed.

Fluticasone and budesonide suppressed prostaglandin output at concentrations as low as 1 nmol/L. In contrast, triamcinolone had no effect at 1 nmol/L. The rank order of potency for corticosteroid inhibition of PGE₂ synthesis in cultured human airway epithelial cells was as follows: fluticasone = budesonide > triamcinolone. This potency order generally correlates with the ranking for

glucocorticoid receptor-binding affinity (ie, fluticasone > budesonide > triamcinolone).²⁵ For all 3 corticosteroids, the time course of action showed a rapid onset of inhibition (within 2 hours), with a maximum effect attained by 14 hours. Of interest, the time course for fluticasone was significantly faster than that of an equimolar concentration of budesonide. Differences between the time courses of fluticasone and budesonide may reflect differences in lipophilicity, which influence cellular uptake. Fluticasone has been shown to be considerably more lipophilic than budesonide.²⁵

Potential mechanism(s) of corticosteroid action in human airway epithelial cells

In our experiments corticosteroid treatment markedly reduced the total tritiated counts released (Table II). At 1 $\mu\text{mol/L}$, this decrease ranged from 67% to 78%. A reduction in total counts could be explained by a corticosteroid-induced decrease in labelled arachidonate incorporation or in subsequent release of labelled arachidonate and eicosanoid products from membrane phospholipids. In our experiments none of the 3 corticosteroids significantly affected the rate or extent of tritiated AA uptake (Table I). Consequently, the reduction in total counts strongly suggests a decrease in net arachidonate/eicosanoid release.

In epithelial^{26,27} and other^{28,29} cell systems, corticosteroids have been shown to inhibit the cytosolic form of PLA_2 . Epithelial cells,^{26,27} including the BEAS-2B cell line,^{16,30} express the ubiquitous 85-kd, Ca^{2+} -sensitive cPLA_2 (Type IV). A similar 85-kd PLA_2 isoform, termed iPLA_2 (or Type VI PLA_2), which occurs as a homotrimer with ankyrin repeats, is found in macrophages.^{21,31,32} In our studies immunoreactivity for both cPLA_2 and iPLA_2 was detected in BEAS-2B cells. However, 10 nmol/L fluticasone did not decrease cPLA_2 or iPLA_2 expression. Therefore at this concentration corticosteroids do not appear to inhibit PGE_2 release by inhibiting PLA_2 expression.

In its native form, CC10 is a potent inhibitor of PLA_2 ³³ and can be upregulated in the rat and rabbit by corticosteroids.³⁴⁻³⁶ To determine whether changes in CC10 expression could explain corticosteroid inhibition of eicosanoid synthesis, we assessed CC10 expression. In our studies CC10 could not be detected in BEAS-2B cell lysates despite loading as much as 70 μg of protein. Failure to detect CC10 immunoreactivity argues against this pathway as a mechanism for the effects of corticosteroid observed in this study. Of note, our studies were performed on unconcentrated, unfiltered whole cell lysates because our interest was in CC10 acting intracellularly in an autocrine fashion. A recent study did detect CC10 release into the medium by BEAS-2B cells when using filtered and 200-fold-concentrated medium.³⁷ Nonetheless, because we did not measure PLA_2 activity, we cannot rule out the possibility that corticosteroid treatment decreased PLA_2 activity without affecting expression. In fact, PLA_2 undergoes extensive posttranslational modification, which affects its activity.²¹

Most cell types express 2 distinct isoforms of COX: a constitutive COX (ie, COX1) and a COX induced by inflammatory mediators, such as LPS, phorbol esters, and cytokines (ie, COX2).^{22,38,39} Walenga et al¹² and Asano et al⁴⁰ report that human airway epithelial cells (eg, primary cells, 16HBE40-) and alveolar epithelial (A549) cells depart from this scheme by constitutively expressing COX2 but little or no COX1. Our results agree with these findings. In BEAS-2B cells, COX2 was the predominant constitutive isoform, suggesting that BK-evoked prostaglandin synthesis is primarily catalyzed by COX2.

Corticosteroid treatment markedly suppressed COX2 expression without affecting COX1 expression. This effect was produced by all 3 corticosteroids, even at a concentration of 10 nmol/L. The profound corticosteroid inhibition of COX2 likely explains the decrease in the prostanoid output. Moreover, because prostanoid release comprised approximately 80% of the total released counts, the 95% inhibition of prostanoid release by corticosteroids (Figs 2 and 4) could explain the 70% to 80% reduction in total radioactivity (Table II).

It could be argued that inhibition of COX expression without an effect on PLA_2 would shunt arachidonate into lipoxygenase or epoxidase pathways or increase the amount of free arachidonate in the medium. However, in medium from BK-stimulated BEAS-2B cells, lipoxygenase and epoxidase products were scant or undetectable, and arachidonate levels were slight and unaffected either by BK or corticosteroids. To account for this, we surmise that much of the "unused," free arachidonate produced in corticosteroid-treated cells may have been retained or reincorporated into the cell membrane and that lipoxygenase/epoxidase activity in BEAS-2B cells is negligible.

Our data showing inhibition of COX2 expression by corticosteroids are in agreement with the results presented by Mitchell et al.¹³ Mitchell et al demonstrated that dexamethasone (1 $\mu\text{mol/L}$) pretreatment inhibited constitutive and cytokine-stimulated COX2 protein expression in primary cultures of human airway epithelial cells. In contrast, our data are at variance with the results presented by Walenga et al¹² in the same cell types. Walenga et al reported that dexamethasone (10 $\mu\text{mol/L}$) did not affect constitutive or phorbol ester-stimulated increases in COX2 mRNA or COX activity (assessed by using radiolabeled arachidonate) in primary cultures of human airway epithelial cells. The explanation for the differing results obtained by Mitchell et al and ourselves compared with those of Walenga et al are unclear.

The clinical consequences of corticosteroid inhibition of prostaglandin release by human airway epithelial cells are of interest but can only be speculated on because PGE_2 has both deleterious and beneficial effects on airway function and inflammation. PGE_2 increases airway irritant receptor activity, tachykinin release, and capsaicin-induced cough in human subjects.⁴¹ However, PGE_2 also dilates airway smooth muscle,⁴² inhibits the release of acetylcholine from parasympathetic motor endplates,⁴³ inhibits mucus secretion,⁴⁴ inhibits the release of proinflammatory cytokines from activated inflammatory

cells,⁴⁵ and, by increasing intracellular cAMP levels, stimulates NO production from airway epithelial cells.⁴⁶ The expression of COX2 and subsequent release of PGE₂ by airway epithelial cells may represent a protective effect against airway inflammation and airway hyperreactivity. However, the proinflammatory effects of PGE₂ released by cells in the airway other than epithelial cells may outweigh any potential beneficial effects of PGE₂ released by epithelial cells. If so, then the inhibitory effects of corticosteroids on COX2 expression and PGE₂ release exert favorable effects on airway function.

In conclusion, this study indicates that prostaglandin output from cultured airway epithelial cells is reduced in dose- and time-dependent fashion by 3 clinically used corticosteroids. The steroids exhibit similar, but not identical, time courses and vary in potency, with fluticasone and budesonide more potent than triamcinolone. An important site of inhibitory action appears to be COX2.

We wish to recognize the expert technical assistance of Ms Carla Tyler in cell culture and Mr Remus Baretta in HPLC analysis.

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