**Objectives:** Tissue remodeling often accompanies diseases such as COPD that are caused by or aggravated by tobacco exposure. Inhaled or systemic corticosteroids are frequently used for the treatment of these illnesses, and their beneficial effects are often ascribed to their anti-inflammatory properties. However, their role in tissue remodeling remains unclear. This study was designed to evaluate the role of corticosteroids in matrix expression in vitro.

**Design:** We investigated the effects of the corticosteroid fluticasone propionate (FP) on the production of fibronectin by fibroblasts before and after stimulation by nicotine, a plant alkaloid found in tobacco. Fibronectin is an extracellular matrix glycoprotein found elevated in the alveolar lining fluid and airway walls of subjects with obstructive lung disease, and is considered a marker of tissue remodeling after injury.

**Results:** FP, 1 μmol/L, inhibited the expression of fibronectin messenger RNA and protein in unstimulated NIH-3T3 cells and primary lung fibroblasts, as well as in fibroblasts stimulated with nicotine. The inhibitory effect of FP occurred at the level of gene transcription as demonstrated in lung fibroblasts expressing a construct containing the human fibronectin promoter connected to a luciferase reporter gene, but posttranscriptional effects also appeared involved. Electrophoresis mobility gel shift assays revealed that FP inhibited phosphorylation and DNA binding by the cyclic adenosine monophosphate response element binding protein, a transcription factor required for constitutive and nicotine-induced fibronectin expression.

**Conclusions:** Together, these data suggest that FP could diminish lung tissue remodeling by inhibiting the production of fibronectin in lung fibroblasts. *(CHEST 2005; 127:257–265)*

**Key words:** cyclic adenosine monophosphate response element binding protein; fibroblasts; fibronectin; gene transcription; matrix; nicotine; remodeling

**Abbreviations:** AP = activator protein; bp = base-pair; cAMP = cyclic adenosine monophosphate; CREB = cyclic adenosine monophosphate response element binding protein; DMEM = Dulbecco modified Eagle medium; EDTA = ethylenediamine tetra-acetic acid; FBS = fetal bovine serum; FP = fluticasone propionate; GAPDH = glyceraldehyde 3-phosphate dehydrogenase; MMP = matrix metalloproteinase; mRNA = messenger RNA; PBS = phosphate-buffered saline solution; PCR = polymerase chain reaction; SDS = sodium dodecyl sulfate

**Tobacco** is an important etiologic factor in the development of COPD, and is considered an aggravating factor in a number of interstitial lung diseases. In general, these pulmonary disorders are characterized by inflammation and the activation of tissue remodeling that result in structural alterations such as airway wall thickening and the destruction of alveolar septae. Corticosteroids are commonly used in the treatment of these disorders, and their beneficial effects are often ascribed to their anti-inflammatory properties. More recently, attention has been given to the potential effects of corticosteroids on tissue remodeling. For example, inhalation of the corticosteroid fluticasone propionate (FP) was found to partially inhibit airway wall thickening and matrix

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deposition in a rat model of allergen-induced airway remodeling, and limited the progression of structural airway changes in this model. These studies suggest that corticosteroids might ameliorate progressive tissue remodeling in diseases characterized by airflow obstruction, but the mechanisms responsible for this effect remain unelucidated. We speculate that corticosteroids might inhibit lung tissue remodeling by directly modulating the expression of extracellular matrix genes in lung fibroblasts.

In an attempt to elucidate the effects of corticosteroids on extracellular matrix gene expression, we studied the expression of fibronectin in fibroblasts treated with FP. Fibronectin is an extracellular matrix glycoprotein that is highly expressed in acute and chronic forms of lung injury including COPD, asthma, and many interstitial lung diseases. Villiger et al demonstrated an increase in the production of fibronectin in alveolar macrophages obtained from smokers when compared to nonsmokers. Alveolar macrophages from patients with chronic bronchitis spontaneously released greater amounts of fibronectin than those from asthmatic patients and control subjects. The levels of fibronectin have been correlated with a decrease in pulmonary function in smokers with COPD. In asthma, airway wall remodeling is not only associated with increased deposition of extracellular matrices, but also with alterations in the composition of the airway wall connective tissue with increased deposition of fibronectin. The aforementioned studies have implicated excessive production of fibronectin as a marker of activation of tissue remodeling and a potential mechanism for promoting fibroproliferation in tobacco-related lung disease.

Herein, we present evidence that suggests that FP can modulate lung tissue remodeling by inhibiting the expression of the fibronectin gene in fibroblasts at baseline and after treatment with nicotine. This effect is related to the ability of FP to inhibit DNA binding by cyclic adenosine monophosphate response element binding protein (CREB), a transcription factor necessary for stimulation of fibronectin gene transcription.

Materials and Methods

Reagents

Nicotine, antifibronectin (F-3648) antibody, goat antirabbit horseradish peroxidase (A-9169) antibody, and albuterol were purchased from Sigma Chemicals (St. Louis, MO). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody was purchased from Abcam, (Cambridge, MA). CREB and phosphorylated CREB (Ser133) antibodies were purchased from Cell Signaling Technologies (Beverly, MA). FP was obtained from Glaxo Group Research (Greenford, Middlesex, UK). Real-time polymerase chain reaction (PCR) primers and probes were synthesized by Epoch Biosciences (Bothell, WA).

Cell Culture and Treatment

Murine NIH-3T3 fibroblasts (CRL No. 1658; American Type Culture Collection; Manassas, VA) were cultured in Dulbecco modified Eagle medium (DMEM) with 4.5 g/L of glucose supplemented with 10% heat inactivated fetal bovine serum (FBS), 1% antibiotic-antimycotic solution (100 U/mL penicillin G sodium, 100 U/mL streptomycin, 0.25 μg/mL amphotericin B), and incubated in a humidified 5% CO2 incubator at 37°C. The cells were harvested by trypsinization with 2.5 × trypsin (Sigma Chemicals), washed twice with phosphate buffered saline solution (PBS) [137 mmol/L NaCl, 2.7 mmol/L KCl, 4.3 mmol/L Na2HPO4, 1.47 mmol/L KH2PO4, pH to 7.4], counted, and plated at 1.5 × 105 cells per well into six-well tissue culture dishes.

Primary lung fibroblasts were harvested from transgenic mice expressing the human fibronectin promoter connected to a luciferase reporter gene, pFN(1.2kb)/LUC. The characterization of these animals indicates that the expression in the lung of the transfected gene is similar to that of the endogenous fibronectin gene (unpublished observations). Primary lung fibroblasts were obtained from mouse lungs by discarding the outer 3 mm of lung periphery and cutting the remaining lung parenchymal tissue into 1-mm sections. Tissue sections were washed twice in sterile PBS, resuspended in DMEM with 4.5 g/L glucose, 10% FBS, 1% antibiotic-antimycotic solution (100 U/mL penicillin G sodium, 100 U/mL streptomycin, 0.25 μg/mL amphotericin B), transferred to a tissue culture dish, and incubated in a humidified 5% CO2 incubator at 37°C for 1 to 3 weeks to allow fibroblasts to migrate out of tissue sections. Primary lung fibroblasts were between three passages and five passages when used in experiments.

Experimental treatments include nicotine (50 μg/mL), fluticasone (0.1 to 50 μg/mL), or albuterol (10 μg/mL), or the combination of these agents. The doses of experimental agents were chosen based on previous experiments. Cell viability was determined by Trypan blue exclusion. No alterations in cell viability were noted under the conditions and treatments described. Also, gross evaluation of cell attachment and appearance by light microscopy did not reveal alterations with the treatments described.

Western Blotting for Fibronectin

Fibroblasts (1.5 × 105/mL) were pretreated with or without FP for 1 h prior to incubation in the presence or absence of nicotine for 48 h, washed with ice-cold PBS, and lysed in 1 mL of homogenization buffer (50 mmol/L NaCl, 50 mmol/L NaF, 50 mmol/L Na2P04·10 H2O, 5 mmol/L ethylenediamine tetra-acetic acid [EDTA], 5 mM ethyleneglycol tetra-acetic acid, 2 mmol/L Na2, sodium [ortho] vanadate, 0.5 mmol/L phenylmethylsulfonyl fluoride, 0.01% Triton X-100, 10 μg/mL leupeptin, 10 mmol/L hydroxethyl piperazine-ethanesulfonic acid, pH 7.4). Pretreatment with FP for 1 h prior to nicotine exposure was chosen because preliminary experiments revealed that concurrent treatment with FP showed inefficient inhibition. The resulting homogenate was centrifuged at 14,000 revolutions per minute for 5 min at 4°C. Protein concentration was determined by the Bradford method. The protein (100 μg) was mixed with an equal volume of 2 × sample buffer (125 mmol/L Tris HCl, pH 6.8, 4% sodium dodecylsulfate [SDS], 20% glycerol, 5 to 10% β-mercaptoethanol, 0.004% bromophenol blue), boiled for 5
min, loaded onto a 5% SDS-polyacrylamide gel (10% SDS-polyacrylamide gel for CREB and phosphorylated CREB) with a 3.5% stacking gel, and electrophoresed for 2 h at 60 mA. The separated proteins were transferred onto nitrocellulose using a BioRad Trans Blot semidy transfer apparatus for 2 h at 1.0 mA/cm², blocked with blotto (1 × Tris-buffered saline solution [10 mmol/L Tris-HCl, pH 8.0, 150 mmol/L NaCl], 5% nonfat dry milk, 0.05% Tween-20) for 1 h at room temperature, and washed twice for 5 min with wash buffer (1 × Tris-buffered saline solution, 0.05% Tween-20). Blots were incubated with a polyclonal antibody raised against human fibronectin (1:1000 dilution), human CREB (1:1000 dilution), or human phosphorylated CREB (Ser133) [1:1,000 dilution] for 24 h at 4°C, washed three times for 5 min with wash buffer, and incubated with a secondary rabbit antibody raised against goat IgG conjugated to horseradish peroxidase (1:15,000 dilution) for 1 h at room temperature. Identified loaded blots used for loading controls were incubated with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) [1:2000 dilution] primary antibody. The blots were washed four times in wash buffer, transferred to freshly made ECL solution (Amersham; Arlington, IL) for 1 min, and exposed to radiograph film.

Protein bands were quantified by densitometric scanning using a GS-800 calibrated laser densitometer (Bio-Rad; Hercules, CA). Western blots were repeated at least four times using samples from four separate experiments.

Detection of mRNAs by Real-time PCR

Total cellular RNA was extracted from NIH-3T3 fibroblasts (1.5 × 10⁶/mL) pretreated with FP (1 μmol/L) for 1 h prior to nicotine (50 μg/mL) treatment for 24 h using a published method¹⁴,¹⁵ and modified as we previously described.¹⁶ The reverse transcription reactions of the extracted RNA were performed by reverse transcription of the extracted RNA was performed by reverse transcription using a Superscript II Reagent System (Invitrogen). One microgram of total RNA was reverse-transcribed to first-strand cDNA in a 20-μL reaction containing 5 mmol/L MgCl₂, 0.2 μg/μL oligo(dT)₁₇, 1 μg/μL RNasin, 0.5 mmol/L dithiothreitol, 200 U RNAsin, 200 U RNase inhibitor (Rnasin; Promega; Madison, WI), and 1 μg extracted RNA in a total volume of 25 μL. Samples were heated to 70°C for 5 min, chilled on ice for 5 min, centrifuged briefly, incubated at 42°C for 1 h followed by a 10-min incubation at 90°C, and chilled on ice for 5 min.

Real-time PCR reactions were set up by adding the following reagents to Smart Cycler Reaction Tubes (Cepheid; Sunnyvale, CA): 5 mmol/L MgCl₂, 0.2 μmol/L forward primer, 0.2 μmol/L reverse primer, 0.2 μmol/L probe (3’ FAM Amidine), 1× platinum quantitative PCR (SuperMix-UDG; Invitrogen), template complementary DNA (500 ng total). Forward, reverse, and probe primers for human fibronectin messenger RNA (mRNA) were 5’ GGAGAGAGGGAAAAAGGAGA, 5’ ACCAAATGGCCGACGAGA CAGGATG, and 5’ AGGAGAGTCGCGGAGA, respectively. Forward, reverse, and probe primers for human β-actin mRNA were 5’ CGGAGATGCCTGACGTCAGG ACGTAG, and 5’ TATGGGGT ACTTCAGGGT, respectively. Negative controls consisted of distilled H₂O in place of template complementary DNA, RNA without reverse transcriptase-PCR products, and standardization was made to the housekeeping gene β-actin. Samples were briefly centrifuged and processed using the following cycle program using the Smart Cycler (Cepheid): hold at 95°C for 120 s followed by 40 cycles at temperatures of 95°C for 15 s, 60°C (50°C for β-actin) for 30 s, and 72°C for 30 s. Results of the log-linear phase of the growth curve were analyzed by use of the mathematical equation of the second derivative, and relative quantification was performed using the 2⁻ΔΔCT method.¹⁷ All products were verified by agarose gel electrophoresis to ensure that the predicted mRNA species was being examined.

Examination of Fibronectin Gene Transcription

To evaluate for fibronectin gene transcription, the pFN(1.2kb)LUC promoter construct was introduced into murine NIH-3T3 fibroblasts via electroporation to create stable transfectants as previously described.¹₂ In addition, primary lung fibroblasts harvested from transgenic mice expressing pFN(1.2kb)LUC were tested. pFN(1.2kb)LUC contains approximately 1.20 base-pairs (bp) of the 5’ flanking region of the human fibronectin gene isolated from the human fibrosarcoma cell line HT1080.¹₆ This construct also contains several previously identified regulatory elements such as three cyclic adenosine monophosphate (cAMP) response elements located at –415 bp, –270 bp, and –170 bp, and a signal protein-1 site at –102 bp from the transcription start site.

The NIH-3T3 fibroblasts were harvested by trypsinization with 2.5 × trypsin and 5.3 mmol/L EDTA (Sigma Chemicals), washed with PBS, counted, and plated at 1.5 × 10⁷/mL in 12-well tissue culture dishes in 10% FBS. Fibroblasts were pretreated with or without FP or albuterol for 1 h prior to incubation with or without nicotine for 16 h. An incubation time of 16 h was chosen based on preliminary time-course experiments that showed maximal nicotine-induced fibronectin gene transcription at that time (not shown). Afterwards, the cells were tested for luciferase activity. For this, the cells were harvested by cell scraper, washed with PBS, resuspended in 100 μL of cell lysis buffer (Promega), sonicated, and a 10-μL aliquot was tested by adding 50 μL of luciferase assay reagent (Promega). Light intensity was measured using a Luminoskan Ascent Plate luminometer (Labsystems; Helsinki, Finland). Results were recorded as normalized luciferase units and adjusted for total protein content that was measured using the Bradford method.¹³

Electrophoretic Mobility Shift Assay

NIH-3T3 fibroblasts (3 × 10⁶/mL) were seeded onto 150-mm² tissue culture flasks and in DMEM with 10% heat inactivated FBS, and incubated the presence or absence of FP or albuterol for 1 h prior to treatment with or without nicotine in for 16 h in a humidified 5% CO₂ incubator at 37°C. Cells were washed with ice-cold PBS, and nuclear binding proteins were extracted by a published method.¹⁹ Protein concentration was determined by the Bradford method using BioRad protein assay reagent. Double-stranded cAMP-responsive element binding protein consensus oligonucleotide (5’ AGAGATTGCTGACGTCAGG ACGTAG) was labeled with ³²P gamma adenosine triphosphate using T4 polynucleotide kinase enzyme. Nuclear protein (5 μg) was incubated with ³²P-labeled CREB for 30 min at room temperature as described previously.²⁰ DNA-protein complexes were separated on 6% native polyacrylamide gel (20:1 acrylamide/bis ratio) in low-ionic-strength buffer (22.25 mmol/L Tris borate, 22.25 mmol/L boric acid, 500 mmol/L EDTA) for 2 to 3 h at 4°C at 10 V/cm. Gels were fixed in a 10% acetic acid/10% methanol solution for 10 min, dried under vacuum, and exposed to radiograph film. All samples were evaluated and cleared for presence of lipopolysaccharide.²¹

Statistical Analysis

Reverse transcriptase-PCR data were analyzed using the 2⁻ΔΔCT method.²⁷ Gene transcription data were analyzed by
Student t test method. Each case data from individual groups were compared to the control group. All experiments were repeated between four times and eight times.

RESULTS

FP Inhibits Fibronectin Expression in Fibroblasts

Like other fibroblasts, cultured NIH-3T3 fibroblasts produce fibronectin in a constitutive fashion, and we have previously demonstrated that nicotine greatly enhances fibronectin expression in these cells.22 To examine the effects of FP on fibronectin protein expression, NIH-3T3 fibroblasts were pretreated in the presence or absence of FP for 1 h, cultured with or without nicotine (50 μg/mL) for 48 h, followed by harvesting and processing for western blotting. As demonstrated in Figure 1, left, A, untreated fibroblasts produced fibronectin protein, and this was greatly enhanced by treatment with nicotine. FP pretreatment inhibited the production of fibronectin protein in both untreated and nicotine-stimulated fibroblasts. In addition, nicotine also stimulated an increase in fibronectin mRNA of > 2.5-fold (p < 0.03) as compared to untreated control cells as demonstrated in Figure 1, right, B. Preincubation of fibroblasts for 1 h with FP was associated with a significant reduction (p < 0.04) in fibronectin mRNA accumulation in nicotine-treated fibroblasts as determined by real-time PCR.

FP Inhibits Fibronectin Gene Transcription in Fibroblasts

The observations described above suggested that FP might exert its effect by inhibiting fibronectin gene transcription. To test this, NIH-3T3 fibroblasts were stably transfected with pFN(1.2kb)LUC, a DNA construct containing the human fibronectin promoter fused to a luciferase reporter gene. As expected, the transfected cells showed constitutive fibronectin gene transcription as determined by measuring luciferase activity (Fig 2, top, A). The transcription of the fibronectin gene was enhanced by treatment with nicotine alone (p < 0.001). Of note, the exposure of fibroblasts to FP inhibited the transcription of the fibronectin gene at baseline and after nicotine stimulation (p < 0.001). The effect of FP pretreatment on nicotine-induced fibronectin protein production was also demonstrated in Figure 1, right, B.

![Figure 1](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/22020/)
gene transcription was exerted in a dose-dependent manner with maximum activity between 1 and 5 μmol/L FP (Fig 2, top, A, insert). The same effect was observed in primary mouse lung fibroblasts containing pFN(1.2 kb)LUC, where FP pretreatment significantly (p < 0.001) inhibited transcription of the fibronectin gene after nicotine stimulation (Fig 2, bottom, B).

We and others have shown that fibronectin gene transcription is dependent on phosphorylation and
DNA binding by the transcription factor CREB. Consequently, we postulated that FP might affect fibronectin expression by inhibiting CREB. Consistent with this idea, we found that nicotine-treated fibroblasts show increased CREB phosphorylation when compared to untreated fibroblasts. FP pretreatment diminished phosphorylated CREB after nicotine stimulation, whereas the amount of total CREB protein remained relatively unchanged (Fig 3, top, A). To determine if FP also inhibited DNA binding by CREB, electrophoresis mobility gel shift assays were conducted with nuclear protein extracted from NIH-3T3 fibroblasts pretreated with or without FP for 1 h prior to exposure with or without nicotine (50 μg/mL). As demonstrated in Figure 3, bottom, B, untreated fibroblasts showed little to no CREB-DNA binding, whereas nicotine-treated cells showed increased binding. FP pretreatment inhibited DNA binding after nicotine treatment. Albuterol was used as control and was found to increase CREB-DNA binding in fibroblasts.

**DISCUSSION**

Corticosteroids are commonly used in the management of lung diseases induced or exacerbated by environmental tobacco smoke and characterized by airflow limitation such as asthma and COPD.23,24 The beneficial effects of corticosteroids in this setting are often ascribed to their powerful anti-inflammatory activity.25,26 However, studies4,5,27 suggest that corticosteroids might affect tissue remodeling as well. Vanacker et al4,5 showed that FP, the corticosteroid used here, inhibited the progression of allergen-induced structural changes in the airways of rats. These effects were associated with inhibition of epithelial cell proliferation, goblet cell hyperplasia, and airway wall thickening. Blyth et al28 showed significant resolution of established subepithelial fibrosis in a murine model of atopia. Moreover, Chetta et al29 showed that fluticasone can reduce the vascular component of airway remodeling. In humans, Hoshino et al30 showed that inhaled corticosteroids can reduce the lamina reticularis of the basement membrane by modulating insulin-like growth factor-I expression in bronchial asthma, and Olivieri et al31 showed that fluticasone might control the intensity of airway remodeling. Together, these studies suggest that corticosteroids can diminish lung tissue remodeling, thereby ameliorating the progression of disease. However, the exact mechanisms by which corticosteroids exert their anti-tissue remodeling effects remain to be fully elucidated.

We hypothesize that corticosteroids modulate lung tissue remodeling by affecting intracellular signaling pathways that control the expression of extracellular matrix genes. The data presented here suggest that this hypothesis is correct. We report that nicotine stimulates the production of fibronectin in transformed and primary lung fibroblasts. The induction of fibronectin was due to increased transcription of the fibronectin gene followed by accumulation of fibronectin mRNA and secretion of its protein. Consistent with our hypothesis, we found that FP inhibited the production of fibronectin in lung fibroblasts by blocking the transcription of the fibronectin gene, thereby decreasing fibronectin protein production. This inhibitory effect was related to blockade of CREB phosphorylation, a transcription factor known to be important in regulation of fibronectin gene transcription. In turn, inhibition of CREB phosphorylation was associated with decreased CREB binding to DNA leading to diminished gene transcription.

Although these studies were carried out in vitro, they have important clinical implications that should be further investigated. First, they suggest that the detrimental effects of tobacco in the lung might be partly related to nicotine, an alkaloid present in tobacco. Therefore, tobacco may affect the lung even when used in smokeless form. Second, they unveil another mechanism by which tobacco can affect the lung; namely, through induction of fibroblast-driven

![Figure 3. FP inhibits CREB phosphorylation and DNA binding. Top, A: NIH-3T3 fibroblasts were pretreated with or without FP (1 μmol/L) for 1 h prior to incubation in the presence or absence of nicotine (50 μg/mL) for 24 h. Note that phosphorylated CREB (p-CREB) [Ser 133] protein expression is increased in presence of nicotine. However, in the presence of FP, nicotine-induced fibronectin protein expression is diminished while the total amount of CREB protein remains unchanged. Bottom, B: FP inhibits CREB-DNA binding. NIH-3T3 fibroblasts (3 × 10^6/mL) were incubated in the presence or absence of FP (1 μmol/L) or albuterol (10 μmol/L) for 1 h prior to treatment with or without nicotine (50 μg/mL) for 16 h. Note that nicotine-treated cells show enhanced CREB-DNA binding as compared to untreated control cells. Albuterol also stimulated CREB-DNA binding; however, FP was able to abrogate CREB-DNA binding in cells exposed to nicotine.](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/22020/)
matrix expression and deposition. In view of the fact that matrix molecules have many effects on inflammation and *vice versa*, it is not difficult to envision how nicotine-induced tissue remodeling may promote inflammation thereby accelerating disease progression. Third, they reveal a novel mechanism by which corticosteroids might ameliorate lung tissue remodeling in the setting of tobacco abuse.

The effects of corticosteroids on fibronectin expression were found to be dose dependent. This is not unexpected, but it is considered an important observation in view of the work of Vanacker and colleagues, who showed that, despite inhibition of the initial increase in airway hyperresponsiveness with mild-to-moderate doses of corticosteroids, tissue remodeling progressed. In the same study, Vanacker et al found that higher doses of corticosteroids were definitely needed to inhibit tissue remodeling, which, in turn, had important consequences on clinical symptoms. Thus, corticosteroid doses higher than those needed to control inflammation appear to be required to significantly impact tissue remodeling. This suggests that, in the clinical arena, it might be beneficial to determine optimal doses of corticosteroids based on markers of tissue remodeling rather than based exclusively on markers of inflammation. Of course, much work is needed to identify sensitive and easily accessible markers of lung tissue remodeling.

The recognition that corticosteroids may impact fibronectin expression directly is important because the reduction in fibronectin noted in airway walls after FP treatment could be ascribed to inhibition of plasma leakage. Since fibronectin is abundant in plasma, inhibition of plasma leakage could explain the reduction in fibronectin tissue deposition. However, our data suggest that corticosteroids can also inhibit fibronectin expression in lung fibroblasts; therefore, we believe that this is likely to be a more important mechanism in the setting of tobacco-related disease.

This study also suggests that corticosteroids affect matrix gene expression by specifically inhibiting the activities of specific transcription factors. The effects of corticosteroids on other transcription factors (e.g., sequestration of nuclear factor-κB and activator protein [AP]-1) have been documented before, and this mechanism has been used to explain the inhibitory effects of corticosteroids on genes encoding for cell adhesion (e.g., vascular cell adhesion molecule) and proinflammatory molecules (e.g., cytokines). However, these transcription factors may also affect genes involved in connective tissue remodeling. For example, activator protein-1 is considered an important modulator of the expression of matrix metalloproteinase (MMP)-9 and other MMPs that have been implicated in the pathogenesis of asthma and COPD. Herein, we show that corticosteroids affect yet another transcription factor, CREB. Following phosphorylation, CREB binds to the cAMP response element consensus sequence, which is present in the promoter of a number of genes including that of fibronectin. Therefore, it is likely that more than a few remodeling genes are affected by corticosteroids through this mechanism, and this may explain the work by Hoshino et al, who showed that in humans, corticosteroids reduced subepithelial collagen deposition, down-regulated the expression of MMP-9, and up-regulated tissue inhibitor of MMP molecules.

This study also suggests that drugs commonly used for the management of tobacco-related diseases might affect tissue remodeling. Although corticosteroids are likely to be beneficial in this setting, other drugs may not. In this regard, we showed that a β2-agonist, albuterol, did not inhibit CREB-DNA binding; instead, it stimulated it. β2-agonists are potent drugs used to induce bronchodilatation in the setting of obstructive lung disease. However, we do not know what effects the long-term use of these drugs have on lung tissue remodeling. Wang and others found that short-acting β2-agonists increased fibronectin deposition in their model of long-term antigen exposure in guinea pigs, and that this effect was ameliorated by fluticasone. This is not surprising when viewed together with data indicating that these agents stimulate the phosphorylation of CREB. This suggests that some of these drugs may have unforeseen effects, and that their functions may be influenced by so-called “cross-talk” between transcription factors such as AP-1, nuclear factor-κB, and CREB. The interactions of these drugs with one another, particularly as they relate to tissue remodeling, should also be investigated. Dong et al showed that β2-agonists increase the number and function of glucocorticoid receptors though a number of mechanisms. Therefore, the use of these drugs in combinations might allow for recipients to benefit from these drugs while diminishing their negative effects. Of course, this speculation needs to be tested in humans.

Finally, it should be highlighted that the inhibitory effects of fluticasone on mRNA expression in the absence of dramatic effects on promoter activity or CREB phosphorylation suggest additional effects at the posttranscriptional level. This is not surprising in view of data by others demonstrating that glucocorticoids can affect the posttranscriptional modification of several proteins including that of keratinocyte adhesion molecules, brain manganese-superoxide dismutase, and myelin basic protein. Thus, our
data suggest that fluticasone might also be exerting posttranscriptional effects that regulate fibronectin protein production.

In summary, we showed that nicotine induced the expression of fibronectin by stimulating the transcription of the fibronectin gene in lung fibroblasts. Constitutive and nicotine-stimulated fibronectin expression was blocked in a dose-dependent manner by FP through inhibition of phosphorylation and DNA binding by the transcription factor CREB. Overall, we speculate that this mechanism may partially explain the beneficial effects of cortico-steroids on tobacco-related obstructive lung disease.

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