Biochemical Pharmacology, Vol. 57, pp. 1003–1009, 1999.

ISSN 0006-2952/99/$–see front matter
PII S0006-2952(99)00008-8

Pharmacological Characterization of Glucocorticoid Receptors in Primary Human Bronchial Epithelial Cells

Tricia D. LeVan,†‡ Elizabeth A. Babin,†‡ Henry I. Yamamura‡ and John W. Bloom*‡§||*

*Respiratory Sciences Center, and Departments of ‡Pharmacology and §Medicine, College of Medicine, University of Arizona, Tucson, AZ 85724, U.S.A.

ABSTRACT. Bronchial epithelial cells play an important role in amplifying and perpetuating airway inflammation and may be a target for inhaled steroids. We have characterized glucocorticoid receptors in primary human bronchial epithelial cells. Northern and western blot analyses demonstrated the expression of glucocorticoid receptor mRNA and protein, respectively, in primary bronchial epithelial cells. The activity of these receptors was shown using a radioligand binding assay. High-affinity binding with pharmacological specificity was demonstrated for [^{3}H]dexamethasone. The equilibrium dissociation constant (K_d) and density of binding sites (B_{max}) for [^{3}H]dexamethasone determined from saturation isotherms were 4.4 nM ×/÷ 0.95 (SEM) and 30.1 fmol/mg protein ± 6.4 (SEM). Glucocorticoid receptors were activated by dexamethasone as assessed using a glucocorticoid-responsive reporter plasmid, pTAT$_3$-CAT. Transfection of primary human bronchial epithelial cells with this reporter plasmid resulted in 35-fold activation of transcription following dexamethasone stimulation (10^{-6} M). The glucocorticoid receptor antagonist RU-486 (mifepristone) significantly counteracted the effect of dexamethasone on glucocorticoid receptor activation, indicating that the dexamethasone effect is specific and is mediated through the glucocorticoid receptor. In summary, our study demonstrated that primary cultures of human bronchial epithelial cells possess glucocorticoid receptors that function as a ligand-activated transcriptional regulator. The presence of glucocorticoid receptors confers their responsiveness to glucocorticoids and indicates that the airway epithelium may be a target for the anti-inflammatory effects of inhaled steroids. BIOCHEM PHARMACOL **57**;9:1003–1009, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. primary human bronchial epithelial cells; glucocorticoid receptors; dexamethasone; radioligand binding; transactivation

Inhaled glucocorticoids are the most effective anti-inflammatory therapy presently available for the treatment of asthma [1]. An important target for the anti-inflammatory effects of inhaled steroids may be the airway epithelial cells [2]. Despite the potential central role of bronchial epithelial cells in asthmatic reactions, and the widespread use of inhaled glucocorticoids in the treatment of asthma, little is known about the GR¶ present specifically on human bronchial epithelial cells. Bronchial epithelial cells not only perform a barrier function but also produce a number of inflammatory mediators, including granulocyte macrophage colony-stimulating factor, RANTES, endothelin, IL-1, IL-6, IL-8, IL-10, IL-11, IL-16, transforming growth factor-β, eotaxin, and TNF-α [2–4], upon exposure to immunologic and nonimmunologic stimuli. Therefore, the bronchial epithelium may play an essential role in initiating and secondarily amplifying inflammation in the airways by mediating chemotaxis, recruitment, activation, and survival of inflammatory cells within the airway.

Binding and activation of specific intracellular GR mediate glucocorticoid effects in responsive cells [5]. The binding of glucocorticoids to GR results in the dissociation of chaperone proteins from the receptor and exposure of the DNA-binding domain. This ligand-activated GR complex then translocates to the nucleus and selectively modulates transcription of steroid-responsive genes. GR-mediated control of gene expression can be due either to transcriptional induction of specific GR target genes as a result of binding to positive GRE and transcriptional activation, or to GR-dependent transcriptional repression. Thus, direct cellular effects of glucocorticoids require the presence of specific GR [6].

Glucocorticoid binding sites have been demonstrated

† These authors have contributed equally to the content of this manuscript.

||Corresponding author: John W. Bloom, M.D., Respiratory Sciences Center, University of Arizona HSC, Tucson, AZ 85724. Tel. (520) 626-4021; FAX (520) 626-6970; E-mail: jbloom@resp-sci.arizona.edu

¶ *Abbreviations*: GR, glucocorticoid receptor(s); Dex, dexamethasone; IL, interleukin; TNF, tumor necrosis factor; GRE, glucocorticoid response element; B_{max}, density of binding sites; K_d, equilibrium dissociation constant; K_{obs}, observed association rate constant; K_{+1}, association rate constant; K_{-1}, dissociation rate constant; NHBE, normal human bronchial epithelial; CAT, chloramphenicol acetyltransferase; PMSF, phenylmethylsulfonyl fluoride; SSC, 0.15 M sodium chloride + 0.015 M sodium citrate; K_i, affinity of the inhibitor for the receptor; and IC_{50}, concentration of the competitor that inhibits 50% of the specific binding.

Received 15 September 1998; accepted 12 October 1998.

previously at a relatively high level in several mammalian lung tissues [7–10]. Only a few studies address the presence of GR in the human lung. Autoradiographic localization of specific [^{3}H]Dex binding sites on lung samples from adult and fetus showed nuclear localization of [^{3}H]Dex in alveolar epithelial cells but no significant nuclear localization in bronchial epithelial cells [11]. These results were further confirmed in the adult human lung by *in situ* hybridization, northern blot analysis, immunolocalization, and western analysis [12]. Specifically, GR mRNA and protein were localized in the alveolar walls, vascular endothelium, and smooth muscle; however, few GR were detected in bronchial epithelial cells. GR mRNA expression was demonstrated in isolated cultures of primary human bronchial epithelial cells, obtained by bronchial brushings (>93% purity) [13]. Further characterization of the binding and activation properties of GR was not presented in these studies.

Here we present data demonstrating the expression of GR by northern and western blot analyses on primary cultures of human bronchial epithelial cells. The activity of these receptors was shown using a radioligand binding assay, where density of binding sites and high-affinity binding with pharmacological specificity were demonstrated for [^{3}H]Dex. These GR function as ligand-activated transcriptional regulators as assessed using a glucocorticoid-responsive plasmid.

MATERIALS AND METHODS

Cell Culture and Transfections

Primary cultures of NHBE cells from three individuals were obtained from Clonetics. NHBE cells were isolated from the main stem bronchi immediately after tissue procurement. The bronchial epithelial cell population was characterized and purity was confirmed by morphology and cytokeratin 19 staining. Primary human bronchial epithelial cells were grown on collagen–fibronectin-coated tissue culture plates in serum-free 50% (v/v) LHC-9 (Biofluids Inc.)/RPMI 1640 (GIBCO BRL) medium supplemented with 100 U/mL each of penicillin/streptomycin and 50 ng/mL of amphotericin B at 37° and 5% CO_2 [14]. Twenty-four hours prior to use, cells were incubated with hydrocortisone-deficient LHC-9/RPMI 1640 medium. Transfection by lipofection was utilized for CAT reporter experiments where the cationic lipid DOTAP:DOPE (Avanti Polar Lipids Inc.) was added to plasmid DNA at a 1:1 wt/wt ratio [15]. Lipid/DNA complexes were allowed to form for 15 min at room temperature prior to incubating with primary human bronchial epithelial cells for 2 hr at 37°. Transient transfection assays were carried out with 3.5 μg/10 cm^2 plate of the internal control plasmid pCMV-β-galactosidase and 5 μg/10 cm^2 plate of the pTAT$_3$-CAT reporter plasmid that contains a minimal promoter with three GRE upstream of the CAT gene (a gift from K. Yamamoto, University of California). Following incubation, the lipid/DNA complexes were removed and the cells were exposed to a 20% glycerol solution for 2 min. Glycerol was removed and replaced with hydrocortisone-deficient LHC-9/RPMI 1640 medium. Twenty-four hours after transfection, primary human bronchial epithelial cells were treated with Dex (Sigma Chemical Co.) and/or RU-486 (mifepristone; Roussel-UCLAF) for an additional 24 hr.

Northern Blot Analysis

mRNA was extracted from primary human bronchial epithelial cells using the guanidinium thiocyanate and oligo-dT method (MicroFastTrack, Invitrogen). mRNA pellets were resuspended in diethyl pyrocarbonate (DEPC)-treated water, denatured, and separated by electrophoresis on a 1% agarose/7% formaldehyde gel. RNA was transferred by capillary action onto a nylon membrane (Nytran; Schleicher & Schuell) and immobilized by UV cross-linking. Membranes were prehybridized at 42° for 3–6 hr in 50% deionized formamide, 5× SSC, 0.2% SDS, 50 mM K_2PO_4, pH 7.0, 2× Denhardt's solution, and 30 μg/mL of salmon testes DNA (Sigma). Sequential hybridization was done overnight at 42° with [α-^{32}P]dCTP-labeled human GR and cyclophilin cDNA probes in prehybridization buffer. Cyclophilin was used as a control for mRNA loading. DNA probes were labeled by incubation with random hexamers and Klenow fragments (Boehringer–Mannheim). RNA for GR was probed with the 700-bp *Bgl*II fragment from full length human GR, pSB-hGR (a gift from R. Evans, Salk Institute for Biologic Studies), and cyclophilin was probed with the 103-bp *Kpn*I–*Bam*HI fragment from pTRI-cyclophilin-Hu (Ambion). Following hybridization, membranes were washed twice for 30 min in 0.1× SSC at room temperature and exposed to Kodak Biomax film (Scientific Imaging Systems) with an intensifying screen for 4–48 hr at −80°.

Western Blot Analysis

Primary bronchial epithelial cells, grown in hydrocortisone-deficient LHC-9/RPMI 1640 medium, were pelleted in PBS. Cell pellets were resuspended in TNES non-ionic protein lysis buffer [50 mM Tris (pH 7.4), 1% Nonidet P-40, 2 mM EDTA, 100 mM NaCl, 1 mM PMSF, 0.02 mg/mL of leupeptin, and 0.02 mg/mL of aprotinin] and centrifuged for 30 min at 4000 *g* and 4°. A 10-μL portion of the supernatant was reserved for protein determination by the bicinchoninic acid assay (Pierce). Samples were boiled for 5 min in SDS-loading buffer and subjected to electrophoresis on a 4% polyacrylamide (37.5:1 bis-acrylamide)-SDS stacking gel and a 7.5% polyacrylamide (37.5:1 bis-acrylamide)-SDS resolving gel. Proteins were transferred to nitrocellulose (Bio-Rad) by electroblotting in transfer buffer (48 mM Tris-base, 39 mM glycine, 0.04% SDS, and 20% methanol at pH 8.0) for 2 hr at 500 mA and 4°. Nonspecific sites were blocked for 1 hr in 3% nonfat dry milk solution in TTBS (100 mM Tris at pH 7.6, 4.5% NaCl, and 0.25% Tween 20). GR was detected using

anti-hGR polyclonal antibodies PA1–512 (1:250 dilution; Affinity Bioreagents) derived from New Zealand White rabbits that had been immunized with a 15-amino acid [245–259] synthetic peptide for which the sequence is from the amino terminus of human GR [16]. Primary antibody incubations (1 hr at room temperature) were followed by peroxidase-labeled goat anti-rabbit immunoglobin G secondary antibody (1:20,000). The membrane was incubated with Lumi Glo substrates (Kirkegaard & Perry) to visualize the bands on film.

Radioligand Binding Assay

Approximately 1.5×10^7 primary bronchial epithelial cells, grown in hydrocortisone-deficient LHC-9/RPMI 1640 medium, were pelleted in PBS and rapidly frozen in liquid N_2. Cell pellets were thawed on ice in TEGN50 buffer [50 mM NaCl, 1 mM EDTA, 12% (v/v) glycerol, 1 mM 2-mercaptoethanol, 10 mM sodium molybdate, 1 mM PMSF, 40 ng/mL of leupeptin, 40 ng/mL of aprotinin, and 10 mM Tris–HCl, pH 7.5, at 4°]. Cell extracts were prepared on ice by ultrasonic disruption using a Heat Systems sonicator and microprobe at setting 2 for 10 pulses followed by centrifugation at 4000 g for 10 min at 4°. Soluble protein concentration was determined in the supernatant using the bicinchoninic acid assay (Pierce). Binding assays were set up in duplicate or triplicate with the appropriate concentration of cell extract, 10 μL of ^{3}H-labeled ligand ([^{3}H]Dex; sp. act. 81 Ci/mmol; Amersham), 10 μL of unlabeled Dex as indicated, and TEGN50 buffer to 100 μL. Nonspecific binding was measured by including 1000-fold molar excess of unlabeled Dex. Samples were incubated at 0° for 120 min or as indicated. Unbound hormone was removed by the addition of an equal volume of a charcoal–dextran suspension in TEGN50 (20 mg/mL of acid-activated charcoal and 1 mg/mL of dextran) followed by passage through a 0.45-μm spin filter. The resulting filtrate was counted by standard scintillation. Receptor-specific binding was calculated by subtracting the mean value for the samples containing excess unlabeled ligand from the mean of the samples containing ^{3}H-labeled ligand only.

CAT Assay

Forty-eight hours after transfection, CAT and β-galactosidase assays were performed on cell lysates. Cell lysate protein concentration, as determined with the bicinchoninic acid assay (Pierce), was normalized for transfection efficiency to β-galactosidase expression. CAT activity was determined by the acetylation of [^{14}C]chloramphenicol during a 4-hr incubation at 37°. Substrate and acetylated products were separated by thin-layer chromatography, and the percent conversion of [^{14}C]chloramphenicol to the acetylated forms was quantitated using a Molecular Dynamics PhosphorImager.

Data Analysis

Experimental data for the saturation and inhibition studies were analyzed for one-site and two-site binding models according to the law of mass action as described by Unnerstall [17] using the nonlinear fitting method of steepest descent to reach the lowest sum of squares supplied by PRISM (GraphPad Software, Inc.). Nonspecific binding was calculated as a fitted parameter by linear regression analysis of the nonspecific binding data points. To facilitate comparisons, IC_{50} values were converted to K_i values using the Cheng and Prusoff equation ($K_i = IC_{50}/[1 + [\text{free ligand}]/K_d]$) [18]. Tests of significant difference between means were performed using the *t*-test. The association rate constant, k_{+1}, was calculated from the pseudo-first-order method as described by Bylund and Yamamura [19]. The k_{+1} was derived from the k_{obs} by nonlinear regression of the specific radioligand bound versus time of incubation. The dissociation rate constant, k_{-1}, was estimated from the linear least-squares regression analysis of the first-order plot of the logarithmically transformed data. The mean values for log normal distributed data (i.e. K_d, K_i, IC_{50}) are reported as the geometric means ×/÷ SEM.

RESULTS

Expression of the Human GR in Primary Human Bronchial Epithelial Cells

To initially characterize the expression of the human GR in primary bronchial epithelial cells, northern and western blot analyses were performed. For northern analysis, mRNA was isolated from primary human bronchial epithelial cells cultured for 24 hr in hydrocortisone-deficient LHC-9/RPMI 1640 medium. Hybridization of filters containing mRNA from primary human bronchial epithelial cells with the GR probe revealed the presence of an approximately 7.0 kb GR specific transcript (Fig. 1). A weaker band was detected at approximately 5.0 kb. Western blot analysis revealed that a GR specific protein was expressed in primary human bronchial epithelial cells and comigrated with the GR protein found in the rat hepatoma cell line HTC 19.611 stably transfected with the cloned rat glucocorticoid cDNA sequence (Fig. 2). Low molecular weight bands found for HTC cell protein may reflect degradation products. The predicted molecular mass of the receptor is ~94 to 97 kDa [16].

Radioligand Binding Activity of the Human GR

Radioligand binding studies were performed to determine the density of binding sites (B_{max}) and affinity (K_d) of the GR expressed in primary human bronchial epithelial cells. The effect of tissue concentration on specific binding of 30 nM [^{3}H]Dex to primary human bronchial epithelial cells was determined in initial experiments. For primary human bronchial epithelial cells, specific binding of the radioligand was linearly dependent on the concentration of tissue

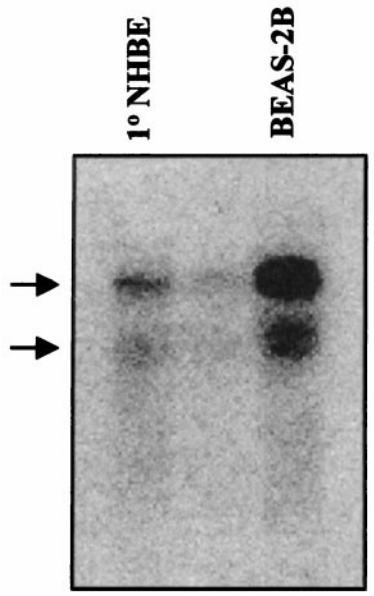

FIG. 1. Northern blot analysis of human GR mRNA in primary bronchial epithelial cells. Primary human bronchial epithelial cells (3×10^6) and BEAS-2B cells (6×10^6, control) were cultured on hydrocortisone-deficient LHC-9/RPMI 1640 medium for 24 hr prior to mRNA isolation. mRNA was isolated and northern analysis was performed as described in Materials and Methods. A representative northern blot is shown. The upper arrow identifies the 7.0 kb transcript, and the lower arrow, the 5.0 kb transcript.

from 150 to 800 μg protein/reaction (Fig. 3, N = 3 experiments). The protein used for all subsequent binding assays was within this linear range (i.e. 200 μg/reaction).

The kinetics of [^{3}H]Dex (0.5 nM) binding to GR were examined. Steady–state binding of [^{3}H]Dex was observed within 60 min at 4° for primary human bronchial epithelial cell lysates (data not shown). The association rate constant (k_{+1}), as determined by pseudo-first-order analysis of association data, was 0.023×10^6 M^{-1} min^{-1}. A time-dependent decrease in receptor-bound radioligand was observed after the addition of 0.5 μM Dex to primary human bronchial epithelial cell lysates that were incubated previously for at least 120 min with 0.5 nM [^{3}H]Dex (data not shown). Dissociation of the ligand–receptor complex occurred with a dissociation rate constant (k_{-1}) of 0.023×10^{-3} min^{-1}. The kinetic K_d (k_{-1}/k_{+1}) determined from primary human bronchial epithelial cell lysates was 1.0 nM for a pseudo-first-order association. The time of incubation for all subsequent radioligand binding experiments was 120 min.

Saturation isotherm experiments with [^{3}H]Dex binding were performed with primary human bronchial epithelial cell lysates (Fig. 4). The equilibrium dissociation constant (K_d) was 4.4 nM (range 2.7 to 7.3) and the density of binding sites (B_{max}) was 30.1 ± 6.4 fmol/mg protein (equivalent to 2.6×10^4 receptors/cell) for N = 3 experiments. The K_d for Dex binding to primary human bronchial epithelial cell lysates was consistent with the kinetic K_d for these cells.

In inhibition studies, the radioligand binding of 5 nM [^{3}H]Dex was inhibited in a concentration-dependent manner by the agonist, unlabeled Dex, and the antagonist, RU-486. Inhibition curves are shown in Fig. 5. The K_i value derived for Dex inhibition of [^{3}H]Dex binding was 6.1 nM ×/÷ 0.9 (SEM), N = 3 experiments. This K_i value is in close agreement with the K_d value determined directly from the saturation binding studies with [^{3}H]Dex ($P > 0.05$). The K_i value derived for RU-486 inhibition of [^{3}H]Dex binding was 59.3 nM ×/÷ 1.2 (SEM).

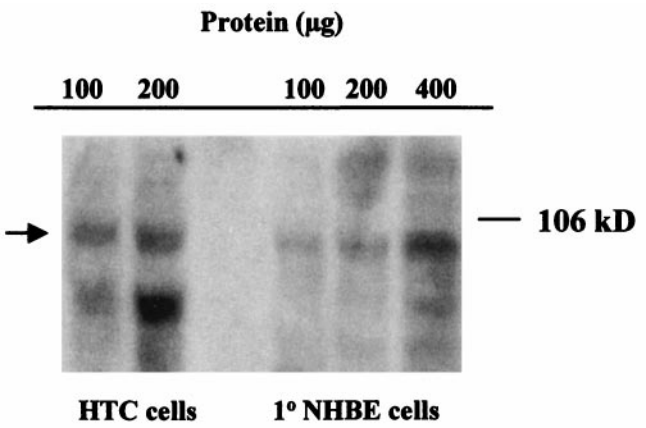

FIG. 2. Western blot analysis of human GR in primary human bronchial epithelial cells. Primary human bronchial epithelial cells were cultured in hydrocortisone-deficient LHC-9/RPMI 1640 medium for 24 hr prior to protein isolation. Western blot analysis was performed using a polyclonal antibody raised against the human GR as described in Materials and Methods. Lanes 1 and 2: (control) HTC cell extract, 100 and 200 μg, respectively; Lanes 4, 5, and 6: primary human bronchial epithelial cell extract, 100, 200, and 400 μg, respectively. The position of a protein standard (106 kDa phosphorylase *b*) run in a separate lane is shown.

GR Transcriptional Transactivation Activity

Activation of endogenous GR by Dex in primary human bronchial epithelial cells was assessed using a glucocorticoid-responsive reporter plasmid, $pTAT_3$-CAT. GR activation resulted in a 35-fold induction of CAT activity over basal in the presence of 10^{-6} M Dex (Fig. 6). Dex was shown to activate GR in a concentration-dependent manner with an EC_{50} of 9.9 (range 6.3 to 15.5 nM). The EC_{50} value was not significantly different from the K_d derived from saturation studies ($P > 0.05$). RU-486, a competitive GR antagonist, significantly counteracted the effect of Dex on GR activation ($P < 0.01$), whereas RU-486 alone had no independent effect on GR activation.

DISCUSSION

To our knowledge, this is the first time that primary cultures of human bronchial epithelial cells have been specifically shown to possess GR, which function as a ligand-activated transcriptional regulator. We hypothesize

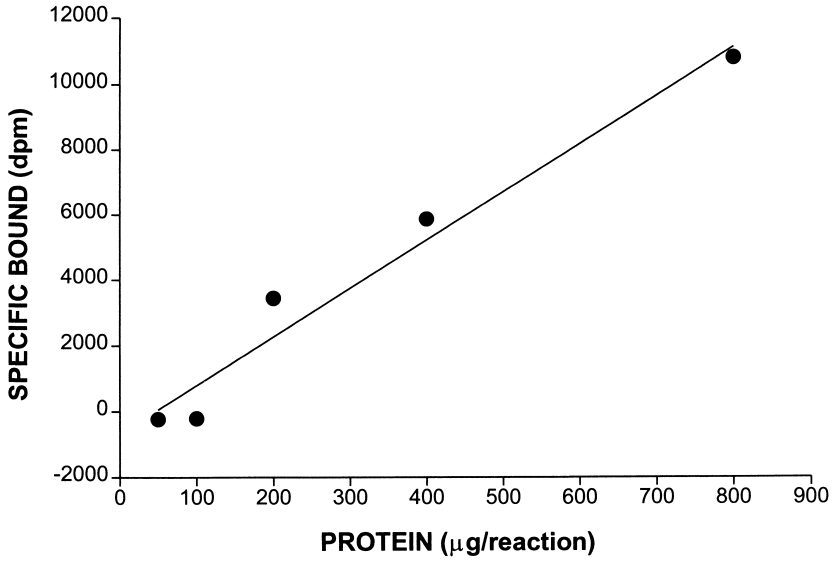

FIG. 3. A representative tissue linearity experiment of 30 nM [^{3}H]Dex binding in primary human bronchial epithelial cell lysates (protein concentration = 100–800 μg/reaction). Data from one of three experiments are shown. Specific binding was experimentally determined as the difference between total binding and nonspecific binding in the absence and presence of 30 μM Dex. The protein concentration used for all subsequent binding assays was within the linear range, i.e. 200 μg/reaction.

that the bronchial epithelium plays an important role in amplifying and perpetuating airway inflammation and is a primary site of glucocorticoid anti-inflammatory activity in the lung.

Glucocorticoid effects are mediated in responsive cells by binding to and activation of functional GR. In this study, we have utilized primary human bronchial epithelial cells isolated from the lung to allow the direct characterization of GR. Expression of GR mRNA and protein in primary bronchial epithelial cells was demonstrated by northern and western blot analysis, respectively. Northern analysis

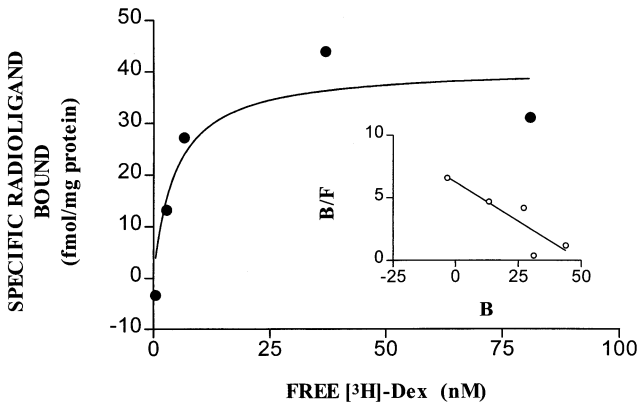

FIG. 4. A representative saturation isotherm of [^{3}H]Dex binding in primary human bronchial epithelial cell lysates (protein concentration = 200 μg/reaction). Data from one of three experiments are shown. Assay conditions were as described under Materials and Methods. Specific binding, best described by a one-site fit, was experimentally determined as the difference between total binding and nonspecific binding in the absence and presence of 30 μM Dex. Specific binding was 37% of total binding (the remainder was nonspecific binding) at the ligand concentration in the range of the dissociation constant. The inset shows a Rosenthal plot of specific [^{3}H]Dex binding data where the abscissa is bound ligand (femtomoles per milligram of protein) and the ordinate is bound over free ligand (femtomoles per nanomolar).

from previous studies has shown 7 and 5 kb mRNA fragments corresponding to the α-GR mRNA and β-GR mRNA, respectively, in various cell types [6, 20]. In this study, a strong band at 7 kb was observed with a weaker band at 5 kb, indicating the predominance of α-GR mRNA in these cells. Western blot analysis revealed the expression and accumulation of human GR in primary bronchial epithelial cells. Furthermore, radioligand binding studies established the affinity (K_d = 4.4 nM) and density (B_{max} = 2.6×10^4 receptors/cell) of GR that specifically bound the ligand [^{3}H]Dex in primary human bronchial epithelial cells. These receptors bind [^{3}H]Dex with a high affinity that is comparable to that found in other cell systems. For example, we and others have reported an affinity for [^{3}H]Dex in a bronchial epithelial cell line (BEAS-2B) ranging from 5.6 to 8.6 nM [21, 22]. An apparent affinity, as measured from autoradiographic localization studies, was calculated for lung tissue, which ranged from 3.5 to 10.2 nM [10]. We found the density of binding sites in primary bronchial epithelial cells (2.6×10^4 receptors/cell or 30.1 fmol/mg protein) to be analogous to that found in the BEAS-2B cell line, i.e. 3.0×10^4 receptors/cell (equivalent to 228 fmol/mg protein) [13, 21]. Peters *et al.* [23] found GR present in rat lung tissue at a density of 60 fmol/mg protein. In human peripheral blood mononuclear cells and fibroblasts, the GR B_{max} was reported to be 191.0 and 94.0 fmol/mg protein, respectively [24]. Consequently, it appears that the affinity of [^{3}H]Dex for GR remains somewhat constant under normal conditions (within the range of 3.5 to 10.2 nM) and is independent of cell type. In contrast, the density of GR binding sites is highly dependent on cell type. Compared with the rat lung, primary bronchial epithelial cells contain approximately the same number of GR [9]. The work of several investigators indicates that glucocorticoids autoregulate GR via transcriptional and posttranscriptional mechanisms and, consequently, the responsiveness of the cell to its own ligand [13, 20, 25]. For

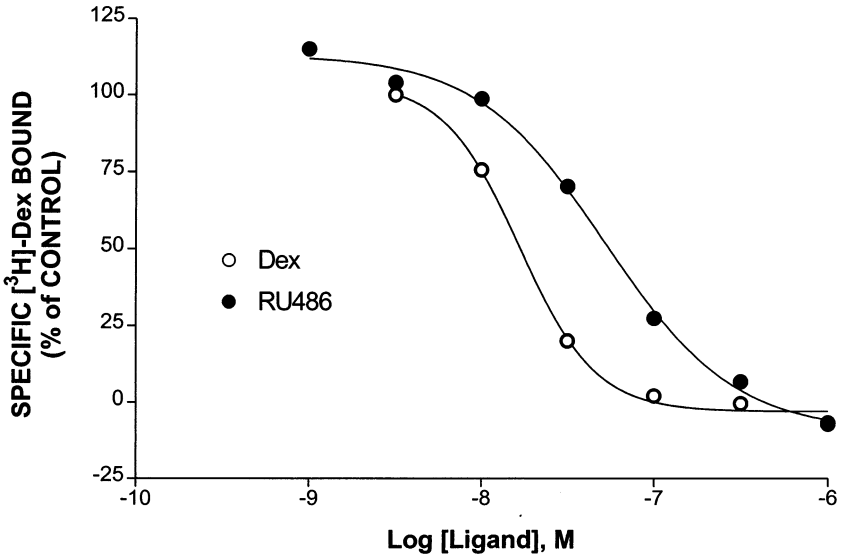

FIG. 5. Inhibition of [^{3}H]Dex (5 nM) by unlabeled Dex or RU-486 in primary human bronchial epithelial cells. Assay conditions were as stated under Materials and Methods. Representative data from one of three experiments are shown. One hundred percent bound was approximately equivalent to 1000 dpm. Nonlinear least-squares regression analysis of the inhibition data were best described by a one-site model.

instance, the presence of glucocorticoids has been reported to cause a 50–75% down-regulation of cellular GR in a number of tissues and cell lines [26]. We have observed a similar pattern of regulation in that removal of Dex from the tissue culture medium for 24 hr resulted in a 100% up-regulation of the GR found in primary bronchial epithelial cells (Babin E, unpublished data).

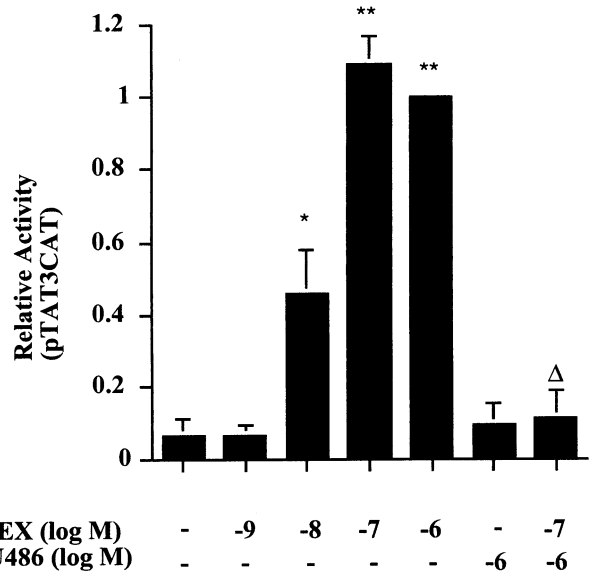

FIG. 6. Effect of Dex and/or RU-486 on GR transcriptional transactivation in primary human bronchial epithelial cells. Transfected primary human bronchial epithelial cells were stimulated with increasing concentrations of Dex and/or 10^{-6} M RU-486 for 24 hr and then were harvested for CAT and β-galactosidase determination. Activity of the GR was calculated relative to the level of CAT activity for primary human bronchial epithelial cells treated with 10^{-6} M Dex (activity = 1). Activity of the GR is expressed as mean ± SEM for N = 3. All measurements were performed in duplicate. Key: (*) $P < 0.05$ and (**) $P < 0.005$, significant difference from basal activity as determined by Student's *t*-test; and (Δ) $P < 0.01$, significant difference from Dex (10^{-6} M)-induced GR activity.

The molecular basis of glucocorticoid action is GR-mediated control of gene expression [5]. This can be due either to transcriptional induction of specific GR target genes as a result of sequence-specific DNA binding and transcriptional activation or to GR-dependent transcriptional repression. In the primary bronchial epithelial cells, transcriptional activation occurred in the presence of Dex. The EC_{50} for transcriptional activation by Dex was comparable to the K_d for [^{3}H]Dex binding to GR, indicating a similar mechanism of activation and binding of GR. Transcriptional activation by Dex was blocked by the GR antagonist RU-486, demonstrating that the Dex effect is specific and mediated through GR. We have reported a similar transactivation activity in the bronchial epithelial cell line BEAS-2B [21]. In addition, Adcock *et al.* [12] reported GR binding to GRE in nuclear extracts from human lung tissue.

In summary, our study demonstrates that the bronchial epithelium contains functional GR, which confers responsiveness to glucocorticoids. Although a variety of inflammatory and resident airway cells could be targets for glucocorticoid-induced effects, these results provide evidence that airway epithelial cells are a target for inhaled glucocorticoids.

This work was supported by grants from the National Heart, Lung and Blood Institute (HL-14136), the Arizona Disease Control Research Commission, and the National Institute of Environmental Health Sciences (T32-ES-07091).

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