Steroid Responsiveness of Renal Epithelial Cells

DISSOCIATION OF TRANSREPRESSION AND TRANSACTIVATION*

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Glucocorticoids modulate cellular and inflammatory responses via stimulation or inhibition of gene transcription. Inhibition of cytokine gene expression is mediated via repression of transcription factors, including NF-κB. Previously we have shown that cytokine production by renal epithelial cells is insensitive to the inhibitory action of dexamethasone. In this study we demonstrate that dexamethasone is unable to inhibit NF-κB activation in the renal epithelial cell line HK-2, as measured by IκB-α degradation and DNA binding activity. Transfection of an NF-κB-inducible reporter gene demonstrated that non-stimulated HK-2 cells contain a high level of constitutively active NF-κB compared with the steroid-sensitive airway epithelial cell line A549, which was not blocked by dexamethasone. Expression and nuclear translocation of the glucocorticoid receptor (GR) was comparable in both cell types. In HK-2 cells, dexamethasone stimulated expression of two glucocorticoid-responsive genes, β₂-adrenoreceptors and angiotensinogen. The capacity of GR to transactivate the native angiotensinogen glucocorticoid-responsive element (GRE) using chromatin-IP was not impaired. Moreover, dexamethasone activation of a GRE-driven reporter construct appeared to be equally effective, although less sensitive compared with A549 cells. In conclusion, we provide evidence that glucocorticoids are unable to repress the activity of NF-κB in renal epithelial cells in the presence of an intact stimulatory pathway.

Glucocorticoids are known to modulate a variety of cellular processes and play an important role in homeostasis and development. Furthermore, glucocorticoids are potent suppressors of the immune system and are therefore used as therapeutic treatment in a broad range of autoimmune and inflammatory diseases. Glucocorticoid action is mediated via binding to the glucocorticoid receptor (GR). The inactive receptor is bound to chaperone proteins, including two heat shock protein 90 (hsp90) subunits, and is located in the cytoplasm (1, 2). Upon ligand binding, the activated GR dissociates from the chaperone complex and translocates to the nucleus to activate or repress transcription of glucocorticoid target genes. Stimulation of gene transcription is mediated via binding of GR to glucocorticoid responsive elements (GREs) in the promoter region of glucocorticoid responsive genes (1–3).

Renal tubular epithelial cells (TEC) are an important source of cytokines and chemokines and are thought to play an active role in the progression of inflammatory processes in the kidney (4). Although glucocorticoids are known to have a profound inhibitory effect on cytokine production by a variety of cell types (5–7), we have previously shown that production of inflammatory mediators by renal tubular epithelial cells is insensitive to the inhibitory action of glucocorticoids (8). Inhibition of cytokine production by glucocorticoids has been attributed to transrepression of NF-κB, a pro-inflammatory transcription factor that regulates the expression of many inflammatory mediators, including IL-6 and IL-8 (9, 10). The mechanism of NF-κB suppression by glucocorticoids has been studied extensively. Initially it was postulated that corticosteroids inhibit NF-κB activation by increasing the transcription of IκB-α, the endogenous inhibitor of NF-κB (11, 12), but this occurs only in certain T-cell lines. Subsequent studies have shown that the activated glucocorticoid receptor can bind to the p65 subunit of NF-κB, thus interfering in the binding of NF-κB to DNA (13, 14). The currently accepted view is that corticosteroids interfere with the transactivating potential of NF-κB via alterations in cofactor recruitment (15–17).

In the present study, we have investigated modulation of gene transcription by dexamethasone in the renal epithelial cell line HK-2 to obtain more insight in the observed steroid insensitivity. We show that dexamethasone is unable to prevent activation of the NF-κB pathway. Furthermore, we demonstrate that glucocorticoid receptor translocation to the nucleus in renal epithelial cells is functional and comparable to the glucocorticoid-sensitive airway epithelial cell line A549. In renal epithelial cells, dexamethasone stimulated the expression of two glucocorticoid-responsive genes, angiotensinogen (AGT) and β₂-adrenoreceptors, but had no effect on cytokine production, clearly demonstrating dissociation of the positive and negative regulatory function of glucocorticoids in this cell type. These results emphasize the cell type-specific characteristics of glucocorticoid action.

EXPERIMENTAL PROCEDURES

Cell Culture—The renal epithelial cell line HK-2 was kindly provided by M. Ryan, University College Dublin, Ireland (18). Cells were cultured in RPMI 1640 medium (Invitrogen) supplemented with 2 mM L-glutamine, 10% heat-inactivated fetal calf serum (ΔFCS) and 100
unit/mL penicillin, 100 μg/mL streptomycin (both from Sigma Chemical Co.). The human airway epithelial cell line A549 was cultured in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% FCS. Prior to stimulation, cells were cultured in serum-free medium for 48 h.

IL-6 and IL-8 ELISA—Prior to stimulation, cells were transferred to 48-well plates (Costar, Corning, NY) at a density of 0.5 × 10^6 cells per well and serum-starved. Cells were stimulated with 5 ng/mL IL-1α (Preprotech, Rocky Hill, NJ). For inhibition experiments, cells were pretreated for 2 h with dexamethasone (Sigma) before addition of IL-1. Production of IL-6 and IL-8 in culture supernatants was measured by specific ELISA as described previously (19). Cytokine production was expressed as the mean concentration ± S.D. from representative experiments. Experiments were repeated at least three times.

**NF-κB Extracts—**IκB-α and β-adenoreceptor protein levels were measured in whole cell extracts. Cells were harvested at different time points after stimulation and incubated in lysis buffer containing 20 mM Tris, pH 7.4, 137 mM NaCl, 10% glycerol, 1% Triton X-100, 2 mM EDTA, and a protease inhibitor mix consisting of 5 units/mL Trasylol (Bayer, Leverkusen, Germany), 1 mM phenylmethylsulfonyl fluoride, 2 μg/mL antipain, 2 μg/mL chymostatin, and 2 μg/mL leupeptin (all from Roche Molecular Biochemicals) for 10 min on ice. Supernatants were collected after centrifugation at 15,000 rpm.

For detection of GR translocation, nuclear, and cytosolic extracts were prepared. After stimulation with dexamethasone (10^{-10} to 10^{-6} M), cells were harvested by scraping in 2 ml of ice-cold Hank's Balanced Salt Solution (Invitrogen) and centrifuged for 5 min. Cytosolic extracts were prepared by addition of buffer A (10 mM Hepes pH 7.9, 1.5 mM MgCl₂, 10 mM KCl, 0.5% Nonidet P-40, 1 mM dithiothreitol, complete protease inhibitor mixture) for 10 min on ice and centrifugation. The pellets containing the nuclear proteins were extracted in buffer B (20 mM Hepes, 1.5 mM MgCl₂, 0.42 mM NaCl, 0.2 mM EDTA, 25% glycerol, 1 mM dithiothreitol, complete protease inhibitor mixture) for 30 min on ice. After centrifugation, the supernatant was mixed with buffer C (20 mM Hepes pH 7.9, 50 mM KCl, 0.2 mM EDTA, 1 mM dithiothreitol, complete protease inhibitor mixture). Protein concentration was determined with a Bradford protein assay kit (Bio-Rad, Munich, Germany).

**Western Blot Analysis—**Cells were size-fractionated by SDS-PAGE using NuPAGE precast gradient gels (Invitrogen) and transferred to Hybond ECL membranes (Amersham Biosciences). The membrane was blocked in Tris-buffered saline, 0.1% Tween 20 containing 5% nonfat dry milk. Primary antibodies used were rabbit anti-human IκB-α (C-21) antibody, rabbit anti-human β-adenoreceptor (H-73) antibody (both from Santa Cruz Biotechnology, Santa Cruz, CA), and mouse anti-human GR antibody (Signal Transduction Laboratories, Lexington, KY). Horseradish peroxidase-conjugated secondary antibodies were obtained from DAKO, Glostrup, Denmark. Blots were developed with ECL chemiluminescence substrate (Amersham Biosciences). Densitometry was performed using a Stratagene Eagle sight analysis system. Efficiency of transfer was verified by staining with Ponceau Red (Sigma). At least three independent experiments were performed.

**RT-PCR—**Total RNA was isolated using RNAzolB (Campro, Veenendaal, The Netherlands) according to the manufacturer's instructions. A_{260}/A_{280} ratios were measured to determine the quantity and purity of RNA preparations. Fixed amounts of total cellular RNA (1 μg) were reverse-transcribed to cDNA by oligo(dT) priming using M-MLV reverse transcriptase (Invitrogen). Expression of IκB-α and AGT mRNA was assessed by RT-PCR with β-actin as an internal control. Primers used for amplification are given in Table I. PCR was performed under standard conditions (50 mM KCl, 10 mM Tris-HCl, pH 8.4, 0.06 mg/mL bovine serum albumin, 0.25 mM dNTPs, 25 pmol of each primer, 1 unit of Taq polymerase, PerkinElmer Life Sciences) with MgCl₃, 2.5 mM for IκB-α and 1.5 mM for AGT and β-actin. The following scheme was used: 5 min 95°C, 30 cycles of 1 min 95°C, 1 min 60°C (IκB-α) or 55°C (β-actin) and 1 min 72°C. The PCR products were analyzed on a 1% agarose gel containing ethidium bromide.

**Detection of NF-κB DNA Binding Activity—**For electrophoretic mobility shift assay (EMSA), nuclear extracts were prepared as previously described (23). Cells were stimulated with IL-1 for 1 h with or without preincubation with dexamethasone. A similar protocol was followed. Nuclear extracts was used to establish DNA binding as described previously (24). The probe used for detection of DNA binding was a 32P-labeled oligonucleotide containing the NF-κB site from HLA-A (5'-GTG GATT CCC CAC TGC A-3') (25). For supershift assays, anti-p65 (sc-114) and anti-p65 (sc-109) antibodies (Santa Cruz Biotechnology) were added to the nuclear extract and protein mixture and incubated for 1 h at 4°C. Subsequently, they were run on a 8% polyacrylamide gel in 0.5× Tris borate/EDTA buffer and analyzed by autoradiography.

NF-κB DNA binding activity was also assessed with Trans-AM NF-κB p65 and p50 transcription factor assay kits (Active Motif Europe, Rixensart, Belgium) according to the manufacturer's instructions. Cells were incubated with 10^{-6} M dexamethasone for 30 min and stimulated with 5 ng/mL IL-1 for 30 min. Whole cell lysates were prepared and 2 μg extracts were added to 96-well plates coated with an oligonucleotide containing the NF-κB consensus site. Binding of NF-κB to the DNA was visualized by anti-p50 and anti-p65 antibodies that specifically recognize activated NF-κB. Antibody binding was measured at 450 nm. Specificity of NF-κB activation was determined by competition experiments using NF-κB wild-type and mutant consensus oligonucleotides that were used to provide the NF-κB consensus site.

**Plasmids—**The reporter plasmids pG1L3-B-Luc and pMMF-Luc were a generous gift from J. W. Bloom, Dept. of Pharmacology, Tucson, AZ. Plasmid pGL3-B-Luc contains 3 NF-κB sites linked to the luciferase gene inserted into the pGL3-basic vector. Plasmid pMMF-Luc is a derivative of pGL3-basic containing the MTVE promoter of the mouse mammary tumor virus (MMTV) linked to the luciferase gene.

**Transient Transfection and Luciferase Assay—**Before transfection, cells were plated at a density of 0.5 × 10^6 cells/well in 6-well plates (Costar) and serum-starved for 24 h. On the day of transfection, culture medium was switched to serum-free Dulbecco's modified Eagle's medium. For inhibition studies, cells were pretreated with 10^{-6} M dexamethasone or 10 μM caffeic acid phenethyl ester (CAPE, Sigma) for 30 min prior to transfection. Cells were then transiently transfected with 1 μl of the MTV-Luc (Promega, Madison, WI) and overnight incubation at 20°C. Lysates were harvested, and luciferase activity was measured using a luciferase assay substrate (Promega) according to the manufacturer's instructions.

**Immunocytochemistry—**A549 and HK-2 cells were cultured in 8-well chamber slides (Falcon, BD Pharmingen, Franklin Lakes, NJ) at a density of 0.5 × 10^6 cells per well. After incubation with dexamethasone, slides were harvested and fixed in ice-cold acetone for 10 min. Cells were permeabilized with 0.8% Nonidet P-40 and blocked for 20 min in phosphate-buffered saline, 20% normal swine serum (DAKO). Primary rabbit anti-human GR (E-20, Santa Cruz Biotechnology) was diluted 1:25 in phosphate-buffered saline, 0.1% bovine serum albumin and incubated for 60 min. After extensive washing in phosphate-buffered saline, biotin-conjugated swine anti-rabbit Ig (DAKO) was incubated for 1 h. After another wash, slides were incubated with streptavidin-fluorescein isothiocyanate (DAKO) for 45 min. Slides were counterstained with 10^{-4} M DAPI (4',6-diamidino-2-phenylindole) (Sigma) for 4 min and washed and mounted in 50% phosphate-buffered saline, 50% glycerol. Slides were analyzed with a Leica TCS-SF confocal laser-scanning microscope.

**Chromatin Immunoprecipitation (ChIP) Assay—**HK-2 cells (1 × 10^6) were stimulated with dexamethasone (10^{-6} M) for 4 h. ChIP assay was performed according to the manufacturer's instructions (Upstate Biotechnology, Lake Placid, NY). Briefly, protein-DNA complexes were fixed in a final concentration of 1% formaldehyde. Cell pellets were resuspended in 5 mM Pipes, pH 8.0, 55 mM KCl, 0.5% Nonidet P-40 containing protease inhibitors (1 μg/mL aprotinin, 1 μg/mL trastuzumab A, and 1 mM phenylmethylsulfonyl fluoride) and incubated on ice for 10 min. After centrifugation, pellets were resuspended in SDS lysis buffer (50 mM Tris, pH 8.1, 1% SDS, 5 mM EDTA, protease inhibitors) and sonicated (nine 10-s pulses). Sonicated samples were incubated overnight with 5 μg of rabbit anti-histone H4 (sc 5) antibody (Serotec, Oxford, UK). A null-antibody immunoprecipitation served as negative control for each sample. 20 μl of antibody material. The soluble chromatin was immunoprecipitated and washed, and histone-DNA complexes were eluted from the antibody by adding elution buffer (1% SDS, 0.1 mM NaHCO₃). Histone-DNA cross-links were reversed in 0.2 M NaCl by heating at 65°C for 4 h. The DNA sample was further purified by 1-h incubation at 45°C in 0.1% EDTA, 0.04 M Tris-HCl, pH 8.1, and 0.4 M NaCl using phenol/chloroform, precipitated with ice-cold 100% ethanol, and resuspended in 50 μl of Tris/EDTA.

The promoter sequence of the human angiotensinogen gene was obtained from GenBank (AF424741). We used the TRANSFAC database (transfac.gbf.de/TRANSFAC) (25) to predict GR-binding sites in the human angiotensinogen gene.
the AGT promoter sequence. Two PCR primer pairs were designed to cover the first (AGT1) or second and third (AGT2) GR-binding sites (Table I). PCR was performed under standard conditions as described for RT-PCR using input samples as positive control. The following scheme was used: 94°C, 4 min; 35 cycles of 94°C, 45 s; 60°C, 45 s; 72°C, 45 s; and 10 min, 72°C. PCR products were run on a 2% agarose gel and visualized with ethidium bromide.

**RESULTS**

**Differential Effect of Dexamethasone on HK-2 and A549 Cells**—Stimulation of the renal epithelial cell line HK-2 with IL-1 (5 ng/ml) for 6 h resulted in increased production of inflammatory mediators, IL-6 and IL-8. To examine the effect of glucocorticoids on cytokine production, cells were preincubated for 2 h with dexamethasone and stimulated with IL-1 in the continuous presence of dexamethasone. No effect of dexamethasone (10−6 M) was observed on either constitutive or IL-1-induced production of IL-6 and IL-8 by HK-2 cells (Fig. 1, A and C). In contrast, dexamethasone completely abolished IL-8 production in the airway epithelial cell line A549 (Fig. 1B). Thus, already at 6 h after stimulation there is a clear discrimination in steroid responsiveness between renal and airway epithelial cells, which is more pronounced after 48 h (Fig. 1C).

**IκB-α Degradation Is Not Inhibited by Dexamethasone**—To identify the mechanism of steroid insensitivity in HK-2, we examined the ability of dexamethasone to repress the activity of NF-κB. Since inhibition of the NF-κB pathway by glucocorticoids has been suggested to be mediated via induction of IκB-α expression (11, 12), this pathway was first investigated. Western blot analysis showed that IL-1 induced degradation of IκB-α 15–60 min after stimulation, whereas protein levels of IκB-α were unaltered after treatment with dexamethasone for 0.5–24 h (Fig. 2A). Furthermore, dexamethasone was unable to prevent IL-1-induced degradation of IκB-α (Fig. 2B). After IκB-α degradation, prolonged stimulation of cells with IL-1 (4–24 h) increased IκB-α protein levels, suggesting the presence of an autoregulatory feedback mechanism (Fig. 2A). In accordance, IL-1 increased mRNA expression of IκB-α, which peaked at 1 h after stimulation (Fig. 2C). Dexamethasone showed no effect on IκB-α mRNA levels, which is compatible with our protein data.

**DNA Binding Activity of NF-κB Is Not Affected by Dexamethasone**—To study the effect of dexamethasone on DNA binding activity of NF-κB we performed EMSA. In non-stimulated HK-2 cells we could already detect NF-κB binding (Fig. 3A). Previously we have shown that primary renal epithelial cells expressed two NF-κB complexes, a p50-p65 heterodimer and a more abundant p50-p50 homodimer (24). Supershift analysis showed that also in HK-2 this p50-p50 complex was the most abundant complex, while the p50-p65 complex was expressed only very weakly. Stimulation of HK-2 for 1 h with IL-1 increased DNA binding activity, which was not inhibited when cells were stimulated in the presence of 1 μM dexamethasone (Fig. 3A, lane 4).
The inhibitory effect of dexamethasone on NF-xB has been specifically attributed to interaction with the p65 subunit of NF-xB (13, 14). To investigate the effect of dexamethasone on p65-mediated DNA binding, we used p65 and p50 transcription factor assay kits, which allow specific detection of activated p65 and p50. As shown in Fig. 3B, a high level of constitutive binding of p65 and p50 to the NF-xB consensus site could be detected in non-stimulated HK-2 cells compared with the steroid-sensitive cell line A549. Stimulation of HK-2 cells with IL-1 induced an increase in p65- and p50-mediated DNA binding activity, which was not blocked by pretreatment with dexamethasone.

**High Constitutive NF-xB Activation in HK-2 Cells**—The high constitutive level of DNA binding prompted us to study transcriptional activation by NF-xB in HK-2 cells using a NF-xB-responsive luciferase reporter gene, pGL-3x-B-Luc, which contains three NF-xB consensus sites. In non-stimulated HK-2 cells, a high constitutive activity of the reporter gene was observed, indicating a high level of constitutively active NF-xB in this cell type (Fig. 4A). In contrast, constitutive levels of reporter gene activity were low in transfected A549 cells. This was not due to a decreased transfection efficiency of A549 cells, which was measured with a control plasmid expressing green fluorescent protein (GFP; data not shown). These results are compatible with the DNA binding studies shown in Fig. 3.

Next, we studied the capability of dexamethasone to transrepress NF-xB-induced transcription of the reporter gene. Fig. 4B shows that in HK-2 cells, reporter gene activity was only minimally blocked by dexamethasone but was strongly repressed (>95%) by CAPE, a known inhibitor of NF-xB activation. In A549 cells, dexamethasone and CAPE suppressed xB-reporter gene activity by, respectively, 65 and 85%.

**Dexamethasone Induces Glucocorticoid Receptor Translocation**—To further analyze the mechanism of steroid action in renal epithelial cells we compared the glucocorticoid receptor signaling pathway in HK-2 and A549 cells. One of the early steps in glucocorticoid receptor signaling is translocation of GR from the cytosol to the nucleus upon activation of the receptor through ligand binding (1, 2). In both cell types, GR expression in control cells was mainly cytosolic as visualized with immunocytochemistry (Fig. 5, A and C). Already at 30 min after stimulation with 10^{-6} M dexamethasone an increase in nuclear staining was observed, which was more pronounced at 4 h (Fig. 5, B and D).

The effect of dexamethasone on GR translocation was also assessed quantitatively with Western blotting. Cytosolic and nuclear extracts were prepared after stimulation with increasing doses of dexamethasone (10^{-10} to 10^{-8} to 10^{-6} M) for 2 h. Glucocorticoid receptor expression in HK-2 cells was comparable to the expression levels in A549 (Fig. 5E). Stimulation of
HK-2 cells with \(10^{-8}\) and \(10^{-6}\) M dexamethasone induced an increase in nuclear GR, which was accompanied by a decrease in cytosolic GR (Fig. 5, E and F). Nuclear translocation induced by dexamethasone was similar in both cell types, suggesting that the first part of the GR signaling pathway in HK-2 cells is functional.

Dexamethasone Stimulates the Expression of Glucocorticoid-responsive Genes—In A549 cells it has been demonstrated that dexamethasone treatment induces an increase in \(\beta_2\)-adrenoreceptor mRNA levels and receptor number (26). Therefore, we analyzed the effect of dexamethasone on the expression of \(\beta_2\)-adrenoreceptor in HK-2 cells with Western blotting. In both cell types, dexamethasone (\(10^{-6}\) M) induced an increase in \(\beta_2\)-adrenoreceptor protein levels (Fig. 6).

Next, we performed semiquantitative ChIP assays to study AGT expression, which is known to be stimulated by glucocorticoids in vitro and in vivo (27–29). Sequence analysis of the AGT promoter (−1223 to +35) identified the presence of three putative GR-binding sites. Two different primer pairs were designed to amplify the first GR-binding site (AGT1) or the second and third GR-binding site (AGT2) as shown in Fig. 7A.

Immunoprecipitation with an antibody against histone H4 (ac 5) showed that in non-stimulated cells, a low level of constitutive histone H4 (Lys-5) acetylation was present (Fig. 7B). Stimulation of HK-2 cells with \(10^{-8}\) M dexamethasone resulted in a marked enrichment of AGT promoter DNA (Fig. 7B). The enrichment for AGT promoter segments was observed for both AGT1 and AGT2, suggesting that more than one GR-binding site within the AGT promoter is acetylated by dexamethasone-activated GR.

In addition we investigated whether the increase in histone acetylation upon stimulation with dexamethasone correlated with an increase in AGT mRNA expression. In accordance with the histone acetylation data, semiquantitative RT-PCR showed that angiotensinogen mRNA levels were increased in cells

![Fig. 4](image1.png)  
**Fig. 4. High constitutive NF-κB activation in HK-2 cells.** A, cells were transfected with 0.1 or 1 μg of a NF-κB-inducible reporter gene (pGL-3x-B-Luc) and assessed for luciferase activity. B, for inhibition studies, cells were pretreated with \(10^{-8}\) M dexamethasone (Dex) or 10 μg/ml CAPE for 1 h. Shown is the stimulation index of five independent experiments.

![Fig. 5](image2.png)  
**Fig. 5. Nuclear translocation of GR is comparable in HK-2 and A549 cells.** A–D, immunocytochemistry of GR localization. Cells were incubated with (B and D) or without (A and C) \(10^{-6}\) M dexamethasone (Dex) for 4 h. GR localization in A549 (A and B) and HK-2 cells (C and D) was analyzed by immunocytochemistry. Results are representative of four independent experiments. E, representative Western blot showing the effect of dexamethasone on GR localization. Cells were stimulated with dexamethasone (\(10^{-10}\) to \(10^{-6}\) M) for 2 h. Cytosolic and nuclear extracts were prepared and analyzed for GR protein levels. F, Western blots were analyzed with densitometry. Shown are mean ± S.D. from three independent experiments. *, \(p < 0.05\) versus control cytosolic extract; **, \(p < 0.01\) versus control cytosolic extract; #, \(p < 0.05\) versus control nuclear extract; ##, \(p < 0.01\) versus control nuclear extract.

![Fig. 6](image3.png)  
**Fig. 6. Increased expression of \(\beta_2\)-adrenoreceptors after stimulation with dexamethasone.** Cells were stimulated with dexamethasone (\(10^{-6}\) M) for 4 h. Expression levels of \(\beta_2\)-adrenoreceptors (\(\beta_2\)-AR) in whole cell lysates were analyzed by Western blot. One representative blot of three independent experiments is shown.
stimulated with dexamethasone for 4 h compared with non-stimulated cells (Fig. 7C).

**GR-mediated Transactivation Is Functional**—To compare the transactivating capacity of GR in HK-2 and A549, cells were transfected with a luciferase reporter gene, which is regulated by a promoter containing glucocorticoid-responsive elements (pMMF-Luc). Constitutive GR activity in HK-2 cells was very low compared with A549 cells (Fig. 8A). This was not due to altered transfection rates as confirmed by co-transfection with GFP-expressing control plasmids. At high concentrations of dexamethasone (10^{-8} M), stimulation of the reporter gene was comparable in both cell types (35-fold), indicating that the positive regulatory pathway of glucocorticoids is intact (Fig. 8B). However, transactivation of the reporter gene in HK-2 cells was less sensitive than in A549 cells with a log shift in EC_{50} for dexamethasone between the cell lines (Fig. 8, A and B).

**DISCUSSION**

In the present study we demonstrate dissociation of transrepression and transactivation by glucocorticoids in renal tubular epithelial cells. Glucocorticoids are potent suppressors of the immune system and are known to inhibit cytokine production by a variety of cell types (5–7). However, we have shown previously that cytokine and chemokine production by activated renal tubular epithelial cells is insensitive to the inhibitory action of dexamethasone (8). The present study was undertaken to investigate the mechanism of steroid action in renal epithelial cells. We compared the effect of dexamethasone on cytokine production by the renal epithelial cell line HK-2 with the airway epithelial cell line A549 and found that already at 6 h after stimulation with IL-1 there is a clear discrimination in steroid responsiveness between renal and airway epithelial cells, suggesting that an early signaling pathway might be involved. Suppression of cytokine production by glucocorticoids is mainly attributed to inhibition of NF-κB (3, 10), for which several mechanisms have been proposed (11–17). In our study, no effect of dexamethasone on NF-κB synthesis and DNA binding activity of NF-κB was observed. Furthermore, transactivation of an NF-κB-inducible reporter gene was not inhibited. The absence of transrepression by dexamethasone prompted us to investigate the glucocorticoid signaling pathway in HK-2. We compared the effect of dexamethasone on cytokine production by the renal epithelial cell line HK-2 with the airway epithelial cell line A549 and found that already at 6 h after stimulation with IL-1 there is a clear discrimination in steroid responsiveness between renal and airway epithelial cells, suggesting that an early signaling pathway might be involved. Suppression of cytokine production by glucocorticoids is mainly attributed to inhibition of NF-κB (3, 10), for which several mechanisms have been proposed (11–17). In our study, no effect of dexamethasone on NF-κB synthesis and DNA binding activity of NF-κB was observed. Furthermore, transactivation of an NF-κB-inducible reporter gene was not inhibited.

The absence of transrepression by dexamethasone prompted us to investigate the glucocorticoid signaling pathway in HK-2. We demonstrated that dexamethasone induced translocation of GR from the cytosol to the nucleus, suggesting that the first part of the signaling pathway is intact. Next, we examined the transactivation capacity of dexamethasone on the expression of AGT, a gene that is positively regulated by glucocorticoids in various cell types, including rat proximal tubular epithelial cells (27–29). Stimulation of gene transcription is thought to be mediated via acetylation of core histones on highly conserved lysine residues (2, 30). Co-activator molecules including the CREB-binding protein (CBP) are able to induce histone acetyl-
This suggests either that it is unlikely that NF-
transcription factor involved in increased cytokine production by HK-2 cells, but rather functions in conjunction with other signaling pathways, including mitogen-activated protein kinases (34, 35) or that a repressor must be removed to enable cytokine transcription to occur efficiently.

Alternatively, it has been shown that inhibition of transcription factors involves only a single GR monomer and that DNA binding is not required (36, 37), whereas activation of gene transcription by glucocorticoids is mediated via binding of GR homodimers to GRE in the promoter of glucocorticoid-responsive genes. Defective interaction of GR monomers with NF-κB might also explain the lack of transpression by glucocorticoids. Inhibition of gene transcription by glucocorticoids can also occur via negative GRE or via transpression of other transcription factors like AP-1. Whether these inhibitory functions of GR are also hampered in renal epithelial cells still has to be determined.

In conclusion, we have demonstrated that renal epithelial cells are responsive to positive but not to negative modulation by glucocorticoids. Decreased GR activity or increased constitutive activation of the NF-κB signaling pathway might contribute to the lack of suppression by glucocorticoids. These results emphasize the cell type-specific characteristics of glucocorticoid action.

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Fig. 8. Transactivation capacity of GR in HK-2 cells is functional but less sensitive than in A549 cells. A, cells were transfected with a GR-inducible reporter gene (pMMF-Luc) and stimulated with increasing concentrations of dexamethasone for 6 h. B, stimulation index of the experiment described in A. n = 3.
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