Biochemical Pharmacology, Vol. 52, pp. 1415–1421, 1996.


ELSEVIER

ISSN 0006-2952/96/$15.00 + 0.00
PII S0006-2952(96)00503-5

# Interference of Corticosteroids with Prostaglandin $E_2$ Synthesis at the Level of Cyclooxygenase-2 mRNA Expression in Kidney Cells

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**ABSTRACT.** In the kidney, prostanoids play a role as vasoactive and immunomodulatory mediators. One of the main biosynthetic enzymes, the inducible cyclooxygenase-2 (EC 1.14.99.1, Cox-2), has been recognized as a target of glucocorticoids. Therefore, we investigated whether the physiologically active corticosteroid aldosterone in the kidney might also interfere with prostaglandin (PG) synthesis. In two cell types, an epithelial cell line of tubular origin (MDCK) and rat renal mesangial cells, $PGE_2$ release, Cox activity and Cox mRNA expression were determined after stimulation with phorbol ester and IL-1β, respectively. An increase in $PGE_2$ release and Cox activity was observed, which correlated with an increase in Cox-2 mRNA expression. In MDCK cells, both dexamethasone and aldosterone were equally effective, suppressing all parameters measured by approximately 60%. A similar effect of aldosterone was also seen in mesangial cells, whereas dexamethasone was far more potent (>90% inhibition at $10^{-6}$ M). Whole cell binding assays showed the same number of receptors for aldosterone in both cell types (approximately 70,000 receptors/cell) but more than ten times higher receptor numbers for dexamethasone in mesangial cells than in MDCK cells (90,000 vs. 6000 receptors/cell). Receptor affinities of the corticosteroids were comparable. Thus, interaction of the corticosteroids with their cognate receptors was not sufficient to explain their different potencies but indicated the involvement of more complex regulatory mechanisms. Pathophysiologically, inhibition of $PGE_2$ synthesis by aldosterone may play a role in the induction of hypertension by high concentrations of aldosterone. BIOCHEM PHARMACOL 52;9:1415–1421, 1996. *Copyright © 1996 Elsevier Science Inc.*

**KEY WORDS.** prostaglandin synthesis; cyclooxygenase-2; mineralocorticoid; glucocorticoid; dexamethasone; aldosterone

Steroid receptors act as transcription factors that positively or negatively regulate gene expression (reviewed in [1–3]). GR† and MR are structurally closely related and can bind to identical consensus responsive elements [4]. Their physiological actions, however, greatly differ. The kidney is one of the main target organs of the naturally occurring mineralocorticoid Aldo and expression of the MR is found in cortex and medulla of rat kidney [5]. GR and MR expression were detectable in all segments of the nephron [6, 7]. The distribution of GR and MR along the nephron differed in accordance with the different functional role of these steroids in kidney physiology. Mineralocorticoids are primarily involved in regulation of ion transport by increasing reabsorption of sodium and secretion of potassium and hydrogen ions in the renal tubules. Glucocorticoids regulate renal gluconeogenesis and ammoniagenesis and are also discussed as mediators of mineralocorticoidlike effects. Pharmacologically, there are effective inhibitors of a wide variety of inflammatory mediators.

Multiple target genes regulated by GRs have been defined; these are either positively regulated, such as acute phase proteins in the liver [8], or negatively regulated, as is the case with cytokines or inflammation-related enzymes, where glucocorticoids interfere with induction [9]. In contrast, very few Aldo-sensitive genes are known, e.g., Na,K-adenosinetriphosphatase (Na,K-ATPase). Na,K-ATPase α1 and β1 mRNA levels are enhanced by aldosterone receptors in rat kidney epithelial cells [10], vascular smooth muscle cells [11–13] and colon epithelium [14], which is consistent with the stimulatory effect of Aldo on sodium and potassium transport.

In the kidney, prostanoids are locally acting hormones involved in the physiological and pathophysiological regulation of vascular resistance and glomerular filtration (reviewed in [15]). $PGE_2$ affects salt balance by enhancing sodium and water secretion. As in other tissues, prostanoids are involved in inflammatory reactions of the glomerulus.

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† *Abbreviations:* Aldo, aldosterone; Cox, cyclooxygenase; Dex, dexamethasone; ELISA, enzyme-linked immunosorbent assay; GAPDH, glyceraldehyde phosphate-3-dihydrogenase; GR, glucocorticoid receptor; IL, interleukin; MDCK, Madin Darby canine kidney cells; MR, mineralocorticoid receptor; PG, prostaglandin; TPA, 12-O-tetradecanoylphorbol 13-acetate.

Received 25 March 1996; accepted 17 June 1996.

Glucocorticoids interfere with PG synthesis at different levels: expression of the secreted and cytosolic forms of phospholipase $A_2$ (EC 3.1.1.4) is impaired by glucocorticoids in different cell types [16–19]. Furthermore, Cox isoforms, especially type 2 (inducible Cox-2, prostaglandin G/H synthase-2, EC 1.14.99.1), are targets of glucocorticoid action (reviewed in [20]).

We previously studied the effects of glucocorticoids on PG synthesis and Cox isozyme expression in monocytic cells [21, 22]. In those experiments, no effect of Aldo on PG synthesis and Cox expression was detected, most likely due to missing or insufficient numbers of MR in these cells [23]. In the present study, we investigated the effect of Aldo on PG synthesis in kidney cells, which are natural targets of Aldo action. As model systems, we used primary cultures of rat mesangial cells and the epithelial cell line MDCK (Madin Darby canine kidney). We and others have shown previously that IL-1β is a potent stimulus for $PGE_2$ synthesis related to Cox-2 mRNA and protein induction in mesangial cells [24, 25]. In MDCK cells, activation of protein kinase C by phorbol ester proved to be a useful model system to investigate regulation of $PGE_2$ synthesis [26]. In the present study, we were able to show that in both systems Cox expression is not only sensitive to glucocorticoid action but is also inhibited by the mineralocorticoid Aldo.

## Materials and Methods

### *Cell Culture*

MDCK cells (ATCC, Rockville, MD, USA) were grown in Dulbecco's modified Eagle's medium (DMEM; Gibco, Eggenstein, Germany) supplemented with 10% fetal calf serum (FCS), 2 mM glutamine, 50 U/mL penicillin and 50 μg/mL streptomycin. Cells were plated in Petri dishes at a cell density of $5 \times 10^5$ cells/mL. After 24 hr, the subconfluent cells were further incubated in DMEM with 0.5% FCS overnight. MDCK cells were stimulated with TPA (Sigma, Deisenhofen, Germany). Rat mesangial cells were isolated and cultured as described previously [27]. Part of the mesangial cell isolates were kindly provided by H. H. Radeke (Hannover Medical School). Cells were grown in DMEM supplemented with 2 mM L-glutamine, 5 μg/mL insulin, 100 U/mL penicillin, 100 μg/mL streptomycin and 10% FCS. For the experiments, cells were used between passages 10 and 25. Mesangial cells ($5 \times 10^5$/10 mL) were plated in 100-mm Petri dishes in medium with 10% FCS and used at subconfluency after 3–5 days.

### *Determination of $PGE_2$*

$PGE_2$ concentration in the cell culture supernatants was determined by a specific ELISA as described elsewhere [28]. In brief, microtiter plates were coated with $PGE_2$ coupled with bovine serum albumin. As standards, 10 different concentrations of $PGE_2$ (500–1 pg) were used in triplicate. The specific monoclonal mouse-anti-$PGE_2$ antibody was kindly provided by K. Brune (Institute of Pharmacology, University of Erlangen–Nürnberg). Complexed antibody was detected by biotinylated goat-anti-mouse antibody and horse radish peroxidase complexed with streptavidin biotin. Substrate was ABTS (2,2′-azino-di-[3-ethylbenzthiazoline-6-sulfonate]). Detection limit of the assay was 3 pg per well, corresponding to 0.03 ng/mL supernatant. Statistical significance was calculated by using the nonpaired two-tailed Student's t test. A $P$ value of less than 0.05 was considered statistically significant.

### *RT-PCR Analysis*

Total RNA was prepared with RNA-Clean (AGS, Heidelberg, Germany); 0.1 μg RNA was used for reverse transcription (RT) with Oligo $dT_{16}$ primers. For amplification by polymerase chain reaction (PCR), aliquots of the RT reaction were amplified with primers specific for Cox-2 and GAPDH (used as internal standard). The following primers were used: Cox-2 [29] sense primer 5′-CAT TCT TTG CCC AGC ACT TCA C-3′, Cox-2 antisense primer 5′-GAG GAG GGT AGA TCA TCT C-3′, GAPDH sense primer 5′-CCT TCA TTG ACC TCA ACT AC-3′ and GAPDH antisense primer 5′-GGA AGG CCA TGC CAG TGA GC-3′. Following agarose gel electrophoresis, the optical density of the ethidium bromide-stained DNA bands was measured by video densitometry (Bioprofil, Fröbel, Wasserburg, Germany). The ratio of Cox-2 to GAPDH absorbance was taken as a measure of Cox-2 mRNA expression. RT-PCR was evaluated as linear in terms of the amount of input mRNA and cycle number. Optimal conditions were: RT reaction: 0.1 μg RNA, 20 min 37°C, 20 min 42°C, 5 min 95°C, 5 min 4°C; PCR reaction: Cox-2: 2 min 95°C (1 min 95°C, 1 min 55°C, 2 min 72°C) × 24, 7 min 72°C, 5 min 4°C; GAPDH: 2 min 95°C (1 min 95°C, 1 min 55°C, 2 min 72°C) × 21, 7 min 72°C, 5 min 4°C.

### *Northern Blot Analysis*

Northern blot analysis was performed essentially as previously described [30]. The specific Cox-2 probe was a 1.156-kb EcoRI fragment from the 5′-end of mouse cDNA [31]. The probe was kindly provided by D. DeWitt (Michigan State University, MI, USA).

### *Determination of Cox Activity*

To determine Cox activity, MDCK cells were incubated with 10 μM arachidonic acid for the final 15 min of the stimulation time. Thereafter, the $PGE_2$ concentration was determined in the cell culture supernatants and corrected for $PGE_2$ release before the addition of exogenous arachidonic acid. Cox activity in mesangial cells was determined in the microsomal membrane fraction as previously described [25]. Microsomal membranes were incubated with $10^{-5}$ M arachidonic acid for 30 min. The specific activity was calculated as pg $PGE_2$ × mg $protein^{-1}$ × $min^{-1}$.

### *Quantitation of Corticosteroid Receptors*

The quantitation of GRs and MRs was performed by radioligand assay [32, 33]. As ligands, [$^3$H]-Dex (3.256 TBq/mmole; NEN, Bad Homburg, Germany) was used for quantitation of GR and [$^3$H]-Aldo (3.108 TBq/mmole, NEN) for quantitation of MR. As competitors, stock solutions (10 mM in ethanol) of unlabeled Dex or Aldo (both from Sigma) were diluted in phosphate buffered saline (PBS). Subconfluent cells were collected in PBS, pelleted and resuspended in PBS to a concentration of $10^6$ cells/mL.

The radioligand assay was performed in 96-well microtiter plates. In a final volume of 25 μL, $10^5$ cells were incubated with different tracer concentrations (40, 20, 10, 5, 2.5 and 1.25 nM final). To determine nonspecific binding, unlabeled corticosteroid was added at 1000-fold excess (final concentration, 40 μM). The microtiter plate was carefully shaken, tightly covered with a self-adhesive plastic wrap and incubated for 90 min at 37°C, which allowed the reaction to reach equilibrium, as determined in control experiments.

Separation of bound and free tracer was performed in a PHD cell harvester (Dunn, Asbach, Germany) by washing 5 times for 1 sec with double-distilled $H_2O$. The filters containing the ligand–receptor complexes bound to DNA were transferred to scintillation vials, and activity was determined by liquid scintillation counting (LS 6000IC, Beckman, Munich, Germany).

The dissociation rate constant ($K_d$), maximum binding ($B_{max}$) and receptor number per cell were estimated by Scatchard plot analysis.

## Results

### *Effects of Corticosteroids on Stimulated $PGE_2$ Synthesis*

Treatment of MDCK cells with TPA led to a concentration- and time-dependent stimulation of $PGE_2$ synthesis, as shown previously [26]. By stimulation with TPA at $10^{-8}$ M for 24 hr, the $PGE_2$ concentration was increased approximately 6-fold from 0.4 ± 0.2 ng/mL (control, n = 19) to 2.7 ± 0.5 ng/mL (n = 8, $P < 0.001$). Preincubation of MDCK cells with the glucocorticoid Dex or the mineralocorticoid Aldo for 2 hr followed by 24-hr treatment with TPA showed a concentration-dependent decrease in $PGE_2$ released into the culture supernatant (Fig. 1A). Effects of mineralocorticoid and glucocorticoid were comparable: at $10^{-6}$ M, the highest corticosteroid concentration used, the TPA-stimulated $PGE_2$ synthesis was reduced by Dex to 1.3 ± 0.3 ng/mL (n = 5, $P < 0.001$), corresponding to an inhibition of 60 ± 14%, and by Aldo to 1.3 ± 0.4 ng/mL (n = 4, $P < 0.001$), corresponding to an inhibition of 62 ± 18%.

For mesangial cells, IL-1β is a potent stimulus for $PGE_2$ [25]. $PGE_2$ concentration in the cell culture supernatants increased from 2.4 ± 1.6 ng/mL (control, n = 11) to 8.1 ± 4.1 ng/mL (n = 8, $P < 0.001$) following stimulation with IL-1β at 500 pg/mL for 20 hr and to 11.6 ± 4.5 ng/mL (n = 6, $P < 0.001$) after stimulation with IL-1β at 5000 pg/mL. In comparison with the MDCK cell line, the variability of $PGE_2$ release was higher because different preparations of primary cultures of mesangeal cells were used.

Preincubation with Dex or Aldo for 2 hr followed by treatment with IL-1β showed a concentration-dependent decrease in $PGE_2$ concentration (Fig. 1B). In these cells, Dex was far more potent than Aldo: at $10^{-6}$ M, Dex inhibited the IL-1β-stimulated $PGE_2$ synthesis by 94 ± 12% (n = 3, $P < 0.05$), whereas inhibition by Aldo was less pronounced, reaching 68 ± 16% (n = 3, $P < 0.05$) at $10^{-6}$ M.

### *Effects of Corticosteroids on Stimulated Cox Activity*

The changes in $PGE_2$ synthesis observed under corticosteroid treatment could be related to diminished Cox activity. In MDCK cells, Cox activity was determined by treatment of the cells for 15 min with a high concentration of exogenous arachidonic acid. Incubation of MDCK cells with

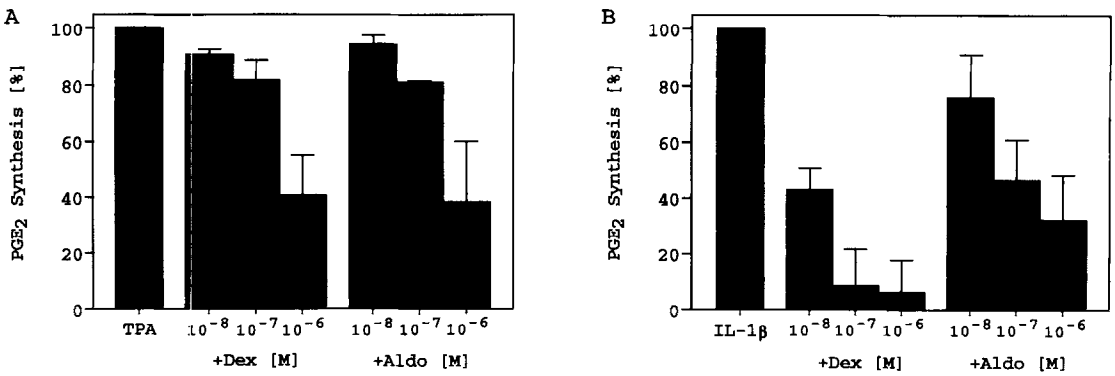


**FIG. 1. Inhibition of stimulated $PGE_2$ synthesis by corticosteroids. (A) MDCK cells were stimulated with TPA ($10^{-8}$ M) for 24 hr alone (black bar) or after preincubation with either Dex (light gray bar) or Aldo (dark gray bar) for 2 hr at the concentrations indicated. (B) Mesangial cells were stimulated with IL-1β (5 ng/mL) for 12 hr alone (black bar) or after preincubation with either Dex (light gray bar) or Aldo (dark gray bar) for 2 h at the concentrations indicated. $PGE_2$ concentration in the supernatants was determined as described in Materials and Methods. Data shown as percentage of stimulated $PGE_2$ synthesis are means ± SD of at least four individual experiments performed in triplicate.**

TPA for 24 hr induced a 6-fold increase in Cox activity (control: 0.5 ± 0.2 ng $PGE_2 \times mL^{-1} \times 15\ min^{-1}$, n = 2; TPA: 3.1 ± 0.7 ng $PGE_2 \times mL^{-1} \times 15\ min^{-1}$, n = 2). The TPA-induced Cox activity was reduced by $10^{-6}$ M Dex to 1.5 ± 0.4 ng $PGE_2 \times mL^{-1} \times 15\ min^{-1}$ (n = 2) and by $10^{-6}$ M Aldo to 1.6 ± 0.6 ng $PGE_2 \times mL^{-1} \times 15\ min^{-1}$ (n = 2), corresponding to an inhibition of approximately 60% by both corticosteroids (Fig. 2).

IL-1β enhances Cox activity in microsomal preparations of mesangeal cells [25]. Cox activity was detectable in unstimulated mesangeal cells, most likely due to activity of Cox-1, and was enhanced by incubation with IL-1β (3.0 ± 1.0 ng $PGE_2 \times mg\ protein^{-1} \times min^{-1}$; control: 1.3 ± 0.5 ng $PGE_2 \times mg\ protein^{-1} \times min^{-1}$, n = 4, $P < 0.05$). IL-1β-induced activity was reduced by 70% by using $10^{-7}$ M Dex and by 35% by using $10^{-7}$ M Aldo (Fig. 2). Neither Dex nor Aldo inhibited Cox activity in unstimulated cells (data not shown).

### *Effects of Corticosteroids on Cox mRNA Expression*

Changes in Cox activity may be due to changes in Cox-1 and/or Cox-2 mRNA expression. In MDCK cells, Cox-1 mRNA could be detected by neither Northern blot analyses using cDNA probes, which detected Cox-1 mRNA obtained from human, mouse and rat cells, nor semiquantitative RT-PCR using primers taken from a consensus sequence of human, mouse and rat cDNA (data not shown). Cox-2 mRNA was barely detectable by semiquantitative RT-PCR in unstimulated MDCK cells. Incubation with TPA revealed a concentration and time-dependent stimulation of Cox-2 mRNA expression (Fig. 3) [26]. Preincubation with Dex or Aldo for 2 hr followed by 24-hr treatment with $10^{-8}$ M TPA revealed a concentration-dependent decrease in Cox-2 mRNA expression (Fig. 4). At a corticosteroid concentration of $10^{-6}$ M, the TPA-stimulated Cox-2 mRNA expression was reduced by Dex to 51 ± 7% and by Aldo to 51 ± 6% (n = 3; Fig. 4). Inhibition of IL-1β-induced Cox-2 mRNA expression in mesangial cells by Dex and Aldo is shown in Fig. 5. In accordance with the effects of Dex and Aldo on Cox activity, Dex was a more potent inhibitor of Cox-2 mRNA expression than was Aldo in mesangial cells.

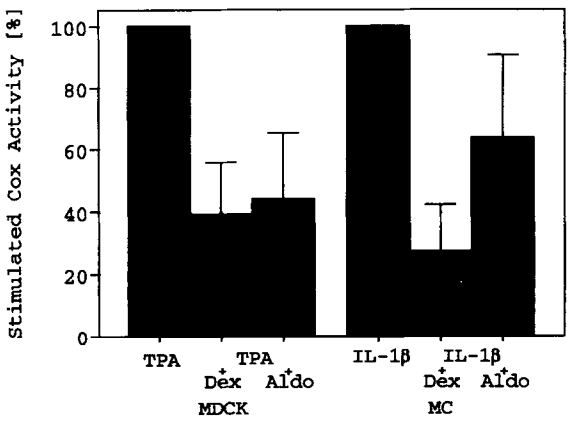


**FIG. 2. Inhibition of stimulated COX activity by corticosteroids. Cox activity was determined as described in Materials and Methods following stimulation with TPA ($10^{-8}$ M for 24 hr; MDCK-cells) or IL-1β (5 ng/mL for 12 hr; mesangial cells [MC]) alone (black bar) or after preincubation with either Dex (light gray bar) or Aldo (dark gray bar) for 2 hr at $10^{-6}$ M. Data shown as percentage of stimulated Cox activity are means ± SD of two individual experiments performed in triplicate.**

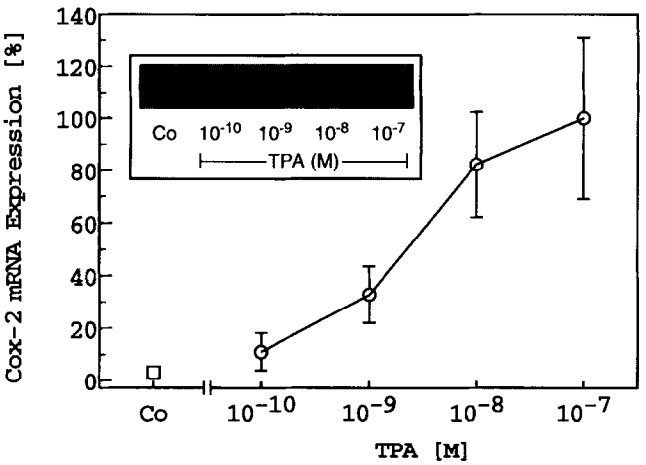


**FIG. 3. Concentration-dependent TPA stimulation of Cox-2 mRNA expression in MDCK cells. MDCK cells were stimulated for 24 hr with TPA at the concentrations indicated. Cox-2 mRNA expression was determined by using RT-PCR as described in Materials and Methods. Data shown as percentage of stimulation with $10^{-7}$ M TPA are means of three individual experiments. (Inset) Agarose gel of a representative RT-PCR showing the ethidium bromide-stained DNA bands of Cox-2.**

### *Quantitation of Corticosteroid Receptors*

GRs and MRs were investigated by a whole cell binding assay with Dex and Aldo as ligands. Competition of the binding of [$^3$H]-Dex to GR by a 1000-fold excess of Aldo and of [$^3$H]-Aldo to MR by a 1000-fold excess of Dex were both less than 10% (data not shown), indicating that $^3$H-Dex and [$^3$H]-Aldo specifically bound to their corresponding receptors.

Typical Scatchard plot analyses for GR and MR are shown in Fig. 6. Both corticosteroid receptors were present in both cell types. In MDCK cells, GR and MR had similar ligand affinities, with a $K_d$ of approximately 20 nM for Dex and Aldo. A major difference was observed with respect to receptor numbers: $B_{max}$ values (Fig. 6A, B) corresponded to approximately 65,000 receptors for Aldo but only 6000 receptors for Dex in MDCK cells. In contrast, the differences between GR and MR in mesangial cells were not significant regarding ligand affinities and receptor concentration (Fig. 6C, D).

## Discussion

In this study, we examined the influence of the glucocorticoid Dex and the mineralocorticoid Aldo on regulation of

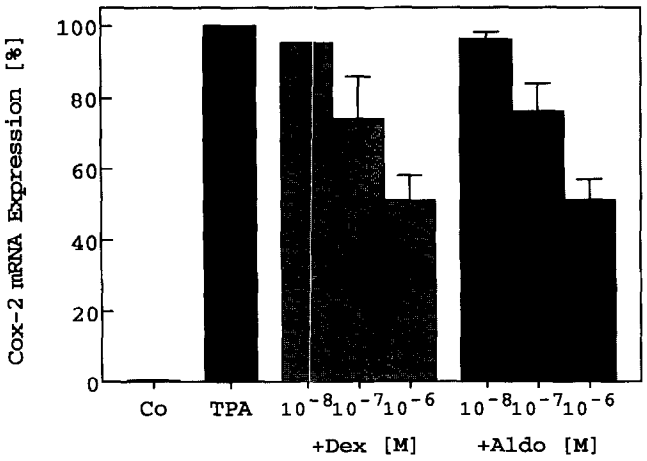



**FIG 4. Inhibition of stimulated Cox-2 mRNA expression in MDCK cells. MDCK cells were stimulated with TPA ($10^{-8}$ M) for 24 hr alone (black bar) or after preincubation with either Dex (light gray bar) or Aldo (dark gray bar) for 2 hr at the concentrations indicated. Cox-2 mRNA expression was determined by using RT-PCR as described in Materials and Methods. Data shown as percentage of TPA stimulation are means ± SD of three individual experiments. The agarose gel shows a representative RT-PCR with ethidium bromide-stained DNA bands of Cox-2 and GAPDH (as internal standard).**

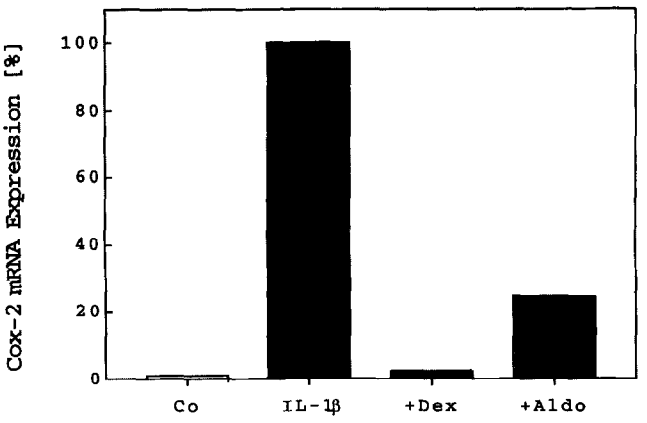



**FIG. 5. Inhibition of stimulated Cox-2 mRNA expression in mesangial cells. Mesangial cells were stimulated with IL-1β (5 ng/mL) for 12 hr alone (black bar) or after preincubation with either Dex (light gray bar) or Aldo (dark gray bar) for 2 hr at $10^{-6}$ M. Cox-2 mRNA expression was determined by using Northern blot analysis as described in Materials and Methods. The autoradiogram shows a representative Northern blot analysis hybridized with cDNAs specific for Cox-2 and GAPDH (as internal standard).**

$PGE_2$ synthesis in two types of renal cell: MDCK cells, i.e. epithelial cells originally obtained from dog distal tubule or collecting duct, and rat mesangial cells. Distal tubule and collecting duct are the main targets of mineralocorticoid action, whereas high levels of GRs are found in the glomerulus [6, 7]. The distal nephron and the glomerulus are also major sites of PG synthesis in the kidney [34]. In both cell types investigated, PG synthesis can be induced by appropriate stimuli. Enhanced PG synthesis is dependent on increased availability of the precursor fatty acid arachidonic acid and on Cox activity for further metabolism. We showed previously that in these cell types enhanced PG synthesis was related to an increase in expression of the inducible Cox isoform, Cox-2 [25, 26]. This enzyme is a target of glucocorticoid action, thus contributing to their anti-inflammatory effects. The molecular mechanism of Cox-2 inhibition by glucocorticoids has not yet been elucidated. In mesangial cells, inhibition of Cox-2 expression was claimed to be not at the mRNA but rather at the translational level [24]. In our experiments, Dex dose-dependently inhibited Cox-2 mRNA expression in both cell types. The extent of inhibition correlated well with the effects observed at the level of enzyme activity but does not exclude additional effects of glucocorticoids on Cox-2 translation. In MDCK cells, inhibition of Cox activity and expression was also reflected at the product level, PG synthesis being inhibited to the same extent (ca. 50–60%) by the highest concentration of Dex. In mesangial cells, PG synthesis was almost completely inhibited by high concentrations of Dex ($10^{-6}$ and $10^{-7}$ M), whereas Cox activity was impaired by 50–60%. These results hint at phospholipases as additional targets of glucocorticoid action. Secreted and cytosolic phospholipase $A_2$ have been described as being involved in PG synthesis in mesangial cells and are sensitive to glucocorticoid action [17].

PG synthesis was not only impaired by the glucocorticoid Dex but also by the mineralocorticoid Aldo. Compared with Dex, a weak effect was observed in mesangial cells: maximal inhibition of PG synthesis was 60%, whereas Cox activity and Cox-2 mRNA expression were impaired by 50%. In MDCK cells, in contrast, Aldo was as effective as Dex with regard to the parameters investigated. Cox-2 could thus be shown to be a target of Aldo action.

The effect of different glucocorticoids and mineralocorticoids is in part regulated by receptor density and affinity. Therefore, we determined the number of specific binding sites for Aldo and Dex in the cell types used. Receptor affinities were approximately 2-fold higher in mesangial cells than in MDCK cells, but no significant differences between Aldo and Dex were detected. Receptor affinities in the namomolar range were also determined with Aldo in the cortical portion of the collecting duct (reviewed in [5]) or in rabbit kidney cytosol [35]. Dex binding was observed

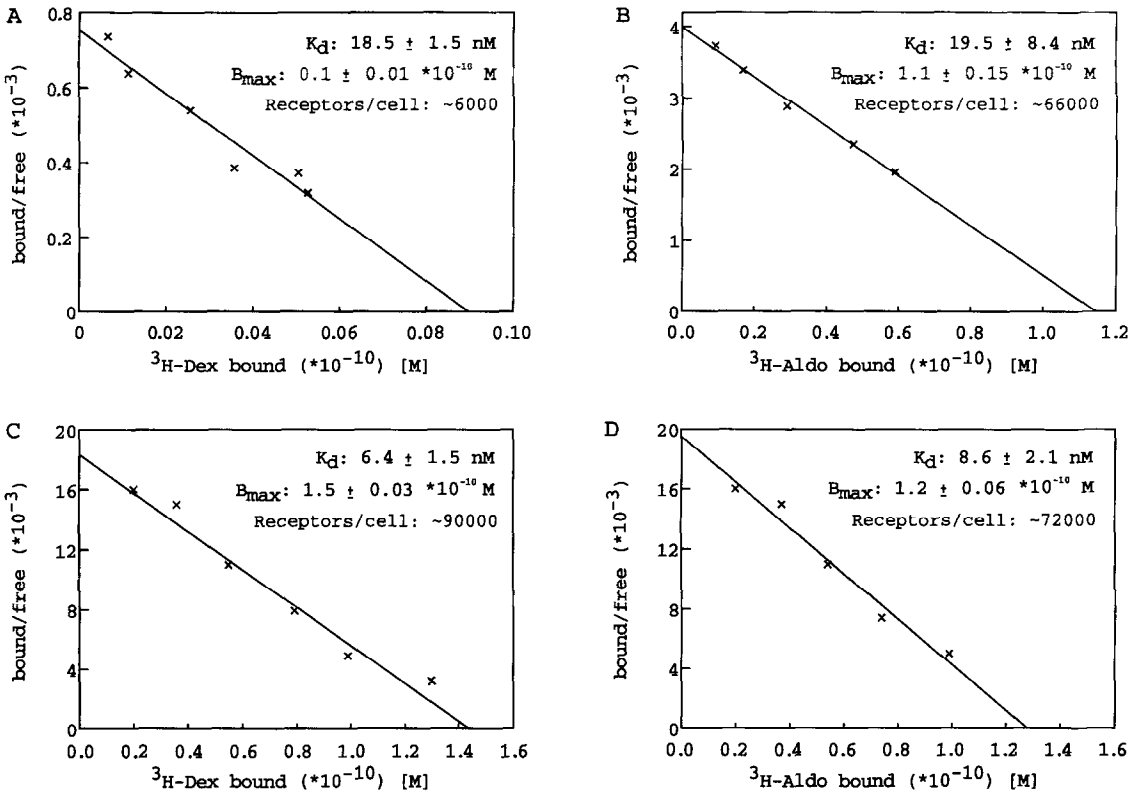


**FIG. 6. Quantitation of GRs and MRs. Determination of GR and MR concentration was performed by radioligand assay and calculated by using Scatchard blot analysis as described in Materials and Methods. Typical Scatchard blots are shown for MDCK cell GRs (A), MDCK cell MRs (B), mesangial cell GRs (C) and mesangial cell MRs (D).**

to be greater in tubular than in glomerular preparations of mouse kidneys [36]. These data are in accordance with the differences observed between mesangial cells and MDCK cells, which are tubular in origin. In mesangial cells, approximately equal numbers of receptors for Aldo and Dex were determined. The greater potency and efficacy of Dex in these cells, therefore, cannot be explained solely by the characteristics of hormone–receptor interaction. Additional mechanisms have to be postulated to add to the different effects on Cox-2 mRNA expression, possibly distinct interactions of both receptors with other transcription factors [37]. In MDCK cells, the number of GRs was more than 10-fold lower than in mesangial cells, whereas the number of MRs was comparable in both cell types. These differences are related to the biological effects: inhibition of Cox-2 mRNA expression was much more strongly impaired by Dex in mesangial cells, whereas the effects of Aldo were similar in both cell types. Taken together, the effect of gluco- and mineralocorticoid within one cell cannot be solely attributed to receptor binding. In contrast, the differences observed between the two cell types correlate well with the different receptor number. These data are in accordance with the general observation that cell specificity of corticosteroids is obtained by different mechanisms, among them receptor expression.

MRs are not only found in kidney cells but also in the gastrointestinal tract, colon, lung, glandular tissues, smooth muscle cell, osteoblasts, human skin and mononuclear lymphocytes [5]. All these tissues and cells are also able, when stimulated appropriately, to synthesize and secrete PGs. It remains to be investigated whether expression of Cox-2 in these cells will also turn out to be a target of mineralocorticoid action.

Functionally, Aldo and $PGE_2$, the predominant form of PG produced in the kidney, counteract: $PGE_2$ stimulates renin production, inhibits sodium reabsorption and inhibits vasopressin-induced water flux across the collecting tubule (reviewed in [38]) and therefore acts as vasodilator, whereas Aldo acts as vasopressor by interfering with similar mechanisms. Inhibition of $PGE_2$ synthesis thus might add to the mechanisms by which hyperaldosteronism.

*The technical assistance of M. Golombek is greatly appreciated. We thank Dr. A. Carter, Institute of Food Research, Norwich, UK, for critical reading of the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft (Ste 196/3-1 TP1 to MGS).*

## References

1. Fuller PJ, The steroid receptor superfamily: Mechanisms of diversity. *FASEB J* **5:** 3092–3099, 1991.
2. Beato M, Gene regulation by steroid hormones. *Cell* **56:** 335–344, 1989.
3. Evans RM, The steroid and thyroid hormone receptor superfamily. *Science* **240:** 889–895, 1988.

4. Lombes M, Binart N, Oblin M-E, Joulin V and Baulieu EE, Characterization of the interaction of the human mineralocorticosteroid receptor with hormone response elements. *Biochem J* **292:** 577–583, 1993.
5. Agarwal MK, Perspectives in receptor-mediated mineralocorticoid hormone action. *Pharmacol Rev* **46:** 67–87, 1994.
6. Farman N, Steroid receptors: distribution along the nephron. *Sem Nephrol* **12:** 12–17, 1992.
7. Todd-Turla KM, Schnermann J, Fejes-Toth G, Naray-Fejes-Toth A, Smart A, Killen PD and Briggs JP, Distribution of mineralocorticoid and glucocorticoid receptor mRNA along the nephron. *Am J Physiol* **264:** F781–F791, 1993.
8. Baumann H, Jahreis GP and Morella KK, Interaction of cytokine- and glucocorticoid-response elements of acute-phase plasma protein genes. *J Biol Chem* **265:** 22275–22281, 1990.
9. Goppelt-Struebe M and Resch K, Molecular mechanisms of the antiinflammatory action of glucocorticoids. In: *Advances in Rheuma and Inflammation* (Eds. Hedquist P, Kalden JR, Müller-Peddinghaus R and Robinson DR), pp. 219–228. Eular Verlag, Basel, 1991.
10. Whorwood CB, Ricketts ML and Stewart PM, Regulation of sodium-potassium adenosine triphosphate subunit gene expression by corticosteroids and 11 beta-hydroxysteroid dehydrogenase activity. *Endocrinology* **135:** 901–910, 1994.
11. Guchi A, Ikeda U, Kanbe T, Tsuruya Y, Yamamoto K, Kawakami K, Medford RM and Shimada K, Regulation of Na-K-ATPase gene expression by aldosterone in vascular smooth muscle cells. *Am J Physiol* **265:** H1167–H1172, 1993.
12. Verrey F, Schaerer E, Zoerkler P, Paccolat MP, Geering K, Kraehenbuhl JP and Rossier BC, Regulation by aldosterone of Na-K-ATPase mRNAs, protein synthesis, and sodium transport in cultured kidney cells. *J Cell Biol* **104:** 1231–1237, 1987.
13. Verrey F, Kraehenbuhl JP and Rossier BC, Aldosterone induces a rapid increase in the rate of Na-K-ATPase gene transcription in cultured kidney cells. *Mol Endocrinol* **3:** 1369–1376, 1989.
14. Wiener H, Nielsen JM, Klaerke DA and Jorgensen PL, Aldosterone and thyroid hormone modulation of alpha 1-, beta 1-mRNA, and Na,K-pump sites in rabbit distal colon epithelium. *J Membr Biol* **133:** 203–211, 1993.
15. Bonventre JV and Nemenoff R, Renal tubular arachidonic acid metabolism. *Kidney Int* **39:** 438–449, 1991.
16. Nakano T, Ohara O, Teraoka H and Arita H, Glucocorticoids suppress group II phospholipase A2 production by blocking mRNA synthesis and post-transcriptional expression. *J Biol Chem* **265:** 12745–12748, 1990.
17. Muhl H, Geiger T, Marki F, van den Bosch H, Cerletti N, Cox D, McMaster G, Fosbeck K and Pfeilschifter J, Transforming growth factors type beta and dexamethasone attenuate group II phospholipase A2 gene expression by interleukin-1 and forskolin in rat mesangial cells. *FEBS Lett* **301:** 190–194, 1992.
18. Gewert K and Sundler R, Dexamethasone down-regulates the 85 kDa phospholipase A2 in mouse macrophages and suppresses its activation. *Biochem J* **307:** 499–504, 1995.
19. Hoeck WG, Ramesha CS, Chang DJ, Fan N and Heller RA, Cytoplasmic phospholipase A2 activity and gene expression are stimulated by tumor necrosis factor: dexamethasone blocks the induced synthesis. *Proc Natl Acad Sci USA* **90:** 4475–4479, 1993.
20. Goppelt-Struebe M, Regulation of prostaglandin endoperoxide synthase (cyclooxygenase) isozyme expression. *Prostagl Leuk Essent Fatty Acids* **52:** 213–222, 1995.
21. Hoff T, DeWitt D, Kaever V, Resch K and Goppelt-Struebe M, Differentiation-associated expression of prostaglandin G/H synthase in monocytic cells. *FEBS Lett* **320:** 38–42, 1993.
22. Hoff T, Spencker T, Emmendoerffer A and Goppelt Struebe M, Effects of glucocorticoids on the TPA-induced monocytic differentiation. *J Leuk Biol* **52:** 173–182, 1992.
23. Koehler L, Hass R, DeWitt DL, Resch K and Goppelt-Struebe M: Glucocorticoid-induced reduction of prostanoid synthesis in TPA-differentiated U937 cells is mainly due to a reduced cyclooxygenase activity. *Biochem Pharmacol* **40:** 1307–1316, 1990.
24. Coyne DW, Nickols M, Bertrand W and Morrison AR, Regulation of mesangial cell cyclooxygenase synthesis by cytokines and glucocorticoids. *Am J Physiol* **263:** F97–F102, 1992.
25. Martin M, Neumann D, Hoff T, Resch K, DeWitt DL and Goppelt-Struebe M, Interleukin-1-induced cyclooxygenase 2 expression is suppressed by cyclosporin A in rat mesangial cells. *Kidney Int* **45:** 150–158, 1994.
26. Schaefers H-J, Haselmann J and Goppelt-Struebe M, Regulation of prostaglandin synthesis in Madin Darby canine kidney cells: Role of prostaglandin G/H synthase and secreted phospholipase A2. *Biochim Biophys Acta* **1300:** 197–202, 1996.
27. Lovett DH, Ryan JL and Sterzel RB, Stimulation of rat mesangial cell proliferation by macrophage interleukin 1. *J Immunol* **131:** 2830–2836, 1983.
28. Schaefer D, Lindenthal U, Wagner M, Bölcskei PL and Baenkler H-W, *Thorax*, Effect of prostaglandin $E_2$ on eicosanoid release by human bronchial biopsy specimens from normal and imflamed mucosa. In press.
29. Appleby SB, Ristimaki A, Neilson K, Narko K and Hla T, Structure of the human cyclo-oxygenase-2 gene. *Biochem J* **302:** 723–727, 1994.
30. Stroebel M and Goppelt-Struebe M, Signal transduction pathways responsible for serotonin-mediated prostaglandin G/H synthase expression in rat mesangial cells. *J Biol Chem* **269:** 22952–22957, 1994.
31. DeWitt DL and Meade EA, Serum and glucocorticoid regulation of gene transcription and expression of the prostaglandin H synthase-1 and prostaglandin H synthase-2 isozymes. *Arch Biochem Biophys* **306:** 94–102, 1993.
32. Rupprecht M, Rupprecht R, Kornhuber J, Wodarz N, Koch HU, Riederer P and Hornstein OP, Elevated glucocorticoid receptor concentrations before and after glucocorticoid therapy in peripheral mononuclear leukocytes of patients with atopic dermatitis. *Dermatologica* **182:** 100–105, 1991.
33. Rupprecht R, Kornhuber J, Wodarz N, Göbel C, Lugauer J, Sinzger C, Beckmann H, Riederer P and Müller OA, Characterization of glucocorticoid binding capacity in human mononuclear lymphocytes: increase by metyrapone is prevented by dexamethasone pretreatment. *J Neuroendocrinol* **2:** 803–806, 1990.
34. Farman N, Pradelles P and Bonvalet JP, PGE2, PGF2a, 6-keto-PGF1a and TxB2 synthesis along the rabbit nephron. *Am J Physiol* **252:** F53–F59, 1987.
35. Rafestin-Oblin ME, Lombes M, Lustenberger P, Blanchardie P, Michaud A, Cornu G and Claire M, Affinity of corticosteroids for mineralocorticoid and glucocorticoid receptors in the rabbit kidney: effect of steroid substitution. *J Steroid Biochem* **25:** 527–534, 1986.
36. Ellis D, Turocy JF, Sweeney WE and Avner ED, Partial characterization and ontogeny or renal cytosolic glucocorticoid receptors in mouse kidney. *J Steroid Biochem* **24:** 997–1003, 1986.
37. Pearce D and Yamamoto KR, Mineralocorticoid and glucocorticoid receptor activities distinguished by nonreceptor factors at a composite response element. *Science* **259:** 1161–1165, 1993.
38. Bonventre JV, Phospholipase A2 and signal transduction. *J Am Soc Nephrol* **3:** 128–150, 1992.