Tumor Necrosis Factor α and Interleukin 1β Enhance the Cortisone/Cortisol Shuttle

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Summary

Endogenously released or exogenously administered glucocorticosteroids are relevant hormones for controlling inflammation. Only 11β-hydroxy glucocorticosteroids, but not 11-keto glucocorticosteroids, activate glucocorticoid receptors. Since we found that glomerular mesangial cells (GMC) express 11β-hydroxysteroid dehydrogenase 1 (11β-OHSD1), which interconverts 11-keto glucocorticosteroids into 11β-hydroxy glucocorticosteroids (cortisone/cortisol shuttle), we explored whether 11β-OHSD1 determines the antiinflammatory effect of glucocorticosteroids. GMC exposed to interleukin (IL)-1β or tumor necrosis factor α (TNF-α) release group II phospholipase A2 (PLA2), a key enzyme producing inflammatory mediators. 11β-hydroxy glucocorticosteroids inhibited cytokine-induced transcription and release of PLA2 through a glucocorticoid receptor–dependent mechanism. This inhibition was enhanced by inhibiting 11β-OHSD1. Interestingly, 11-keto glucocorticosteroids decreased cytokine-induced PLA2 release as well, a finding abrogated by inhibiting 11β-OHSD1. Stimulating GMC with IL-1β or TNF-α increased expression and reductase activity of 11β-OHSD1. Similarly, this IL-1β– and TNF-α–induced formation of active 11β-hydroxy glucocorticosteroids from inert 11-keto glucocorticosteroids by the 11β-OHSD1 was shown in the Kiki cell line that expresses the stably transfected bacterial β-galactosidase gene under the control of a glucocorticosteroids response element. Thus, we conclude that 11β-OHSD1 controls access of 11β-hydroxy glucocorticosteroids and 11-keto glucocorticosteroids to glucocorticoid receptors and thus determines the anti-inflammatory effect of glucocorticosteroids. IL-1β and TNF-α upregulate specifically the reductase activity of 11β-OHSD1 and counterbalance by that mechanism their own proinflammatory effect.

IL-1β and TNF-α often act synergistically and cause a wide array of in vitro and in vivo immune inflammatory responses such as the secretion of phospholipase A2 (PLA2)[1], a key enzyme that releases arachidonic acid and therefore boosts prostaglandin production and secretion (1–3). This inflammatory reaction is regulated by 11β-hydroxy glucocorticosteroids; for instance, glucocorticoid deficiency increases, whereas physiological and pharmacological doses of glucocorticosteroids suppress the enhanced expression of group II PLA2 during inflammation (4–7). The biological activity of glucocorticosteroids depends on their dose, metabolism, local access to their cognate receptors, and on the responsiveness of the target cells (8, 9). Traditionally the 11-keto–glucocorticosteroid molecules are believed to have hardly any biological activity because of their negligible affinity to glucocorticoid receptors. In the present investigation, we demonstrate that during inflammation, 11-keto steroids exhibit antiinflammatory properties. This effect is dependent on the activity of the enzyme 11β-hydroxysteroid dehydrogenase (11β-OHSD), which interconverts the 11-keto and the corresponding 11β-hydroxy glucocorticosteroids by the so-called cortisone/cortisol shuttle (8, 10–15; Fig. 1).

Two isoenzymes accounting for 11β-OHSD activity have been cloned and characterized: 11β-OHSD1 (11) is dependent on the reduced form of nicotinamide adenine dinucleotide phosphate [NADP(H)] and catalyses both the oxidation and the reduction reactions, whereas 11β-OHSD2 requires nicotinamide adenine dinucleotide (NAD) as a cofactor and exhibits only oxidative activity (12). The biological role of 11β-OHSD2 is most likely to provide selective access of aldosterone to the mineralocorticoid receptor by inactivating cortisol (8, 13–15). The absence of 11β-
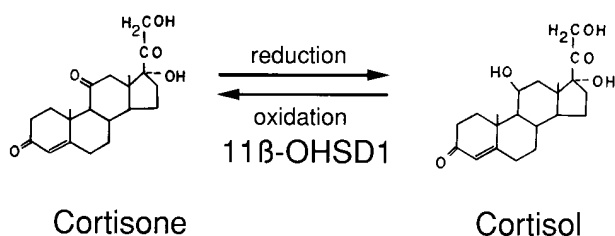

Figure 1. Cortisone/cortisol shuttle. The endogenous hormones cortisol and corticosterone, as well as the pharmacologically used prednisolone, are biologically active 11β-hydroxy glucocorticosteroids because they can bind to the cognate receptor. The corresponding 11-keto glucocorticoids cortisone, dehydrocorticosterone, and prednisone are unable to do so. The enzyme 11β-OHSD1 converts 11-keto glucocorticosteroids to 11β-hydroxy glucocorticosteroids and vice versa, and thus regulates local intracellular access of the steroids to the receptors. 11β-OHSD activity can be inhibited by glycyrrhetinic acid, a compound found in licorice and anise. Corticosterone and dehydrocorticosterone differ from cortisol and cortisone because of the absence of a hydroxyl group at position C_{17}, whereas prednisolone and prednisone have an additional double bond in the A ring.

OHSD2 results in apparent mineralocorticoid excess with hypertension and hypokalemia. Because specific inactivation of cortisol is relevant only in distal tubular cells of the kidney, salivary glands, and colon (the target cells of aldosterone), 11β-OHSD2 is almost exclusively expressed in this subset of cells. 11β-OHSD1, on the other hand, is expressed in a wide variety of tissues, but its function is still not clear. In this report, we studied the role of 11β-OHSD1 in glomerular mesangial cells (GMC). These proinflammatory cells were chosen because they play a pivotal role in certain forms of glomerular diseases. During inflammation, these cells release active substances such as enzymes, vasoactive endobiotics, extracellular matrix components, prostaglandins, and cytokines such as IL-1β and TNF-α, which cause local glomerular tissue damage (6, 16–18). In the present investigation, it is demonstrated that the activity of the 11β-OHSD1 determines the antiinflammatory effect of 11β-hydroxy glucocorticosteroids and that the proinflammatory endobiotics IL-1β and TNF-α upregulate the reductase activity of 11β-OHSD1, and thus, these cytokines display a dual mode of action in that they induce concomitantly inflammation and an antiinflammatory response.

Materials and Methods

Supplies. For cell culture and 11β-OHSD assay, corticosterone, dehydrocorticosterone, glycyrrhetinic acid, transferrin, and insulin were obtained from Sigma Chemical Co. (Buchs, Switzerland), and NAD phosphate (NADP), NADPH, and NAD were from Boehringer Mannheim (Rotkreuz, Switzerland). [1,2,6,7 ³H]corticosterone with a specific activity of 83 Ci/ mM and [³H]oleic acid (specific activity 10 Ci/mM) were purchased from Amersham Intl. (Buckinghamshire, U.K.). [³H]dehydrocorticosterone was prepared as already described (19). Bicinchonic acid protein assay reagent was from Pierce Chemical Co. (Rockford, IL). Triton X-100 and TLC plates (60 fluorescence indicator 254) coated with silica gel were from Merck (Schweiz) AG (Dietilcon, Switzerland). RPMI-1640, penicillin G (100,000 U/liter) and

streptomycin sulfate (100,000 μg/liter) were obtained from GIBCO BRL (Basel, Switzerland); FCS was from Biological Industries (France). Tissue culture plates (24-well plates) were obtained from Becton Dickinson Labware (Basel, Switzerland), IL-1β and TNF-α were from Pharma Biotechnologie (Hannover, Germany), and forskolin was from Calbiochem-Novabiochem (Luzern, Switzerland). RU 486 was a gift from Dr. B. Stadler (Institute of Immunology, Berne, Switzerland). For reverse transcription and polymerase chain reaction, deoxynucleotides (dNTPs), RNAse inhibitor, avian myeloblastosis virus reverse transcriptase, and BSA were obtained from Boehringer Mannheim. Primers were ordered from Microsynth (Balgach, Switzerland) and Thermus aquaticus DNA polymerase from Perkin-Elmer Cetus Instrs. (Norwalk, CT). The enhanced chemiluminescence detection kit was purchased from Amersham Intl.

Cell Cultures. GMC were cultured from isolated rat (Sprague-Dawley) glomeruli (20). In brief, the cells were grown in RPMI-1640 supplemented with 10% FCS, penicillin (100,000 U/liter), streptomycin (100,000 μg/liter), transferrin (5 mg/liter), insulin (5 mg/liter), and sodium selenite (5 μg/liter). For the experiments, passages 20–30 were used. For 11β-OHSD assays, confluent GMC cultures in 15-mm–diameter wells were incubated with 500 μl RPMI-1640 medium containing 10% FCS and increasing concentrations of IL-1β, TNF-α, or both for 48 h in a CO_2 incubator maintained at 37°C. The medium was removed and conversion by stimulated GMC of corticosterone to dehydrocorticosterone and dehydrocorticosterone to corticosterone was analyzed in situ by incubating the cells for 4 h with 200 μl medium containing 5 nCi [³H]corticosterone or [³H]dehydrocorticosterone and 0.5 μM corticosterone or dehydrocorticosterone. After incubation, the medium was extracted with 200 μl ethyl acetate and TLC were performed as described below. Protein was extracted from the cells by adding 200 μl 0.4 N NaOH at 37°C for 1 h after neutralization with the same volume of 0.4 N HCl. Specific activity was expressed as the percentage of conversion of corticosterone to dehydrocorticosterone, and dehydrocorticosterone to corticosterone, respectively, per milligram of total protein during 4 h. For PLA2 assays, confluent GMC cultures in 15-mm–diameter wells were incubated with 500 μl RPMI-1640 medium containing 10% FCS and 1 nM of IL-1β with and without indicated concentrations of corticosterone and/or glycyrrhetinic acid, and forskolin or RU 486. 24 h later, the medium was removed, centrifuged for 5 min at 4,000 rpm and used for PLA2 assay. COS-1 cells were cultured and transfected with the cDNA of 11β-OHSD1 as previously described (19).

Kiki cells are a cell line derived from rat embryonal 3Y1 cells (21). They were engineered to carry a bacterial β-galactosidase gene (lacZ) under control of the mouse mammary tumor virus promoter. This promoter contains essential glucocorticoid response elements. Thus, Kiki cells express β-galactosidase when exposed to sufficient doses of 11β-hydroxy steroids. Kiki cells were cultured in DMEM supplemented with 10% FCS, 60 mg/liter kanamycin, and 30 mg/liter hygromycin B. For steroid activity assays, the cells were incubated for 72 h with a combination of steroids and cytokines, and then subjected to the in situ β-galactosidase assay as described (21).

Assay for 11β-OHSD1. The assay was performed as previously described by Monder et al. (22). Oxidation or reduction at C-11 was determined by measuring the rate of conversion of corticosterone to 11-dehydrocorticosterone in the presence of NADP or dehydrocorticosterone to corticosterone in the presence of NADPH. GMC were extracted with 10 mM Tris-HCl (pH 7.5), 5 mM EDTA (pH 8), 1% Triton X-100, 2 mM PMSF, and 100

μg total protein were used for the reaction. The assay was performed in 0.25 mM NADP or NADPH, 100 mM Tris (pH 8.3), 10 nCi [³H]corticosterone or [³H]dehydrocorticosterone, and 5 μM corticosterone or dehydrocorticosterone. Samples were incubated for 3 h at 37°C, reaction was stopped on ice, and steroids were extracted with 500 μl ethyl acetate. The organic layer was separated by centrifugation at 13,000 rpm and evaporated under a stream of nitrogen. The steroid residue was dissolved in 20 μl methanol containing a mixture of 20 μg each of unlabeled corticosterone and dehydrocorticosterone. This was quantitatively transferred to thin-layer plates and developed in chloroform-methanol (90:10 vol/vol). The spots corresponding to the steroids were located under a UV lamp, cut out, transferred to scintillation vials, and counted in scintillation fluid in a Kontron (Zurich, Switzerland) Betamatic fluid scintillation counter. Specific activity was expressed as nanomolar of product formed per microgram protein per hour.

Assay for 11β-OHSD2. The assay was performed as previously described by Albitson et al. (12). Homogenization of cells for measurement of 11β-OHSD2 activity was performed in homogenization buffer containing 250 mM sucrose and 10 mM Tris-HCl (pH 7.5). Protein extract was incubated for 3 h at 37°C with 1 mM NAD, 10 nM corticosterone, and 50 nCi [³H]corticosterone in 500 μl homogenization buffer. The subsequent steps were the same as those described for 11β-OHSD1.

Assay for 17β-OHSD. The stimulation experiments were performed in parallel with those for 11β-OHSD1 measurements. 17β-OHSD activity was performed as already described (23) by measuring in situ the conversion of estradiol to estrone or estrone to estradiol. GMC were incubated for 4 h with 200 μl medium containing 5 nCi [³H]estradiol or [³H]estrone and 0.5 μM estradiol or estrone. After incubation, the medium was extracted with 1 vol diethyl ether and frozen. The unfrozen organic layer was poured into a fresh tube, evaporated under a stream of nitrogen, and TLC were performed in 4:1 dichloromethane/ethyl acetate, using 20 μg unlabeled estradiol and estrone. The next steps were the same as for 11β-OHSD1.

Assay for PLA2 Activity. [³H]oleic acid–labeled *Escherichia coli* were prepared as already described (24, 25). PLA2 was assayed using [³H]oleate-labeled, autoclaved *E. coli* as the substrate (26). The reaction mixture of 350 μl contained 100 mM Tris-HCl (pH 8.0), 5 mM Ca²⁺, 2.85 × 10⁸ cells of autoclaved *E. coli* (corresponding to 10,000 cpm and 5.0 nm lipid phosphorus), and tissue acid extracts or supernatants of GMC. The amount of protein was chosen such that 6–15% hydrolysis of substrate was obtained when incubated at 37°C for 2 h. The reaction was stopped by adding 100 μl of 2N HCl. 100 μl of fatty acid–free BSA (100 μg/ml) was added, and the tubes were vortexed and centrifuged at 13,000 rpm for 5 min. An aliquot (140 μl) of the supernatant containing released [³H]oleic acid was mixed with scintillation cocktail and counted in a liquid scintillation counter.

Reverse Transcription of Messenger RNA and PCR. Total RNA was extracted from GMC after the guanidium thiocyanate method (27). The RNA concentration was determined by measuring the absorption at 260 nm and its quality was controlled by loading 1 μg on a 1% formaldehyde gel. Reverse transcription was performed in 20 μl containing 50 mM Tris-HCl (pH 8.2), 6 mM MgCl₂, 10 mM dithiothreitol, 100 mM NaCl, 200 μM dNTPs, 11 U of ribonuclease inhibitor RNAsin, 1 U avian myeloblastosis virus reverse transcriptase, 10 pmol 3′ primer of the corresponding cDNA position (852–873 for 11β-OHSD1, 1271–1295 for 11β-OHSD2, 335–359 for group I PLA2, 695–719 for group II PLA2, 980–1004 for the internal standard glyceraldehydephosphate dehydro-

genase [GAPDH]), and 2 μg total RNA (4, 11, 12, 24, 28). Initially, the 3′ primer was incubated with total RNA for 5 min at 65°C and cooled at room temperature for 15 min. The remaining reaction components were added and incubated for 1 h at 42°C.

PCR was performed in a total volume of 30 μl with 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 200 μM dNTPs, 10 pmol of the appropriate 3′ and 5′ cDNA primers (5′ primer: 117–137 for 11β-OHSD1, 381–406 for 11β-OHSD2, 38–62 for group I PLA2, 58–82 for group II PLA2 II, 66–90 for GAPDH), 6 μg BSA, 1 μCi [α-³²P]desoxy CTP (dCTP), 2 μl of reverse transcribed cDNA, 1 U Thermus aquaticus DNA polymerase. The mixture was overlaid with mineral oil and cDNA was amplified with a DNA thermal cycler (Perkin Elmer Cetus) for 35 cycles. The amplification profile involved denaturation at 94°C for 1 min and 15 s, primer annealing at 60°C for 2 min, and elongation of annealed primers at 72°C for 3 min. 10 μl of each PCR reaction mixture was mixed with 2 μl of sixfold concentrated loading buffer and applied on a 0.9% agarose gel containing ethidium bromide. Electrophoresis was carried out with a constant voltage of 8 V/cm for 40 min. Bands were visualized under UV light and excised from the gel. The radioactivity was measured in a liquid scintillation β-counter using a Cerenkov program.

Western Blot Analysis. Electrophoresis was performed using a 12.5% polyacrylamide gel under reducing conditions. Prestained protein standards were used as markers. Probes (25 μg of total protein) were heated along with SDS sample buffer at 95°C for 15 min. After electrophoresis, the gel was equilibrated in transfer buffer (20 mM Tris, 190 mM glycine, 20% methanol) for 10 min. The transfer of protein to an Immobilon membrane (Millipore Corp., Bedford, MA) was performed with a constant voltage of 60 V for 1 h on ice. The membrane was blocked in 5% BSA in PBS for 2 h, washed with PBS, and incubated over night with a rabbit polyclonal antibody for 11β-OHSD1 (gift from Carl Monder, The Population Council, New York, NY). The Immobilon membrane was washed with PBS, saturated again for 2 h in 5% BSA, and incubated for 1 h at room temperature with a goat anti–rabbit IgG horseradish peroxidase conjugate (Bio Rad Labs., Hercules, CA). After washing, the detection was performed with the enhanced chemiluminescence kit. The bands on the x-ray film were scanned with a transmittance scanning densitometer (Scanalytics, Billerica, MA).
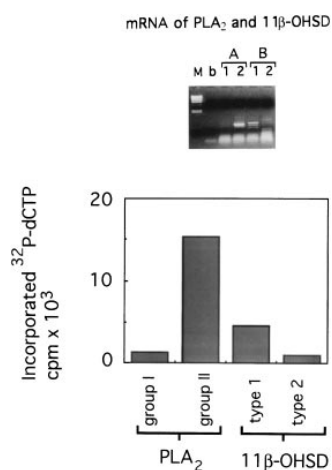

Figure 2. GMC contain 11β-OHSD1 and group II PLA2 transcripts. (*Top*) Agarose gel electrophoresis of PCR products of GMC. The mRNA of PLA2 and 11β-OHSD isoenzymes was reverse transcribed from 5 μg of total RNA using appropriate primers, and one sixth of that mixture was amplified by PCR. *M*, molecular weight marker (λ-HindIII, GIBCO BRL); *b*, blank (no cDNA); *A1*, group I PLA2; *A2*, group II PLA2; *B1*, 11β-OHSD1; *B2*, 11β-OHSD2. (*Bottom*) Incorporation of [α³²P]-dCTP (cpm) into cDNA of PLA2 and 11β-OHSD. Each value represents the average of two determinations. These results indicate negligible transcription of group I PLA2 and 11β-OHSD2, and substantial transcription of group II PLA2 and 11β-OHSD1.
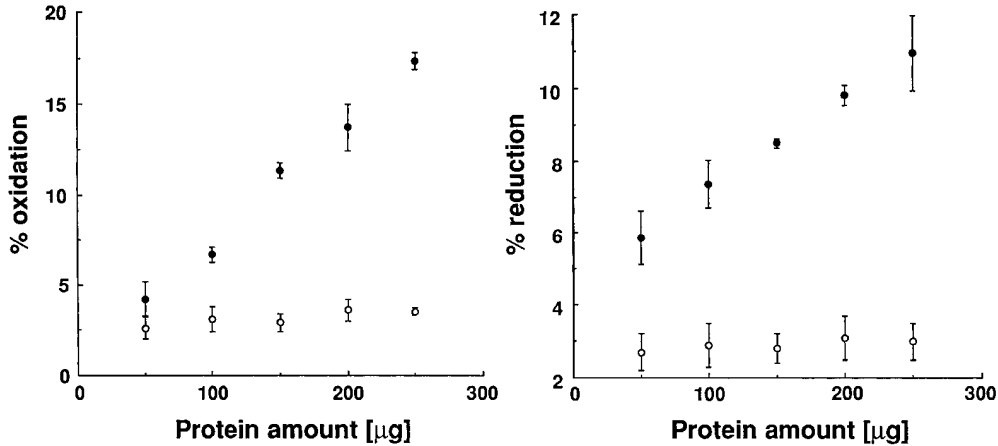
Figure 3. GMC contain 11β-OHSD1 activity. The amount of protein extract from GMC versus 11β-OHSD1 oxidation (*left*) and reduction (*right*) activity is displayed. Enzyme activity is expressed as percentage of substrate converted into product in 3 h. Closed and open circles represent values of 11β-OHSD1 activity without and with 5 μM glycyrrhetinic acid, respectively. Each value is the average (± SEM) of triplicate determinations. Both oxidation and reduction activities increased with increasing amounts of the protein extracts. Glycyrrhetinic acid completely abrogated both activities.

Results

To establish the presence of 11β-OHSD1 and 11β-OHSD2, as well as PLA2 group I and group II, their messenger RNA (mRNA) levels were measured in GMC by reverse transcribed PCR (RT-PCR) using specific primers. The PCR products on the gel revealed signals for group II PLA2 and 11β-OHSD1, but no signal for group I PLA2 (pancreatic), and only a very faint signal for 11β-OHSD2 (Fig. 2, *top*). These signals were also quantified by including [α^{32}P]-dCTP nucleotide during PCR (Fig. 2, *bottom*).

Oxidation of the hydroxyl group of corticosterone and reduction of the keto group of dehydrocorticosterone by 11β-OHSD1 were measured using NADP and NADPH as cofactors, respectively, in Triton X-100 extracts of GMC. The enzyme activity was plotted as a function of the amount of protein (Fig. 3). A dose-dependent increase in the oxidation (Fig. 3, *left*) and reduction (Fig. 3, *right*) activ-

ity was observed. Glycyrrhetinic acid (GA), a known inhibitor of 11β-OHSD1 and 11β-OHSD2 (12, 29), completely inhibited oxidation and reduction activity of GMC 11β-OHSD1. The GMC extract had no 11β-OHSD2 oxidation activity with NAD as cofactor (data not shown), an observation in line with the weak signal observed by RT-PCR. It has to be noted that 11β-OHSD2 has only oxidation and no reduction activity (12).

During glomerular inflammation, PLA2 levels rise sharply in GMC after stimulation with IL-1β, TNF-α, or forskolin (30–32). Therefore, we studied the role of 11β-OHSD1 on the inhibitory effect of glucocorticoids on PLA2. For that purpose, GA was used to modulate the activity of 11β-OHSD1. The level of group II PLA2 was first assessed by RT-PCR (Fig. 4). Stimulation of GMC with IL-1β markedly enhanced the level of group II PLA2. The rise was less pronounced in the presence of corticosterone and was diminished further when GA was added. GA alone, however, does not affect group II PLA2 expression induced by IL-1β. The quantitatively assessed mRNA content of GAPDH in GMC was not affected in these different experiments (data not shown).
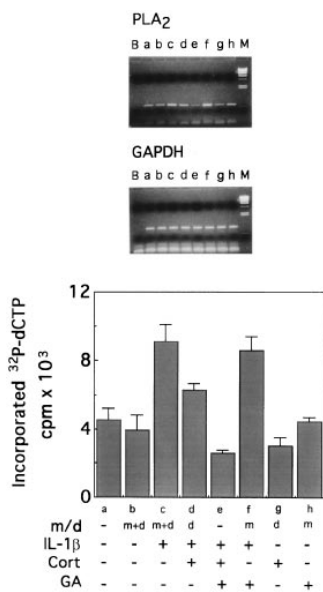

Figure 4. Inhibition of 11β-OHSD1 activity by GA enhances the ability of corticosterone to decrease IL-1β–induced PLA2 mRNA levels. Agarose gel of PCR products of group II PLA2 and GAPDH from GMC stimulated with IL-1β and modulated by corticosterone (*Cort*) and GA. Lane *B* indicates the PCR blank and lane *M* the molecular weight marker. Lanes *a–h* in the upper panel correspond with the columns in the lower panel. In the lower panel, each value represents the average (± SD) of three determinations. Corticosterone was dissolved in methanol (*m*) and GA in DMSO (*d*). These solvents were also added whenever corticosterone and/or GA were not used. GA increased the capacity of corticosterone to inhibit IL-1β–stimulated expression of group II PLA2 (*e*). GA itself had no effect (*f* and *h*) and corticosterone reduced only negligibly the mRNA content of unstimulated GMC (*g*).
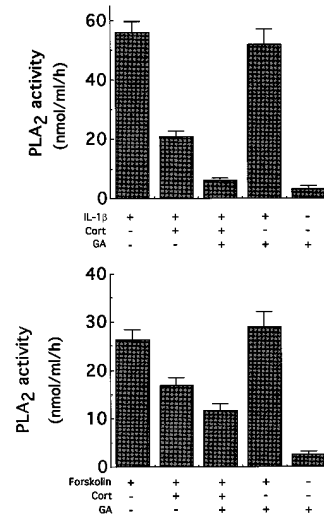

Figure 5. GA enhances the ability of corticosterone (*Cort*) to inhibit IL-1β– and forskolin–induced phospholipase (PLA2) activity. (*Top*) GMC were incubated for 48 h with combinations of IL-1β (5 nM), corticosterone (50 nM), and glycyrrhetinic acid (5 μM), and PLA2 assays were performed. Results represent the mean (± SD) of three assays. (*Bottom*) Inhibition of group II PLA2 enzyme activity in forskolin stimulated GMC. Each column represents the mean (± SD) of three determinations. The inhibition of the enzyme activity by corticosterone was enhanced by the addition of GA.
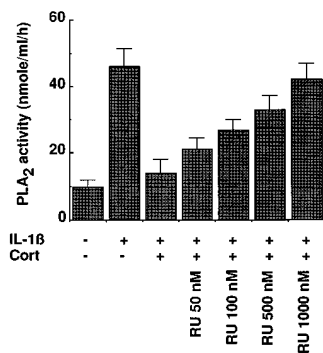
Figure 6. Corticosterone inhibits PLA2 expression through glucocorticoid receptors. GMC were incubated for 48 h with combinations of IL-1β (5 nM), corticosterone (50 nM), and increasing concentrations of the glucocorticoid antagonist RU 486 (*RU*). With increasing concentrations of RU 486, the inhibitory effect of corticosterone was abolished. PLA2 was measured as the release of [³H]oleic acid from *E. coli* membranes as described. Each value is the average (± SD) of triplicate determinations.

Figure 7. (*Top*) IL-1β and TNF-α increase steady-state 11β-OHSD1 mRNA in GMC. The mean (± SEM, $n = 6$) incorporation of [α³²P]-dCTP (cpm) into cDNA of 11β-OHSD1 after incubation of GMC with TNF-α and/or IL-1β was standardized to that of GAPDH mRNA. (*Bottom*) IL-1β and TNF-α increase 11β-OHSD1 protein levels in GMC. Western blot analysis of 11β-OHSD1 in GMC stimulated with and without TNF-α and/or IL-1β. *A*, 11β-OHSD1 from COS cells transfected with the cDNA of 11β-OHSD1; *B*, GMC; *C*, GMC with 5 nM of TNF-α; *D*, GMC with 5 nM of IL-1β; *E*, GMC with 5 nM of TNF-α and 5 nM of IL-1β. Quantification of 11β-OHSD1 protein by densitometry analysis of the Western blot. Without cytokines, no 11β-OHSD1 protein was detected. Both IL-1β and TNF-α increased the relative transmission from a signal of below the detection limit to a value of 2–4.

In line with these observations, the enzymatic activity of PLA2 increased upon stimulation with IL-1β or forskolin, to be then decreased by the action of corticosterone and corticosterone combined with GA (Fig. 5). Corticosterone reduced the IL-1β–stimulated PLA2 enzyme activity by 60% and corticosterone in combination with GA diminished the activity by 90% (Fig. 5, *top*). GA alone did not alter the secretion of PLA2 induced by IL-1β (Fig. 5, *top*). Similar results were obtained when forskolin, instead of IL-1β, was used for stimulaton of group II PLA2 activity (Fig. 5, *bottom*).

To establish whether the inhibiton of group II PLA2 enzyme by corticosterone is mediated through glucocorticoid receptors, the PLA2 activity was assayed in the presence of the glucocorticoid antagonist RU 486. As shown in Fig. 6, the effect of corticosterone was diminished by increasing concentrations of RU 486, indicating that corticosterone exerts its effect through glucocorticoid receptors.

Since it was shown previously that IL-1β increased corticosteroid levels, the hypothesis was tested whether IL-1β and TNF-α regulate the 11β-OHSD1 (33, 34). For that purpose, GMC were incubated with TNF-α, IL-1β, or a combination of both, and the mRNA content was quantified by RT-PCR for 11β-OHSD1 (Fig. 7, *top*). Both IL-1β and TNF-α only slightly enhanced the mRNA content in GMC. The combination of both, in contrast, yielded a strong additive effect (Fig. 7, *top*). The increased mRNA content of GMC was reflected by a sharp increase in the amount of 11β-OHSD1 protein (Fig. 7, *bottom*). The cytokine-induced increase of protein levels was more marked than the effect on steady-state mRNA levels, suggesting that the modulation may take place mainly at the translational level. When the specific activity of 11β-OHSD1 was analyzed, no change was seen with respect to oxidation (results not shown). However, the reductive activity of 11β-OHSD1 increased as a function of the concentrations of IL-1β and/or TNF-α (Fig. 8). The combination of TNF-α and IL-1β stimulated the reductive activity more than either of the cytokines added alone; this was shown when either increasing concentrations of TNF-α or IL-1β were used, or when a fixed amount of TNF-α with an increasing amount of IL-1β were given to the cell cultures (Fig.
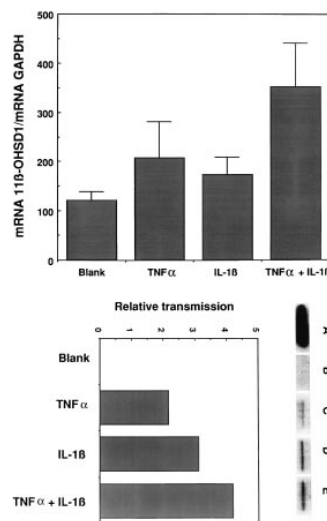
8). A similar pattern was observed when the specific activity (percentage of conversion of dehydrocorticosterone to corticosterone per milligram protein per 4-h period) was calculated (data not shown). To exclude that the increased reductive activity after stimulation with cytokines was due to an increased availability of cofactors, incubations of stimulated GMC with dehydrocorticosterone in the presence of 5 mM NADPH were performed. The results from these studies revealed that the increased reductase activity induced by cytokines was not attributable to a higher availability of NADPH (data not shown).

Besides IL-1β and TNF-α, other cytokines such as IL-3 and IL-6, and other stimulating agents including platelet-derived growth factor, PMA, and forskolin were added to the GMC cultures. These agents exhibited neither an increase nor a decrease of the oxidative or reductive activity of 11β-OHSD1. To exclude a nonspecific effect of TNF-α and/or IL-1β on oxidoreductase activity in general, the activity of 17β-hydroxysteroid dehydrogenase was determined in GMC with and without the addition of TNF-α and IL-1β using estradiol and estrone as substrates. The activity of 17β-OHSD was not affected by these cytokines. Thus, the enhancement of 11β-OHSD1 by IL-1β and TNF-α is specific.

The biological relevance of the increased reductase activity of 11β-OHSD1 after stimulation with IL-1β and/or TNF-α was assessed first on the inhibition of PLA2 activity in GMC. Unstimulated GMC or GMC treated with GA alone or with the 11-keto–glucocorticosteroid dehydrocorticosterone alone displayed only background PLA2 activity (Fig. 9). Stimulation of GMC with IL-1β enhanced PLA2 production at least fivefold. Dehydrocorticosterone reduced the IL-1β–induced PLA2 activity. This effect was abrogated with increasing concentrations of GA (Fig. 9). In
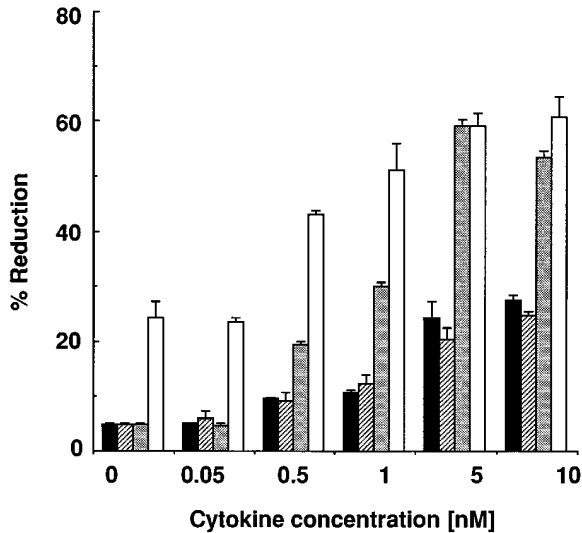
Figure 8. IL-1β und TNF-α increase 11β-OHSD1 reductase activity in GMC. The percentage of conversion of dehydrocorticosterone to corticosterone was determined as a function of increasing concentrations of TNF-α (*black columns*), IL-1β (*hatched columns*), TNF-α and IL-1β combined (*stippled columns*), and of a fixed amount of TNF-α (5 nM) in the presence of increasing concentrations of IL-1β (*white columns*). A dose-dependent increase in the reductive activity was observed. Each column represents the mean (± SD) of three determinations.

a second set of experiments, the relevance of the interconversion of biologically inactive 11-keto glycocorticosteroids into active 11β-hydroxy glucocorticosteroids was demonstrated in the Kiki cell line (21). We found that Kiki cells contain high levels of 11β-OHSD1, but low levels of 11β-OHSD2 transcripts (data not shown). TNF-α and IL-1β induce the reductase activity of 11β-OHSD1 in these cells (data not shown). Kiki cells express the bacterial β-galactosidase gene under the control of glucocorticoid-responsive elements of the mouse mammary tumor virus promoter. This cell line therefore expresses β-galactosidase only when biologically active 11β-hydroxy glucocorticosteroids are present. The experiments in Fig. 10 demonstrate that although 11β-hydroxy glucocorticosteroids directly drive gene expression, 11-keto glucocorticosteroids are unable to do so unless the cells are concomitantly stimulated by IL-1β. This effect is mediated by 11β-OHSD activity, because it can be abolished with GA in a dose dependent fashion (Fig. 10 *b*). Furthermore, this effect is specific for 11-keto gluco-
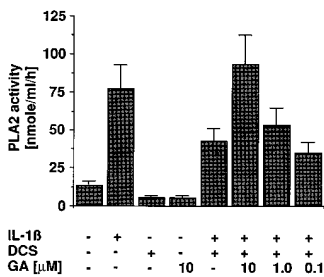

Figure 9. The 11-keto–glucocorticosteroid dehydrocorticosterone inhibits IL-1β–induced PLA2 release by a process that can be inhibited by GA. PLA2 activity was measured as the release of [³H]oleic acid from *E. coli* membranes as described. Results represent the mean value (± SD) of three independent assays. *GA*, 0.1, 1.0, 10 μM; *DCS*, dehydrocorticosterone, 0.1 μM; IL-1β, 5 nM. GMC were treated with GA alone, DCS alone, IL-1β alone, IL-1β and DCS, IL-1β, and DCS and different concentrations of GA.
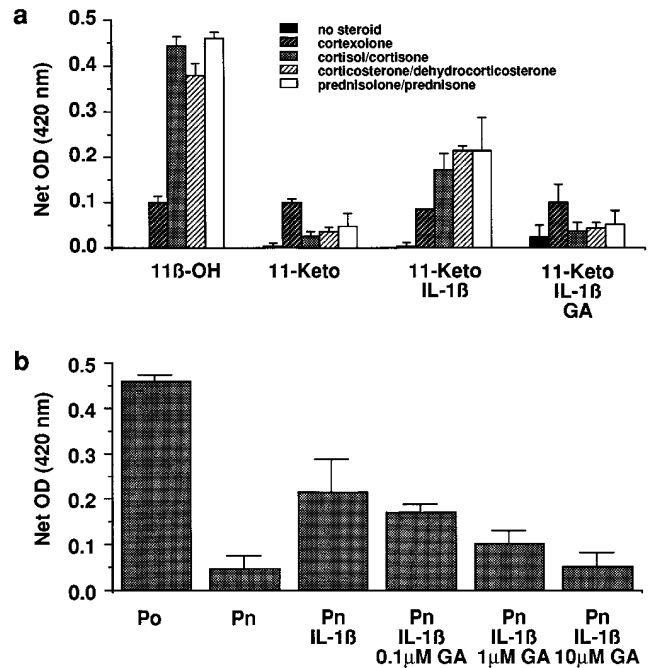

Figure 10. (*a*) 11-keto glucocorticosteroids are converted into active compounds and drive glucocorticoid-dependent gene expression in Kiki cells. Kiki cells were incubated with 11β-hydroxy glucocorticosteroids (*far left histogram*), the corresponding 11-keto glucocorticosteroids alone (*middle left histogram*), and the 11-keto glucocorticosteroids with IL-1β in absence (*middle right histogram*) and presence (*far right histogram*) of GA. Expression of the bacterial β-galactosidase reporter gene was detected by an in situ assay as described (21). Results represent the mean value (± SD) of three independent assays. Concentrations: steroids, 0.1 μM; IL-1β, 5 nM; GA, 50 μM. 11β-hydroxy glucocorticosteroids: cortisol, corticosterone, prednisolone. 11-keto glucocorticosteroids: cortisone, dehydrocorticosterone, prednisone. Cortexolone, included in both keto and hydroxy groups, does not carry a functional group at position 11, and is therefore not a substrate for 11β-OHSD1. (*b*) Effect of GA on conversion of prednisone (*Pn*) into prednisolone (*Po*). Kiki cells were incubated in the presence of 0.1 μM prednisolone alone (column *1*), 0.1 μM prednisone alone (column *2*), 0.1 μM prednisone + 5 nM IL-1β without GA (column *3*), and with 0.1, 1.0, and 10 μM GA (columns *4*, *5*, and *6*). Results represent the mean value (± SD) of three determinations.

corticosteroids as cortexolone, a steroid without a functional group at position 11, displays an 11β-OHSD– and cytokine–independent constitutive activity, albeit weak (Fig. 10 *a*) The relevance of the conversion of cortisone to cortisol was furthermore shown when these two endogenous glucocorticosteroids are present in the low physiological concentration range using Kiki cells (data not shown).

Discussion

The present study revealed for the first time 11β-OHSD activity in GMC. The activity was attributable to 11β-OHSD1 as shown by measurement of mRNA, protein, and activity using NADP as a cofactor. The absence of appreciable expression of 11β-OHSD2 in GMC is in line with previous immunohistochemical data on kidney cortex (35, 36) showing only a weak staining for 11β-OHSD2 in the visceral epithelial cells of the outer capillary loop of the glomerulus.

The impact of 11β-OHSD1 on glucocorticoid action during inflammation was analyzed by studying the corticosterone-induced inhibition of group II PLA2 release in GMC. As recently shown, GMC stimulated with IL-1β or TNF-α enhance the eicosanoid formation concomittantly with an increased 14-kD group II PLA2 gene expression and secretion (30–32, 37). A similar effect was observed when GMC were stimulated with forskolin (37, 38). Forskolin and IL-1β stimulate PLA2 through two distinct mechanisms: activation of adenyl cyclase and protein kinase C (38). The enhanced expression of PLA2 in inflammation is suppressed by glucocorticosteroids (39). In the present study, the inhibitory effect of corticosterone on the release of group II PLA2 stimulated by IL-1β or forskolin was enhanced by inhibiting the 11β-OHSD1 by GA. This effect was most likely mediated through glucocorticoid receptors as shown by abrogating the glucocorticoid inhibitory effect with RU 486. When GA was added to the GMC, no direct inhibition of group II PLA2 transcription and release was observed; the inhibitory effect of GA on PLA2 was only detected in the presence of corticosterone. This indicates that the previously reported antiinflammatory (40) and antiallergic effects of GA (41), the active pharmacological component of licorice (*Glycyrrhiza glabra*), is attributable to an enhanced effect of endogenous glucocorticoids as a consequence of the inhibition of 11β-OHSD.

GMC occupy a central position within the glomeruli (16–18). They are important for the regulation of glomerular filtration rate, and furthermore, GMC participate in the process of glomerular injury. For both processes, arachidonic acid–derived metabolites are relevant. Group II PLA2 plays a pivotal role for the arachidonic acid release. PLA2 is regulated by glucocorticosteroids, since glucocorticoid deficiency enhances and pharmacological doses of glucocorticosteroids suppress group II PLA2 transcription (4, 6, 16). This high sensitivity of group II PLA2 to exogenously prescribed or endogenously released steroid hormones underlines the relevance of glucocorticoids in health and disease states for prostaglandin release. The present study demonstrates that not only the concentrations of the glucocorticosteroids but, in addition, the intracellular disposition determines their antiinflammatory effect.

The addition of IL-1β and TNF-α enhanced the expression of 11β-OHSD1. The activity of the reductase, but not that of the oxidase, of 11β-OHSD increased severalfold. This observation has been made in two different cell lines. The mechanism accounting for the enhanced reduction is a specific phenomenon because the activity of another oxido-reductase, the 17β-hydroxysteroid dehydrogenase, was not affected by the cytokines. The enhanced activity of 11β-OHSD1 cannot be explained by mere proliferation of the GMC induced by these cytokines, since the specific activity of 11β-OHSD1 reductase increased and other GMC-stimulatory agents did not enhance the 11β-OHSD1 reductive capacity in GMC. The increased reductive capacity is biologically relevant as evidenced first by the activation of a glucocorticosteroid response element–dependent reporter gene by biologically inert 11-keto steroids such as corti-

sone, dehydrocorticosterone, or prednisone, and by the inhibition of PLA2 through a glucocorticoid receptor–dependent pathway by dehydrocorticosterone.

The mechanism for the enhanced reductive but not oxidative activity, despite increased concentrations of 11β-OHSD1 protein after stimulation with IL-1β and TNF-α, is unknown. One possible mechanism is a different posttranslational modification. Such modifications exist for 11β-OHSD1, as shown by Agarwal et al. who incubated TK-143B human osteosarcoma cells transfected with the cDNA of rat 11β-OHSD1 in the presence of the glycosylation inhibitor A1-tunicamycin and observed a 50% decrease in dehydrogenase activity without affecting the reductase activity (15). Similarly, in the present investigation, a clear decline in the oxidase activity, but a less pronounced decline in the reductase activity was seen when GMC were incubated with A1-tunicamycin (data not shown). Thus, a possible explanation of the altered oxidative/reductive ratio induced by IL-1β and TNF-α, is a posttranslational modification. Nominally, however, we can not rule out the possibility that the relative increase in reductase activity relates to the activation of a yet undiscovered and hypothetical 11β-OHSD3.

IL-1β and TNF-α often exert various synergistic in vitro and in vivo effects including immune inflammatory responses (1–3, 42, 43). In glomerular inflammation, increased expression of the inflammatory cytokines IL-1β and TNF-α has been observed (44–49). IL-1β and TNF-α induce, among others, contraction, proliferation, expression of receptors, and metabolic effects in GMC. The present novel observation, that IL-1β and TNF-α modulate the 11β-OHSD activity in such a way that the formation of active 11β-hydroxy glucocorticoids is favored, is a biologically interesting phenomenon for the following reasons. First, Besedowsky et al. and Sapolsky et al. presented evidence for an interaction between IL-1β and glucocorticoids (33, 34). The injection of IL-1β activated the hypothalamo-pituitary-adrenal axis with an enhanced secretion of adrenocorticotropic hormone and corticosterone due to an IL-1–stimulated release of corticotropin-releasing factor (33). This observation was interpreted to mean that during infections, cortisol or corticosterone appear in higher concentrations in serum to overcome the stress due to an infectious challenge. The present observation that IL-1β favors the formation of biologically active glucocorticoids during inflammatory reactions within the target cells, is in line with the seminal observations of Besedovsky et al. and Sapolsky et al. (33, 34). Second, IL-1β and TNF-α are known to induce apoptosis in some cells (50, 51). Similarly, 11β-hydroxy glucocorticosteroids induce apoptosis (52). Since TNF-α and IL-1β stimulate the intracellular formation of active glucocorticosteroids, they enhance their own apoptoting potential. Third, inflammatory processes are limited with respect to time in most situations. The mechanisms accounting for such limitation are not completely understood. Glucocorticosteroids are powerful inhibitors of IL-1β and TNF-α secretion and of many of the inflammatory effects of these cytokines (53–56). IL-1β and TNF-α can

mediate the onset of inflammation and, by inducing the reductase activity of 11β-OHSD, enhance the GMC susceptibility to glucocorticoids. It is interesting to note that the two processes are temporally distinct. It has been already shown that PLA2 levels rise sharply as early as 12 h after exposure to proinflammatory cytokines (37). In contrast, we have observed that a consistent increase in 11β-OHSD reductase activity is detectable only after 24 h. After 1, 4, 6, and 12 h, no significant increase in reductase activity was

detected (data not shown). By this dual action, IL-1β and TNF-α may counterbalance their proinflammatory effect, and contribute to the ultimately necessary arrest of inflammation. A comparable dual effect of proinflammatory and antiinflammatory actions has recently been described for leukotriene B4 (57). Leukotriene B4 sustains and amplifies inflammation, while concomitantly activating the peroxisome proliferator-activated receptor, which drives peroxisomal β-oxidative degradation of leukotriene B4 itself.

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