Dexamethasone increases eNOS gene expression and prevents renal vasoconstriction induced by cyclosporin

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Dexamethasone increases eNOS gene expression and prevents renal vasoconstriction induced by cyclosporin. Am. J. Physiol. 277 (Renal Physiol. 46): F464-F471, 1999.—Cyclosporin A (CsA)-induced renal vasoconstriction (RV) is attributed to an imbalance in vasoactive factors release. Dexamethasone (Dex) exerts a renal vasodilatory effect by a mechanism not yet characterized. This study evaluates whether the effect of Dex is mediated by NO and whether it prevents CsA-induced RV. Micropuncture studies were performed in six groups of uninephrectomized rats treated for 7 days with the following: vehicle (Veh); Veh + 4 mg/kg dexamethasone (Veh + Dex); 30 mg/kg CsA; CsA + Dex; vehicle + 10 mg/kg nitro-L-arginine methyl ester (Veh + L-NAME); and Veh + Dex + L-NAME. NO synthase (NOS) isoforms mRNA levels were evaluated in renal cortex and medulla by semiquantitative RT-PCR analysis in the first four groups. Dex produced renal vasodilation, which was blocked by concomitant L-NAME administration, and the effect of Dex was associated with higher cortical and medullary endothelial NOS (eNOS) and cortical inducible NOS (iNOS) mRNA levels. In the CsA group, Dex prevented RV, restoring glomerular hemodynamics to control values. These changes were associated with further enhancement of eNOS and restoration of medullary iNOS and neuronal NOS (nNOS) expression. We conclude that Dex prevents CsA-induced RV, and its vasodilator effect could be mediated by increased intrarenal generation of NO, secondary to enhanced expression of eNOS and iNOS.

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sheep that the increase in renal flow induced by cortisone is suppressed when NO synthesis is inhibited, suggesting that NO is the principal mediator of the vasodilator effect exerted by glucocorticoids. Thus the major goals of the present study was to evaluate whether renal vasodilator effect of Dex is mediated by NO and whether Dex can prevent renal vasoconstriction induced by CsA. For this purpose, we evaluated the effect of Dex on glomerular hemodynamics in control and CsA- and nitro-L-arginine methyl ester (L-NAME)-treated rats, as well as changes in neuronal NOS (nNOS), inducible NOS (iNOS), and eNOS gene expression in renal cortex and medulla during Dex or CsA administration. Our data suggest that renal vasoconstriction induced by Dex prevents CsA renal vasoconstriction. This effect was partially mediated by a NO-dependent mechanism, which is associated with an increment of eNOS gene expression.

METHODS

Male Wistar rats, weighing 300–350 g, with right nephrectomy were used for the study. Fifteen days after nephrectomy, animals were randomly divided into four groups, which were subjected to the following daily treatments during 7 days: group I included 12 rats that received 0.1 ml olive oil as vehicle (Veh); group II consisted of 12 rats treated with vehicle plus 4 mg/kg sc dexamethasone (Veh+Dex); group III consisted of 12 rats that received 30 mg/kg sc CsA (CsA); group IV consisted of 12 rats treated with CsA and Dex (CsA+Dex); group V consisted of 6 rats treated with 10 mg/kg L-NAME in drinking water for 2 days before micropuncture studies were performed (Veh+L-NAME); and group VI consisted of 6 rats that received Veh+Dex+L-NAME. The Veh, Veh+Dex, and Veh+L-NAME groups were pair fed.

Micropuncture studies. Hemodynamic studies were performed in seven rats of groups I, II, III, and IV, as well as in six rats of groups V and VI. Rats were anesthetized with pentobarbital sodium (30 mg/kg ip), and supplemental doses were instilled as required. The rats were placed on a thermoregulated table, and temperature was maintained at 37°C. Trachea, both jugular veins, femoral arteries, and the left ureter were catheterized with polyethylene tubing (PE-240, PE-50, and PE-10). The left kidney was exposed, placed in a Lucite holder, sealed with elastomer (Xantropen, Bayer), and covered with Ringer solution. Mean arterial pressure (MAP) was monitored with a pressure transducer (model p23 db; Gould, San Juan, PR) and recorded on a polygraph (Grass Instruments, Quincy, MA). Blood samples were taken periodically and replaced with blood from a donor rat.

Rats were maintained under euolemic conditions by infusion of 10 ml/kg of body weight of isotonic rat plasma during surgery, followed by an infusion of 25% polyfructosan, at 2.2 ml/h (Inunest, Laevosan-Gesellschaft). After 60 min, five to six 3-min collection samples of proximal tubular fluid were obtained to determine flow rate and polyfructosan concentration. Intratubular pressure under free-flow and stop-flow conditions and peritubular capillary pressure were measured in other proximal tubules with a servo-null device (Servo Nulling Pressure System; Instrumentation for Physiology and Medicine, San Diego, CA). Polyfructosan was measured in plasma samples. Glomerular capillary osmotic pressure was estimated from protein in blood of the femoral artery (Cp) and surface effluent afferent arterioles (Ca). Polyfructosan concentrations were determined by the technique of Davidson and Sackner (8). Tubular fluid volume was estimated as previously desribed (16). Concentration of tubular polyfructosan was measured by the method of Vurek and Pegram (38). Protein concentration in afferent and efferent samples was determined according to the method of Vienot et al. (37).

MAP, GFR, single-nephron GFR (SNGFR), glomerular capillary hydrostatic pressure (Pc), single-nephron filtration fraction, single-nephron plasma flow (Qf), afferent (Ra) and efferent (Re) resistances, Kf, and oncotic pressure (ø) were calculated according to equations given elsewhere (2).

RNA isolation. Kidneys were obtained from five rats of groups I, II, III, and IV. Rats were anesthetized by intraperitoneal injection of pentobarbital sodium, and their left kidneys were excised, macroscopically divided into renal cortex and medulla, frozen in liquid nitrogen, and kept at −80°C until used. Total RNA was isolated from each cortex or medulla following the guanidine isothiocyanate-ceesium chloride method (30). Integrity of isolated total RNA was examined by 1% agarose gel electrophoresis, and RNA concentration was determined by the ultraviolet (UV) light absorbance at 260 nm (model DU640; Beckman, Brea, CA). All RNA samples were incubated with RNase-free DNase I (Boehringer, Mannheim, MD) for 15 min at 37°C and extracted with the phenol-chloroform technique.

Relative quantitation of NOS mRNA. The relative level of NOS mRNA expression was assessed in the renal cortex and medulla by semiquantitative RT-PCR, as previously described (4, 5). Briefly, NOS primer sequences were custom obtained from GIBCO-BRL (Life Technologies, Gaithersburg, MD). nNOS primers were as follows: sense 5′- GAAACCCCA-AGACCATC 3′ and antisense 3′-GCTTTTGCCTCCACAGTT 5′, which amplified a fragment of 308 bp on the amino-terminal domain of nNOS (from base 692 to 999 of rat cerebellum nNOS sequence). NOS primers were as follows: sense 5′- GTGTTCC-CACGAGAGATGGT 3′ and antisense 3′- CTCTCCGCAC-TGAGTTGCTC 5′, which amplified a fragment of 570 bp on the amino-terminal domain of nNOS (from base 1407 to 1977 of the murine macrophages NOS sequence), and eNOS primers were as follows: sense 5′- TACGAGGAGCAGAACAT- CAC 3′ and antisense 3′- CAGGCTCGAGTTCCTTGGATC 5′, which amplified a fragment of 819 bp on the amino-terminal domain of eNOS (from base 1923 to 2742 of rat eNOS). To monitor nonspecific effects of the experimental treatment and to semiquantitate NOS isoforms expression, we amplified a fragment of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), using primers previously described (27). Genomic DNA contamination was checked by treating all RNA samples with DNase and by carrying samples through PCR procedure without adding reverse transcriptase.

Reverse transcription (RT) was carried out using 10 µg of total RNA from the renal cortex or medulla of each rat. Prior to RT reaction, the RNA was heated at 65°C for 10 min. RT was performed at 37°C for 60 min in a total volume of 20 µl containing 1 µg of random hexamers, 0.5 mM of each dNTP (Sigma Chemical, St. Louis MO), and 1× RT buffer (75 mM KCl, 50 mM Tris-HCl, 3 mM MgCl2, and 10 mM DTT, pH 8.3). Samples were warmed at 95°C for 5 min to inactivate the reverse transcriptase and diluted to 40 µl with PCR grade water. One-tenth of the RT individual samples of each group was used for each NOS isoform or GAPDH amplification in 20-µl final volume reactions containing 1× PCR buffer (10 mM Tris-HCl, 1.5 mM MgCl2, and 50 mM KCl, pH 8.3), 0.1 mM of each dNTP, 0.2 µCi of [32P]dCTP (~3,000 Ci/mmole, 9.25 MBq, 250 µCi), 10 µM of each primer, and 1 U of Taq DNA polymerase (GIBCO-BRL). The samples were overlaid with 30 µl mineral oil, and PCR cycles were performed in a DNA thermal cycler.
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(M.J. Research, Watertown, MA), with the following profile: denaturation 1 min at 94°C, annealing 1 min at 55°C for nNOS primers and 60°C for iNOS and eNOS primers, and 1 min extension step at 72°C. The last cycle was followed by a final extension step of 5 min at 72°C. The control gene was amplified simultaneously in each reaction. Amplification kinetics of the three NOS isoforms and housekeeping gene in renal cortex and medulla total RNA and the optimal number of cycles for quantitation for each NOS isoform were previously reported (4). To analyze the PCR products, one-half of each reaction was electrophoresed in a 5% acrylamide gel. Bands were ethidium bromide stained and visualized under UV light, cut out, suspended in 1 ml of scintillation cocktail (Ecolume; ICN, Aurora, OH), and counted by liquid scintillation (model LS6500; Beckman, Fullerton, CA). To semiquantitate each NOS isoform, we performed all reactions individually from each cortex or medulla total RNA in duplicate.

Statistical analysis. Statistical significance is defined as two-tailed P < 0.05, and the results are presented as means ± SE. NOS isoforms expression is shown as the ratio between SE. NOS isoforms expression is shown as the ratio between groups by one-way ANOVA and Student-Newman-Kuels for multiple comparisons.

RESULTS

Hemodynamic studies. Table 1 summarizes the results obtained in glomerular hemodynamic studies on groups I to IV. Dex administration produced a clear vasodilator effect. In Veh+Dex group, compared with Veh group, glomerular plasma flow increased 39.8%, due to a 34.2% fall in afferent resistance, while efferent resistance was not modified; preglomerular vasodilation was characterized by increases in MAP to glomerular capillaries, and Pgc increased significantly. The fall in Rg resulted in a 39.8% increase in glomerular plasma flow, whereas Kf values remained unchanged. Thus significant elevations in SNGFR and total GFR (26.4 and 48%, respectively) were observed. These results confirm the ability of Dex to produce renal vasodilation under normal conditions.

Chronic CsA administration produced marked renal vasoconstriction, as evidenced by 51% reduction in glomerular plasma flow produced by sharp increases in both Rg and Rf. Glomerular plasma flow was reduced, Pgc remained unchanged, and Kf was significantly reduced. The fall in two of the four determinants of GFR, that is Qf and Kf, was responsible for the decrease in SNGFR to almost one-half of the values obtained in Veh animals. Thus the CsA group exhibited the characteristic renal vasoconstriction observed during chronic treatment.

In the CSA+Dex group, Dex administration produced a marked vasodilator effect and restored renal hemodynamics to control values. Pre- and postglomerular vasodilation were observed; thus Rg and Rf decreased significantly (61.5 and 48.7%, respectively). The fall in Rg allowed the transmission of a greater fraction of MAP to glomerular capillaries in this group, causing a rise in Pgc. Arteriolar vasodilation induced an important rise in Qf (171.3%) compared with CsA-treated rats. The percent increase of Qf in CsA+Dex vs. CsA group was fourfold higher than that observed in Veh+Dex vs. Veh alone (171.3 vs. 39.8%). An increase in Kf was also observed. Elevations in Qf, Pgc, and Kf cause a significant increase in SNGFR and total GFR of 104.8 and 116.0%, respectively, reestablishing completely glomerular dynamics to control values. These results clearly demonstrate that Dex administration prevented renal vasoconstriction induced by CsA.

To evaluate whether the vasodilatory effect induced by Dex was due to increased NO generation, the glomerular hemodynamics in rats treated with Dex were assessed with and without administration of the NO synthesis inhibitor L-NAME, as well as in a group treated with L-NAME alone. Figure 1 shows the results of these series of experiments. As we also shown in Table 1, Dex administration produced renal vasodilation characterized by increases in Pgc, Qf, and SNGFR. L-NAME produced renal vasoconstriction characterized by a reduction of Qf due to a profound elevation of Rg and Rf resistances (6.3 ± 0.5 and 4.1 ± 0.4 dyn·s·cm−2, respectively), together with an increase in Pgc and a decrease in Kf. These changes resulted in significant fall in SNGFR. However, in the presence of NO synthesis inhibition by L-NAME, the vasodilatory effect of Dex was completely suppressed, as indicated by the similar values of Qf, Kf, and SNGFR in Veh+Dex+L-NAME group, as in rats treated with L-NAME alone. Thus Dex renal vasodilation seems to be dependent on NO synthesis.

Expression of NOS mRNA. Figure 2 shows the results of each NOS isoform gene expression in renal cortex and medulla determined by semiquantitative RT-PCR analysis in groups I, II, III, and IV. Dex administration was associated with a fivefold increase of eNOS expression in renal cortex (Fig. 2A) and a slightly lower, but nevertheless significant, increment

Table 1. Dexamethasone effect on glomerular hemodynamics during CsA nephrotoxicity

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP, mmHg</th>
<th>Qf, nl/min</th>
<th>Resistance, ×10−10 dyn·s·cm−2</th>
<th>Pgc, mmHg</th>
<th>Kf, nl·s−1·mmHg−1</th>
<th>SNGFR, nl/min</th>
<th>GFR, nl/min</th>
</tr>
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<tbody>
<tr>
<td>Veh</td>
<td>125 ± 4.4</td>
<td>130 ± 9.4</td>
<td>2.4 ± 0.3</td>
<td>1.4 ± 0.1</td>
<td>50.4 ± 0.8</td>
<td>34.1 ± 2.8</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Veh+Dex</td>
<td>121 ± 3.7</td>
<td>183 ± 15.4†</td>
<td>1.5 ± 0.1†</td>
<td>1.3 ± 0.1†</td>
<td>57.4 ± 1.5†</td>
<td>43.3 ± 1.8†</td>
<td>1.4 ± 0.1†</td>
</tr>
<tr>
<td>CsA</td>
<td>115 ± 4.1</td>
<td>63.7 ± 72*</td>
<td>4.7 ± 0.8*</td>
<td>2.7 ± 0.2*</td>
<td>46.6 ± 1.5*</td>
<td>17.1 ± 1.9*</td>
<td>0.6 ± 0.1*</td>
</tr>
<tr>
<td>CsA+Dex</td>
<td>121 ± 4.1</td>
<td>172 ± 28.5†</td>
<td>1.8 ± 0.3†</td>
<td>1.4 ± 0.2†</td>
<td>54.7 ± 1.9†</td>
<td>35.0 ± 5.8†</td>
<td>1.3 ± 0.1†</td>
</tr>
</tbody>
</table>

Values are means ± SE. CsA, cyclosporin A; Veh, vehicle; Dex, dexamethasone; MAP, mean arterial pressure; Qf, single-nephron plasma flow; Rg and Rf, afferent and efferent arteriolar resistance, respectively; Kf, ultrafiltration coefficient; Pgc, glomerular capillary pressure; GFR, glomerular filtration rate; SNGFR, single-nephron GFR. *P < 0.05 vs. vehicle. †P < 0.05 vs. CsA.
In renal medulla (Fig. 2B). In addition, Dex also induced a threefold increase of iNOS expression in renal cortex (Fig. 2C), but not in renal medulla (Fig. 2D). No changes were observed in nNOS levels in either cortex or medulla (Fig. 2, E and F).

In CsA-treated rats, we found that eNOS mRNA levels increased in renal cortex (Fig. 2A), whereas nNOS and iNOS mRNA levels were not modified. In contrast, in medullary total RNA, eNOS did not change (Fig. 2B), whereas nNOS and iNOS decreased significantly (Fig. 2, D and F). Interestingly, Dex administration in rats receiving CsA was associated with a further increase in cortical eNOS mRNA (Fig. 2A). Also, in the group treated with CsA + Dex, we observed an increase in eNOS levels in medullary RNA (Fig. 2B) and in iNOS in cortical total RNA (Fig. 2C). In addition, compared with CsA, Dex administration restored nNOS and iNOS mRNA levels in renal medulla (Figs. 2, D and F). Figure 3 shows a representative autoradiography containing eNOS and GAPDH amplification products in

![Fig. 1. Effect of NO synthesis inhibition on glomerular hemodynamics during dexamethasone (Dex) administration. Q_a, single-nephron plasma flow (A); P_gc, glomerular capillary hydrostatic pressure (B); K_f, ultrafiltration coefficient (C); and SNGFR, single-nephron glomerular filtration rate (D). L-NAME, nitro-L-arginine methyl ester. *P < 0.05 vs. vehicle (Veh) group. ‡P < 0.05 vs. CSP

![Fig. 2. Semiquantitative PCR analysis of renal NO synthase (NOS) isoforms. A, C, and E: endothelial NOS (eNOS)-, inducible NOS (iNOS)-, and neuronal NOS (nNOS)-to-GAPDH ratios in renal cortex total RNA. B, D, and F: eNOS-, iNOS-, and nNOS-to-GAPDH ratios in renal medulla total RNA. All ratios were obtained by dividing the amount of PCR products (cpm) of each NOS by the level of GAPDH. *P < 0.05 vs. Veh group. ‡P < 0.05 vs. cyclosporin A (CsA) group. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.](http://ajprenal.physiology.org/Downloadedfrom)
renal cortex of each rat from the four studied groups. The increase in eNOS expression in Veh+Dex and CsA+Dex groups is evident.

**DISCUSSION**

The major findings of this study are that renal vasodilation induced by Dex is associated with an upregulation of cortical and medullary eNOS mRNA levels. This vasodilator effect was blocked by NO synthesis inhibition. In addition, Dex administration prevented CsA-induced renal vasoconstriction and enhanced eNOS mRNA levels and restored medullary nNOS and iNOS expression.

In the present study, we found that chronic Dex administration produced marked renal vasodilation, which appeared to be limited to preglomerular vessels. In fact, afferent resistance decreased, whereas efferent resistance remained unchanged. These changes were associated with a significant increment in both glomerular capillary pressure and flow that resulted in a striking elevation of SNGFR.

It has been known for years that glucocorticoid administration results in renal vasodilation, which is associated with an increase in GFR (3), this vasodilator effect is observed solely in the kidney. However, the mechanism responsible for this effect is not known. The participation of vasoconstrictors is not clear, since the renin-angiotensin system and endothelin seem to be not altered by glucocorticoids. A novel potential mechanism to explain the renal vasodilator effect of glucocorticoids is the liberation of NO in renal arteries. De Matteo and May (9) demonstrated in sheep that the inhibition of NO synthesis markedly attenuates the renal vasodilator response to cortisol, suggesting that glucocorticoid action depends on NO release. In this study, we observed that the vasodilatory effect of Dex was completely suppressed by concomitant L-NAME administration. In fact, glomerular hemodynamics in Dex+L-NAME group were similar to that observed in rats treated with L-NAME alone, also suggesting that NO could be implicated in this vasodilatory effect. More recently, it was reported that renal vasodilation induced by cortisol is partially prevented by indomethacin, suggesting that prostaglandins are, in addition to NO, another locally released factor involved in mediating the renal vasodilation induced by glucocorticoids (10). Therefore, renal vasodilation induced by Dex could be the result of an imbalance between vasoconstricting and vasodilating factors, secondary in part to increase in the liberation of vasodilatory substances such as NO and prostaglandins. More studies are necessary to establish other mechanisms implicated in this effect.

In CsA-treated rats, Dex administration produced a marked renal vasodilation, raising P<sub>GC</sub>, Q<sub>a</sub>, and SNGFR to normal values. Thus Dex completely reversed renal vasoconstriction induced by CsA.

In the clinical transplantation, CsA nephrotoxicity is a relatively common complication despite the fact that CsA is routinely administrated in combination with glucocorticoids. However, the most commonly used drugs are prednisone and prednisolone, which are unlikely to have a significant renal vasodilatory effect at the low doses that are given in the late stage of transplant, when CsA toxicity occurs.

Locally released NO plays a key role in maintaining renal function, its effect however, is determined by the pattern of expression of each NOS isoform. In the kidney, NO is produced by at least three NOS isoforms that are constitutively expressed and located in specific nephron structures (for review, see Ref. 18). nNOS is expressed in macula densa cells and inner medullary collecting duct; NO produced by this isoform regulates the tubuloglomerular feedback system activation and renin release. iNOS is present in the proximal tubular epithelium, afferent arteriole wall, and in immunostimulated mesangial cells. The role of iNOS under normal conditions is still unknown. eNOS is located in
forms gene expression in renal cortex and medulla. CsA treatment induced striking changes in the pattern of NOS isoforms in both cortex and medulla, suggesting that increased cortical NO counterbalances the renal vasoconstriction induced by CsA. Interestingly, in control and CsA-treated rats, chronic Dex administration induced striking changes in the pattern of NOS isoforms in both cortex and medulla.

In Veh+Dex animals, Dex administration resulted in a significant increase in eNOS gene expression in both cortex and medulla, suggesting that renal vasodilation could be mediated by a greater NO release. In CsA-treated rats, in which the pattern of NOS isoforms expression was altered by increased cortical eNOS and decreased medullary nNOS and iNOS, Dex enhanced even more the expression of eNOS in the cortex and medulla and reestablished nNOS and iNOS gene expression to control levels. Thus the renal vasodilator effect of Dex was associated with an increase in eNOS mRNA levels in both renal cortex and medulla. Since, eNOS isoform is located in the endothelium of glomerular vessels and NO released by this isoform diffuses into vascular smooth muscle cells, it is quite likely that the increase in eNOS expression could be in part responsible for the vasodilator effect. However, further studies are required to determine the type of cells in the renal cortex and medulla in which the increase of eNOS expression occurs.

The mechanism by which Dex induces an increase in eNOS mRNA level can be direct or indirect. There is no evidence for the existence of a glucocorticoid responsive element in the promoter region of eNOS gene. Alternately, increased eNOS expression could result from the induction of other transcription factors acting on the promoter region of this isoform gene or by increasing eNOS mRNA stability. A possible explanation could be related to the Dex effect on the heat shock proteins, especially the Hsp90, which recently has been shown to interact with eNOS, resulting in a marked increase in eNOS activity and stability (14). It is evident that more studies are necessary to establish the molecular mechanism responsible for the induction of eNOS expression by Dex.

In our study, Dex increased iNOS mRNA levels in renal cortex. This finding differs with previous studies in which Dex inhibited iNOS transcription (19, 31). We believe that our observations cannot be attributed to DNA contamination, since it was carefully assessed. Several possibilities to explain this discrepancy can be considered. First, most of the studies in which iNOS expression was reduced by Dex were made under immunostimulated conditions, whereas we are showing the effect of Dex on the fraction of iNOS that is constitutively expressed. Thus it is possible that Dex blocks the immunostimulation of iNOS expression, rather than the constitutive expression of iNOS. Second, our observation was made using total RNA from renal cortex. Since we studied the constitutive expression of iNOS, it is highly likely that this represent iNOS from proximal tubules and renal blood vessels. It is possible that regulation of iNOS expression is very different among different types of cells. Third, preliminary evidence suggests that there could be two iNOS isoforms in the rat kidney: one that is predominantly expressed in renal tubule segments, with the highest expression in the medullary thick ascending limb, and the other one in vascular smooth muscle cells from the afferent arteriole, glomeruli, and interlobular and arcuate arteries (21, 35). If indeed two isoforms of iNOS exist, then it is possible that they could be regulated by different mechanisms. Since we only observed upregulation of iNOS in renal cortex where the vascular smooth muscle cell iNOS isoform has been located (21), our results agree with the finding of Perrella et al. (25), that Dex does not inhibit nitrate production in interleukin-1β-induced smooth muscle cells and increases transcriptional rate and iNOS mRNA half-life. The contribution of NO generated by this isoform is difficult to evaluate, since under nonimmunostimulated conditions the expression of iNOS in the kidney is relatively small. More studies are necessary to establish the regulation of NOS isoforms in the kidney by glucocorticoids.

We have shown in the present and in a previous study (4) that CsA reduces iNOS and nNOS expression in renal medulla. Furthermore, in the present study Dex administration prevents this reduction in CsA-treated rats. Chronic renal ischemia induced by CsA administration is associated with increased apoptosis (33), expression of osteopontin (26), and reduction of iNOS (4, 36). In addition, it has been reported recently that renal injury produced by CsA involves a hypoxia-reoxygenation mechanism (39), suggesting that tubulointerstitial fibrosis resulted from renal hypoxia. Thus, in the present study, preservation of nNOS and iNOS levels in renal medulla by Dex in CsA-treated rats was probably mediated by restoration of the renal blood flow that preventing medullary hypoxia.

In summary, the present study suggests that the renal vasodilator effect of Dex is partially mediated by increased NO generation that could be secondary to an increase of cortical and medullary eNOS expression. Our results confirm that CsA-induced renal vasoconstriction is associated with an increased cortical eNOS and decreased medullary nNOS and iNOS expression. Finally, our findings indicate that prevention of CsA renal vasoconstriction by Dex was associated with further enhancement of eNOS. Restoration of renal perfusion by Dex probably prevented the suppression of medullary nNOS and iNOS expression induced by CsA.

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