Role of NO in cyclosporin nephrotoxicity: effects of chronic NO inhibition and NO synthases gene expression

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Bobadilla, Norma A., Gerardo Gamba, Edilia Tapia, Romeo García-Torres, Alexis Bolio, Pedro López-Zetina, and Jaime Herrera-Acosta. Role of NO in cyclosporin nephrotoxicity: effects of chronic NO inhibition and NO synthases gene expression. Am. J. Physiol. 274 (Renal Physiol. 43): F791–F798, 1998.—The role of nitric oxide (NO) during cyclosporin renal vasoconstriction was evaluated by glomerular hemodynamic and histological changes produced during cyclosporin renal vasoconstriction. NO synthase gene expression and NO synthases mRNA in renal cortex and medulla. Uninephrectomized rats treated during 7 days with vehicle (Veh), cyclosporin A (CsA) 30 mg/kg, CsA + nitro-o-arginine methyl ester (L-NAME), and Veh + L-NAME (10 mg/dl) in the drinking water were studied. A decrease in arterial pressure and afferent and efferent resistances, as well as decrease in glomerular plasma flow, ultralfiltration coefficient, and single-nephron glomerular filtration rate were significantly greater with CsA + L-NAME than with CsA alone. The increase in afferent resistance was higher with CsA + L-NAME than with Veh + L-NAME. In addition, glomerular thrombosis, proximal tubular vacuolization, and arteriolar thickening were more prominent in renal cortex, eNOS mRNA expression exhibited a 2.7-fold increase in CsA, whereas, in medulla, nNOS and iNOS expression were lower in CsA than in Veh, while eNOS tended to increase. Our results support the hypothesis that NO synthesis is enhanced at cortical level during CsA nephrotoxicity, counterbalancing predominantly preglomerular vasoconstriction. Higher NO production could be the result of increased eNOS mRNA expression.

micropuncture studies; preglomerular vasoconstriction; renal histopathology; reverse transcription-polymerase chain reaction; nitric oxide

CYCLOSPORIN A (CsA) is a potent immunosuppressive agent with indubitable efficacy in the prevention of organ allograft rejection; however, nephrotoxicity is a serious complication of the therapy. CsA nephrotoxicity is characterized by renal vasoconstriction that often progresses to chronic injury with irreversible structural renal damage (20, 27). Renal vasoconstriction is attributed to an imbalance in the release of vasoactive substances: on the one hand, increased release of vasoconstricting factors, such as thromboxane (22), endothelin (15), and angiotensin II (21); and, on the other, a decrease in vasodilating factors such as prostacyclin (21) and nitric oxide (NO) (10, 30). Participation of NO, however, has not been well defined. Some studies suggest that cyclosporin impairs NO production. Zoja et al. (39) showed that endothelial cell cultures developed structural damage when the cells were exposed to cyclosporin. Diederich et al. (10) and Takenaka et al. (30) reported a deficient response to the endothelium-dependent agonist in vascular beds of cyclosporin-treated animals. This response, however, was attributed to an enhanced generation of free radicals, which inactivate NO in mesenteric cyclosporintreated arteries (9). In contrast, more recent studies demonstrated that NO synthesis is well preserved during cyclosporin nephrotoxicity (6, 17, 29). We have shown that, in rats treated with cyclosporin, the acute renal hemodynamic responses to arginine or NO synthase inhibition were similar to those obtained in vehicle-treated rats, suggesting that the ability of the renal endothelium to produce NO was maintained and appeared to attenuate the renal vasoconstriction induced by cyclosporin (6). In addition, in bovine aortic endothelial cells in culture, López-Ongil et al. (17) recently demonstrated enhanced NO production in the presence of cyclosporin, which correlated with an increase in endothelial nitric oxide synthase (NOS) mRNA, protein, and activity. Stroes et al. (29), studying forearm blood flow of healthy volunteers, found that CsA increases NO activity.

In the kidney, NO is a vasoactive factor that plays a key role in maintaining vascular tone. NO is produced from L-arginine by the action of NOS isoforms, of which at least three molecular-level isoforms have been identified. These isoforms are the products of three different genes: neuronal NOS (nNOS) that encodes for a calcium-calcmodulin-dependent enzyme, which is markedly expressed in the brain; iNOS that encodes a second isoform expressed in macrophages after appropriate immunological and inflammatory stimuli; and eNOS, a third isoform, that is expressed primarily in endothelial cells (14). The three NOS isoforms are present in the kidney. In renal cortex, nNOS exhibits a macula densa cell-specific expression, iNOS has been observed in mesangial and proximal tubule cells, and eNOS is expressed mainly in endothelial cells of the afferent and efferent arterioles and glomerular capillaries. In renal medulla, nNOS is expressed in inner medullary collecting duct; iNOS is detected in medullary thick ascending limb of Henle’s loop (MTAL), and eNOS is located in thick ascending limb and collecting duct (for review, see Ref. 16).

NO produced by the nNOS in macula densa cells modulates renal vascular tone by direct relaxation of the afferent arteriole, thus attenuating the vasoconstric-
tion mediated by tubuloglomerular feedback activation (31, 35); in addition, NO produced by macula densa cells enhances renin secretion in yuxtaglomerular cells of the afferent arteriole (4, 26), whereas the NO produced by eNOS contributes to maintain the vascular tone and regulates the glomerular plasma flow through vasodilation of the glomerular vasculature (3, 37). In normal and during immunostimulatory conditions, NO produced by the iNOS in mesangial cells can also modulate the vascular tone by an indirect mechanism due to mesangial cell relaxation (7, 19). Thus the source of NO determines its effect on the glomerular function in normal and pathophysiological conditions.

The present study was undertaken to characterize the contribution of NO during renal vasoconstriction induced by cyclosporin administration. For this purpose, we evaluated 1) the renal hemodynamic and histological changes during chronic inhibition of NO synthesis and 2) the level of mRNA expression in the cortex and medulla of each NOS isoforms during cyclosporin administration.

**METHODS**

Male Wistar rats weighing 300–350 g, with right nephrectomy, were used for the study. Fifteen days after surgery, animals received daily subcutaneous injections of either cyclosporin (30 mg/kg of body wt) or vehicle (0.1 ml olive oil). Rats receiving vehicle were pair fed and served as controls. Renal hemodynamic and histological studies were performed 7 days after cyclosporin or vehicle administration in four groups of six rats each. Group I included rats that received vehicle, group II consisted of rats treated CsA, and group III was formed by rats that received CsA plus the inhibitor of the NO synthesis, nitro-L-arginine methyl ester (CsA + L-NAME), in the drinking water at 10 mg/dl, which provides a daily ingestion of ~6 mg/kg for a period of 7 days. This dose of L-NAME has been previously demonstrated to be enough to produce systemic and renal NO blockade (11). Group IV was composed of rats treated with vehicle and chronicL-NAME.

Micropuncture studies. For micropuncture studies, rats were anesthetized with pentobarbital sodium, and their left kidneys were excised, macroscopically divided into renal cortex and medulla. Histological studies. After micropuncture studies, the kidney was perfused through the femoral catheter with phosphate buffer, conserving the MAP of each animal. The kidney was excised, fixed in alcoholic Bouin’s solution, and processed for light microscopy. After appropriate dehydration, kidney slices were embedded in paraffin, sectioned at 4 µm, and stained with routine methods: hematoxylin/eosin, periodic acid-Schiff, Jones’ methenamine silver, Masson trichromic, and Weigert’s resorcin-fuchsin with a Van Gieson counterstain for elastic fibers demonstration. The whole colored slides containing at least 100 glomeruli each were analyzed. Morphological analyses were performed using a semiquantitative scale with values from 0 to 3 for each of the following alterations: for tubular vacuolization, 1 = scant, 2 = abundant and focal, and 3 = abundant and diffuse. For arteriolar lesions, such as wall thickening, lumen narrowing, and elastic fibers folding, 0 = none, 1 = slight, 2 = moderate, and 3 = severe. In addition, the percents of glomerular thrombosis and focal fibrinoid necrosis were evaluated. Histological samples were analyzed blindly.

**RNA isolation.** Kidneys were obtained from five rats of either CsA- or vehicle-treated groups. Rats were anesthetized by intraperitoneal injection of pentobarbital sodium, and their left kidneys were excised, macroscopically divided into cortex and medulla, frozen in liquid nitrogen, and kept at −80°C until used. Total RNA was isolated from individual cortex or medulla, following the guanidine isothiocyanate-cesium chloride method (24). 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 (Beckman DU640; Beckman, Brea, CA).

Relative quantitation of NOS mRNA. The relative level of NOS mRNA expression was assessed at renal cortex and medulla by semiquantitative reverse transcription-polymerase chain reaction (RT-PCR), as previously described (5). Briefly, Table 1 shows the primers sequence used to amplify a fragment of nitric oxide synthase isoforms by polymerase chain reaction.

**Table 1.** Primers sequences to amplify a fragment of nitric oxide synthase isoforms by polymerase chain reaction

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Primers Sequence</th>
<th>Size, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuronal NOS</td>
<td>5’-GAACCCCCAAGACCATCC3’</td>
<td>308</td>
</tr>
<tr>
<td></td>
<td>3’-GGCCTTTGCTCCCAAGCTTTG5’</td>
<td></td>
</tr>
<tr>
<td>Inducible NOS</td>
<td>5’-GTTCTTACCAAGATGATCTGG3’</td>
<td>570</td>
</tr>
<tr>
<td></td>
<td>3’-CTCTCGGCCAATGCTGGT5’</td>
<td></td>
</tr>
<tr>
<td>Endothelial NOS</td>
<td>5’-TACGGAGCAGAAATCCAC3’</td>
<td>819</td>
</tr>
<tr>
<td></td>
<td>3’-CAGGCTGCACTGTCCTTGA5’</td>
<td></td>
</tr>
</tbody>
</table>

NOS, nitric oxide synthase.
fragment of nNOS, iNOS, and eNOS. The NOS primers were custom obtained from Life Technologies. The nNOS primers were designed from a region of low identity between the three NOS isoforms (14). The iNOS and eNOS primers have been previously reported (19, 32).

The specificity of the primers was demonstrated by sequencing PCR products in both directions by the dideoxy chain termination method (25), using the Sequenase V 2.0 DNA sequencing kit (U.S. Biochemical). To monitor nonspecific effects of the experimental treatment and to semiquantitate NOS isoform expression, we amplified a fragment of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), using primers previously described (23), which yield a PCR product of 515 bp. Genomic DNA contamination was checked by carrying samples through PCR procedure without adding reverse transcriptase.

RT was carried out using 10 µg of total RNA from 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, using 200 U of the Moloney murine leukemia virus reverse transcriptase (Life Technologies). 100 pmol of random hexamers (Life Technologies), 0.5 mM of each dNTP (Sigma), and 1× RT buffer (75 mM KCl, 50 mM Tris·HCl, 1.5 mM MgCl2, 50 mM MgCl2, 10 mM dithiothreitol, pH 8.3). Samples were heated 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 were 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, 50 mM KCl, pH 8.3), 0.1 mM of each dNTP, 0.2 µCi of [32P]dCTP (≈3,000 Ci/mmol, 9.25 MBq, 250 µCi), 10 µM of each primer, and one unit of Taq DNA polymerase (Biotecnologías Universitarias, Mexico City, Mexico). The samples were overlaid with 30 µl mineral oil, and PCR cycles were performed in a DNA thermal cycler (MJ Research, Watertown, MA) with the following profile: denaturation 1 min at 94°C, annealing 1 min at 55°C for NOS 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 coamplified simultaneously in each reaction.

Preliminary studies were performed to determine the optimum number of cycles for quantitation. To analyze the PCR products, one-half of each reaction was electrophoresed in 5% acrylamide gel. Bands were ethidium bromide stained and visualized under UV light, cut out, suspended in 1 ml of scintillation cocktail (Ecolume, ICN), and counted by liquid scintillation (Beckman LS6500). The amount of radioactivity recovered from the excised bands was plotted in a log scale against the number of cycles. Figure 1 shows amplification kinetics of the three NOS isoforms and housekeeping gene in renal cortex and medulla total RNA that was pooled from vehicle group. From these curves, we chose the optimal number of cycles for each primers pair as follows: in renal cortex, 28 for nNOS, 30 for iNOS, 32 for eNOS, and 20 for GAPDH; in renal medulla, we chose 24, 31, 23, and 18, respectively. To semiquantitate each NOS isoform, all reactions were performed individually from each cortex or medulla total RNA from vehicle- and CsA-treated rats in quadruplicate.

Statistical analysis. Statistical significance is defined as two-tailed P < 0.05, and the results are presented as means ± SE. For the hemodynamics data, the significance of the differences between groups were tested by two-way ANOVA with multiple comparison, using Student-Neumann-Kuels correction. The histopathological data were analyzed using one-way ANOVA with multiple comparison using Bonferroni's correction. NOS level of expression is shown as the ratio between NOS/GAPDH PCR product (means ± SE of 5 rats/group) and was analyzed using Student’s unpaired two-tailed t-test or Mann-Whitney U-test, as needed.

RESULTS

Hemodynamic studies. Table 2 summarizes the results obtained in glomerular hemodynamics studies. Although chronic CsA administration did not change MAP, marked renal hemodynamic effects were observed. In the CsA group, the decrease in total GFR was associated with glomerular vasoconstriction. Glomerular plasma flow was reduced due to a sharp rise in afferent and efferent resistances. Because MAP was not increased, PFR remained unchanged, and a significant reduction was observed in Kf; since Kf is determined in part by mesangial cells tone, its decrease suggests that CsA induces mesangial cell contraction. The fall in two of the determinants of glomerular filtration rate, that is, Qg and Kf, was responsible for the decrease in
unchanged values of $P_{gc}$. The decrease in $Q_A$ and sure to glomerular capillaries, as disclosed by the prevented transmission of elevated systemic blood pres-

3.15-fold. The higher preglomerular vasoconstriction rose 1.8-fold, and afferent resistance rose as much as 

further increased by L-NAME administration, as shown 

by the marked increase in afferent and efferent resist-

ances. However, despite the rise in $P_{gc}$, SNGFR fell as a 

proportional elevation of afferent and efferent resis-

tances. Thus the CsA group exhibited the characteris-

tic renal vasoconstriction observed during chronic cyclo-

sporin nephrotoxicity.

The simultaneous administration of L-NAME with CsA enhanced the effects observed with CsA alone. 

In the CsA + L-NAME group, the marked elevation of MAP demonstrated the effect of systemic NO inhibition. Total kidney GFR was further reduced, although the change did not reach statistical significance. Con-

striction of glomerular vasculature induced by CsA was further increased by L-NAME administration, as shown by the marked increase in afferent and efferent resist-

ances, producing a fall in glomerular plasma flow to 

~50% of the flow observed in rats treated with CsA alone. It is noteworthy that the vasoconstriction induced by L-NAME was significantly different in affer-

ent and efferent arterioles, whereas efferent resistance rose 1.8-fold, and afferent resistance rose as much as 3.15-fold. The higher preglomerular vasoconstriction prevented transmission of elevated systemic blood pressure to glomerular capillaries, as disclosed by the unchanged values of $P_{gc}$. The decrease in $Q_A$ and $K_f$ values resulted in a further decrease in SNGFR compared with that observed with CsA alone.

The vehicle + L-NAME group was designed to demon-

strate that the dose of L-NAME used was enough to inhibit NO synthesis and to induce systemic and renal vasoconstriction, as previously reported (11). Indeed, we found that this was the case. Table 2 shows that the dose of L-NAME used in vehicle-treated rats induced a rise in MAP and significant renal vasoconstriction, as demonstrated by a decrease in $Q_A$. The pattern of glomerular vasoconstriction induced by NAME in vehicle-treated rats was, however, different from that observed in CsA-treated rats. In the vehicle group, afferent and efferent resistances rose in the same proportion (2-fold), whereas, in CsA-treated rats, L-

NAME produced a predominant afferent vasoconstriction. A rise in intraglomerular capillary pressure second-

ary to the elevation in MAP was observed in response to 

SNGFR to almost half of the values obtained in control 

animals. Thus the CsA group exhibited the characteris-

tic renal vasoconstriction observed during chronic cyclo-

sporin nephrotoxicity.

Histological studies. Table 3 summarizes the light microscopy findings observed in the four groups studied. CsA administration produced glomerular thrombosis in 1.0% of the glomeruli, abundant and focal prox-

imal tubular vaculization, and moderate arteriolar thickening. Addition of the NO synthesis inhibitor to CsA resulted in greater structural changes. Glomerular thrombosis was present in 11% of the glomeruli, prox-

imal tubular vaculization was abundant and diffuse, and arteriolar thickening was severe. Figure 2 shows a representative light microscopy image of a glomerulus and arterioles of a CsA + L-NAME-treated rat showing glomerular thrombosis and arteriolar thickening. These lesions were not observed in vehicle-treated rats and appeared in much lesser proportion in CsA alone and vehicle + L-NAME groups.

Expression of NOS mRNA. The level of gene expres-

sion of each NOS isoform was individually determined by semiquantitative RT-PCR analysis from renal cortex and medulla total RNA of each vehicle- and CsA-

treated rats. Results obtained on kinetic amplification 

experiments (Fig. 1) show that the three NOS isoforms in renal medulla reached plateau phase earlier than in renal cortex, suggesting that renal medulla has greater capacity to generate NO than renal cortex, as was recently proposed by Kone and Baylis (16). Therefore, we used more cycles to amplify each NOS from renal 

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cortex than medulla.

Figure 3 shows that in renal cortex, mRNA levels of 
nNOS and iNOS were similar in vehicle and CsA groups. In contrast, eNOS expression was 2.7-fold higher in CsA-treated rats than in the control group. The difference was statistically significant ($P < 0.05$). In the renal medulla, however, mRNA levels of nNOS and iNOS were 47 and 75% lower in CsA than in vehicle 

group, respectively. The differences were statistically significant ($P < 0.05$). In contrast, eNOS expression 

Table 2. Glomerular hemodynamics during chronic nitric oxide inhibition

<table>
<thead>
<tr>
<th>Group</th>
<th>GFR, ml/min</th>
<th>MAP, mmHg</th>
<th>$Q_A$, ml/min</th>
<th>$R_A$</th>
<th>$R_E$</th>
<th>$P_{gc}$, mmHg</th>
<th>$K_f$, nl/s·mmHg</th>
<th>SNGFR, nl/min</th>
<th>SNFF, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>1.0±0.2</td>
<td>122.5±4.6</td>
<td>133.7±11.3</td>
<td>2.3±0.3</td>
<td>1.4±0.1</td>
<td>50.3±10</td>
<td>0.049±0.007</td>
<td>35.3±3.3</td>
<td>0.26±0.03</td>
</tr>
<tr>
<td>CsA</td>
<td>0.6±0.1</td>
<td>118.2±4.3</td>
<td>68.3±7.2</td>
<td>4.0±0.5</td>
<td>2.6±0.3</td>
<td>47.2±18</td>
<td>0.031±0.003</td>
<td>18.4±18</td>
<td>0.27±0.04</td>
</tr>
<tr>
<td>CsA + L-NAME</td>
<td>0.3±0.1</td>
<td>162.4±6.0</td>
<td>32.2±5.6</td>
<td>16.6±2.7</td>
<td>7.2±1.6</td>
<td>47.8±19</td>
<td>0.010±0.002</td>
<td>8.5±1.4</td>
<td>0.27±0.04</td>
</tr>
<tr>
<td>Vehicle + L-NAME</td>
<td>0.6±0.1</td>
<td>162.0±5.5</td>
<td>69.3±6.2</td>
<td>6.9±0.9</td>
<td>4.2±0.5</td>
<td>60.4±1.9</td>
<td>0.014±0.002</td>
<td>18.9±16</td>
<td>0.27±0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 6$. GFR, glomerular filtration rate; MAP, mean arterial pressure; $Q_A$, single-nephron plasma flow; $R_A$ and $R_E$, afferent and efferent resistance; CsA, cyclosporin A; L-NAME, nitro-l-arginine methyl ester. *$P < 0.05$ vs. vehicle; †$P < 0.05$ vs. CsA; ‡$P < 0.05$ vs. vehicle + L-NAME.

Table 3. Histopathological findings

<table>
<thead>
<tr>
<th>Alteration</th>
<th>Vehicle</th>
<th>CsA</th>
<th>CsA + L-NAME</th>
<th>Vehicle + L-NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular thrombosis, %</td>
<td>0</td>
<td>1.3 ± 0.9*</td>
<td>11 ± 3.2**</td>
<td>1.0 ± 0.5*</td>
</tr>
<tr>
<td>Proximal tubular vaculization</td>
<td>Scant</td>
<td>Abundant and diffuse</td>
<td>Abundant and diffuse</td>
<td>Abundant and focal</td>
</tr>
<tr>
<td>Arteriolar thickening</td>
<td>None</td>
<td>Moderate</td>
<td>Severe</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

Values for glomerular thrombosis are means ± SE. *$P < 0.05$ vs. vehicle. †$P < 0.05$ vs. CsA. ‡$P < 0.05$ vs. vehicle + L-NAME.
was twofold higher in CsA group, but this difference did not reach statistical significance (P = 0.09).

DISCUSSION

The mechanisms involved in CsA-induced renal vasoconstriction have not been completely elucidated. Previous studies demonstrated that administration of the drug stimulates the production of vasoconstrictor factors such as endothelin, thromboxane A2, and angiotensin II (15, 21, 22). In addition, CsA inhibits the release of the vasodilator prostacyclin (21). However, participation of NO, an important renal vasodilator that maintains the low vascular resistances in the kidney, is not well defined. Studies on endothelial cell cultures showed that exposure of cells to cyclosporin results in structural damage (39), and several in vitro studies reported that acetylcholine-induced vasodilation is impaired in vascular beds of cyclosporin-treated animals, suggesting a deficient endothelial NO synthesis (10, 30), although these findings can also be explained by enhanced generation of free radicals that inactivate NO (9).

Previous studies from our laboratory evaluating the acute hemodynamic renal response to NO synthesis inhibition with L-NAME or stimulation with arginine infusion in cyclosporin-treated rats showed that the responses to both L-NAME and arginine were similar to that observed in controls, suggesting that the ability of the renal endothelium to produce NO is preserved (6). Moreover, Amore et al. (1) and López-Ongil et al. (17) recently found in kidney homogenates and in bovine aortic endothelial cells, respectively, that NOS activity is increased in the presence of cyclosporin. Thus, as a whole, these studies suggest that during cyclosporin nephrotoxicity NO release is normal or even increased.

To characterize the contribution of NO during renal vasoconstriction induced by cyclosporin administration in the rat, we evaluated the effect of chronic NO inhibition on the renal hemodynamics and histology in uninephrectomized rats treated with CsA, a model with functional and hypertrophic compensatory changes similar to those observed in renal transplant recipients (12, 36). In addition, we determined the renal expression level of each NOS isoforms mRNA in cyclosporin-treated rats.

To determine whether renal vasoconstriction induced by cyclosporin was mediated by NO synthesis defi-
creased, L-NAME will markedly accentuate renal vaso-
moderate, but, if NO synthesis is preserved or in-
vivo by changes in the Kf, which was further reduced by NO synthesis inhibi-
tion. L-NAME produced similar changes in Kf values of
control animals, suggesting that NO participates in
maintaining mesangial tone to the same extent in
control and CsA groups. Taken all together, these
findings support the statement that NO synthesis is
preserved and plays an important role in maintaining
the vascular tone and renal function during cyclosporin
nephrotoxicity.

As an alternative approach, to discern the pathway of
NO production during vasoconstriction induced by cyclo-
sporin, the gene expression levels of NOS in cortex and
medulla were determined by RT-PCR. We chose the
semiquantitative RT-PCR approach because this stra-
egy offers the possibility to detect changes in the
expression level of low abundant mRNAs, as in the case
of NO synthases in the kidney (28).

In the renal cortex, synthesis of NO due to nNOS
activity is mainly produced at macula densa cells, which
is directly involved in the local control of vascu-
lar tone and renin secretion (4, 26, 31, 35). Changes in
NaCl concentration sensed by the macula densa cells
determine nNOS expression: low-NaCl delivery to the
distal nephron stimulates nNOS gene expression,
whereas high-NaCl delivery suppresses it (28). In
CsA-treated rats, we previously found that, although
SNGFR was markedly reduced, proximal fractional
sodium reabsorption was also decreased (6). Therefore,
we reasoned that nNOS expression should not change
because the delivery of NaCl to macula densa cells did
not fall as much as SNGFR. In the present study, nNOS
mRNA levels in the renal cortex were no different in
CsA treated, compared with control group, suggesting
that NO production in macula densa cells was not
suppressed by CsA. In addition, iNOS mRNA levels in
renal cortex were not affected by CsA administration.

NO release by endothelial cells is responsible for the
low vascular resistances that characterize renal circula-
tion and contributes to the regulation of glomerular
plasma flow. Shear stress on the luminal surface of the
endothelium by the streaming blood is considered to be
the most important physiological stimulus for the
release of NO from endothelial cells (7, 14). In this
study, we found that renal cortex expression of eNOS
was 2.7-fold higher in CsA-treated rats, suggesting
increased NO synthesis in endothelial cells, which
agrees with our hemodynamic and histological results.

The increase in eNOS mRNA can be due to either an
indirect or direct effect. Indirect stimulation can result
from renal vasoconstriction induced by CsA, which
decreases arteriolar diameter at constant flow rate,
increasing shear stress to which the endothelial cell
layer is exposed, thereby eliciting an increase in eNOS
expression. In this regard, in cerebral blood vessels,
eNOS was markedly upregulated during cerebral ische-
ia (38), suggesting that NOS expression can be indi-
rectly enhanced by shear stress resulting from vaso-
constriction. Direct effect of CsA can be at transcriptional
level and/or the stabilization of eNOS transcript. In
bovine aortic endothelial cells, López-Ongil et al. (17)
recently showed that cyclosporin enhances NO produc-

In CsA-treated rats, NO synthesis inhibition pro-
duced elevation of arterial pressure and further in-
creased renal vasoconstriction, which was disproportio-
nally enhanced in preglomerular vessels. The rise in
afferent resistance was significantly greater than the
increment in efferent resistance and prevented the
transmission of systemic pressure to glomerular capil-
laries. These findings contrast with the effect observed
in control animals, in which there was a proportional
rise in pre- and postglomerular resistances and eleva-
tion in glomerular capillary pressure. This unique
effect of L-NAME suggests that, in cyclosporin nepho-
toxicity, NO plays a proportionally greater role in
maintaining vascular tone of preglomerular vessels.
Supporting this suggestion, light microscopy analysis
demonstrated that vascular structural changes and
glomerular thrombosis were enhanced by NO inhibi-
tion in cyclosporin-treated rats.

Mesangial cells tone can be indirectly evaluated in
vivo by changes in the Kf, which is the product of
glomerular permeability and filtration area that is
determined by mesangial cells tone (2). Cyclosporin
nephrotoxicity was associated with mild reduction of

Fig. 3. Representative experiments showing mRNA level expression
of nNOS, iNOS, and eNOS in renal cortex or medulla, expressed as
the ratio between each NOS isofrom and GAPDH as a housekeeping
gene, in vehicle (open bars) and cyclosporin (closed bars)-treated rats.
*P < 0.05 vs. vehicle group.
tion that correlates with an increase in endothelial NOS mRNA, protein, and activity, suggesting that cyclosporin itself can stimulate eNOS gene expression.

In this study, we found that CsA administration induced differential changes in the expression pattern of each NOS isoforms between cortex and medulla. At cortical level, nNOS and iNOS mRNA levels did not change, whereas eNOS increased significantly. In contrast, at medullary level, nNOS and iNOS expression were diminished by CsA, whereas eNOS tended to increase. In support of our observation of iNOS mRNA reduction in renal medulla, Marumo et al. (18) reported that CsA inhibits iNOS mRNA expression and nitrile products in vascular smooth muscle cells.

This different expression of NOS isoforms pattern observed in CsA-treated rats could result from several factors, such as vascular changes and/or tubular damage induced by CsA; however, more studies are necessary to investigate the mechanisms involved in the different regulation of NOS isoforms by CsA.

In summary, the results obtained in our hemodynamic and histological studies, as well as the change in renal cortex eNOS expression, support the notion that NO synthesis is enhanced at cortical level during CsA nephrotoxicity, counterbalancing predominantly preglomerular vasoconstriction induced by CsA. The increase in NO production could be secondary to an increase in medullar vasoconstriction induced by CsA. The increase in nephrotoxicity, counterbalancing predominantly preglomerular vasoconstriction induced by CsA. This different expression of NOS isoforms pattern observed in CsA-treated rats could result from several factors, such as vascular changes and/or tubular damage induced by CsA; however, more studies are necessary to investigate the mechanisms involved in the different regulation of NOS isoforms by CsA.

We thank Juan A. Alvarado for technical assistance and the members of the Molecular Physiology Unit for their suggestions and stimulating discussion.

This work was supported by research grant no. 0356PM (to J. Herrera-Acosta) and nos. 2036 and 3900 (to G. Gamba) and a scholarship grant to N. A. Bobadilla from the Mexican Council of Science and Technology.

Parts of this work were presented at the 27th meeting of the American Society of Nephrology, Orlando, FL, 1994, and at the Xth Inter-American Society of Hypertension Meeting in Mexico City, Mexico, in 1997.

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Received 13 March 1997; accepted in final form 01 December 1997.

REFERENCES


