Effect of cyclosporin A on the expression of tissue kallikrein, kininogen, and bradykinin receptor in rat

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Wang, Cindy, Philbert Ford, Caroline Chao, Lee Chao, and Julie Chao. Effect of cyclosporin A on the expression of tissue kallikrein, kininogen, and bradykinin receptor in rat. Am. J. Physiol. 273 (Renal Physiol. 42): F783–F789, 1997.—The tissue kallikrein-kinin system is involved in vasodilation and blood pressure regulation. In the present study, we investigated the effects of chronic cyclosporin A (CsA) administration on blood pressure and the expression of tissue kallikrein, kininogen, and bradykinin receptor in normotensive Wistar rats. Chronic administration of CsA significantly increased systolic blood pressure compared with control rats (n = 6, P < 0.01), although body weight was significantly lower than control rats (n = 6, P < 0.01). The development of hypertension was accompanied by the altered expression of kallikrein-kinin system components. Immunoreactive renal kallikrein and urinary excretion of tissue kallikrein levels were increased by chronic administration of CsA (n = 5 or 6, P < 0.05). Levels of N-tosyl-l-phenylalanine chloromethyl ketone-trypsin and kallikrein-releasable kininogens in sera increased in response to chronic CsA treatment (n = 5 or 6, P < 0.05). Chronic CsA treatment significantly increased renal kallikrein, bradykinin B₂ receptor, and hepatic kininogen mRNA levels. The increased levels of tissue kallikrein-kinin system components were accompanied by significant increases in 24-h urine excretion and water intake after chronic CsA treatment (n = 5, P < 0.05). These results suggest that enhanced activity of the tissue kallikrein-kinin system may compensate for the CsA-induced vasoconstriction and hypertension.

blood pressure; Northern blot; radioimmunoassay; Wistar rat

Tissue kallikrein (EC 3.4.21.35) cleaves low-molecular-weight (low-Μₑ) kininogen to release the vasoactive bradykinin peptide (6). Kinins bind to bradykinin B₁ and B₂ receptors in target tissues and exert a broad spectrum of biological effects, including vasodilation, blood pressure reduction, increased renal blood flow, natriuresis, and diuresis (6, 19). Kininogens are multifunctional proteins involved in the kinin-generating pathway, cysteine proteinase inhibition, and the acute-phase response (23).

Cyclosporin, a potent immunosuppressant, is associated with the development of hypertension and nephrotoxicity (4, 24). In humans, cyclosporin A (CsA) treatment increases blood pressure compared with age-matched control subjects. The elevation of blood pressure was associated with a normal or mildly reduced cardiac output and an elevation of total peripheral resistance (16). The precise mechanisms by which CsA contributes to these phenomena are not well characterized. Studies have shown that chronic administration of CsA produces decreases in renal blood flow and glomerular filtration rate, impaired urea secretion, and tubular dysfunction with preserved sodium reabsorption (16) and increases in renal vascular resistance (25). It has been speculated that renal vasoconstriction, with resultant extracellular volume expansion caused by retention of salt and water, provides the probable hypertensive mechanism (3). It has been hypothesized that increased sensitivity to a number of vasoconstrictor substances in isolated blood vessels by cyclosporin may contribute to the development of hypertension (17). The increased activity of the renin-angiotensin system in humans and animals plays a central role in the pathogenesis of cyclosporin-induced functional and structural lesions (22). However, the potential role of the vasodepressive kallikrein-kinin system in CsA-induced hypertension and nephrotoxicity remains unclear.

Extensive studies have shown that the tissue kallikrein-kinin system regulates blood pressure and renal hemodynamics. Epidemiological studies have shown that urinary kallikrein levels are inversely correlated with blood pressure in essentially hypertensive patients (21). An allele expressing high urinary kallikrein excretion may be associated with a reduced risk of essential hypertension (5). Reduced urinary kallikrein excretion has been described in a number of genetically hypertensive rats (20). In rats in which hypertension is induced by high-salt diet, renal kallikrein expression is suppressed, whereas renal kallikrein expression is induced in hypertension induced by chronic inhibition of nitric oxide synthase (8, 31). Renal kallikrein has been implicated in the control of renal blood flow, water and electrolyte balance, and vascular resistance (6). It has been suggested that increases in papillary blood flow, urine volume, and sodium excretion induced by kinin are mediated by kinin B₂ receptors (13). Because the major functional side effects of cyclosporine are systemic vasoconstriction and renal vasoconstriction, the tissue kallikrein-kinin system could be involved.

The present study examined the regulation of the kallikrein-kinin system in cyclosporin-induced hypertension in Wistar rats. We showed that the expression of the renal kallikrein, bradykinin B₂ receptor, and the hepatic kininogen genes is upregulated by chronic administration of cyclosporin, which is associated with an elevation in blood pressure and diuresis.

MATERIALS AND METHODS

Animal treatment. Male Wistar rats (200 g) were obtained from Harlan Sprague Dawley (Indianapolis, IN). The rats were divided into two groups, i.e., control and CsA. Each group consisted of six age-matched rats. The CsA group received daily subcutaneous injections of CsA (Sandimmune; Sandoz, East Hanover, NJ) solubilized in sesame oil at a dose...
of 25 mg·kg⁻¹·day⁻¹ for 28 days (n = 6). A control group (n = 6) was injected subcutaneously with sesame oil. Both groups were given standard commercial rat chow and tap water ad libitum. Throughout the study, the rats were housed in a room that was kept at constant temperature (25 ± 1°C) and humidity (60 ± 5%) and was lighted automatically for 12 h daily. Procedures and protocols were in accordance with our institutional guidelines.

Blood pressure measurement. Systolic blood pressure was measured with a manometer-tachometer (model KN-210–1; Narco Bio-Systems), using the tail cuff method as previously described (31). Calibration of the blood pressure device was carried out as described by the manufacturer. Unanesthetized rats were introduced into a plastic holder mounted on a thermostatically controlled warm plate that was maintained at 37–38°C during measurement. On the average, five readings were taken for each animal.

Tissue homogenate preparation and protein determination. Rats were anesthetized with pentobarbital sodium (50 mg/kg), the heart was exposed, and 5 ml of blood were withdrawn. Heparin (100 units/rat) was injected into the left ventricle. After 30 s, the vena cava was cut, and the circulation was perfused via cardiac puncture with at least 50 ml of normal saline until tissue appeared blood free. Tissues were removed, minced, and homogenized as described previously (14). Protein concentrations of the tissue homogenates were determined by the Lowry method (18). Sera of these rats were collected and used to measure kallikrein levels by radioimmunoassay (RIA).

RNA preparation. Wistar rats were anesthetized and perfused with normal saline via cardiac puncture. Tissues of interest were removed, and the RNAs were extracted by the guanidine isothiocyanate-cesium chloride ultracentrifugation method (27). The extracted RNA was quantified spectrophotometrically by absorbance at 260 nm, dissolved in diethyl pyrocarbonate-treated water, and stored at −80°C until further use.

Tissue kallikrein radioimmunoassay. Tissue kallikrein levels were measured by a direct RIA as described previously (28). Purified tissue kallikrein (5 µg) was labeled with ¹²⁵I using the iodogen method, and the labeled tissue kallikrein was separated from the reaction mixture with a GF-S column (Pierce, Rockford, IL). In the antibody titration curve, tissue kallikrein antiserum was diluted in assay buffer (1% bovine serum albumin in phosphate-buffered saline, pH 7.0) from 1/1,000 to 1/640,000. One hundred microliters of ¹²⁵I-labeled tissue kallikrein [10,000 counts·min⁻¹·100 µl⁻¹ (cpm/µl)] and 100 µl of diluted antibody were added to 100 µl of sample. The assay mixtures were brought to a final volume of 400 µl with assay buffer and incubated at room temperature for 18 h. Antibody-bound tissue kallikrein was separated from free tissue kallikrein through centrifugation in an optimum combination of 25% polyethylene glycol and 1% bovine gamma-globulin. The final antiserum dilution was 1:480,000, and the standard ranged from 80 pg to 10 ng.

Determination of kallikrein levels. Fifty microfilters of serum were added to 0.45 ml of 0.02 M Tris(hydroxymethyl)-aminomethane hydrochloride (Tris·HCl), pH 8.0, and boiled for 30 min to eliminate kininase activity. Aliquots (100 µl) of the supernatants were collected after a 5-min centrifugation in a microfuge. N-tosyl-L-phenylalanine chloromethyl ketone (TPCK)-trypsin (TPCK-trypsin, 40 µg) or rat tissue kallikrein (1 µg) in 0.4 ml of 0.02 M Tris·HCl, pH 8.0, was added to each aliquot for releasing the kinin moiety from kinogens. The samples were then incubated at 37°C for 30 min, and the reaction was stopped by boiling for 10 min. Aliquots were used in a kinin RIA (29), which utilized rabbit antiserum against bradykinin and ¹²⁵I-labeled [Tyr]bradykinin. Levels of TPCK-trypsin and kallikrein-releasable kininogens were expressed as micrograms of kinin equivalent per milliliter of serum. TPCK-trypsin was purchased from Sigma Chemical (St. Louis, MO), and rat tissue kallikrein was purified from rat salivary gland according to a previously described method (10).

Northern blot analysis. The effects of chronic CsA administration on mRNA levels of tissue kallikrein in the kidney and kininogen in the liver were examined with Northern blot analysis using specific cDNA probes. cDNA probes encoding tissue kallikrein (14), rat kininogen (9), and rat α₁-antitrypsin (11) were used for Northern blot analysis. A 2,000-bp human β-actin cDNA insert was purchased from Clontech Laboratories (Palo Alto, CA). Total kidney RNA (25 µg) or liver RNA (10 µg) from salt-treated and control rats was separated on a 1.5% agarose gel containing 0.66 M formaldehyde. RNA samples were transferred in 20× SSC (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0) for 12–16 h by capillary action and immobilized onto an Immobilon-N membrane. The membrane was prehybridized in hybridization buffer [5× SSPE (1× SSPE is 0.15 M NaCl, 0.01 M NaH₂PO₄, and 0.001 M EDTA, pH 7.4), 10× Denhardt’s solution, 0.5% sodium dodecyl sulfate (SDS), and 100 µg/ml tRNA] at 60°C for 4 h. The following cDNA probes were prepared by restriction enzyme digestion and purified by agarose gel electrophoresis and electrospraying: tissue kallikrein, kininogens, and α₁-antitrypsin. All cDNA probes were labeled with ³²P (NEN Research Products, Boston, MA), using a nick translation kit (Bethesda Research Laboratories, Bethesda, MD) according to the protocols recommended by the manufacturer. Unincorporated label was removed by spin column (G-50; 5 Prime — 3 Prime, Boulder, CO), and the specific activity of each probe was ~1–2 × 10⁶ cpm/µg DNA.

Membranes were hybridized for 16–18 h with kallikrein or tissue kallikrein cDNA probes. Membranes were washed with 0.1× SSPE at room temperature and exposed to X-ray film at −70°C. Then membranes were stripped and reprobed with nick-translated α₁-antitrypsin or β-actin cDNA probes. The X-ray film was scanned into Adobe Photoshop 2.5 with a Hewlett Packard Scan Jet IICX/T, and the mean intensity of respective bands was quantified by Image 1.47. Densitometric intensities of three independent blots were averaged and expressed as the mean ± SEM.

Reverse transcription-polymerase chain reaction Southern blot analysis. Semi-quantitative reversetranscription-polymerase chain reaction (RT-PCR) assays were used to estimate the abundance of bradykinin B₂ receptor mRNA in the kidney of CsA-treated and control rats. The RT mixture contained 1 µg of total kidney RNA, 4 µl of 10× RT buffer, 8 µl of 2.5 mM dNTP, 10 pmol of 3′ primer, 2 µl of 0.1 M dithiothreitol, and 200 U of Moloney murine leukemia virus reverse transcriptase Superscript II (BRL, Gaithersburg, MD) in a total volume of 20 µl. RT was performed at 37°C for 60 min, and the RT enzyme was inactivated by heating at 95°C for 5 min. The bradykinin B₂ receptor and β-actin cDNA was amplified by PCR, using 10 pmol of 5′ primer, 0.5 U of Taq polymerase, and 5 µl of 10× PCR buffer in 50 µl total volume for 25 cycles at 94°C, 1 min; 60°C, 1 min; and 72°C, 1 min, using a PTC-100 Programmable Thermal Controller (M Research, Watertown, MA). The 5′ primer corresponds to nucleotides of rat B₂ receptor cDNA (5′-CGTCTGGACCTCCTTGAAC-3′) and the 3′ primer is complementary to the sequence of rat B₂ receptor cDNA (5′-GCTGAGGACAAAGATGTTC-3′). The RT-PCR primers for β-actin (5′ primer, 5′-GAACCCATAGGGCAACC-GTG-3′; 3′ primer, 5′-TGGCATAGGGTTTACCCAG-3′) are derived from the reported rat cytoplasmic β-actin gene sequence (26). The predicted sizes of the amplified PCR products are 572 bp for rat B₂ receptor and 560 bp for β-actin. One-half of the PCR mixture was submitted to Southern blot...
analysis followed by hybridization with an end-labeled rat bradykinin B2 receptor (5'-TACTCCTCTATGGTCCGGAA-CACCA 3') or β-actin (5'-CGACGATTTCCTCTACGC 3') probes in 6× SSC, 5× Denhardt's solution, 0.5% SDS, 100 µg/ml herring sperm DNA at 60°C for 18 h. The membranes were washed four times in a solution containing 6× SSC and 0.5% SDS at 60°C. Signals were detected by autoradiography at −80°C and scanned into Adobe Photoshop 2.5 with the Hewlett Packard ScanJet II CX/T. The mean intensity of respective bands was quantified by Image 1.47, and densitometric intensities were averaged and expressed as means ± SD.

Urine collection and electrolytes analysis. Twenty-four hour urine of rats was collected using metabolic cages after 21 and 28 days of chronic CsA administration. The rats were maintained in metabolic cages that allowed for the separate collection of urine and measurement of water intake. To eliminate contamination of urine samples, animals received only water during the collection period. The animals had 2 h daily (1600–1800) access to food and continuous access to water.

Statistical analysis. Group data are expressed as means ± SE. Intergroup differences were then analyzed by the Student's t-test for unpaired data. Serial measurements were analyzed by analysis of variance (ANOVA). Differences were considered significant at a value of P < 0.05.

RESULTS

Blood pressure and body weight changes in response to chronic CsA treatment. Chronic administration of CsA significantly increased systolic blood pressure of Wistar rats at 14–28 days compared with vehicle-treated control rats (Fig. 1A). The average body weight of the CsA group during the treatment period (7–28 days) was significantly lower than control rats (Fig. 1B).

Effect of CsA on tissue kallikrein expression. Tissue kallikrein gene expression in the kidney of Wistar rats after chronic administration of CsA was analyzed at the mRNA and protein levels. Tissue kallikrein mRNA in the kidney appeared as a single 1.0- to 1.1-kb band on Northern blot (Fig. 2). Quantitative densitometry of the Northern blot showed that tissue kallikrein mRNA in the kidney was significantly increased by chronic administration of CsA (104.6 ± 6.7 densitometric units) compared with control rats (59.1 ± 6.8 densitometric units). Tissue kallikrein mRNA levels in the kidney of CsA-treated rats were 1.8-fold of control rats. When the same blot was stripped and reprobed with the β-actin cDNA probe, no changes of β-actin mRNA were detected (Fig. 2).

The increase in tissue kallikrein mRNA was accompanied by an increase in the amounts of immunoreactive tissue kallikrein in the kidney and urinary excretion of Wistar rats (Table 1). Immunoreactive tissue kallikrein levels in the kidney were measured with a direct radiomunnoassay specific for tissue kallikrein. The results showed that tissue kallikrein levels in the kidney of Wistar rats at 28 days after chronic administration of vehicle (sesame oil) or CsA were 27.36 ± 4.98 and 51.17 ± 3.73 ng/mg protein, respectively. A similar increase of tissue kallikrein levels by chronic administration of CsA was observed in urinary excretion of tissue kallikrein. At 21 days after chronic administration of vehicle or CsA, tissue kallikrein levels in the 24-h urine were 33.56 ± 3.82 and 63.41 ± 6.77 µg, respectively. At 28 days after chronic administration of vehicle or CsA, tissue kallikrein levels in the 24-h urine were 45.50 ± 5.81 and 69.72 ± 6.12 µg, respectively. To evaluate whether urinary flow rate contributes to the

Fig. 1. Effect of chronic cyclosporin A (CsA) treatment on systolic blood pressure (A) and body weight (B) in Wistar rats. Values are expressed as means ± SE (n = 6). Bars are standard deviation. †P < 0.01 between CsA vs. control rats. Control rats received same amount of vehicle.

Fig. 2. Northern blot analysis of tissue kallikrein and β-actin mRNA expression in kidneys of control (C) and CsA-treated (CsA) rats. Twenty-five micrograms of total kidney RNA were used for each lane. Rats were killed at 28 days after chronic CsA treatment.
Table 1. RIA measurements of intrarenal kallikrein, urinary excretion of tissue kallikrein, and kinin equivalent of kininogens in CsA-treated Wistar rats

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<th>21 Days</th>
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<td>Control CsA</td>
<td>Control CsA</td>
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<td>Intrarenal kallikrein, ng/mg protein</td>
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<tr>
<td>Urinary kallikrein, µg/24-h urine excretion</td>
<td>33.56 ± 3.82</td>
<td>27.36 ± 4.98</td>
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<td>TPCK-trypsin-releasable kininogens, µg kinin eq/ml serum</td>
<td>6.48 ± 0.76</td>
<td>5.57 ± 0.29</td>
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<td>Kallikrein-releasable kininogens, µg kinin eq/ml serum</td>
<td>1.30 ± 0.07</td>
<td>1.31 ± 0.09</td>
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<td>51.17 ± 3.73*</td>
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|                      | Values are means ± SE; n = 5 or 6. Levels of N-tosyl-L-phenylalanine chloromethyl ketone (TPCK)-trypsin-releasable kininogens were determined by measuring amount of released kinin following TPCK-trypsin treatment. Levels of kallikrein-releasable kininogens were determined by measuring amount of released kinin following tissue kallikrein treatment. Kininogen levels were expressed as µg kinin eq/ml serum, and kinin levels were measured by a radiimmunoassay (RIA). Tissue kallikrein levels in kidney extracts and urinary excretion were measured by a direct RIA. CsA, cyclosporin A. *P < 0.01.

Effect of CsA on kininogen expression. Kininogen mRNA expression in the liver of Wistar rats after chronic administration of CsA was analyzed at the mRNA and protein levels. Kininogen mRNA in the liver appeared as a single 1.6- to 1.8-kb band on a Northern blot (Fig. 3). Quantitative densitometry of the Northern blot showed that kininogen mRNA in the liver was signifi-
cantly increased by chronic administration of CsA (168.5 ± 15.7 densitometric units) compared with control rats (81.1 ± 7.7 densitometric units). Kininogen mRNA levels in the liver of CsA-treated rats were 2.1-fold of control rats. When the same blot was stripped and reprobed with the α1-antitrypsin cDNA probe, no changes of α1-antitrypsin mRNA were detected (Fig. 3).

For total kininogen assays, TPCK-trypsin was added to heat-denatured rat sera to convert kininogens to bradykinin. Tissue kallikrein is able to liberate bradykinin from high-M r kininogen, while tissue kallikrein releases kinin from high-M r kininogen at a lower affinity. In addition, high-M r kininogen level is reduced in serum because plasma kallikrein cleaves high-M r kininogen during blood clotting. Therefore, rat tissue kallikrein preferentially cleaves low-M r kininogen in rat sera to liberate kinin. Levels of TPCK-trypsin and kallikrein-releasable kininogens in sera were determined by measuring kinin equivalents by a kinin RIA. At 21 days after CsA treatment, levels of TPCK-trypsin-releasable kininogens were 6.48 ± 0.76 and 19.61 ± 1.31 µg kinin eq/ml serum in control and CsA-treated Wistar rats, respectively. At 28 days after CsA treatment, levels of TPCK-trypsin-releasable kininogens were 5.57 ± 0.29 and 17.25 ± 2.41 µg kinin eq/ml serum in control and CsA-treated Wistar rats, respectively. These results showed that chronic CsA treatment upregulates the expression of hepatic-derived kininogen and increases total kininogen levels in circulation about threefold in Wistar rats. The increase of low-M r kininogen was demonstrated by incubating sera with rat tissue kallikrein, which preferentially cleaves low-M r kininogen to liberate kinin (Table 1). At 21 days after CsA treatment, kallikrein-releasable kininogen levels were 1.30 ± 0.07 and 2.62 ± 0.33 µg kinin eq/ml serum in control and CsA-treated Wistar rats, respectively. At 28 days after CsA treatment, kallikrein-releasable kininogen levels were 1.31 ± 0.09 and 2.40 ± 0.24 µg kinin eq/ml serum in control and CsA-treated Wistar rats, respectively. Therefore, both low-M r kininogen and T-kininogen levels increased in response to chronic CsA treatment.

Effect of CsA on bradykinin B2 receptor expression. The expression of bradykinin B2 receptor was analyzed with RT-PCR followed by Southern blot analysis, using three gene-specific oligonucleotides. Figure 4 shows the expression of rat bradykinin B2 receptor mRNAs in the kidney of Wistar rats after chronic administration of CsA. Quantitative densitometry of RT-PCR Southern blot analysis showed that chronic administration of CsA increased bradykinin B2 receptor mRNA (160.9 ± 28.1 densitometric units) in the kidney compared with control rats (106.6 ± 25.6 densitometric units, n = 4, P < 0.05). Bradykinin B2 receptor mRNA levels in the kidney of CsA-treated rats increased by 50% compared with control rats. However, bradykinin B1 receptor mRNA levels in the kidney of CsA-treated rats were not

Fig. 3. Northern blot analysis of kininogen and α1-antitrypsin (α1-AT) mRNA expression in livers of control (C) and CsA-treated (CsA) rats. Ten micrograms of total liver RNA were used for each lane. Rats were killed at 28 days after chronic CsA treatment.
affected by chronic administration of CsA as determined by RT-PCR Southern blot analysis (data not shown). Rat β-actin mRNA levels were not affected by chronic CsA treatment.

Effect of CsA on urine excretion and water intake. Figure 5 shows the results of urine excretion and water intake of Wistar rats at 28 days after chronic administration of CsA. Twenty-four-hour urine excretion was significantly higher in the CsA-treated group (27.6 ± 2.5 ml/day, n = 6, P < 0.01) compared with the control rats (12.5 ± 2.2 ml/day, n = 6). Similarly, water intake in the CsA-treated group (27.6 ± 3.4 ml/day, n = 6, P < 0.01) was significantly higher compared with the control rats (10.7 ± 2.1 ml/day, n = 6). Similarly, urine excretion and water intake of Wistar rats at 21 days after chronic administration of CsA were significantly increased compared with control rats. At 21 days after chronic administration of CsA, 24-h urine excretion was significantly higher in the CsA-treated group (30.1 ± 6.3 ml/day, n = 6, P < 0.05) compared with the control rats (10.8 ± 2.1 ml/day, n = 6). Similarly, water intake in the CsA-treated group (33.5 ± 7.8 ml/day, n = 6, P < 0.05) was significantly higher compared with the control rats (10.0 ± 1.5 ml/day, n = 6) (data not shown). In accord with the increased level of water intake and urine excretion, chronic CsA administration significantly increased the level of urinary kallikrein excretion (Fig. 5).

DISCUSSION

The present study demonstrates that chronic administration of CsA modulates the expression of tissue kallikrein-kinin system components. Chronic administration of CsA increased the expression of renal kallikrein and hepatic kininogens at both protein and mRNA levels. In addition, bradykinin B2 receptor mRNA levels were significantly increased in the kidney of CsA-treated Wistar rats. Collectively, these results illustrate that chronic administration of CsA upregulates the synthesis and release of renal kallikrein and hepatic kininogen and the expression of renal bradykinin B2 receptor, which was accompanied by the development of hypertension and diuresis. However, the role of increased activity of the tissue kallikrein-kinin system in the development of hypertension and nephrotoxicity induced by chronic CsA treatment awaits further studies.

Cyclosporin has been shown to possess intrinsic vasoconstrictor properties and to stimulate the renin-angiotensin system with an increase in plasma renin activity (22, 33). In the present study, we show that tissue kallikrein gene expression in the kidney and urinary excretion increases in response to chronic CsA treatment. The increased expression of tissue kallikrein may produce an elevated level of kinins locally in the kidney. Because kinins are natriuretic/diuretic and vasodilatory, the upregulation of renal kallikrein may compensate for the vasoconstrictor activity of cyclosporine. In accord with the increased level of urinary kallikrein excretion, chronic CsA administration significantly increased water intake and urine excretion at 21 and 28 days after treatment. The elevated level of renal kallikrein may contribute to produce the diuretic effect in CsA-treated rats. The mechanisms by which chronic administration of CsA regulates tissue kallikrein expression are not clear at the present time. However, recent studies have shown that short-term cyclosporin treatment (20 mg·kg⁻¹·day⁻¹ for 3 days) suppresses kallikrein and bradykinin B2 receptor mRNAs in the rat renal cortex (7). This suggests that increased tissue kallikrein expression is indirectly caused by hypertension and nephrotoxicity in chronic CsA-treated rats.

The kallikrein binding protein binds to active tissue kallikrein and inhibits its enzymatic activities. Our previous study has shown that the kallikrein binding protein gene is downregulated in arterial hypertensive rats (8). The levels of kallikrein binding protein mRNA and protein in the circulation were not affected by chronic administration of CsA (data not shown).

The upregulation of kininogen expression has been described in other hypertensive rat models induced by
high-salt diet or by nitric oxide synthase inhibitor (8, 31). In this study, we have shown that kininogen mRNA and its protein levels in the serum increased in response to chronic administration of CsA. The increase in serum kininogen could cause kininogen levels to increase locally in the kidney. Because low-M₉ kininogen is converted to kinin by tissue kallikrein, kinin levels may be elevated as a result of increased levels of both tissue kallikrein and its substrate, low-M₉ kininogen, in the kidney. These results suggest that the increase in kininogen levels may have a protective role in CsA-induced hypertension.

Kinins exert their biological effect by interacting with bradykinin B₁ and B₂ receptors (19). The bradykinin B₂ receptor mediates most of the actions of kinins, and the important role of the bradykinin B₂ receptor has been indicated in hypertensive animal models. Several lines of evidence indicate that the bradykinin B₂ receptor participates in blood pressure regulation. In two-kidney, one-clip (2K-1C) Goldblatt hypertensive rats, bradykinin B₂ receptor binding sites increased (12). This increase was associated with a decreased intrarenal level of kallikrein. A high-salt diet has been shown to induce hypertension in mice that are homozygous for the disruption of the B₂ receptor gene (1). In addition, intrarenal infusion of bradykinin could cause an acute elevation of renal blood flow, urine volume, and urinary sodium excretion (15). In concert with increased renal kallikrein and kininogen levels, the increased level of bradykinin B₂ receptors in CsA-treated rats may compensate for CsA-induced nephrotoxicity and vasoconstriction. Studies have shown that subacute CsA treatment selectively impairs renal endothelium-dependent relaxation related to prostaglandin and nitric oxide release (2, 30). Because activation of bradykinin B₂ receptor leads to the formation of the potent vasodilators, nitric oxide and prostacyclin, upregulation of bradykinin B₂ receptors may be the result of feedback control from an impaired signal-transduction pathway.

Several pathophysiological mechanisms have been proposed to explain the hypertensive effect of cyclosporine, such as direct and sympathetically mediated vasoconstriction. Studies suggest that the balance between the vasodilators and vasoconstrictors in the kidney maintains blood pressure homeostasis. Therefore, increased levels of tissue kallikrein-kinin system components in the kidney by CsA may counterbalance hypertension and nephrotoxicity induced by increased activities of vasoconstrictors, e.g., endothelins and angiotensin II (22). This hypothesis is supported by an in vitro study showing that the pretreatment of kidney epithelial cells with CsA was associated with tolerance against subsequent lethal CsA exposure (34).

In summary, the results of the present study demonstrated that chronic administration of CsA upregulates renal kallikrein, renal bradykinin B₂ receptor, and hepatic kininogen gene expression. The increased activity of the tissue kallikrein-kinin system in response to CsA treatment may compensate for CsA-induced vasoconstriction and nephrotoxicity. The precise mechanisms whereby the tissue kallikrein-kinin system components are regulated by chronic CsA treatment require further investigation. The potential of tissue kallikrein in the attenuation of CsA-induced hypertension and nephrotoxicity needs to be explored.

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