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Effect of cyclosporine administration on renal hemodynamics in conscious rats

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Effect of cyclosporine administration on renal hemodynamics in conscious rats. The effect of acute and chronic administration of cyclosporine on systemic and renal hemodynamics was studied in conscious rats. Infusion of cyclosporine in a dose of 20 mg/kg (Cy 20) resulted in a significant fall in renal blood flow (RBF) (3.4 vs. 6.5 ml/min/g, $P < 0.05$) and a rise in renal vascular resistance (RVR) (36.9 vs. 20.6 mm Hg/ml/min/g, $P < 0.05$). Infusion of cyclosporine at a dose of 10 mg/kg (Cy 10) did not result in a significant change in RBF or RVR. Both doses of cyclosporine resulted in stimulation of plasma renin activity (PRA) from control values of 5.6 ± 0.8 ng/ml/hr to 11.6 ± 2.0 with 10 mg/kg and 26.7 ± 5.6 with 20 mg/kg. Urinary 6-keto-$PGF_{1\alpha}$ excretion increased from control values of 14.0 ± 2.0 ng/6hr to 22.7 ± 2.2 with 10 mg/kg and 25.0 ± 2.0 with 20 mg/kg. Similar effects on RBF, RVR, PRA, and 6-keto-$PGF_{1\alpha}$ excretion were seen after chronic administration of cyclosporine (20 mg/kg i.p. for 7 days). Pretreatment of animals with captopril did not prevent the fall in RBF after cyclosporine, suggesting that the vasoconstriction was not mediated by angiotensin II. Animals treated with meclofenamate demonstrated reduction in RBF with 10 mg/kg cyclosporine (4.3 vs. 7.0 ml/min/g, $P < 0.05$), suggesting that prostaglandins protect against the vasoconstrictor effect of cyclosporine. Administration of phenoxybenzamine after cyclosporine improved RBF (5.0 vs. 3.4 ml/min/g) and restored RVR to normal. Similarly, renal denervation dramatically reduced the fall in RBF after cyclosporine (innervated right kidney 3.6 vs. denervated left kidney 6.0 ml/min/g, $P < 0.001$). We conclude that cyclosporine causes renal vasoconstriction, which is mediated by the renal sympathetic nervous system, and that vasoconstriction is exacerbated by the administration of cyclooxygenase inhibitors.

Effet de l'administration de cyclosporine sur l'hémodynamique rénale de rats éveillés. L'effet d'une administration aiguë ou chronique de cyclosporine sur l'hémodynamique systémique ou rénale a été étudié chez des rats éveillés. Une perfusion de cyclosporine à la dose de 20 mg/kg (Cy 20) a entraîné une chute significative du débit sanguin rénal (RBF) (3,4 vs. 6,5 ml/min/g, $P < 0,05$) et une élévation des résistances vasculaires rénales (RVR) (36,9 vs. 20,6 mm Hg/ml/min/g, $P < 0,05$). Une perfusion de cyclosporine à la dose de 10 mg/kg (Cy 10) n'a pas modifié significativement RBF ni RVR. Les deux doses de cyclosporine ont stimulé l'activité rénine plasmatique (PRA) de valeurs contrôles de 5,6 ± 0,8 ng/ml/hr à 11,6 ± 2,0 avec 10 mg/kg, et à 26,7 ± 5,6 avec 20 mg/kg. L'excrétion urinaire de 6-céto-$PGF_{1\alpha}$ a augmenté de valeur contrôle de 14,0 ± 2,0 ng/6 hr à 22,7 ± 2,2 avec 10 mg/kg, et 25,0 ± 2,0 avec 20 mg/kg. Des effets identiques sur RBF, RVR, PRA et excrétion de 6-céto-$PGF_{1\alpha}$ ont été observés après administration chronique de cyclosporine (20 mg/kg ip, pendant 7 jours). Le prétraitement des animaux avec du captopril n'a pas empêché la chute de RBF après cyclosporine, ce qui suggère que la vasoconstriction n'était pas médiée par l'angiotensine II. Les animaux traités avec du méclofénamate ont présenté une réduction de RBF avec 10 mg/kg de cyclosporine (4,3 vs. 7,0 ml/min/g, $P < 0,05$) ce qui suggère que la prostaglandine protège des effets vasoconstricteurs de la cyclosporine. L'administration de phénoxybenzamine après la cyclosporine a amélioré RBF (5,0 vs. 3,4 ml/min/g) et a ramené RVR à la normale. De même, la dènervation rénale a réduit de facon marquée la chute de RBF après la cyclosporine (rein droit innervé 3,6 contre rein gauche dénervé 6,0 ml/min/g, $P < 0,001$). Nous concluons que la cyclosporine entraîne une vasoconstriction rénale, qui est médiée par le système nerveux sympatique rénal, et que cette vasoconstriction est exacerbée par l'administration des inhibiteurs de la cyclohoxygénase.

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Cyclosporine has become increasingly popular as the immunosuppressive of choice for renal, cardiac, pancreatic, and hepatic transplantation [1–5]. However, one of the major factors limiting its use is nephrotoxicity. A number of adverse effects of cyclosporine on renal function have been described. These include a reversible, dose-dependent increase in serum creatinine [6], hyperkalemia [7, 8], metabolic acidosis [9], hypertension [1–3], and chronic interstitial nephritis with irreversible deterioration in renal function [10]. The exact mechanisms involved in these toxic effects of cyclosporine on the kidney are not fully understood, although they may reflect tubular toxicity, especially in the straight segment of the proximal tubule [11]. However, the possibility that cyclosporine may affect renal blood vessels has recently been raised [12]. A decrease in the "renal perfusion index" derived from nuclear medicine studies following CSA administration to humans [13] and a vascular lesion thought to be specific for cyclosporine have been described [14]. Recently, Powell-Jackson et al described a syndrome in liver transplant recipients receiving intravenous cyclosporine characterized by oliguria and a low fractional excretion of sodium [15]. These findings suggest that cyclosporine may have a direct effect on the renal circulation. We, therefore, studied the effects of acute and chronic administration of cyclosporine on renal blood flow in the rat and examined several systems controlling the renal circulation, namely, the renin-angiotensin, prostaglandin, and the sympathetic nervous systems.

Methods

Male Sprague-Dawley rats (Harlan, Madison, Wisconsin, USA) 250 to 350 g in weight were maintained on Purina rat chow and tap water ad lib. Cyclosporine (provided by Sandoz Pharmaceuticals, East Hanover, New Jersey, USA) was prepared by dissolving in ethanol and then diluting with 10% fat

emulsion (Intralipid, Miles Labs, Berkeley, California, USA) to give a solution containing 5 to 10 mg/ml of cyclosporine.

Effect of cyclosporine infusion on systemic and renal hemodynamics

Rats were anesthetized briefly with ether, and polyethylene cannulas (PE-50) were inserted into the femoral artery, vein and the left ventricle via the right carotid artery. Volume replacement was given as 0.5% of body wt as isotonic saline. Rats were placed in acrylic restrainers and allowed 45 min to recover from anesthesia. The following groups were studied: Group I ($N = 8$) received an infusion of the fat emulsion vehicle only. Group II ($N = 8$) received an infusion of cyclosporine (10 mg/kg), and Group III ($N = 6$) received cyclosporine (20 mg/kg). The infusions were given at the rate of 0.034 ml/min through the femoral vein catheter. Thirty min after the end of the infusion, systemic and renal hemodynamics were measured using radiolabelled microspheres [16]. The microspheres used were 9 ± 0.7 μ in diameter and labelled with ^{85}Sr. Mean arterial pressure (MAP) was monitored using a pressure transducer (P23D6, Gould Statham Inc., San Juan, Puerto Rico) connected to a chart recorder (Hewlett Packard 7702B, Elkhart, Indiana, USA). Cardiac output was expressed as cardiac index by correcting for body wt in kilograms. Renal blood flow was corrected for kidney wt in g.

Effect of cyclosporine infusion on plasma renin activity (PRA) and urinary prostaglandins

Plasma renin activity was determined by radioimmunoassay [17] at pH 5.7 using a commercially available kit (New England Nuclear, Boston, Massachusetts). Samples of heparinized plasma were obtained from the femoral arteries of conscious rats 15 min after intravenous administration of cyclosporine (20 mg/kg), cyclosporine (10 mg/kg) and vehicle, and before injection of microspheres. To determine the effect of cyclosporine infusions on prostaglandin excretion, three separate groups of rats received either the vehicle ($N = 10$), cyclosporine 10 mg/kg ($N = 8$), or cyclosporine 20 mg/kg ($N = 8$). At the conclusion of the infusions, the rats were given 2 ml of 5% dextrose by vein to ensure adequate urine flow, placed in metabolic cages and their urine collected for 6 hrs. PGE_2 and 6-keto-$PGF_{1\alpha}$ in the urine were measured by radioimmunoassay [18].

Effect of captopril on the renovascular response to cyclosporine infusion

Thirteen rats were given captopril (Squibb, Princeton, New Jersey, USA), 50 mg/kg i.v., 15 min after insertion of vascular catheters and allowed a further 30 min to recover. Group IV ($N = 7$) then received an infusion of the vehicle, while Group V ($N = 6$) received cyclosporine (20 mg/kg). Thirty min after completion of the infusion, systemic and renal hemodynamics were measured.

Effect of meclofenamate on the renovascular response to cyclosporine infusion

Fourteen rats were given meclofenamate, 5 mg/kg i.v., 15 min after insertion of vascular catheters and allowed a further 30 min to recover. Group VI ($N = 6$) then received an infusion of the vehicle, while Group VII ($N = 8$) received cyclosporine (10 mg/kg). Thirty min after completion of the infusion, systemic and renal hemodynamics were measured.

Effect of phenoxybenzamine on the renovascular response to cyclosporine infusion

Fourteen rats were prepared for microsphere study as outlined previously. Group VIII ($N = 8$) received an infusion of the vehicle 45 min after surgery, while Group IX ($N = 6$) received cyclosporine (20 mg/kg). Fifteen min after completion of the infusion, both groups were given an intravenous injection of the α-adrenengic blocker, phenoxybenzamine, 1 mg/kg in 5% dextrose. Fifteen min later, mean arterial pressure was recorded and systemic and renal hemodynamics were measured.

Effect of renal denervation on the renovascular response to cyclosporine

Twelve rats underwent unilateral denervation of the left renal artery, accomplished by exposing the renal nerve and painting it with 10% phenol. Three days later the rats were divided into two groups and prepared for microsphere study. Group X ($N = 6$) received an infusion of the vehicle while Group XI ($N = 6$) received an infusion of cyclosporine 20 mg/kg. Thirty min after completion of the infusions, systemic and renal hemodynamics were measured.

Effect of chronic cyclosporine administration on renal hemodynamics

Rats housed in individual metabolic cages received daily intraperitoneal injections of cyclosporine (20 mg/kg) in 10% fat emulsion ($N = 6$) or vehicle alone ($N = 6$). Because preliminary experiments revealed that this dose of cyclosporine resulted in decreased food intake, cyclosporine and control rats were pair-fed. Urine was collected daily for measurement of prostaglandin excretion. After 1 week the rats were prepared as above for study of renal hemodynamics. Shortly before microsphere injection, 0.5 ml of whole blood was obtained for measurement of PRA and plasma creatinine. This blood was replaced with an equivalent volume of normal saline.

Statistical analysis

All data are expressed as mean ± standard error. Multiple groups were compared using one-way analysis of variance and the studentized range test was then used for intergroup comparisons. Comparisons of only two groups were made by unpaired t test, with the exception of the renal denervation studies where differences in blood flow between right and left kidneys were tested by paired t test.

Results

Effect of cyclosporine infusion on systemic and renal hemodynamnics

Infusion of cyclosporine in a dose of 20 mg/kg resulted in a slight fall in MAP, but no changes in cardiac index (CI) or systemic vascular resistance (SVR) (Table 1). Nevertheless, there was a marked decrease in renal blood flow and a rise in renal vascular resistance (Fig. 1). When given in a lower dose of 10 mg/kg, there was no change in MAP, CI, or SVR. Although there was a trend toward a lower renal blood flow and higher

Table 1. Effect of cyclosporine infusion on systemic hemodynamics

Group	MAP *mm Hg*	CI *ml/min/kg*	SVR *mm Hg/ml/min kg*
I Control ($N = 8$)	126 ± 3	418 ± 27	0.29 ± 0.01
II Cy 10 (N = 8)	126 ± 3	430 ± 9	0.29 ± 0.01
III 20 (N = 8)	114 ± 3	405 ± 64	0.31 ± 0.05
P value			
I vs. II	NS	NS	NS
I vs. III	< 0.05	NS	NS
II vs. III	< 0.05	NS	NS

Abbreviations are: MAP, mean arterial pressure; CI, cardiac index; SVR, systemic vascular resistance; Cy 10, cyclosporine 10 mg/kg; Cy 20, cyclosporine 20 mg/kg.

renal vascular resistance, these changes were not statistically significant.

Effect of cyclosporine infusion on PRA and renal prostaglandin excretion

Both doses of cyclosporine resulted in a dose-dependent activation of the renin-angiotensin system (Fig. 2). Similarly, infusion of cyclosporine resulted in an increase in urinary excretion of 6-keto-$PGF_{1\alpha}$ (Fig. 3) while excretion of PGE_2 was not significantly increased (data not shown). After treatment with meclofenamate, plasma renin levels were suppressed in both control and cyclosporine (10 mg/kg)-treated animals, but PRA remained significantly higher in the cyclosporine-treated animals than in controls (6.6 ± 0.6 vs. 2.5 ± 0.4 ng ml/hr, $P < 0.01$).

Effect of captopril on the response to cyclosporine infusion

To investigate the role of the renin-angiotensin system in the decreased renal blood flow after cyclosporine, rats were pretreated with the converting enzyme inhibitor, captopril, before receiving either cyclosporine or vehicle. This dose of captopril resulted in greater than 90% inhibition of the pressor response to angiotensin I. After captopril, cyclosporine infusion still caused a fall in RBF and a rise in RVR (Table 2). This suggests that the decrease in RBF following cyclosporine infusion was not mediated by angiotensin II.

Effect of meclofenamate on the response to cyclosporine infusion

To investigate the role of prostaglandins in maintaining renal blood flow following cyclosporine infusion, we pretreated rats with meclofenamate to inhibit prostaglandin synthesis. Cyclosporine was then given in a dose (10 mg/kg) that did not cause a fall in renal blood flow in prostaglandin-intact rats. Control animals received meclofenamate and vehicle only. Meclofenamate did not significantly affect RBF in control animals (Table 3). On the other hand, cyclosporine decreased renal blood flow and raised renal vascular resistance in rats with prostaglandin inhibition.

Effect of phenoxybenzamine on the renovascular response to cyclosporine infusion

To investigate a possible role of increased renal or circulating catecholamines in the renovascular response to cyclosporine, phenoxybenzamine was administered to rats who had received infusions of either cyclosporine (20 mg/kg) or vehicle. The dose of phenoxybenzamine used, 1 mg/kg, reduced the pressor effect of norepinephrine by greater than 90%. In both the control and cyclosporine-treated animals, phenoxybenzamine lowered mean arterial pressure and systemic vascular resistance (Table 4). In control animals, there was a small, nonsignificant fall in renal blood flow, but renal vascular resistance was no different from control animals not given phenoxybenzamine. On the other hand, phenoxybenzamine increased renal blood flow in cyclosporine-treated rats so that it was not significantly different from that of phenoxybenzamine-treated controls. Furthermore, phenoxybenzamine decreased renal vascular resistance to normal values in cyclosporine-infused animals.

Effect of renal denervation on renovascular response to cyclosporine infusion

Table 5 summarizes the effect of renal denervation on the renovascular response to cyclosporine (20 mg/kg). Denervation of the left kidney did not significantly affect renal blood flow in control animals. On the other hand, in cyclosporine-treated animals, a significant fall in renal blood flow occurred only in the intact innervated right kidney. Renal blood flow in the denervated left kidney was not different from controls. Therefore, renal denervation prevented the cyclosporine-induced decrease in renal blood flow and increase in renal vascular resistance.

Effect of chronic cyclosporine administration

Table 6 summarizes the results of hemodynamic studies in rats given cyclosporine, 20 mg/kg daily, for 1 week. Mean arterial pressure was slightly lower in cyclosporine-treated animals compared to controls, but there was no significant difference in cardiac index or systemic vascular resistance. Cyclosporine-treated animals had lower renal blood flow and higher renal vascular resistance compared to pair-fed control animals. This fall in blood flow was specific for the kidney, since liver blood flow (hepatic arterial blood flow) was not significantly different between control and cyclosporine-treated animals (control 0.53 ± 0.12 vs. cyclosporine 0.83 ± 0.21 ml/min/g; NS). Chronic administration of cyclosporine, like acute infusion of cyclosporine, caused stimulation of both PRA and 24-hr urinary excretion of 6-keto-$PGF_{1\alpha}$ (Table 7). Finally, cyclosporine-treated animals also had evidence of decreased glomerular filtration since plasma creatinine levels were higher and creatinine clearance values lower than in controls (Table 8).

Discussion

Since the introduction of cyclosporine in human transplantation in 1976, nephrotoxicity has been a recurrent and worrisome problem [19, 20]. A number of studies have examined cyclosporine nephrotoxicity in rats and several investigators have found decreases in glomerular filtration rate after chronic [11, 21] or acute [22] administration of cyclosporine. However, few direct measurements of renal blood flow have been made in

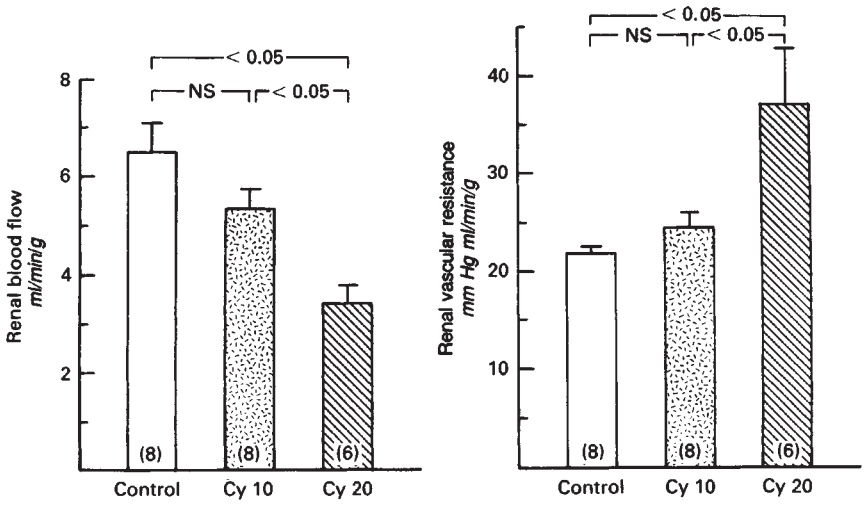

Fig. 1. *Effect of cyclosporine infusion on renal hemodynamics.* Abbreviations are: Cy 10, cyclosporine 10 mg/kg; Cy 20, cyclosporine 20 mg/kg; NS, not significantly different. Numbers inside bars are number of animals.

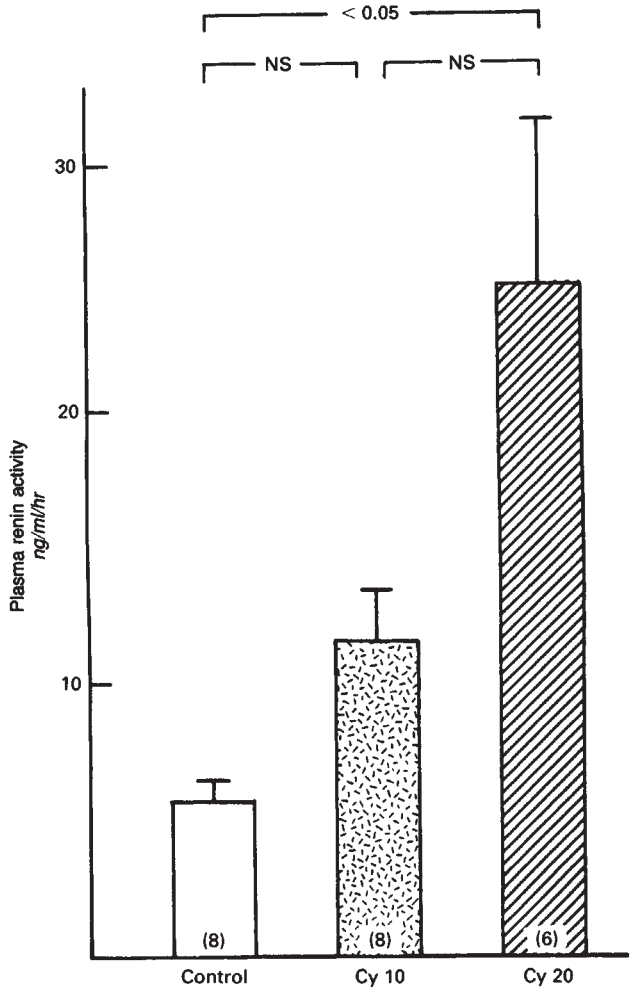

Fig. 2. *Effect of cyclosporine infusion on plasma renin activity.*

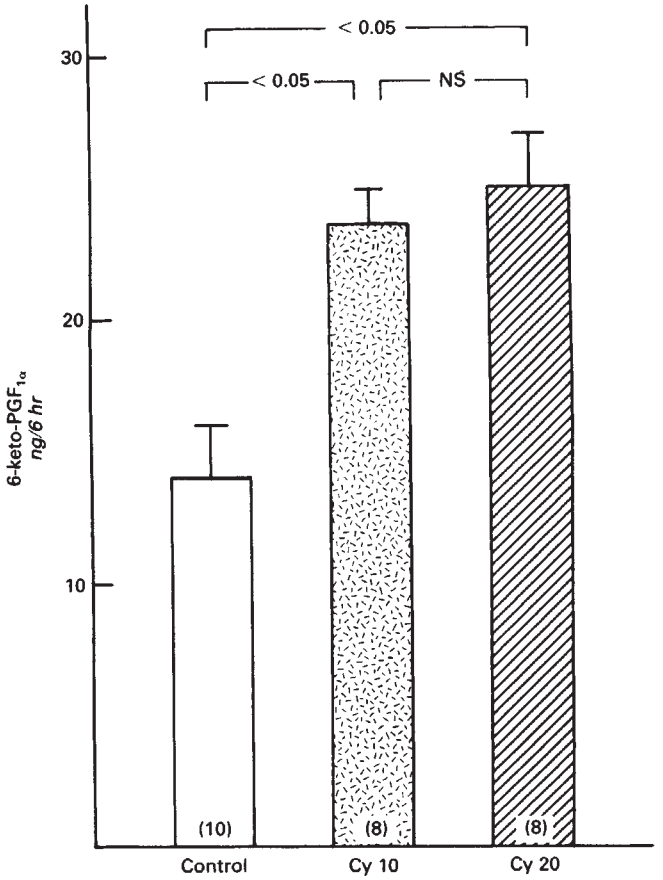

Fig. 3. *Effect of cyclosporine infusion on urinary excretion of 6-keto-PGF$_{1\alpha}$.*

either humans or animals receiving cyclosporine. Siegl et al, using the clearance of PAH, reported decreased renal plasma flow in the spontaneously hypertensive rat after cyclosporine [23], although the actual magnitude of this effect was not reported. Since tubular handling of PAH is impaired by cyclosporine [10], PAH clearance must be adjusted for the reduced extraction to obtain an accurate assessment of renal perfusion. Myers et al found decreased renal plasma flow in heart transplant recipients after 1 year of cyclosporine therapy, but this occurred at a time when histologic damage was present [10]. From this data, it is difficult to know if the effects of

Table 2. Effect of cyclosporine on systemic and renal hemodynamics after captopril

Group	MAP *mm Hg*	CI *ml/min/kg*	SVR *mm Hg/ml/min kg*	RBF *ml/min/g*	RVR *mm Hg/ml/min g*
I Control ($N = 8$)	126 ± 3	418 ± 27	0.29 ± 0.01	6.5 ± 0.6	20.6 ± 1.9
IV Control + captopril ($N = 6$)	113 ± 3	393 ± 12	0.28 ± 0.01	7.1 ± 0.4	15.9 ± 0.8
III Cy 20 mg/kg ($N = 6$)	114 ± 3	405 ± 64	0.31 ± 0.05	3.4 ± 0.4	36.9 ± 5.6
V Cy 20 mg/kg + captopril ($N = 7$)	103 ± 3	361 ± 25	0.29 ± 0.01	4.2 ± 0.6	27.6 ± 3.2
P value					
I vs. III	< 0.05	NS	NS	< 0.05	< 0.05
I vs. V	< 0.05	NS	NS	< 0.05	NS
IV vs. V	NS	NS	NS	< 0.05	NS

Abbreviations are: RBF, renal blood flow; RVR, renal vascular resistance.

Table 3. Effect of cyclosporine on systemic and renal hemodynamics after meclofenamate

Group	MAP *mm Hg*	CI *ml/min/kg*	SVR *mm Hg ml/min kg*	RBF *ml/min/g*	RVR *mm Hg/ml/min g*
I Control ($N = 8$)	126 ± 3	418 ± 27	0.29 ± 0.01	6.5 ± 0.6	20.6 ± 1.9
VI Control + meclofenamate ($N = 6$)	126 ± 3	402 ± 22	0.32 ± 0.02	7.0 ± 0.5	18.7 ± 1.9
II Cy (10 mg/kg) ($N = 8$)	126 ± 2	430 ± 0	0.28 ± 0.01	5.3 ± 0.5	23.9 ± 1.9
VII Cy (10 mg/kg) + meclofenamate ($N = 8$)	117 ± 4	374 ± 22	0.32 ± 0.02	4.3 ± 0.4	28.0 ± 1.9
P value					
I vs. II	NS	NS	NS	NS	NS
I vs. VII	NS	NS	NS	< 0.05	< 0.05
VI vs. VII	NS	NS	NS	< 0.05	< 0.05

Table 4. Effect of cyclosporine on systemic and renal hemodynamics after phenoxybenzamine

	MAP *mm Hg*	CI *ml/min/kg*	SVR *mm Hg ml/min kg*	RBF *ml/min/g*	RVR *mm Hg ml/min g*
I Control ($N = 8$)	126 ± 3	418 ± 27	0.29 ± 0.01	6.5 ± 0.6	20.6 ± 1.9
VIII Control + POB ($N = 8$)	99 ± 4	514 ± 34	0.20 ± 0.01	5.4 ± 0.4	18.6 ± 1.1
III Cy 20 mg/kg ($N = 6$)	114 ± 3	405 ± 64	0.31 ± 0.05	3.4 ± 0.4	36.9 ± 5.6
IX Cy 20 mg/kg ($N = 6$) + POB	89 ± 4	496 ± 15	0.18 ± 0.01	5.0 ± 0.5	18.8 ± 1.5
P value					
I vs. III	NS	NS	NS	< 0.05	< 0.05
I vs. IX	< 0.05	NS	< 0.05	NS	NS
VIII vs. IX	NS	NS	NS	NS	NS

Abbreviation: POB, phenoxybenzamine.

cyclosporine on RBF are direct or occur only after there has been renal tubular damage. In the present study, we found a marked decrease in renal blood flow following both the acute infusion of cyclosporine and 1 week of chronic administration to rats. The rapidity with which these changes were seen after cyclosporine infusion suggests that renal vasoconstriction is a primary effect of cyclosporine.

The renin-angiotensin system was activated in rats given cyclosporine both acutely and chronically (Fig. 2 and Table 7) in agreement with other investigators [24, 25]. Baxter et al have recently shown that cyclosporine can stimulate renin release from cortical slices incubated in vitro [26]. To investigate the possibility that increased angiotensin II might mediate the renal vasoconstriction seen after acute cyclosporine infusion, we

Table 5. Effect of unilateral renal artery denervation on the renovascular response to cyclosporine infusion

Group	MAP *mm Hg*	CI *ml/min/kg*	SVR *mm Hg/ml/min kg*	RBF *ml/min/g* RK innervated	RBF *ml/min/g* LK denervated	RVR *mm Hg/ml/min g* RK innervated	RVR *mm Hg/ml/min g* LK denervated
X Control ($N = 6$)	122 ± 3	423 ± 14	0.29 ± .01	7.5 ± .09	7.5 ± .09	17.1 ± 1.6	17.3 ± 1.9
XI Cy 20 ($N = 6$)	105 ± 5	392 ± 30	0.27 ± .01	3.6 ± 0.6	6.0 ± 0.7	32.3 ± 5.0	18.3 ± 1.9
				—— $P < 0.001$ ——		—— $P < 0.005$ ——	
P value	< 0.05	NS	NS	< 0.01	NS	< 0.01	NS

Table 6. Effect of chronic cyclosporine administration on systemic and renal hemodynamics

	MAP *mm Hg*	CI *ml/min/kg*	SVR *mm Hg/ml/min kg*	RBF *ml/min/g*	RVR *mm Hg/ml/min g*
Control ($N = 6$)	132 ± 3	367 ± 20	0.38 ± 0.02	5.4 ± 0.4	26.4 ± 2.4
Cyclosporine ($N = 6$)	120 ± 4	355 ± 44	0.37 ± 0.04	3.0 ± 0.5	48.1 ± 8.3
P value	< 0.05	NS	NS	< 0.01	< 0.05

Table 7. Effect of chronic cyclosporine administration on plasma renin activity and urinary prostaglandins

Group	PRA *ng ml/hr*	6-keto-$PGF_{1\alpha}$ *ng/24 hr*	PGE_2 *ng/24 hr*
Control ($N = 6$)	8.4 ± 1.9	58.6 ± 8.2	118.7 ± 10.4
Cyclosporine ($N = 6$)	24.0 ± 6.6	107.7 ± 17.3	78.7 ± 11.5
P value	< 0.05	< 0.05	NS

Table 8. Effect of chronic cyclosporine administration on plasma creatinine and creatinine clearance

Group	Plasma creatinine mg/dl	Creatinine clearance *ml/min*
Control ($N = 6$)	0.34 ± .03	2.09 ± .12
Cyclosporine ($N = 6$)	0.53 ± .05	1.20 ± .11
P value	< 0.01	< 0.01

treated animals with captopril before administering a dose of cyclosporine, known to cause renal vasoconstriction. Captopril did not prevent the decrease in renal blood flow caused by cyclosporine, suggesting that activation of the renin-angiotensin system was probably the result, rather than the cause, of decreased renal perfusion.

Acute and chronic administration of cyclosporine to rats resulted in increased urinary excretion of 6-keto-$PGF_{1\alpha}$ (Fig. 3 and Table 7). Increased urinary excretion of 6-keto-$PGF_{1\alpha}$ has also been seen in other conditions characterized by renal hypoperfusion and increased renin secretion. It has been postulated that the role of increased prostacyclin production in these states is to maintain renal blood flow and stimulate renin secretion [27]. To investigate whether prostacyclin might have a protective effect in cyclosporine-mediated renal vasoconstriction, we treated rats with meclofenamate prior to cyclosporine infusion. The dose of cyclosporine used for this experiment, 10 mg/kg, did not cause a significant decrease in RBF in prostaglandin-intact animals, although it did increase 6-keto-$PGF_{1\alpha}$ excretion (Fig. 3). Following meclofenamate, cyclosporine in this dose decreased renal blood flow, suggesting that prostaglandin production attenuates cyclosporine-induced renal vasoconstriction. It also suggests that the use of cyclooxygenase inhibitors in patients receiving cyclosporine could exacerbate the toxic effects of this drug on the kidney.

Stimulation of the renal sympathetic nervous system can result in renal vasoconstriction and stimulation of renin production [28]. To test the possibility that renal vasoconstriction in response to cyclosporine might be mediated by increased levels of circulating catecholamines or increased renal nerve activity, we administered the α-adrenergic blocker, phenoxybenzamine, to rats infused with cyclosporine. Phenoxybenzamine did not affect renal blood flow or vascular resistance in control animals. However, in cyclosporine-treated animals, phenoxybenzamine increased renal blood flow and prevented an increase in renal vascular resistance (Table 4). These findings suggest that the renal vasoconstriction after cyclosporine infusion is mediated by catecholamines.

To define the role of the sympathetic nervous system further, we studied the effect of renal denervation on the renovascular response to cyclosporine. We elected to denervate only the left kidney so that the contralateral right kidney would serve as a paired control. In the control animals receiving vehicle, denervation did not affect renal blood flow (Table 5). However, in the animals infused with cyclosporine, significant vasoconstriction occurred only in the innervated right kidney. In the denervated left kidney, renal blood flow and vascular resistance were not significantly different from controls. Therefore, renal

vasoconstriction following cyclosporine infusion was mediated through increased renal nerve activity.

Primary renal vasoconstriction could result in the decreased glomerular filtration rate (GFR) seen after acute cyclosporine infusion [22]. However, Whiting et al [11] have proposed that primary tubular injury results in activation of the tubuloglomerular feedback (TGF) mechanism with a secondary fall in renal blood flow and GFR. We do not believe that such a mechanism explains the decreased renal blood flow seen in our studies. The renal nerves are not felt to mediate tubuloglomerular feedback [29], yet renal denervation completely corrected the decrease in renal blood flow seen after cyclosporine infusion. Also, Diepirink, Starklint, and Leyssac found that in rats given cyclosporine chronically, proximal tubular sodium reabsorption was enhanced and distal delivery of sodium decreased [21]. Increased, rather than decreased, distal sodium delivery would be required to stimulate tubuloglomerular feedback. Our studies, however, do not prove that a fall in glomerular filtration rate after cyclosporine is due solely to decreased renal blood flow. Restoration of renal blood flow might not completely restore glomerular filtration rate to normal if cyclosporine also had effects on the glomerular ultrafiltration coefficient, for example.

Most studies of cyclosporine nephrotoxicity to date have focused on its tubular toxicity with little attention being paid to its effect on the renal vasculature. While extrapolation from the rat to humans is hazardous, our findings may be relevant to certain aspects of cyclosporine nephrotoxicity in humans. The closest parallel in humans is the occurrence of acute renal dysfunction following heart or liver transplantation [15, 30]. Acute onset of oliguria and increased creatinine have been seen within a few days of transplantation in the absence of identifiable prerenal or postrenal causes. In this setting, urinary sodium excretion is generally low, suggesting intact tubular function, and PRA levels are elevated. This syndrome has been seen most commonly when cyclosporine was administered intravenously [15]. Our findings strongly support the hypothesis that this syndrome is the result of cyclosporine-induced renal vasoconstriction. Such hypoperfusion, if prolonged, could also result in acute tubular necrosis or slow recovery from postoperative acute renal failure.

The relevance of these studies to cyclosporine nephrotoxicity in human renal transplants is less clear. Initially, the renal allograft is denervated, so that any vasoconstriction in the early post-transplant period would have to be mediated by circulating catecholamines. However, there is evidence that grafts begin to reinnervate within 1 month, and that innervation is extensive by 5 months [31]. Patients treated with cyclosporine also have higher serum creatinine levels than patients treated with conventional immunosuppression, and switching patients from cyclosporine to azathioprine results in a prompt fall in creatinine levels in the absence of superimposed rejection [32]. Furthermore, in the first 6 months after transplant, cyclosporine nephrotoxicity is not characterized by any particular histological finding [6, 33]. Renal vasoconstriction by cyclosporine could account for this functional renal failure. Further studies will be required to define the role of the sympathetic nervous system in chronic cyclosporine nephrotoxicity and whether α-adrenergic blocking agents such as prazosin are helpful in limiting the nephrotoxic effects of cyclosporine.

In summary, both acute and chronic administration of cyclosporine in rats resulted in increased renal vascular resistance. Plasma renin activity and urinary 6-keto-$PGF_{1\alpha}$ were increased following cyclosporine, but angiotensin II was not the cause of the renal vasoconstriction. Prostaglandin synthesis inhibitors potentiated cyclosporine-induced renal vasoconstriction. Cyclosporine was found to produce renal vasoconstriction by activation of the sympathetic nervous system via the renal nerves since either phenoxybenzamine or renal denervation prevented cyclosporine-mediated decreases in renal blood flow and increases in renal vascular resistance.

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References

1. European Multicentre Trial Group: Cyclosporine in cadaveric renal transplantation: One-year follow-up of a multicentre trial. *Lancet* 2:986–989, 1983
2. Najarian JS, Ferguson RM, Sutherland DE, Rynasiewicz JJ, Simmons RL: A prospective trial of the efficacy of cyclosporine in renal transplantation at the University of Minnesota. *Transplant Proc* 15:438–441, 1983
3. Canadian Multicentre Trial Group: A randomized clinical trial of cyclosporine in cadaveric renal transplantation. *N Engl J Med* 309:809–815, 1983
4. Starzl TE, Klintmalm GB, Porter KA, Iwatsuki S, Schroder GPJ: Liver transplantation with use of cyclosporine A and prednisone. *N Engl J Med* 305:266–269, 1981
5. Oyer PE, Stinson EB, Jamieson SW: Cyclosporine in cardiac transplantations: a 2½ year follow-up. *Transplant Proc* 15(Suppl 1):2546–2552, 1983
6. Klintmalm GB, Iwatsuki S, Starzl TE: Nephrotoxicity of cyclosporin A in liver and kidney transplant recipients. *Lancet* 1:470–471, 1981
7. Adu D, Turney J, Michael J, McMaster P: Hyperkalemia in cyclosporine treated renal allograft recipients. *Lancet* 2:370–372, 1983
8. Bantle JP, Nath KA, Sutherland DE, Najarian JS, Ferris TF: Effects of cyclosporine on the renin-angiotensin-aldosterone system and potassium excretion in renal transplant recipients. *Arch Intern Med* 145:505–508, 1985
9. Hamilton DV, Evans DB, Henderson RG, Thiru S, Calne RY, White DJG, Carmichael DJS: Nephrotoxicity and metabolic acidosis in transplant patients on cyclosporin A. *Proc Eur Dial Transplant Assoc* 18:400–409, 1981
10. Myers BD, Ross J, Newton L, Luetscher J, Perloth M: Cyclosporine-associated chronic nephropathy. *N Engl J Med* 311:699–705, 1984
11. Whiting PH, Thomson AW, Blair JT, Simpson JG: Experimental cyclosporin A nephrotoxicity. *Br J Exp Path* 63:88–94, 1982
12. Osman EA, Barrett JJ, Bewick M, Parsons V: Does cyclosporine affect renal blood vessels? *Lancet* 1:1470, 1984
13. Hilson AJW, Maisey MN, Brown CB, Ogg CS, Bewick MS: Dynamic renal transplant imaging with Tc-99m DTPA (Sm) supplemented by a transplant perfusion index in the management of renal transplants. *J Nucl Med* 19:985–1000, 1978
14. Mihatsch MJ, Thiel G, Spichtin MP, Oberholzer M, Brunner FP, Harder F, Olivieri V, Brenner R, Ryffel B, Stockler E, Torhorst J, Gudat F, Zollinger HU, Loertscher R: Findings in kidney transplants after treatment with cyclosporine. *Transplant Proc* 15(Suppl 1):2821–2835, 1983
15. Powell-Jackson PR, Young B, Calne RY, Williams R: Nephrotoxicity of parenterally administered cyclosporine after orthotopic liver transplantation. *Transplantation* 36:505–508, 1983

16. Hsu CH, Kurtz TW, Waldinger TP: Cardiac output and renal blood flow in glycerol-induced acute renal failure in the rat. *Circ Res* 40-178–182, 1977
17. Haber E, Koern T, Page B, Klinian B, Purnode A: Application of a radioimmunoassay for angiotensin I to the physiologic measurements of plasma renin activity in normal human subjects. *J Clin Endocrinol Metab* 29:1349–1355, 1968
18. Ferris TF, Weir EK: Effect of captopril on uterine blood flow and prostaglandin E synthesis in the pregnant rabbit. *J Clin Invest* 71:809–815, 1983
19. Calne RY, White DJ, Thiru S, Evans DB, McMaster P, Dunn DC, Craddock GN, Pentlow BD, Rolles K: Cyclosporin A in patients receiving renal allografts from cadaver donors. *Lancet* 2:1323–1327, 1978
20. Bennett WM, Pulliam JP: Cyclosporine nephrotoxicity. *Ann Intern Med* 99:851–854, 1983
21. Diepirink M, Starklint M, Leyssac PP: Nephrotoxicity of cyclosporine—an animal model: study of the nephrotoxic effect of cyclosporine on overall and renal tubular function in conscious rats. *Transplant Proc* 15(Suppl 1):2736–2741, 1983
22. Tonnesen AS, Hammer RW, Weinmann EJ: Cyclosporine and sodium and potassium excretion in the rat. *Transplant Proc* 15(Suppl 1):2730–2735, 1983
23. Siegl H, Ryffel B, Petric R, Shoemaker P, Muller A, Donatsch P, Mihatsch M: Cyclosporine, the renin-angiotensin-aldosterone system and renal adverse reactions. *Transplant Proc* 15(Suppl 1):2719–2725, 1983
24. Siegl H, Ryffel B: Effect of cyclosporine on the renin-angiotensin-aldosterone system. *Lancet* 2:1274, 1982
25. Baxter CR, Duggin GG, Willis NS, Hall BM, Horvath JS, Tiller DJ: Cyclosporin A-induced increases in renin storage and release. *Res Commun Biochem Pathol Pharmacol* 37:305–312, 1982
26. Baxter CR, Duggin GG, Hall BM, Horvath JS, Tiller DJ: Stimulation of renin release from rat renal cortical slices by Cyclosporine A. *Res Comm Biochem Pathol Pharmacol* 43:417–423, 1984
27. Dunn MJ: Renal prostaglandins, in *Renal Endocrinology,* edited by Dunn MJ, Baltimore, Williams and Wilkins, 1983, pp 1–74
28. di Bona G: The function of the renal nerves. *Rev Physiol Biochem Pharmacol* 94:75–181, 1982
29. Takabatake T: Feedback regulation of glomerular filtration rate in the denervated rat kidney. *Kidney Int* 22(Suppl 12):S129–S135, 1982
30. Devineni R, McKenzie N, Duplan J, Keown P, Stiller C, Wallace AC: Renal effects of cyclosporine: clinical and experimental observations. *Transplant Proc* 15(Suppl 1):2695–2698, 1983
31. Gazdar AF, Dammin GJ: Neural degeneration and regeneration in human renal transplants. *N Engl J Med* 283:222–224, 1970
32. Chapman JR, Griffiths A, Harding NGL, Morris PJ: Reversibility of cyclosporin nephrotoxicity after three months treatment. *Lancet* I:-128–129, 1985
33. Verani RR, Flechner SM, Van Buren CT, Kahan BD: Acute cellular rejection or cyclosporine A nephrotoxicity? A review of transplant renal biopsies. *Am J Kidney Dis* 4:185–191, 1984