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Glomerular hemodynamics and hormonal participation on cyclosporine nephrotoxicity

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Glomerular hemodynamics and hormonal participation on cyclosporine nephrotoxicity. The mechanism of cyclosporine A (CyA) nephrotoxicity is unclear. In order to evaluate renal microcirculation seven euvolemic Munich–Wistar (MW) rats were studied after acute CyA treatment (50 mg/kg, i.v.). Both total glomerular filtration rate (GFR, 0.96 ± 0.04 vs. 0.47 ± 0.07 ml/min) and single nephron GFR (27.90 ± 3.39 vs. 14.02 ± 3.49 nl/min) declined significantly ($P < 0.001$). It was observed an increase in afferent (R_A, ↑ 188%) and efferent (R_E, ↑ 360%) arteriolar resistances that caused a decrease on glomerular plasma flow rate (Q_A) from 100.99 ± 17.09 to 44.37 ± 13.37 nl/min ($P < 0.001$). Mean glomerular capillary hydraulic pressure ($\bar{P}_{GC}$) increased from 45 ± 1 to 55 ± 4 mm Hg ($P < 0.05$) and the glomerular ultrafiltration coefficient (K_f) decreased by 70% (0.096 ± 0.030 to 0.031 ± 0.010 nl/sec · mm Hg, $P < 0.05$). Additionally, in order to study hormonal participation in this nephrotoxicity, other three groups of MW rats were previously treated with captopril (2 mg/kg, i.v.), verapamil (20 μg/kg/min, i.v.) or indomethacin (2 mg/kg, i.v.). Both captopril and verapamil minimized the renal effects of CyA, with a decline of ~25% instead of ~50% on GFR and RPF. Moreover, two groups of Brattleboro rats were studied. Acute CyA administration in homozygote Brattleboro rats produced a decline of only ~22% and ~31%, respectively, in GFR and renal plasma flow (RPF), when compared with MW rats ($P < 0.05$). Similar results were observed in heterozygote Brattleboro rats when compared with MW rats, disclosing differences due to a different strain of rats. According to these data, acute CyA administration caused a reduction in SNGFR due to an increase on R_A and R_E with decreases on Q_A and K_f values. These studies suggest that, at least, the renin–angiotensin system and ADH may participate in this glomerular function impairment. Furthermore, indomethacin did not alter the effect induced by CyA, indicating that prostaglandins may be not an important factor in these alterations. The protective action of verapamil may indicate a potential use of Ca channel blockers in order to minimize CyA nephrotoxicity.

Cyclosporine A (CyA) is a potent immunosuppressive drug which has afforded benefit in transplantation and in the treatment of several immune–mediated diseases [1–3]. However, a number of side effects are observed, with nephrotoxicity being the most common and important [4–6].

The impairment of glomerular filtration rate (GFR) and thus, acute renal failure (ARF) observed with CyA, appears to be related with renal hemodynamic alterations [7, 8]. The ARF is reversible when the dosage of CyA is diminished or withdrawn [6]. Moreover, no significant glomerular lesions have been found by light, immunofluorescence or electron microscopy [4, 9]. It has been suggested that hormonal factors may participate in this acute nephrotoxicity. Both activation of renin–angiotensin (RAS) and suppression of prostaglandin (PG) systems have been observed [9–11].

Therefore, to study the renal microcirculation in response to acute CyA infusion, Munich Wistar rats were submitted to glomerular hemodynamic studies. Also, in order to evaluate hormonal factors in CyA toxicity, additional experimental groups were treated with an angiotensin 1-converting enzyme inhibitor (captopril), prostaglandin synthesis inhibitor (indomethacin), and calcium channel blocker (verapamil). The final experimental group involved acute administration of CyA to Brattleboro rats, a genetic diabetes insipidus strain lacking endogenous vasopressin (ADH).

Methods

Studies were performed on 45 adult male, Munich Wistar rats weighing between 250 and 320 g during two experimental periods, as follows: Group 1, control rats ($N = 7$). After the initial study period, a second period was performed with the cyclosporine–vehicle, cremophor (Sandoz, Basle, Switzerland), 0.4 to 0.6 ml, i.v. Group 2 was CyA group ($N = 10$) treated with CyA, 50 mg/kg body wt i.v. in the second period study. Group 3 ($N = 8$) rats were treated with captopril (SQ 14,225, E.R. Squibb and Sons, Princeton, New Jersey, USA), 2 mg/kg body wt per hour i.v. during both experimental periods. In the second period, CyA (50 mg/kg i.v.) was simultaneously administered. Group 4, the verapamil group ($N = 7$), was treated with 20 μg/kg/min (Dl-verapamil, Knoll, USA) during both experimental periods. Similarly to group 3, CyA was administered in the second period. Group 5 was the indomethacin group ($N = 7$). A dose of 2 mg/kg i.v. was administered in bolus, after canulation of jugular vein, 45 minutes before the initial period, and then CyA (50 mg/kg i.v.) was given in the second period.

The experiments were also performed on rats of the Brattleboro strain (National Institutes of Health, Maryland, USA), weighing between 200 to 310 g, to evaluate the role of ADH in CyA nephrotoxicity. In this Group 6, seven Brattleboro heterozygote rats served as control group. After the first period, CyA (50 mg/kg body wt i.v.) was administered and after 30 minutes, the second period was performed. Group 7 had nine Brattleboro homozygotes rats that received CyA in the same manner as Group 6.

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Each rat was allowed free access to water and a standard rat pellet diet until the morning of study. Rats were anesthetized with Inactin, 100 mg/kg i.p., and placed on a temperature–regulated micropuncture table. Immediately after anesthesia was induced the left femoral artery was catheterized with a PE-50 polyethylene tubing, and approximately 70 μl of arterial blood was collected for baseline hematocrit (Hct) determination. This arterial catheter was used for subsequent periodic blood sampling and estimation of mean femoral arterial–pressure ($\overline{AP}$), with an electronic transducer (model P23 Db, Stathan Instruments Div., Gould Inc., Hato Rey, Puerto Rico) connected to a direct–writing Gould Recorder (model 2200, Cleveland, Ohio, USA). Polyethylene catheters were also inserted into the right and left jugular veins for infusion of inulin, p-aminohippuric acid (PAH), CyA vehicle, verapamil, indomethacin, captopril, saline and isoncotic rat serum. An i.v. infusion of 7.5% inulin and 2% PAH solution in 0.9% sodium chloride was then started at a rate of 1.2 ml/hour. Following tracheostomy, rats were prepared in routine fashion for micropuncture study [12–14]. After an initial equilibration period of 45 minutes, appropriate measurements and collections needed to characterize glomerular dynamics and K_f were obtained in Group 3. Throughout the period of surgical preparation and experimental studies, all rats received a continuous i.v. infusion of isoncotic rat serum to maintain circulating plasma volume at conscious (or euvolemic) levels, since plasma volume of rats prepared for micropuncture is reduced by approximately 20% relative to unanesthetized animal [12]. The following protocol for maintaining the euvolemic state was used. Soon after collection of the baseline arterial blood sample, isoncotic rat serum was infused for 45 minutes at the rate of 7 to 10 ml/kg/hr, followed by reduction in infusion rate to 1.5 ml/kg/hr for the remainder of each experiment to maintain the hematocrit value at the baseline level measured immediately after induction of anesthesia [12, 15].

In Brattleboro rats during the experiments the animals were given a continuous intravenous infusion of normal saline at a mean rate of 4 ml/100 g body wt/hr in homozygote rats in order to replace the urinary losses. In heterozygote rats, saline was infused for 45 minutes at the rate of 7 to 10 ml/kg/hr, followed by reduction in infusion rate to 1.5 ml/kg/hr for the remainder of each experiment. In both groups Hct were evaluated during the experimental periods. A catheter (PE-10) was also inserted into the left ureter for urine sampling and flow rate determination. Following these procedures the left kidney was exposed and prepared for micropuncture study. After 30 to 45 minutes equilibration the initial study period was begun. Two 15 minute urine samples and blood samples of about 60 μl each were collected for determinations Hct and total protein concentration of inulin and PAH in plasma and urine. CyA was then administered and the same procedures were performed in the second study period.

Micropuncture studies

In Group 3, initial micropuncture measurements were performed in seven Munich Wistar rats. Exactly timed (1 to 3 min) samples of fluid were collected from surface proximal convolutions of at least three nephrons for determinations of flow rate and inulin concentration, and calculation of single–nephron glomerular filtration rate (SNGFR). Coincident with these tubule fluid collections, two or three samples of femoral arterial blood were obtained in each period for determination of systemic arterial Hct, total protein, inulin and PAH concentration in plasma.

Hydraulic pressures were measured in single capillaries within surface glomeruli by a continuous recording servonull micropipette–transducer system (IPM Inc, San Diego, California, USA). Micropipettes with outer tip diameters of 2 to 4 μm and containing 2.0 M sodium chloride were used. Hydraulic output from the servo-system was channeled via an electronic transducer (Statham model P23Db) to a second channel of the recorder. Direct measurements of hydraulic pressure in single glomerular capillaries ($\overline{P}_{GC}$), proximal tubules (P_T), efferent arterioles (P_{EA}) and third–order peritubular capillaries (P_C) were recorded in each rat.

To obtain estimates of colloid osmotic pressure (π) of plasma entering and leaving glomerular capillaries, we measured protein concentrations (C) in femoral arterial (C_A) and efferent arteriolar (C_E) blood plasma as described previously [16]. C_A was taken as a measure of protein concentration in the afferent arteriole. These estimates of preglomerular and postglomerular plasma protein concentrations permit calculation of colloid osmotic pressures, single nephron filtration fraction (SNFF), initial glomerular capillary plasma flow rate (Q_A), afferent (R_A), efferent (R_E) and total ($R_T = R_A + R_E$) arteriolar resistance and glomerular capillary ultrafiltration coefficient (K_f), using equations given elsewhere [17].

Analytical procedures

The volume of tubule fluid collected was estimated from the length of the fluid column in a constant–bore capillary tube of known internal diameter. The concentration of inulin in the tubule fluid was measured, usually in duplicate, by the microfluorescence method of Vurek and Pegram [18]. Inulin concentration in plasma and urine was determined by the macroanthrone method of Führ, Kaczmarczyk and Kruttgen [19]. Protein concentrations in efferent arteriolar and femoral arterial blood–plasmas were determined, usually in duplicate, with an ultramicrocolorimeter by the method of Viets et al [16].

Glomerular filtration rate (GFR) was evaluated by inulin clearances and renal plasma flow (RPF) by PAH clearances. Plasma and urinary PAH concentrations were determined by the method of Smith et al [20]. Total renal vascular resistance (TRVR) was calculated as the $\overline{AP}$/RPF ratio. Filtration fraction (FF) was calculated as the quotient of GFR and RPF. Values of GFR and RPF were corrected for kidney weight. Urinary Na concentration was determined by flame photometry (Tecnow, São Paulo, Brasil). Plasma protein levels were determined with a refractometer and corrected on the basis of a standard curve constructed by the method of Lowry, modified by Schachterle [21].

Statistical analysis

Data were analyzed statistically by the Student unpaired and paired two–tailed test. Statistical significance was taken as $P < 0.05$, all data were reported as means ± SEM.

Changes between the groups were statistically evaluated with the Mann–Whitney test for unpaired and two tailed observation.

Table 1. Summary of general data

Group	Body wt	Kg weight	$\overline{AP}$	Hct_o	Hct	C_{Ao}	C_A
	g		*mm Hg*	%		*g/dl*	
1	304 ± 11[a]	1.24 ± 0.04	112 ± 3	48 ± 1	49 ± 1	5.5 ± 0.1	5.4 ± 0.1
2	277 ± 6	1.12 ± 0.10	108 ± 3	49 ± 1	49 ± 1	5.4 ± 0.1	5.3 ± 0.3
3	276 ± 8	1.13 ± 0.08	110 ± 5	48 ± 1	49 ± 1	5.6 ± 0.1	5.7 ± 0.1
4	265 ± 7	1.10 ± 0.03	92 ± 3[b]	48 ± 1	48 ± 1	5.6 ± 0.3	5.7 ± 0.3
5	265 ± 6	1.16 ± 0.03	114 ± 3	49 ± 1	56 ± 1	5.5 ± 0.1	5.7 ± 0.1
6	276 ± 5	0.86 ± 0.03[b]	125 ± 1	47 ± 1	50 ± 1	5.5 ± 0.1	4.9 ± 0.1[c]
7	250 ± 11	0.75 ± 0.06[b]	101 ± 4	46 ± 1	45 ± 1	5.6 ± 0.1	4.3 ± 0.1[c]

Subscript o refers to baseline values at start of anesthesia
[a] $\overline{X}$ ± SE
[b] $P < 0.05$ vs. groups
[c] C_A vs. C_{Ao}

Table 2. Summary of several measures of whole kidney function

	GFR *ml/min*	RPF *ml/min*	FF %	TRVR *mm Hg · min/ml*
Group 1				
Saline	1.00 ± 0.06[a]	2.75 ± 0.30	38 ± 3	44 ± 5
Vehicle	1.03 ± 0.07	2.82 ± 0.31	37 ± 1	41 ± 4
Group 2				
Saline	0.96 ± 0.04	2.91 ± 0.19	34 ± 2	39 ± 3
CyA	0.47 ± 0.07[b]	1.30 ± 0.23[b]	38 ± 2[b]	129 ± 40[b]
Group 3				
Captopril	1.05 ± 0.05	3.06 ± 0.22	35 ± 3	38 ± 3
Captopril + CyA	0.82 ± 0.04[b,c]	2.50 ± 0.15[b,c]	33 ± 2	41 ± 3[c]
Group 4				
Verapamil	0.92 ± 0.06	2.91 ± 0.29	33 ± 3	34 ± 3
Verapamil + CyA	0.65 ± 0.06[b,c]	2.31 ± 0.17[b,c]	29 ± 2[b]	38 ± 3[c]
Group 5				
Indomethacin	1.03 ± 0.07	3.27 ± 0.31	32 ± 2	38 ± 5
Indomethacin + CyA	0.55 ± 0.09[b]	1.49 ± 0.26[b]	37 ± 3	86 ± 15[b]

[a] Values are expressed as the mean ± SEM
[b] $P < 0.05$ before vs. after CyA
[c] $P < 0.05$ second period of groups 3, 4 and 5 vs. group 2

Results

General

Mean values of general data obtained in rats given vehicle or CyA (Groups 1 and 2); CyA after captopril, verapamil and indomethacin (Groups 3 to 5) and in Brattleboro rats (Groups 6 and 7) are summarized in Table 1. All groups were similar in mean values for body and kidney weights and $\overline{AP}$. However, in Brattleboro rats, mean kidney weight was significantly lower than for Munich Wistar rats ($P < 0.05$). Also, during verapamil administration, mean $\overline{AP}$ was significantly reduced ($P < 0.02$). The reduction was slight and the values were maintained well within renal autoregulation levels (Table 1). Mean values for initial and experimental periods for arterial hematocrit (Hct_o, Hct) and plasma protein concentration (C_{Ao}, C_A) are also similar in all studied groups, but for Groups 6 and 7 a decline of C_A was observed ($P < 0.05$, Table 1). However, the protein concentrations were maintained stable during all experimental periods (Table 1). Table 2 summarizes several whole kidney functions in Groups 1 to 5. Vehicle infusion, Group 1, did not change TRVR. CyA treatment (Group 2) induced significant declines in GFR, 0.96 ± 0.04 vs. 0.47 ± 0.07 ml/min ($P < 0.005$), and in RPF, 2.91 ± 0.19 vs. 1.30 ± 0.23 ml/min ($P < 0.005$), with an increase in FF, 34 ± 2 vs. 38 ± 2 ($P < 0.01$), respectively. Also, CyA infusion caused a marked rise in TRVR, from 39 ± 3 to 129 ± 40 mm Hg · min/ml ($P < 0.025$).

Although not shown, CyA (Group 2) induced declines on urinary sodium excretion, $U_{Na}V$, from 0.65 ± 0.17 to 0.23 ± 0.04 μEq/min ($P < 0.025$) and in potassium excretion, U_KV, from 1.04 ± 0.06 to 0.33 ± 0.06 μEq/min ($P < 0.001$), with maintenance of urine flow rate, 3.23 ± 0.55 vs. 3.10 ± 0.27 μl/min ($P < 0.20$).

Micropuncture studies

The micropuncture data obtained in Group 2 before and after CyA infusion are shown in Table 3. Mean SNGFR and Q_A were reduced after CyA infusion, 27.90 ± 3.39 to 14.02 ± 3.49 nl/min ($P < 0.02$) and 100.99 ± 17.09 to 44.37 ± 13.37 nl/min ($P < 0.001$), respectively. SNFF increased from 0.30 ± 0.03 to 0.34 ± 0.03, but these values did not reach statistical significance. Since $\overline{P}_{GC}$ increased from 45 ± 1 to 55 ± 4 mm Hg ($P < 0.02$), and P_T did not change, $\overline{\Delta P}$ increased (Table 3). Both P_{EA} and P_C were not affected by CyA (Table 3). R_T rose significantly. This increase was accounted for by the rise in both R_A and R_E, but mainly R_E ($P < 0.05$) as shown in Table 3.

Table 3. Glomerular hemodynamic measurements before and after cyclosporine (CyA) administration in Group 2 rats

	SNGFR	Q_A	SNFF	$\overline{P}_{GC}$	P_T	$\overline{\Delta P}$	P_{EA}	P_C
	nl/min		%	*mm Hg*				
Before	27.90[a] ± 3.39	100.99 ± 17.09	0.30 ± 0.03	45 ± 1	14 ± 3	30 ± 2	14 ± 2	8 ± 1
After	14.02[b] ± 3.49	44.37[b] ± 13.37	0.34 ± 0.03	55[b] ± 4	14 ± 3	41[b] ± 3	14 ± 2	9 ± 2

[a] $\overline{X}$ ± SE
[b] $P < 0.05$ before vs. after CyA

Mean values for C_A and thus π_A were similar before and after CyA administration. However, C_E and thus π_E increased but without significance ($P < 0.05$) from 7.83 ± 0.18 to 8.27 ± 0.39 g/dl and 30.88 ± 1.12 to 33.81 ± 2.54 mm Hg ($P < 0.10$), respectively. This increase was responsible for the slight increase in SNFF. This increase was in similar proportion (~10%) as seen in whole kidney FF.

Since filtration pressure disequilibrium is defined as $\pi E/\overline{\Delta P} < 0.95$ [13], in the period before CyA administration this condition was obtained in four of seven rats. However, despite the euvolemic condition, after CyA treatment, filtration pressure equilibrium was obtained in five of seven rats. When the data were analyzed statiscally (equilibrium or desiquilibrium), an important declined on K_f of ~70% was observed (Table 3).

Effects of the angiotensin I-converting enzyme inhibitor, captopril

Whole kidney GFR and RPF declined after CyA treatment during captopril infusion (Table 2), from 1.05 ± 0.05 to 0.82 ± 0.04 ml/min ($P < 0.005$) and from 3.06 ± 0.22 to 2.50 ± 0.15 ml/min ($P < 0.005$), respectively. These effects of CyA in this group were reduced when compared with CyA treatment alone ($P < 0.001$). Moreover, the effects of CyA on TRVR were blunted by captopril infusion, 38 ± 3 vs. 41 ± 3 mm Hg · min/ml ($P < 0.05$), Table 2.

Effects of the Ca channel blocker, verapamil

Similar to captopril treatment, verapamil administration lessened the decline on GFR by CyA by ~30%, from 0.92 ± 0.06 to 0.65 ± 0.06 ml/min ($P < 0.001$). The changes in RPF and RVR induced by CyA were also blunted by verapamil (Table 2).

Effects of prostaglandin inhibitor, indomethacin

Different from the observed results with captopril and verapamil, indomethacin failed to modify the renal alterations induced by CyA. GFR and RPF declined to the same extent after CyA in the presence or absence of PG inhibition. Also, TRVR increased despite the presence of indomethacin.

Effects of CyA in Brattleboro rats

The infusion of CyA in Brattleboro heterozygote rats (Group 6) led to marked falls in GFR and RPF, from 0.99 ± 0.06 to 0.42 ± 0.06 ($P < 0.01$) and from 2.67 ± 0.26 to 0.94 ± 0.12 ($P < 0.01$), respectively (Table 4). Total RVR increased from 51 ± 4 to 147 ± 21, (Table 3). The changes in these parameters were similar to those seen in Munich Wistar rats, indicating no differences in the overall nephrotoxic susceptibility to CyA between both strains. The treatment of CyA in Brattleboro homozygotes rats, however, did not alter renal function as extensively as observed for Brattleboro heterozygotes or Munich Wistar rats (Table 4). GFR and RPF declined from 0.88 ± 0.04 to 0.69 ± 0.06 ml/min ($P < 0.05$) and from 2.76 ± 0.20 to 1.91 ± 0.13 ml/min ($P < 0.05$), respectively. Again, both values were significantly less affected than observed for CyA in heterozygotes and Munich Wistar rats ($P < 0.05$). Total RVR increased but to a lesser extent than for Group 6 ($P < 0.05$).

Discussion

CyA has become a commonly–employed immunosupressive drug for cardiac, hepatic, renal and other organ transplantation [3, 22]. It has been demonstrated that patient and allograft survival is enhanced by its use [2]. Moreover, an increased number of immune mediated diseases are now being treated with CyA [3, 23]. However, several adverse effects limit its use, nephrotoxicity being one of the most important of them [5]. In humans, the nephrotoxicity is mainly characterized by diffuse interstitial fibrosis, arteriolopathy and tubulopathy [4, 5, 24]. No significant glomerular lesions are found. However, acute tubular necrosis is the most restrictive alteration for the use of CyA [25]. Myers et al [22] observed in patients with cardiac transplantation that the chronic use of CyA produced a reduction in renal plasma flow associated with a variable degree of tubulo-interstitial injury. These lesions were accompanied by focal glomerular sclerosis. Similar functional and morphological alterations were found in rat, but less pronounced [24]. In addition, doses of 50 to 100 mg/kg in rats precipitate ARF caused by proximal tubular damage [25]. Also, acute and chronic administration of CyA alters glomerular hemodynamics [7, 8]. A significant fall in total RPF together with a marked rise in TRVR were the major reported findings [7].

In the present study, acute CyA administration caused an important fall in GFR. This fall was caused by a marked increase in TRVR which in turn determined a prominent decrease in RPF. Therefore, whole kidney hemodynamics suggest an increase of renal vascular resistance, predominantly postglomerular since FF also regularly increases.

Moreover, acute administration of CyA in euvolemic rats leads to a marked decrease in urinary sodium and potassium excretion ($P < 0.05$). We found an important decrease in fractional and absolute potassium excretion. The decreased potassium excretion was probably determined by a reduction on distal delivery of sodium. These data are also observed by Tonnesen, Hammer and Weinmann [26] that found a decrease in fractional and absolute sodium and potassium excretion with CyA.

Table 3. *Continued*

	R_A	R_E	R_T	C_A	C_E	π_A	π_E		K_f
	X10^{10} dyn · sec · cm^{-5}			*g/dl*		*mm Hg*		$\pi_E/\overline{\Delta P}$	*nl/(sec · mm Hg)*
Before	2.91[a] ± 0.42	1.48 ± 0.29	4.39 ± 0.64	5.41 ± 0.16	7.83 ± 0.18	17.82 ± 1.01	30.88 ± 1.12	1.04 ± 0.08	0.096 ± 0.030
After	8.38 ± 3.75	6.82[b] ± 2.04	15.21[b] ± 5.61	5.43 ± 0.17	8.27 ± 0.39	17.55 ± 0.81	33.81 ± 2.54	0.84[b] ± 0.06	0.031[b] ± 0.010

To clarify the pathophysiological mechanism(s) of acute renal failure induced by CyA, glomerular hemodynamic studies were performed in euvolemic Munich–Wistar rats. We observed a marked increase in both afferent and efferent arteriolar resistances; the greater increment in efferent arteriolar resistance determined a significant increase on $\overline{P}_{GC}$. Since P_T remained unaltered it is possible to conclude that in CyA nephrotoxicity, not only can tubular obstruction be excluded but also that a significant increase in mean $\overline{\Delta P}$ occurs. Moreover since mean P_C and mean P_{AE} were maintained at values similar to control, an important increase on interstitial pressure could be excluded.

Despite the observed increase in mean $\overline{\Delta P}$ ($\overline{\Delta P} = \overline{P}_{GC} - P_T$), augmented values for SNGFR were not found. Actually, SNGFR was reduced. The arteriolar vasoconstriction determined a fall on mean Q_A. Nevertheless, the fall on Q_A was not the only factor responsible for the decline in SNGFR. Our data indicate that SNGFR was also decreased due to a significant fall in K_f. The decline on K_f was also suggested in man by Myers et al [22] who observed a 36% reduction of glomerular membrane pore density in patients after heart transplantation [22, 27]. Moreover, they also deduced by indirect methodology an increase in $\overline{\Delta P}$, similar to that observed by direct micropuncture data in rats in the present paper. These findings in glomerular hemodynamics all taken together resemble those produced by endogenous or exogenous angiotensin II [13, 28]. In fact, angiotensin II is capable to produce glomerular mesangial cell contraction and has an effect of reducing K_f [13, 29]. Moreover, it has been suggested that renal functional changes induced by CyA on total renal blood flow and glomerular filtration rate observed in rats may be caused by stimulation of the renin–angiotensin system [9, 11]. Actually, CyA has been shown to stimulate the renin–angiotensin system both in vitro and in vivo [9, 11, 30, 31]. In in vivo experiments, with spontaneous hypertensive rats (SHR), Siegel et al [11] induced acute renal failure and renin–angiotensin system activation with CyA administration. In vitro, Baxter et al [30] have shown stimulation of renin release from rat renal cortical slices by CyA. In the present study in rats treated with captopril, we observed that the renal vasoconstriction induced by CyA was completely abolished (Table 2), the fall in GFR and RPF being largely prevented. These findings suggest that angiotensin II may play important role in the pathogenesis of renal vasoconstriction produced by CyA. Angiotensin II could have altered renal function in this model of nephrotoxicity by several mechanisms. One of them was through a stimulation of tubuloglomerular feedback mechanism, leading to an increase in renin and angiotensin II production [11, 32]. Another possibility could have been the direct action of angiotensin II in decreasing K_f [13]. The benefical effects of captopril in rats receiving CyA infusion could also be due to a stimulation of kallikrein–kinin and/or on prostaglandin systems [33]. On the other hand, Murray, Paller and Ferris (7) found no effect of captopril on CyA induced fall in RPF despite the fact that an increase on plasma renin activity was also observed. The relationship between the stimulation of the renin–angiotensin system and the observed functional abnormalities is not clear. Gerkens et al [34] showed that high salt intake protects from CyA nephrotoxicity while a low Na intake increases it. The stimulation of renin by CyA may increase renal prostaglandin and thereby modulate CyA nephrotoxicity. However, several studies have suggested that renal PGs are depressed by CyA [10, 11, 35]. A decrease in prostacyclin synthesis factor (PSF) has been described in CyA treated rabbits [10]. In rats receiving CyA, prostacyclin (PGI_2) has been shown to be decreased in both rat skin allografts and activated macrophages [36]. However, Paller and Murray [27] observed that CyA administration is associated with a stimulation of renal prostaglandin synthesis, resulting in increased PGI_2 urinary excretion. Inhibition of prostaglandin synthesis by indomethacin or meclofenamate led to an increase in CyA renal toxicity [7, 27]. In addition, Baxter et al [37] have suggested that renal cortical concentration of PGE_2, PGF_2, 6-keto-PGF_1 and TXB_2 are not different from normal levels in rats receiving either acute or chronic doses of CyA. Recently it was suggested that PGs affect CyA toxicity by blocking intestinal absorption of this drug [38]. Thus, the conflicting and inconclusive studies did

Table 4. Summary of several measures of whole kidney function in Brattleboro rats before and after CyA administration

	GFR *ml/min*	RPF *ml/min*	FF %	TRVR *mm Hg · min/ml*
Homozygotes, $N = 9$				
Before	0.88[a] ±0.04	2.76 0.20	33 ±2	38 ±3
After	0.69[b] ±0.06	1.91[b] 0.13	38 ±4	55[b] ±4
Heterozygotes, $N = 7$				
Before	0.99 ±0.06	2.67 0.26	38 ±3	51 ±4
After	0.42[b] ±0.06	0.94[b] 0.12	45[b] ±3	147[b] ±21

[a] $\overline{X}$ ± SE
[b] $P < 0.05$ after vs. before CyA

not permit a definitive statement to be made concerning the role of prostaglandin on CyA nephrotoxicity. But, in the present experimental model, the use of indomethacin maintained essentially the same results as found with CyA alone, implying that the prostaglandin system has a minor if any effect in impairing glomerular hemodynamics during acute CyA treatment. It is possible that CyA has a PG inhibition–like effect and thus, simultaneous treatment with indomethacin and CyA did not enhance the nephrotoxicity.

Since it is clear that CyA caused an increase in RVR, it became important to evaluate the effect of a vasodilator Ca-channel blocker drug. Iaina et al [39], observed that CyA plus ischemia produces acute renal failure which can be attenuated by the use of verapamil. Verapamil reduces the severity of renal insufficiency as well as hystological damage. In the present study, verapamil attenuated CyA effects on RVR and RPF leading to an improvement in GFR. This protective action could be explained either by arteriolar vasodilation and/or by interaction with the renin–angiotensin system, since calcium is a final common pathway for both mechanisms [13].

Antidiuretic hormone (ADH) is another vasoactive substance which is capable of influencing the process of glomerular ultrafiltration, mainly by reducing K_f [13]. In the present study, acute CyA administration in Brattleboro homozygote rats produced a decline of only 22% and 31%, respectively, in GFR and RPF, when compared with the observed reductions of 51% and 55% ($P < 0.05$) seen in Munich Wistar rats. Perhaps, different sensitivities of these rat strains to CyA could explain our findings. To further elucidate this possibility, we performed the same protocolol using heterozygote Brattleboro rats. In this setting, results were not different from Munich Wistar rats, denying therefore strain differences for CyA nephrotoxicity. Therefore, we can postulate that the presence of ADH in some way is necessary for that full nephrotoxic action of CyA to be observed in the rat kidney. It is also clear that in the absence of this hormone it is still possible to observe a significant nephrotoxic action of this drug. It is possible that ADH participated in this pathophysiology either by its vasoconstrictor effect or its known action to reduce K_f. The mechanism of the ADH-associated fall in K_f is independent of a pathway involving angiotensin II since it acts selectively on K_f, presumably via mesangial cell contraction [13]. It is also possible that an interelationship between anesthesia, ADH and renin secretion could interfere with CyA nephrotoxicity. However, in Brattleboro rats, the absence of ADH disclose the effect of anesthesia on ADH release and thus on renin secretion.

Finally, the influence of CyA on renal function in experimental animals is milder and, in order to produce a uniform nephrotoxicity it was necessary to employ relatively high doses [40]. Thus, in the present experimental protocol it is possible that CyA causes an unique effect in the rat that may not occur in man.

In summary, according to these data, acute CyA administration caused a reduction in single nephron filtration rate due to an increase on afferent and efferent arteriolar resistance with a decrease of Q_A and K_f values. These studies suggest that at least the renin–angiotensin system and ADH may participate in this glomerular function impairment. Furthermore, indomethacin did not alter the effect induce by CyA, indicating that PGs may be not an important factor in these alterations. Finally, the protective action of verapamil may indicate a potential use of Ca channel blockers in order to minimize CyA nephrotoxicity.

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