Intravenous cyclosporine activates afferent and efferent renal nerves and causes sodium retention in innervated kidneys in rats

(autonomic nervous system/renal function/renal denervation/cyclosporine toxicity)

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ABSTRACT The effect of acute intravenous infusion of cyclosporine (10 mg/kg) on efferent renal and genitofemoral nerve activity and afferent renal nerve activity was studied in anesthetized rats. All animals were studied after unilateral renal denervation and extracellular fluid volume expansion. Activity of both efferent sympathetic nerves was increased significantly by cyclosporine infusion (renal, 69%; genitofemoral, 60%). Afferent renal nerve activity was increased 82% after cyclosporine ($P < 0.05$). Urine flow rate and both absolute and fractional sodium excretion from the innervated kidney were reduced 50% after cyclosporine infusion ($P < 0.01$). Absolute and fractional sodium excretion from the denervated kidney were significantly increased after cyclosporine. Infusion of vehicle had no significant effect on any measured variable in innervated or denervated kidneys. These studies demonstrate the capacity of cyclosporine to increase efferent sympathetic nerve activity and afferent nerve activity. It is also shown that sodium retention resulting from acute infusion of cyclosporine can be attributed to the increase in efferent renal nerve activity.

Cyclosporine (cyclosporin A) is a potent immunosuppressive agent that has significantly improved graft survival in organ and bone marrow transplant recipients. However, toxic side effects are common. Nephrotoxicity and hepatotoxicity have been most often reported as complications of cyclosporine use (1, 2), but clinically important effects such as hypertension, tremors, and tachycardia are also frequently observed (3, 4). These latter indications of toxicity have been attributed to stimulation of the sympathetic nervous system (5). In view of the known effects of renal efferent nerve activity on renal excretory function (6, 7), we postulated that reports of decreased glomerular filtration rate (GFR) and retention of salt and water associated with an acute infusion of cyclosporine (8, 9) were consistent with increased sympathetic nerve activity to the kidneys. Furthermore, the excitatory action of cyclosporine on the sympathetic nervous system may be the result of an activation of peripheral afferent nerves. These hypotheses were tested in rats by measuring the effects of an acute infusion of cyclosporine (total dose 10 mg/kg) on renal function, efferent renal and genitofemoral nerve activity (ERNA and EGNA), and afferent renal nerve activity (ARNA).

METHODS AND MATERIALS

Preparation of Animals. Experiments were performed on 30 male Sprague-Dawley rats (Charles River Breeding Laboratories). Animals were 12-14 weeks old and weighed 248 ± 5 g (mean $\pm$ SEM, range 210-290 g) after an overnight fast.

Anesthesia was induced by intraperitoneal pentobarbital (50 mg/100 g of body weight; Sigma) and maintained by

intravenous pentobarbital whenever the corneal reflex reappeared. Extracellular volume expansion was produced by a continuous infusion of isotonic saline into an external jugular vein at a rate of 3% of body weight per hr. Inulin (Sigma) and *p*-aminohippurate (Eastman Kodak) were added to provide plasma concentrations of approximately 100 mg/dl and 3 mg/dl, respectively.

Both femoral arteries were cannulated, one with polyethylene-50 tubing for measurement of blood pressure and heart rate (Gould Db 23 strain gauge transducer and Hewlett-Packard 7414A polygraph) and the other with polyethylene-10 tubing for blood sampling. Renal venous blood was obtained from the left renal vein by venipuncture with a 30-gauge needle. The retroperitoneal space was opened on the left side and filled with mineral oil, and the kidney was retracted to expose the renal pedicle. The left ureteropelvic junction and the urinary bladder were cannulated with polyethylene-50 tubing for collection of urine from the left and right kidney, respectively.

In the course of preparing renal nerves for activity recording, all nerve bundles from the left kidney were cut and the adventitia associated with the renal pedicle was removed. However, the use of phenol in alcohol to ensure complete destruction of renal nerve fibers was avoided because this would have endangered the dissected nerve bundles intended for activity recordings.

Nerve Activity Recording. Multifiber activity was recorded from the renal and genitofemoral nerves, both of which contain sympathetic efferent and visceral afferent fibers. In each nerve, activity from afferent or efferent fibers was selectively recorded on bipolar hook electrodes by cutting the nerve and recording at the distal or proximal end for afferent or efferent fibers, respectively. Two simultaneous recordings were attempted in each rat. ERNA and EGNA were monitored during clearance experiments. In a separate group of rats, ARNA was recorded together with genitofemoral afferent activity or, if this preparation was not successful, hypogastric afferent activity.

The electrical signal was preamplified with a differential amplifier, passed through a band-pass filter with frequency cutoffs set at 100 Hz and 1000 Hz, and displayed on an oscilloscope. Compound potentials resulting from simultaneous firing of different axons were minimized by dissecting the nerve bundles to a point where the recorded activity was composed of discrete impulses from individual axons. Nerve activity was recorded continuously on magnetic tape during each experiment. Impulses exceeding the background noise level were counted by nerve activity monitors and displayed on the polygraph as counts/sec. For purposes of analysis, activity was expressed as the mean frequency (Hz) in each clearance period.

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Abbreviations: GFR, glomerular filtration rate; RPF, renal plasma flow; ERNA, efferent renal nerve activity; EGNA, efferent genitofemoral nerve activity; ARNA, afferent renal nerve activity.

Experimental Protocol. The cyclosporine used in these studies was Sandimmune I.V. (Sandoz Pharmaceutical). In this product, 50 mg of cyclosporine is dissolved in 278 mg of 95% ethanol and 650 mg of Cremophor EL, a surfactant. The vehicle was prepared for use in control experiments which, in all other respects, duplicated those in which cyclosporine was used (we are grateful to A. L. Jacobs of Sandoz for the gift of Cremophor EL).

Basal levels of renal function and efferent nerve activity were obtained in two 20-min clearance periods before cyclosporine (0.67 mg/ml, 10 rats) or vehicle (1.3% vol/vol, 10 rats) was added to the maintenance infusion. These solutions were infused over 30-min to provide a total dose of 10 mg of cyclosporine/kg of body weight or an equivalent volume of vehicle. A 30-min equilibration period was followed by two final 20-min clearance periods. ERNA and EGNA were monitored throughout the experiment. At the end of each experiment a ganglionic blocker, trimethaphan camsylate (Arfonad, Roche Laboratories), was injected i.v. (2 mg/kg) to verify the postganglionic nature of the efferent nerve preparations and the threshold settings on the two nerve-activity monitors. If this procedure revealed a threshold that was inappropriately high or low, the nerve activity was reanalyzed from the tape recording made during the experiment.

ARNA was recorded in 10 rats during two 20-min control periods, the 30-min period of cyclosporine (10 mg/kg, 5 rats) or vehicle (5 rats) administration, and a subsequent 30-min equilibration period. One additional 20-min experimental period followed the equilibration period. These rats underwent the same degree of volume expansion as those used in clearance studies. In addition to ARNA, afferent genitofemoral nerve activity (3 rats) or afferent hypogastric nerve activity (2 rats) was recorded in the cyclosporine experiments. At the end of each experiment, the origin and response of each multifiber preparation were tested. The afferent genitofemoral nerve was activated by palpating the left testis; the hypogastric nerve responded to traction on the urinary bladder. The responsiveness of renal afferent fibers was tested by backflowing 200 mM KCl into the left renal pelvis. This procedure has been shown to activate renal R2 chemoreceptors which, in our rats, constitute the bulk of renal afferent nerve fibers (10).

Analytical Techniques. GFR was measured as the clearance of inulin, which was assayed in plasma and urine by the anthrone technique (11). Renal plasma flow (RPF) was derived from the clearance of *p*-aminohippurate. An extraction ratio obtained from the left renal arteriovenous difference in *p*-aminohippurate concentration was used to correct for incomplete clearance. *p*-Aminohippurate was assayed in plasma by the colorimetric technique of Bratton and Marshall (12). Sodium concentrations in plasma and urine were measured by flame-emission photometry (Perkin Elmer, model 51Ca), and plasma and urine osmolalities by vapor pressure osmometry (Wescor, model 5100C).

Values are expressed as mean $\pm$ SEM. Differences between pre- and postinfusion values from the same kidney were tested by paired *t*-test. Changes in nerve activity over the course of experiments were tested by analysis of variance and Duncan's multiple range test (13, 14). When Bartlett's test revealed heterogeneity of variances between treatments, the data were normalized by a logarithmic transformation.

RESULTS

Efferent Activity. The absolute level of ERNA ranged from 48 Hz to 208 Hz and that of EGNA, from 28 to 368 Hz. This variability is an inevitable consequence of differences in the size of nerve bundles and unpredictable damage caused by nerve dissection. For this reason the mean activity during the

FIG. 1. ERNA in rats infused with cyclosporine (●, $n = 10$) or vehicle (○, $n = 10$). Values are presented as mean $\pm$ SEM of percent change from the first control period (C1). E1 and E2 are the experimental periods. **, significantly different ($P < 0.01$) from the second control period (C2).

first clearance period was used as a reference level against which subsequent changes were assessed.

The effects of cyclosporine or vehicle infusion on ERNA and EGNA are shown in Figs. 1 and 2. In both cases, basal activity increased (mean latency of 2.3 ± 0.5 min) after the infusion of cyclosporine and continued to increase throughout the post-drug equilibration period. The mean percentage increase in ERNA between the first two and the last two clearance periods was $68.6\% \pm 10.8\%$. EGNA showed a similar increase of $59.9 \pm 10.1\%$ over the same time period. In both nerves, infusion of vehicle did not cause efferent sympathetic activity to deviate significantly from control values (Figs. 1 and 2).

Afferent Activity. The response of ARNA to cyclosporine or vehicle infusion is shown in Fig. 3. These data are presented in the same way as the efferent activity and represent the percentage change from control activity recorded in the initial 20-min measurement period. Mean ARNA during the two control periods was increased by $81.6 \pm 30.7\%$ ($P < 0.05$, paired *t*-test) during the equilibration period after cyclosporine. ARNA tended to decrease toward basal levels in the experimental period, in contrast to ERNA, which remained high after cyclosporine. By examination of the oscilloscope trace of ARNA, it was possible to identify

FIG. 2. EGNA in rats infused with cyclosporine (●, $n = 7$) or vehicle (○, $n = 9$). Data are presented as in Fig. 1.

FIG. 3. ARNA in rats infused with cyclosporine (●, $n = 5$) or vehicle (○, $n = 5$). Data are presented as in Fig. 1. These experiments used one experimental period (E1).

impulses from single afferent nerve fibers that were activated by both cyclosporine infusion and by backflow of KCl into the renal pelvis. Thus, these fibers were renal R2 chemoreceptors, previously described in this laboratory (15). Their pattern of excitation after cyclosporine was characterized by frequent surges of activity which contrasted with stable levels of activity prior to cyclosporine infusion.

Infusion of vehicle had no effect on the level of ARNA, which did not deviate significantly from basal levels throughout the experiment (Fig. 3). All of the renal afferent nerves studied in vehicle experiments responded to backflow of KCl, thus the lack of response to vehicle infusion was not due to a generalized unresponsiveness of these afferent fibers.

In all cases, afferent genitofemoral nerve activity and afferent hypogastric nerve activity became activated after cyclosporine with a mean increase of 65.7% for the genitofemoral nerves and 57.5% for the hypogastric nerves.

Renal Function. Before cyclosporine or vehicle treatment, basal GFR and RPF in the acutely denervated left kidney were significantly higher than those in the innervated right kidney (Table 1). Acute renal denervation also resulted in a considerably increased basal urine flow rate and solute excretion from the left kidney compared to the innervated right kidney (Table 2).

Cyclosporine induced a small but statistically significant decrease in GFR in innervated right kidneys that was not associated with a statistically significant change in RPF. In contrast, RPF and GFR were not altered in the denervated left kidney of the same animals after cyclosporine (Table 1). Infusion of cyclosporine caused a 50% reduction in urine flow rate, sodium excretion, and osmolar excretion from the innervated kidney. In the denervated kidney, sodium and

osmolar excretion were significantly increased after cyclosporine ($P < 0.05$, Table 2).

GFR and RPF were not altered by infusion of vehicle in either the innervated or the denervated kidney. Similarly, urine flow rate, sodium, and osmolar excretion from both the innervated and denervated kidney were not affected by vehicle infusion.

Blood Pressure and Heart Rate. The effects of cyclosporine on arterial blood pressure and heart rate are shown in Table 3. Blood pressure was significantly lower after vehicle infusion, as a result of a steady decrease throughout the experiment. Conversely, heart rate rose and showed a significant, inverse correlation with the falling blood pressure ($r = 0.59$, $P < 0.001$, $n = 30$). In contrast to the effects of vehicle, cyclosporine caused a prompt and significant increase in blood pressure and heart rate during its administration (Table 3). This hypertensive effect was transient, and blood pressure returned to control levels during the 30-min equilibration period that followed cyclosporine infusion. Nevertheless, a persistent effect on blood pressure was apparent since, unlike the blood pressure in vehicle-treated rats, pressure did not fall below control values during the latter stages of the protocol.

DISCUSSION

Intravenous infusion of cyclosporine has a sympathoexcitatory action. The degree of excitation of efferent nerve activity was similar in genitofemoral and renal nerves, suggesting a generalized sympathetic activation. This is further suggested by the increase in blood pressure and heart rate during cyclosporine infusion, a finding similar to that of Tonnesen *et al.* (8).

The excitation of afferent nerves by cyclosporine raises many new possibilities concerning the neurotoxicity of this drug. Our data indicate that the effect is not confined to renal afferent nerves, though the nature of the preparation restricted us to afferent nerves from the urogenital system. The response was not due to an increase in tissue perfusion pressure, since we were able to identify R2 chemoreceptors among the activated afferent renal nerves. These receptors have been shown to respond to alterations in the intrarenal chemical environment rather than to increased renal perfusion pressure (15). Further, because afferent activity was recorded from denervated kidneys, we can dismiss the possibility of an interaction between efferent and afferent nerve endings as a possible cause of the afferent excitation. Thus, the effect was a direct one on afferent nerve endings, though it is unclear whether the responsible agent was cyclosporine itself, a metabolite, or a released endogenous substance.

One can postulate that afferent excitation was the primary neurological event, which resulted in a reflex excitation of efferent sympathetic activity. Activation of renal R2 chemoreceptors is known to elicit reflex excitation of both ipsilateral and contralateral efferent renal nerves (16, 17).

Table 1. GFR and RPF in rats before and after infusion of cyclosporine or vehicle alone

Group	Kidney	GFR, ml/min		RPF, ml/min	
		Preinfusion	Postinfusion	Preinfusion	Postinfusion
Cyclosporine	Denervated	1.425 ± 0.069	1.443 ± 0.077	4.703 ± 0.238	4.906 ± 0.364
	Innervated	1.184 ± 0.058	1.033 ± 0.041*	3.911 ± 0.284	3.766 ± 0.329
Vehicle	Denervated	1.466 ± 0.080	1.496 ± 0.112	5.024 ± 0.320	5.061 ± 0.212
	Innervated	1.185 ± 0.068	1.179 ± 0.089	4.124 ± 0.278	4.111 ± 0.250

Data are presented as mean ± SEM for 10 animals in each group. All values are corrected for kidney weight (g).

*Significantly different ($P < 0.005$) from preinfusion value. All other preinfusion/postinfusion comparisons showed no significant difference.

Table 2. Renal excretory function in rats before and after infusion of cyclosporine or vehicle

Kidney	Urine flow, $\mu\text{l}/\text{min}$		Urinary Na^+ , $\mu\text{eq}/\text{min}$		FE*, Na^+		Total urinary solutes, $\mu\text{osmol}/\text{min}$		FE*, total urinary solutes	
	Before	After	Before	After	Before	After	Before	After	Before	After
Cyclosporine group										
Dener- vated	51.0 ± 3.3	55.7 ± 2.6	9.09 ± 0.55	11.40 ± 0.77	4.89 ± 0.34	6.09 ± 0.38	24.81 ± 1.28	29.06 ± 1.29	5.91 ± 0.31	6.83 ± 0.23
	(NS)		$(P < 0.05)$		$(P < 0.01)$		$(P < 0.05)$		$(P < 0.001)$	
Inner- vated	22.1 ± 1.4	12.3 ± 0.7	3.60 ± 0.38	1.78 ± 0.15	2.29 ± 0.23	1.34 ± 0.13	12.91 ± 0.66	8.16 ± 0.53	3.67 ± 0.15	2.64 ± 0.11
	$(P < 0.0001)$		$(P < 0.001)$		$(P < 0.0001)$		$(P < 0.0001)$		$(P < 0.0001)$	
Vehicle group										
Dener- vated	56.2 ± 2.6	55.6 ± 4.3	9.54 ± 0.66	10.05 ± 1.01	5.00 ± 0.43	5.23 ± 0.50	26.58 ± 1.37	27.53 ± 1.91	6.36 ± 0.37	6.47 ± 0.40
	(NS)		(NS)		(NS)		(NS)		(NS)	
Inner- vated	22.5 ± 1.9	20.4 ± 1.6	3.26 ± 0.33	3.22 ± 0.29	2.06 ± 0.16	2.17 ± 0.20	12.82 ± 0.96	12.34 ± 0.85	3.72 ± 0.17	3.72 ± 0.27
	(NS)		(NS)		(NS)		(NS)		(NS)	

Data are presented as mean $\pm$ SEM for 10 rats in each group. All values are corrected for kidney weight (g). Significance of difference between values measured before and after infusion is given in parentheses; NS, not significant.

*Fractional excretion.

However, we have no reason to exclude other causes, including possible derangements in the baroreflex control of sympathetic nerve activity (18) or a direct action of cyclosporine on the central nervous system (4).

In the present studies, in which rats with expanded extracellular fluid volume received an acute infusion of cyclosporine, reduced salt and water excretion occurred only from the innervated kidney. Since ERNA exerts a salt-retaining effect on the kidney (19, 20), salt and water retention after cyclosporine infusion can be attributed to activation of efferent renal nerves. It is probable that this is also the explanation for the sodium retention and reduced GFR and RPF reported by others after acute cyclosporine administration (8, 9). Whether this action of the renal nerves is significant in long-term cyclosporine administration to animals with innervated kidneys cannot be assessed from our data. In a chronic-administration study on rats with innervated kidneys, Dieperink *et al.* (21) gave cyclosporine by gastric intubation at daily doses of 12.5, 25, and 50 mg/kg. After 13 days with all doses, GFR, urine flow rate, and sodium clearance were reduced significantly below control levels. A comparison of sodium and lithium clearances suggested enhanced reabsorption of sodium from the proximal tubule, which is consistent with increased nerve activity to the kidneys. Gerkens *et al.* (22) found that sodium excretion and urine flow rate were unchanged over a 3-week period in rats fed a low salt diet and given oral doses of cyclosporine (100 mg/kg) at 48-hr intervals. In contrast, the same treatment in rats fed a high sodium diet caused a significant increase in sodium excretion. Thus, the state of

extracellular volume could be an important consideration in the renal actions of cyclosporine, as is the case for other nephrotoxins (23). The nephrotoxic action of cyclosporine is a complicating factor that could influence sodium excretion independently of the renal nerves. We cannot exclude the possibility of a direct inhibitory action of cyclosporine on the reabsorption of tubular fluid; indeed sodium excretion from the denervated kidney increased after cyclosporine. In this acute setting, we interpret this as a compensatory mechanism that maintained sodium balance during salt retention by the innervated kidney.

The clinical implications of this study are several. It is clear that activation of the sympathetic nervous system is an important component of cyclosporine toxicity, and hypertension is a well-known side-effect of cyclosporine administration. In cyclosporine-treated patients, the incidence of hypertension is 80% in heart-transplant recipients and 60% in bone marrow-transplant patients. These values are significantly greater than in control patients treated with other modalities (24–26). This phenomenon may be initiated by a generalized activation of the sympathetic nervous system and exacerbated by salt and water retention in the innervated kidneys. The incidence of hypertension is less in recipients of denervated renal allografts receiving cyclosporine therapy (27) but, when present, this too may be caused by the systemic effects of a generalized sympathetic activation. The renal nerves cannot contribute to salt retention in these patients, and sodium retention through other mechanisms must be invoked. Nevertheless, adrenergic supersensitivity within the chronically denervated allograft would result in exaggerated responses to

Table 3. Heart rate and blood pressure in rats before, during, and after infusion of cyclosporine or vehicle

Group	Time of measurement	Heart rate, beats per min		Blood pressure, mm Hg*	
Cyclosporine	Before	394 $\pm$ 8	$P < 0.005$	114 $\pm$ 4	$P < 0.001$
	During	412 $\pm$ 10		122 $\pm$ 3	
	After	412 $\pm$ 7		111 $\pm$ 4	
Vehicle	Before	398 $\pm$ 5	$P < 0.005$	120 $\pm$ 3	$P < 0.001$
	During	404 $\pm$ 6		113 $\pm$ 3	
	After	417 $\pm$ 5		109 $\pm$ 3	

Data are presented as mean $\pm$ SEM for 10 rats in each group. NS, not significant.

*1 mm Hg = 133 Pa.

circulating catecholamines (28, 29). The commonly observed hand tremor is another complication of cyclosporine treatment that may be a manifestation of an activated sympathetic nervous system. Whether all of these phenomena are linked to a common central nervous system toxicity, which has been reported in 8% of patients receiving cyclosporine (4), or to the reflex consequences of activation of peripheral afferent nerves is yet to be established.

The autonomic nervous system innervates several lymphatic organs including the thymus, the spleen, and the lymph nodes. Functional relationships between sympathetic nerves, their receptors, and immune reactivity have been established (30). In view of the apparent generalized effect of cyclosporine on the sympathetic nervous system, the possibility that neural excitation plays a contributing role in the modulation of the immune response by cyclosporine must be considered.

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