CYCLOSPORINE AUGMENTS REACTIVITY OF ISOLATED BLOOD VESSELS

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Summary

Administration of cyclosporine (CS) as an immunosuppressive agent in clinical transplantation is associated with multiple side effects including nephrotoxicity and hypertension. These two effects could be related in that the renal changes may be secondary to alterations in organ blood flow. The present studies investigate the ability of CS to augment contractile responsiveness in blood vessels from normotensive rats. Isometric force generation was measured in isolated tail arteries and portal veins. CS (8.3x10^-6M) potentiated tail artery contractile responses to sympathetic nerve stimulation, exogenous norepinephrine, and increases in extracellular potassium concentration. Portal veins undergo spontaneous contractions which are related to the firing of calcium-driven action potentials in the smooth muscle cells. CS significantly increased the frequency of these spontaneous contractile events. These results suggest that components of CS toxicity may involve a direct action on vascular smooth muscle and/or on vascular adrenergic neurotransmission.

Cyclosporine (CS), an orally-active endecapeptide with potent immunosuppressive properties, has significantly improved survival in organ and bone marrow transplant recipients. Despite this success, the value of the drug has been lessened by its clinically significant side effects. Prominent among these effects, which have been extensively studied in both humans and in experimental animals, are nephrotoxicity and hypertension (1-5). Other serious problems include: hepatic dysfunction, tremor, convulsions, hirsutism and gingival hyperplasia (see 1, 6-8 for review).

Changes in renal function appear rapidly following CS administration and persist during chronic treatment. Acute intravenous administration of CS (20mg/kg) to rats caused a 26% reduction in glomerular filtration rate and a transient increase in blood pressure (9) as well as a 48% decrease in renal blood flow and an 80% increase in renal vascular resistance (8). An intravenous dose of 10mg/kg caused a 69% increase in efferent renal nerve activity (10), suggesting that at least part of the effect of CS on renal function may be indirect. Chronic CS treatment is associated with elevations of serum urea and creatinine, depressed inulin clearance (1,2,5-8,11), and a reduction in renal blood flow (12). Although structural changes have been reported in the proximal tubule of the rat (11) and human (13), CS nephrotoxicity appears to be readily reversible upon withdrawal of the drug.
These observations have led several authors to speculate that the nephrotoxic effect of CS is mediated primarily through a hemodynamic action of the drug (6,8,14).

The etiology of the elevation of arterial blood pressure is unknown and this change appears to develop independently of known cardiovascular risk factors (3). It has been postulated that generalized vascular damage is at least partially responsible. Pathologic changes have been observed in both arteriolar smooth muscle (5) and endothelial cells (15). Other authors have suggested that the renin-angiotensin-aldosterone system is involved. This speculation is based on the observation of elevations in plasma renin activity (PRA) in CS-treated rats (5,7) as well as augmentation by CS of renin release from isolated renal cortical slices (16). However, consistent elevations in PRA have not been observed in man (3,4,17), and PRA may actually be significantly depressed (18). Finally, Thompson and coworkers (3) have reported that following cardiac transplantation CS administration is associated with a failure of total peripheral resistance to fall appropriately when cardiac output is increased upon replacement of the failing heart. These patients develop hypertension in association with this elevated vascular resistance.

The observation of concurrent alterations in renal function, renal blood flow and systemic vascular resistance led us to hypothesize that cyclosporine alters vascular function, either directly or through an effect on adrenergic neurotransmission. This hypothesis was tested by measuring the effect of CS on force development in isolated segments of vascular smooth muscle responding to physiologic stimuli.

**Methods**

Adult male Sprague-Dawley rats (300-350g) were anesthetized with sodium pentobarbital (50mg/kg) and tail arteries and portal veins were removed. The vessels were cleaned of adherent fat and connective tissue under a dissecting microscope. Tail arteries were cut helically into strips (0.7 x 10mm), portal veins were slit longitudinally. The tissues were mounted vertically in an organ chamber containing physiologic salt solution (PSS) for measurement of isometric force generation as described elsewhere (19).

Transmural nerve stimulation (TNS) was delivered via parallel platinum wire electrodes. A stimulator (Grass model S4) delivered 9 volt square wave pulses of 1 msec duration over a range of frequencies. Ascorbic acid (10^-4M) was added to the buffer during stimulation periods in order to prevent cellular damage due to free radical formation at the electrodes. Cyclosporine was delivered from the IV preparation (Sandimmun) and control experiments were performed using the vehicle compound, cremophor EL plus ethanol (provided by Sandoz Pharmaceuticals). Other drugs used were norepinephrine bitartrate (Levophed, Sterling Drug Inc., New York, NY) and phentolamine mesylate (Regitine, CIBA Pharmaceutical Co., Summit, NJ).

Statistical analysis was performed using either a Student's t-test with the Bonferroni correction or a one-way analysis of variance with a least significant difference test. A p-value of less than 0.05 was considered to be statistically significant.

**Results**

Prolonged incubation (2 hours) of unstimulated tail artery strips in CS (8.3 x 10^-6M) did not elicit a contractile response. However, the presence of CS did alter the response to known vasoconstrictor stimuli whereas the
Responses of tail artery strips to electrical stimulation (1 msec, 9V, 0.1-16Hz) under control conditions (solid line) and in the same strips following a 10 min incubation in CS (8.3 x 10^{-6}M, broken line). Time control repetitions of the frequency response were not significantly different from control. Mean maximal response to norepinephrine was 1730 ± 155 mg. Asterisks indicate a significant difference at p 0.05 (N=8).

Contractile responses (3 min) of tail artery strips to 5.9 x 10^{-9}M norepinephrine before, during and after exposure to 8.3 x 10^{-6}M CS. Bars represent results obtained from eight animals. The tracing depicts a typical experiment. Asterisk indicates a significant difference at p 0.05, (N=8).
vehicle solution had no such properties. Figure 1 shows the response of the isolated tail artery to adrenergic nerve stimulation as the frequency of TNS is varied from 2.1 to 16 Hz. These neural responses can be blocked completely by tetrodotoxin or by phentolamine. Following a 10 min incubation in CS, a significant increase is observed in the responsiveness to TNS. Appropriate time control studies were also performed and no significant changes were observed in the frequency response curve.

Since CS augmented responses to endogenously released norepinephrine, the effect of CS on responses to exogenously applied norepinephrine (figure 2) was evaluated. Tail arteries were made to contract repeatedly (3 min contractions) in response to a low concentration of norepinephrine (5.9 x 10^-9M, an approx. ED10 dose) until a stable control response was established. Following a 10 min incubation in CS a significant potentiation of the contractile response to an identical concentration of norepinephrine was observed. This effect was reversed following washout of CS.

![Graph showing responses of phenolamine-treated (10^-6M) tail arteries to potassium chloride-induced depolarization before (solid line) and following a 10 min exposure to CS (8.3 x 10^-6M, broken line). Asterisks indicate a significant difference at p 0.05, (N=6).]
An attempt was made to distinguish the site of action of cyclosporine in potentiating response to exogenous norepinephrine. In order to discern between effects on calcium influx and intracellular calcium handling, tail arteries were stimulated with norepinephrine \(5.9 \times 10^{-8}, 5.9 \times 10^{-7}\) in the presence of calcium-free PSS containing \(1.0 \text{ mM ethylene glycol bis (B-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA)}\). Under these conditions norepinephrine evokes a transient contractile spike. The magnitude of this spike is not increased by cyclosporine \(8.3 \times 10^{-6} \text{ M, table I}\).

**TABLE I**

<table>
<thead>
<tr>
<th>Concentration of Norepinephrine</th>
<th>Control</th>
<th>Cyclosporine</th>
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<tbody>
<tr>
<td>(5.9 \times 10^{-8} \text{ M})</td>
<td>18 ± 12 mg</td>
<td>17 ± 9 mg</td>
</tr>
<tr>
<td>(5.9 \times 10^{-7} \text{ M})</td>
<td>203 ± 57 mg</td>
<td>119 ± 44 mg</td>
</tr>
</tbody>
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Norepinephrine-induced phasic contractions in calcium-free PSS containing \(1.0 \text{ mM EGTA}\). Tissues were incubated in cyclosporine \(8.3 \times 10^{-6} \text{ M}\) for 10 minutes, \(N=4\).

Figure 1 illustrates contractile responses of tail artery strips which were induced by changing the potassium concentration of the PSS \(10^{-5} - 50 \text{mM}\). The presence of CS \(8.3 \times 10^{-6} \text{M}\) augmented the contractile response observed at a given concentration of potassium. These experiments were performed in the presence of phentolamine in order to prevent an effect of norepinephrine released from depolarized adrenergic nerve endings \(20\).

The portal vein of the rat undergoes spontaneous phasic contractile events under isolated conditions. Figure 4 demonstrates that increasing concentrations of CS \(8.3 \times 10^{-7} - 8.3 \times 10^{-5} \text{M, 10min incubations}\) increased the frequency of these myogenic events.

**Discussion**

These studies support our preliminary observations \(21\) and suggest that CS can exert direct effects upon vascular smooth muscle. This action leads to an augmentation of contractile responsiveness which may contribute directly to CS-induced hypertension or may help to initiate the changes in renal blood flow and subsequent nephrotoxicity associated with CS administration \(6,8,13\).

The ability of CS to potentiate responses to stimulation of the vascular adrenergic nerve endings could be explained by either an action of CS to augment adrenergic neurotransmission or a direct effect of CS on smooth muscle cells. Our experiments which demonstrate a CS-induced increase in responses to exogenous norepinephrine support the latter conclusion. However, CS is known to augment renal sympathetic nerve activity \(10\) and to increase heart rate \(5\), therefore a combination of effects on both nerves and smooth muscle remains an attractive hypothesis.

The effect of CS on responses of the tail artery to depolarizing concentrations of potassium suggests that CS may alter resting membrane potential, bringing the tissue closer to threshold for contraction. This conclusion is supported by the ability of CS to increase the frequency of spontaneous contractions in the portal vein. These spontaneous events have been shown to be associated with firing of a calcium-driven action potential in the smooth muscle cells. The frequency of their firing is increased...
Spontaneous contractile responses in the rat portal vein under control conditions and during exposure to varying concentrations of CS ($8.3 \times 10^{-7}$ - $8.3 \times 10^{-5}$M). Results are expressed as the number of contractions occurring within a 5 min period. Upper panel shows the response of one vein. Asterisks indicate a significant effect at $p < 0.05$, (N=4).

Following depolarization (22), however, unlike potassium-induced depolarization, cyclosporine did not increase the amplitude of portal vein contractions. The reason for this difference is unclear. An ability of cyclosporine to cause partial depolarization would also be consistent with the observed alteration in response to endogenous and exogenous norepinephrine. A decrease in membrane potential has been shown to increase both release of norepinephrine from adrenergic nerve endings (20) and smooth muscle responses to applied norepinephrine (23).

Whatever the action of cyclosporine, it appears to be acting at the level of the plasma membrane. Responses of the blood vessel in calcium-free solution depend upon the release of intracellular calcium stores (24).
our experiments, cyclosporine had no effect on the magnitude of these phasic contractions.

Although this electrophysiological explanation of CS's action on vascular smooth muscle seems reasonable, CS has previously been demonstrated to possess a number of other cellular actions which could potentially alter vascular function. CS inhibits phospholipase A2 activity in both rat and human white blood cells (25,26) and can alter prostanoid release from human peripheral blood mononuclear cells (27). CS had no effect on cytosolic free calcium concentration in unstimulated rat hepatocytes or on resting calcium uptake into mouse lymphocytes. However, the drug was able to augment the receptor-mediated rise in intracellular calcium when the hepatocytes were stimulated with vasopressin and to increase the uptake of calcium into collagen-stimulated lymphocytes (28,29). These observations parallel our results where despite an apparent lack of vasoconstrictor properties, CS is able to increase vascular reactivity to known constrictor stimuli.

The clinical significance of these experiments is unclear. The present studies were performed primarily using 10 min exposures to cyclosporine at a concentration of $8.3 \times 10^{-5}$ M ($10^{-5}$ g/ml). Clinical results (30,31) have suggested that optimal trough plasma levels of cyclosporine in man are somewhat lower than this, in the range of $2 \times 10^{-7}$ g/ml ($10^{-6}$ g/ml of whole blood). However, the rat has been shown to be relatively resistant to the immunosuppressive effects of cyclosporine compared to man (11). In addition, while these in vitro studies involve very short exposures to this compound, clinical treatment with cyclosporine may continue for months or years. Regardless, these experiments demonstrate a direct vascular effect which may be associated with the hypertensive and nephrotoxic side effects of cyclosporine.

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References