Direct Augmentation by Cyclosporin A of the Vascular Contractile Response to Nerve Stimulation

MICHAEL S. GOLUB AND MORRIS E. BERGER

SUMMARY  Cyclosporin A administration is associated with an increased incidence of hypertension. To evaluate the direct effects of the drug on the contractile responses of vascular tissue to adrenergic stimuli, rat caudal artery ring segments were studied before and after the addition of cyclosporin A or its ethanol vehicle in vitro. In a dose-related manner, cyclosporin A augmented the contractile response to transmural nerve stimulation, with a highly significant (p<0.001 relative to that produced by the vehicle) lowering of the stimulation rate, a 50% of maximum contractile response (ED50) that elicited. The difference between pretreatment and treatment maximal responses to transmural nerve stimulation was also significantly greater (p<0.01) in the cyclosporin A–treated preparations than in those receiving the vehicle. In similar experiments, the responses to exogenous norepinephrine were not significantly affected. The effect of cyclosporin A on transmural nerve stimulation was demonstrated at several extracellular calcium concentrations. The results suggest that cyclosporin A enhances nerve stimulation responses by a presynaptic mechanism.

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KEY WORDS  • cyclosporin A • nerve stimulation • sympathetic nervous system • hypertension • vascular reactivity

THE fungal undecapeptide cyclosporin A (CSA) has found widespread utility as an immunosuppressant in the treatment of organ transplantation rejection and in the therapy of other immunological medical conditions. However, significant side effects, especially hepatic and renal toxicity and an increased incidence of hypertension, are of concern. The tendency for the drug to raise blood pressure in humans has been noted in transplant1 and in non-transplant2 patients. Cyclosporin A also significantly raises blood pressure in the spontaneously hypertensive rat.3,4 In CSA-treated rats receiving 5 and 20 mg/kg/day by gavage for 14 days, there was a significant increase in systolic blood pressure, as measured by tail cuff. We found that the tail arteries from animals given either dose of CSA (5 or 20 mg/kg/day) showed an enhanced response to transmural nerve stimulation (TNS), whereas the response to exogenous norepinephrine (NE) was significantly affected only in the animals receiving the higher dose of CSA.3 The present study was performed to evaluate the direct effect of CSA on adrenergic stimulation of vascular tissue in vitro.

Methods

Rat Caudal Artery Preparation

Thirty Sprague-Dawley male rats (weight, 350–500 g) obtained from Bantin-Kingman (Laboratory Animal Consultants, Fremont, CA, USA) were used to provide pairs of adjacent caudal artery segments. On each experimental day, one or two rats were decapitated and their blood removed by rapid exsanguination, a procedure approved for rodents by the institutional animal use committee. A proximal portion (approximately 6 cm) of the caudal artery was rapidly dissected and transferred to a petri dish containing Krebs bicarbonate buffer (millimolar composition: Na+, 144.2; K+, 4.9; Ca2+, 1.3; Mg2+, 1.2; Cl−, 126.7; HCO3−, 25; SO42−, 1.2; glucose, 11.1; EDTA, 0.024; ascorbic acid, 0.1) under continuous oxygenation (95% O2, 5% CO2). Each vessel was carefully cleaned of loose fat and connective tissue and was cut into 4-mm ring segments.

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using a dissection microscope equipped with a calibrated ocular scale. Two stainless steel mounting wires (0.008-in. diameter) were passed through the lumen of each ring. The segments were transferred to 64-ml tissue baths equipped with a water warming jacket (37.5°C), sintered disk oxygenators, and two parallel platinum-field electrodes. Each segment preparation was secured in a specially fabricated assembly modified after that of Bevan and Osher6 and consisting of a strain-gauge transducer (UC-2, Gould/Statham, Ox-
nard, CA, USA) mounted on a vernier micrometer stage (Velmx, East Bloomfield, NY, USA). An opti-
mal passive tension of 0.5 g was determined in pre-
liminary experiments with similar vessel segments by finding the stretched tension that produced the maxi-
mal contractile response to an estimated mean effec-
tive dose (ED₉₀) of NE (Sigma, St. Louis, MO, USA).

Contractile Responsiveness to Exogenous
Norepinephrine

Nine pairs of ring preparations were exposed to NE (1 × 10⁻⁶ to 6.7 × 10⁻³ M) added to the tissue baths in a cumulative fashion in 0.1 to 0.3-ml aliquots. In five of the arteries, a third segment was also studied as an untreated time control. Following the initial NE dose-
response curve, repeated washes with fresh Krebs buf-
fer returned tissue tension to baseline. Transmural nerve stimulation responses to 4 pulses/sec were ob-
tained prior to and 30 minutes after the addition of CSA (6 × 10⁻⁶ M) or ethanol vehicle (0.2 ml) in the
nine paired segments. The NE dose-response curve was then repeated in these preparations and in the five segments that had received 0.2 ml of buffer.

Effect of Different Calcium Concentrations on
Transmural Nerve Stimulation Response

In order to evaluate whether the effect of CSA on the
TNS contractile response was affected by alterations in the extracellular calcium concentration, five pairs of taill artery ring preparations were studied in the pres-
ence of several different concentrations of calcium in the
bath. Following the 90-minute equilibration in nor-
mal calcium buffer (1.3 mM), the tissues were washed
twice in zero-calcium Krebs buffer. Twenty minutes
later, in the presence of continuous TNS at 4 pulses/
sec, a cumulative-dose contractile response curve was
obtained with the addition of calcium chloride in 0.1 to
1.0-m1 volumes to achieve concentrations of 0.0625 to
1.5 mM calcium. After obtaining a maximal response,
the TNS was terminated and tissue tension allowed to
return to baseline. The segments were washed twice in
zero calcium buffer and incubated in this solution for
20 minutes. Preparations were incubated for 30 min-
utes with CSA (10⁻⁶ M) or ethanol vehicle (0.2 ml)
prior to obtaining a second calcium chloride dose-
response curve in the presence of continuous TNS (4
pulses/sec).

Statistical Analysis

The study design in each experiment compared adja-
cent segments from the same vessel treated with either
CSA or its vehicle. Therefore, paired t calculations
were used. When the preparations were studied before
and after the addition of the drugs, the change from
control was used as the major determinant of a differ-
eence between the groups. Means are expressed as ± 1
SE. A p value of <0.05 was considered statistically
significant. The ED⁹₀ and slope values were deter-

Effects of Cyclosporin A Dose on Contractile Response

Four doses of CSA (3 × 10⁻⁷ M, 3 × 10⁻⁶ M,
6 × 10⁻⁶ M, 1.5 × 10⁻⁵ M) and appropriate volumes
of ethanol vehicle (0.1–0.3 ml) were evaluated for their
effect on TNS at 4 pulses/sec in five pairs of ring
preparations. Each segment’s response was recorded at
baseline (control) and after a 30-minute incubation
at each dose of CSA or vehicle. The mean of two
stirrations performed 10 minutes apart was used to
determine the response.
mined for each curve by plotting the percentage of maximal response versus a logarithmic transformation of the dose for those values (usually 4 points) falling between 15 and 85% of maximum, using least-squares linear regression calculations.

Results

Contractile Responsiveness to Transmural Nerve Stimulation

The $ED_{50}$, slope, and maximum responses to TNS before and after the addition of CSA (3 x 10^{-6} M) or vehicle are shown in Table 1. A significant reduction ($p<0.001$) in $ED_{50}$ was evident in the CSA-treated group compared to the vehicle-treated group. There also was a small but statistically significant ($p<0.05$) increase in the slope of the curves after the addition of the vehicle compared to slope plotted from the CSA response. In the second curve, maximum response fell after the addition of the vehicle, but not after the addition of CSA; the difference in these responses was significant ($p<0.01$). When the second curves (after the addition of CSA or vehicle) were plotted as a percentage of the maximal response to a large dose of NE, significant differences were noted at multiple TNS frequencies (Figure 1). The maximal responses to NE in the two groups were identical (9.1 ± 0.4 vs 9.1 ± 0.4 g), and the dry weight of the vessels did not differ significantly (0.44 ± 0.02 mg with CSA vs 0.42 ± 0.02 mg with vehicle).

Effects of Cyclosporin A Dose on Contractile Response

The effect of several different doses of CSA on a single frequency (4 pulses/sec) of TNS are shown in Figure 2. In this experiment, the two higher doses of CSA enhanced the TNS response significantly in comparison to the ethanol vehicle.

Contractile Responsiveness to Exogenous Norepinephrine

Table 2 presents the $ED_{50}$, slope and maximum response data for the dose-response curves generated before and after the addition of CSA (6 x 10^{-6} M) or vehicle. The second curves plotted after the addition of the vehicle showed an increase in the $ED_{50}$, a decrease in the slope, and a decrease in the maximal responses. Although the vessel preparations treated with CSA had smaller changes in $ED_{50}$ and maximum responses, these differences were not significant. On the other

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**TABLE 1. Effect of Cyclosporin A on Transmural Nerve Stimulation-Induced Contractile Responses in Rat Tail Artery Segments**

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$ED_{50}$ (pulses/sec)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>5.6 ± 0.6</td>
<td>5.9 ± 0.5</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>CSA</td>
<td>5.1 ± 0.5</td>
<td>3.9 ± 0.5</td>
<td>-1.2 ± 0.2</td>
</tr>
<tr>
<td>Slope (%/ln[pulses/sec])</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>25.4 ± 1.1</td>
<td>28.8 ± 1.1</td>
<td>3.3 ± 1.0</td>
</tr>
<tr>
<td>CSA</td>
<td>24.7 ± 1.0</td>
<td>24.1 ± 1.2</td>
<td>-0.6 ± 0.7</td>
</tr>
<tr>
<td>Maximum response (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>6.8 ± 0.4</td>
<td>6.5 ± 0.4</td>
<td>-0.4 ± 0.1</td>
</tr>
<tr>
<td>CSA</td>
<td>7.3 ± 0.3</td>
<td>7.4 ± 0.3</td>
<td>0.1 ± 0.1</td>
</tr>
</tbody>
</table>

*CSA, 3 x 10^{-6} M.

†$p<0.001$; ‡$p<0.05$; §$p<0.01$, vs vehicle.
Transmural Nerve Stimulation Response

Effect of Different Calcium Concentrations on Transmural Nerve Stimulation Response

The effect on the TNS response (4 pulses/sec) of adding calcium to a zero-calcium medium before and after CSA or vehicle treatment is shown in Figure 3. A significant difference between the CSA and vehicle responses was evident at most calcium concentrations, particularly the lower ones. The change in maximal response caused by the addition of vehicle was significantly greater than the change caused by adding CSA (−0.8 ± 0.1 g with vehicle vs. −0.1 ± 0.1 g with CSA, p<0.01). This decline in responsiveness of the tissues may be attributed to the repeated washing with zero-calcium medium and the continuous nature of the TNS stimulation. CSA essentially prevented this attenuation. The vessel segments used in this experiment had similar dry weights (0.35 ± 0.02 mg with CSA vs. 0.34 ± 0.01 mg with vehicle).

Discussion

The present study sought to determine whether CSA has a direct effect on vascular responses to nerve stimulation and exogenous NE. Although a trend in the data suggested increased responsiveness to NE after CSA treatment, these differences were not statistically significant. On the other hand, the data for TNS was very consistent and showed a highly significant decrease in the ED₅₀ and a small but significant increase in the maximal response. These results would suggest that CSA has a direct effect on presynaptic mechanisms.

Although the enhancement of TNS responses by CSA was dose-related, the actual concentrations of the material in the bath or in the tissues was not determined. The drug is very insoluble in aqueous solutions, so that in vitro and in vivo studies are not necessarily comparable. However, our studies of CSA administration to the spontaneously hypertensive rat yielded very compatible results. In those experiments, the tail arteries of animals treated with 5 mg/kg/day CSA showed changes analogous to those reported here. The ED₅₀ for the TNS contractile response was significantly decreased in comparison to controls, whereas the response to exogenous NE was not significantly affected. Interestingly, at a higher CSA dose of 20 mg/kg/day, both TNS and NE responses were significantly changed from control responses. This would suggest that both presynaptic and postsynaptic responses can be enhanced by CSA at high doses.

In the present study, data about calcium concentrations demonstrated that the enhanced response to TNS caused by CSA was present at low as well as normal extracellular calcium concentrations. Soltis and Field recently demonstrated that the enhanced contractile response to potassium chloride and NE in the femoral artery of the deoxycorticosterone acetate (DOCA)-salt hypertensive rat was abrogated in a low calcium concentration bath. They interpreted these results to suggest that DOCA-salt increased sensitivity to extracellular calcium. No such effect was evident in our study.

The molecular mechanisms of CSA are still being elucidated. In lymphoid cells, where it is presumed to have its immunological effects, CSA binds to a specific intracellular protein. Within the cell, CSA has been reported to bind to calmodulin and to inhibit calmodulin function. Cyclosporin A is also reported to inhibit the early activation of membrane phospholipid metabolism in rabbit lymphocytes. In rat hepatocytes, however, CSA has been found to increase plasma membrane permeability to calcium and to expand the

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**Table 2. Effect of Cyclosporin A on Exogenous Norepinephrine-Induced Contractile Responses in Rat Tail Artery Segments**

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED₅₀ (× 10⁻⁶ M NE)†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>1.9±0.3</td>
<td>2.8±0.3</td>
<td>0.9±0.2</td>
</tr>
<tr>
<td>CSA</td>
<td>1.6±0.2</td>
<td>2.1±0.4</td>
<td>0.4±0.2 (NS)</td>
</tr>
<tr>
<td>Slope (%/ln NE dose)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>22.2±1.1</td>
<td>20.5±0.7</td>
<td>−1.7±1.0</td>
</tr>
<tr>
<td>CSA</td>
<td>21.7±0.9</td>
<td>19.8±0.9</td>
<td>−1.9±0.7 (NS)</td>
</tr>
<tr>
<td>Maximum response (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>8.2±0.4</td>
<td>7.8±0.4</td>
<td>−0.5±0.2</td>
</tr>
<tr>
<td>CSA</td>
<td>8.7±0.6</td>
<td>8.5±0.6</td>
<td>−0.3±0.2 (NS)</td>
</tr>
</tbody>
</table>

*CSA, 6 × 10⁻⁶ M. †Exogenous norepinephrine.
intracellular calcium pool.\textsuperscript{12} Because calcium is critical to neurotransmitter release,\textsuperscript{13} such a mechanism at the sympathetic nerve synapse could be functionally important. It is worth noting that another small peptide, angiotensin II, has effects on intracellular calcium\textsuperscript{14} and is capable of enhancing nerve stimulation responses in the rat tail artery.\textsuperscript{15}

References

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