Mechanism of Cyclosporine-Induced Sympathetic Activation and Acute Hypertension in Rats

Teresa Lyson, David M. McMullan, LeAnn D. Ermel, Barbara J. Morgan, Ronald G. Victor

Abstract

Although intravenous cyclosporine A (CsA) previously has been shown to cause a robust sympathetically mediated increase in blood pressure in the rat, the underlying mechanism by which CsA increases the activity of the sympathetic nervous system is unknown. To determine the relative contributions of central neural versus peripheral reflex mechanisms in causing this sympathetic activation, we recorded efferent renal sympathetic nerve activity and blood pressure during intracerebroventricular or intravenous infusion of CsA, the latter performed in intact rats and in those with sinoaortic denervation, cervical or subdiaphragmatic vagotomy, or dorsal rhizotomy (T10 through L1). In intact rats, intravenous CsA (5 mg/kg), as expected, tripled renal sympathetic nerve activity and increased mean arterial pressure by 27±4 mm Hg (P<.05). The new findings are that this sympathoexcitatory effect of intravenous CsA (1) was not duplicated by central administration (either into the cerebroventricular system or directly onto the ventrolateral surface of the medulla), (2) was unaffected by sinoaortic denervation, but (3) was greatly attenuated by either cervical or subdiaphragmatic vagotomy or by dorsal rhizotomy. In additional experiments, we found that intravenous cyclosporine increased the multiunit activity of subdiaphragmatic but not cardiopulmonary vagal afferents. From these data, we conclude that in the rat CsA-induced increases in sympathetic activity and blood pressure are caused mainly by activation of excitatory neural reflexes arising in the subdiaphragmatic region. These reflex mechanisms use at least two different afferent neural pathways: one involving the subdiaphragmatic vago-sympathetic reflexes and the other involving the low thoracic dorsal spinal roots. (Hypertension. 1994;23:667-675.)

Key Words • cyclosporine • sympathetic nervous system • hypertension, acute • afferent pathways

The immunosuppressive drug cyclosporine A (CsA) has markedly improved both long-term survival after organ transplantation and the treatment of autoimmune diseases.1-6 However, CsA also has emerged as a new cause of hypertension.7-13 Two syndromes have been described: acute hypertension with the initiation of a high dose of CsA or with CsA overdose7-8 and chronic hypertension with maintenance immunosuppression.9-13 Although the causes of the hypertension are undoubtedly multifactorial,14-18 there is evidence to suggest that sympathetic overactivity is involved.19-25

In some patients with chronic CsA-induced hypertension, baseline levels of muscle sympathetic nerve activity were found to be elevated.21,26 In anesthetized rats, acute administration of CsA was found to evoke robust increases in renal and lumbar sympathetic nerve activity and in blood pressure,19,22,23,25 but the underlying mechanism causing this sympathetic excitation is unknown.

Accordingly, we recorded renal sympathetic nerve activity and blood pressure in anesthetized rats to determine the relative contributions of central neural versus peripheral reflex mechanisms in causing the increased sympathetic activity during acute administration of CsA. Because CsA is known to cross the blood-brain barrier and cause central nervous system toxicity,27,33 one possibility is that CsA might directly activate central sympathetic neurons. We therefore asked if the sympathoexcitatory effect caused by intravenous CsA is duplicated by central administration.

An alternative possibility is that CsA might reflexively increase efferent sympathetic activity by acting on afferent nerve endings located in the peripheral circulation. CsA could suppress inhibitory baroreceptor reflexes or it could stimulate excitatory neural reflexes, such as those arising in abdominal visceral (eg, renal) afferents.19 We therefore asked if the sympathoexcitatory response to intravenous CsA is attenuated by surgical interruption of either sinoaortic, cardiopulmonary, or abdominal visceral afferents.

Methods

General Methods

CsA (Sandimmune) was provided by Sandoz Corporation. Experiments were performed on female Sprague-Dawley rats weighing 220 to 300 g. Anesthesia was induced with ketamine HCl (80 mg/kg IM) and was maintained with α-chloralose (60 mg/kg IV followed by supplemental doses of 10 mg/kg). The trachea was cannulated, and the animal was artificially ventilated. A femoral vein was cannulated for infusion of drugs. Arterial pressure was measured via a femoral artery catheter. Renal sympathetic nerve activity was recorded from a branch of the intact left renal nerve affixed to a bipolar platinum electrodes according to the technique of Schad and Seller.14 Nerve action potentials were detected by a high-impedance probe (model 511, Grass Instruments), amplifi-
of 20,000-fold (Grass P511 amplifier), filtered (bandwidth, 100 to 2000 Hz), and counted using a window discriminator, as previously described.

Data were recorded continuously on a Gould RS 3600 physiological recorder and stored on FM tape. Because CsA increases afferent as well as efferent renal nerve activity, the component of CsA-induced increase in renal nerve activity that was sensitive to ganglionic blockade. At the end of each protocol, the ganglionic blocking agent chlorisondamine (5 mg/kg IV) was administered 1 hour after infusion of CsA. In these whole nerve recordings, the overwhelming majority of the increase in electric activity counted in the window discriminator after CsA was fast-ganglionic efferent sympathetic activity, not afferent nerve activity, because all but a tiny fraction (9% to 5%) of the activity was eliminated by ganglionic blockade, which is consistent with previous reports from this laboratory.

**Sinoaortic Denervation**

Sinoaortic denervation (SAD) was performed according to the method of Krieger by cutting the aortic depressor nerves at the junction of the superior laryngeal nerves and by stripping the arterial walls in the carotid sinus region and painting them with 10% phenol. The cervical sympathetic chains and superior laryngeal nerves were also cut. The completeness of SAD was confirmed using the following criteria: in rats with SAD (1) the dose of phenylephrine required to raise mean arterial pressure by approximately 45 mm Hg was one fourth that required to cause a comparable increase in arterial pressure in intact rats and (2) phenylephrine-induced increases in arterial pressure (44 ± 5 mm Hg) had no effect on sympathetic activity (A = 12 ± 12%) and caused a small increase, not decrease, in heart rate (+ 14 ± 5 beats per minute), whereas in intact rats comparable increases in arterial pressure that were sensitive to ganglionic blockade. At the end of each protocol, the ganglionic blocking agent chlorisondamine (5 mg/kg IV) was infused into the cerebroventricular system by placing the tip of a 10-µL syringe (Hamilton Co) through a hole in the cranium into the left lateral cerebral ventricle (coordinates, 1.4 mm lateral to the midline at the level of bregma − 0.8 mm caudally and 3.4 mm vertically). CsA was infused over 2 minutes, and data were collected for the next 30 minutes, after which we performed intravenous infusion of the same dose of CsA (0.8 mg/kg) in the same rats. In 3 additional rats the same experiments were performed but with the CsA dose decreased from 0.8 to 0.05 mg/kg body weight dissolved in 4 µL of vehicle) was infused into the cerebroventricular system by placing the tip of a 10-µL syringe (Hamilton Co) through a hole in the cranium into the left lateral cerebral ventricle (coordinates, 1.4 mm lateral to the midline at the level of bregma − 0.8 mm caudally and 3.4 mm vertically). CsA was infused over 2 minutes, and data were collected for the next 30 minutes, after which we performed intravenous infusion of the same dose of CsA (0.8 mg/kg) in the same rats. In 3 additional rats the same experiments were performed but with the CsA dose decreased from 0.8 to 0.05 mg/kg body weight; the latter dose was calculated to approximate 5 mg/kg brain tissue. In 6 more rats an equal volume of vehicle alone was infused to exclude nonspecific effects. In 6 rats CsA (0.8 mg/kg) was infused over 2 minutes onto the ventrolateral surface of the medulla through a PE-10 catheter placed through the cisterna magna via an incision in the atlanto-occipital membrane. The same experiments were performed using vehicle in 4 more rats.

After intracerebroventricular infusion of CsA or vehicle, in each experiment we documented Evans blue dye staining of the entire ventricular system postmortem; after infusion of CsA or vehicle through the cisterna magna infusion, we documented blue dye staining of the ventrolateral surface of the medulla. In addition, we documented drug access to excitatory cardiovascular centers by increases in sympathetic activity and blood pressure with intracerebroventricular or intracisternal magna but not intravenous infusion of capsaicin (5 to 10 µg/kg), which is known to stimulate excitatory neural circuits in the medulla.

Protocol 2: Comparative Effects of Sinoaortic Denervation, Cervical or Subdiaphragmatic Vagotomy, or Dorsal Rhizotomy on Increases in Renal Sympathetic Activity and Blood Pressure Produced by Intravenous Infusion of Cyclosporine A

The aim of this protocol was to examine the relative contributions of sinoaortic baroreceptors, cardiopulmonary vagal afferents, and renal and subdiaphragmatic visceral afferents in mediating the sympathoexcitatory effect of intravenous CsA.
We recorded renal sympathetic activity and blood pressure during and for 60 minutes immediately after intravenous infusion of CsA (5 mg/kg infused over 20 minutes) in 10 rats with intact afferent nerves, 5 with SAD, 8 with cervical vagotomy, 6 with subdiaphragmatic vagotomy, 4 with dorsal rhizotomy, and 4 with sham rhizotomy. After performing SAD, vagotomy, or dorsal rhizotomy, we waited for 60 minutes to achieve stable baseline levels of sympathetic activity and blood pressure before beginning the experimental protocols. Although cervical and subdiaphragmatic vagotomies frequently were accompanied by transient changes in sympathetic activity and blood pressure, within 60 minutes sympathetic nerve activity and mean arterial pressure were indistinguishable from the initial baseline values: cervical vagotomy, change in sympathetic nerve activity, +26±28% and change in mean arterial pressure, 0±2 mm Hg; subdiaphragmatic vagotomy, change in sympathetic nerve activity, -1±4% and change in mean arterial pressure, -2±2 mm Hg.

Protocol 3: Effects of Muscarinic Blockade on the Sympathetic Nerve and Blood Pressure Responses to Intravenous Cyclosporine A (5 mg/kg)

To determine if vagotomy attenuates CsA-induced increases in renal sympathetic activity and blood pressure by interrupting efferent as well as afferent vagal activity, in 4 rats we infused atropine sulfate (0.25 mg/kg IV) before intravenous infusion of CsA (5 mg/kg).

Protocol 4: Effects of Intravenous Cyclosporine A (5 mg/kg) on Subdiaphragmatic and Cardiopulmonary Vagal Afferent Nerve Activity

In 10 rats we performed multiunit recordings of subdiaphragmatic (n=4) or cardiopulmonary (n=6) vagal afferent activity during and for 10 minutes immediately after bolus intravenous infusion of CsA (5 mg/kg dissolved in 0.2 mL of saline). In 3 additional rats we performed multiunit recordings of subdiaphragmatic vagal afferent activity during and immediately after bolus intravenous infusion of vehicle. In this protocol the CsA was infused rapidly and the overall observation period compressed from 80 minutes in protocol 2 to 10 minutes to account for the shorter duration of stable record-
tricular and intracisternal magna infusions, respectively \((P<.05)\). Whereas sympathetic activity and blood pressure remained elevated for 10 to 20 minutes after central administration of capsaicin, cyclic increases and decreases in sympathetic activity and blood pressure of much smaller magnitude were seen with intravenous administration of capsaicin.

The effects of SAD, cervical and subdiaphragmatic vagotomy, and dorsal (and sham) rhizotomy on the sympathetic nerve and blood pressure responses to intravenous CsA \((5 \text{ mg/kg})\) are shown in Table 3 and Figs 3 and 4. We previously have shown that intravenous infusion of the same volume of vehicle \((1.5 \text{ mL, infused over 20 minutes})\) causes only a transient increase in blood pressure and does not affect renal sympathetic activity.\(^{22}\) In intact rats this dose of CsA, as expected, tripled renal sympathetic activity and increased mean arterial pressure by 27±4 mm Hg. In rats with SAD, CsA caused increases in sympathetic activity and blood pressure that were indistinguishable from those in intact rats. In contrast, in rats with either cervical or subdiaphragmatic vagotomy and in those with dorsal rhizotomy the CsA-induced increases in sympathetic activity and blood pressure were greatly attenuated \((P<.05)\). In rats with sham rhizotomy the CsA-induced increases in sympathetic activity were indistinguishable from those in intact rats, but the increases in blood pressure were smaller \((P<.05 \text{ sham rhizotomy versus intact rats})\); however, the increases in blood pressure after CsA were approximately two times larger in rats with sham rhizotomy versus dorsal rhizotomy \((P<.05)\). Both the initial (first 15 minutes) and the late component (last 45 minutes) of the renal sympathetic nerve response to CsA were significantly attenuated in rats with either vagotomy or dorsal rhizotomy. Although there was a tendency for vagotomy to have greater effect on the late component and rhizotomy to have a greater effect on the early component, this tendency did not reach statistical significance (no group x time interaction and no group differences in the sympathetic responses to CsA at any point).

Muscarinic blockade with atropine had no effect on the increases in renal sympathetic activity \((273±108\% \text{ versus } 225±32\%, P>.1 \text{ with versus without atropine})\) and blood pressure \((23±9 \text{ mm Hg versus } 27±4 \text{ mm Hg, } P>.1 \text{ with versus without atropine})\).

The effects of rapid intravenous infusion of CsA on subdiaphragmatic and cardiopulmonary vagal afferent activity are shown in Figs 5 and 6. Intravenous CsA \((5 \text{ mg/kg})\) rapidly increased subdiaphragmatic vagal afferent activity by 54±11\% \((P<.05)\) but had no effect on cardiopulmonary vagal activity \((\Delta = -7±4\%, P>.1)\); CsA increased blood pressure comparably in both sets of experiments \((\Delta = 21±6 \text{ mm Hg versus } \Delta = 16±2 \text{ mm Hg, } P>.1 \text{ subdiaphragmatic versus cardiopulmonary})\). In contrast to CsA, capsaicin caused an explosive, transient increase in the activity of both groups of vagal afferents. Vehicle had no effects on the discharge of vagal subdiaphragmatic afferents \((\Delta = 105±1\%, P>.1)\).

**Discussion**

Intravenous CsA previously has been shown to cause robust increases in efferent sympathetic activity and thereby acute hypertension in the rat,\(^{19,22,23,25}\) but the underlying mechanisms by which CsA increases the activity of the sympathetic nervous system have not been determined. Although CsA previously has been shown to markedly stimulate renal, genitofemoral, and hypogastric afferent nerves,\(^{19}\) the importance of such
TABLE 3. Effects of Sinoaortic Denervation, Cervical and Subdiaphragmatic Vagotomy, and Dorsal and Sham Rhizotomy on Sympathetic Nerve and Blood Pressure Responses to Cyclosporine A (5 mg/kg IV)

<table>
<thead>
<tr>
<th></th>
<th>Renal Sympathetic Nerve Activity, %</th>
<th>Mean Arterial Pressure, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 10</td>
<td>Baseline</td>
</tr>
<tr>
<td>Intact</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>SAD</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>Cerv VGX</td>
<td>8</td>
<td>100</td>
</tr>
<tr>
<td>SubD VGX</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>Dorsal RZ</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>Sham RZ</td>
<td>4</td>
<td>100</td>
</tr>
</tbody>
</table>

SAD indicates sinoaortic denervation; Cerv VGX, cervical vagotomy; SubD VGX, subdiaphragmatic vagotomy; and RZ, rhizotomy. Data are mean±SEM.

*P<.05 vs baseline value.
†P<.05 vs responses in intact rats.
‡P<.05 responses in rats with dorsal RZ vs sham RZ.

afferent neural stimulation relative to other potential mechanisms, such as those involving baroreceptors or central neural activation, in causing sympathetically mediated hypertension is unknown. The principal new findings of this study are that the sympathoexcitatory effect of intravenous CsA is (1) not duplicated by central administration, (2) unaffected by sinoaortic denervation, but (3) greatly attenuated either by cervical or subdiaphragmatic vagotomy or by dorsal rhizotomy. We also found that intravenous CsA increases the discharge of subdiaphragmatic but not cardiopulmonary vagal afferents. From these data, we conclude that in the rat CsA-induced increases in renal sympathetic activity and blood pressure are caused mainly by activation of excitatory neural reflexes arising in the subdiaphragmatic region. These reflex mechanisms use at least two different afferent neural pathways: one involv-

![Fig 3. Continuous recordings of arterial pressure and renal sympathetic nerve activity (SNA), the latter displayed as a time frequency histogram, before, during, and for 60 minutes after intravenous infusion of cyclosporine A (CsA) (5 mg/kg) in a rat with intact afferent nerves (A), in one with cervical (Cerv) vagotomy (VGX) (B), and one with dorsal rhizotomy (RZ) (C). In the intact rat, arterial pressure and renal SNA increased during the period of CsA infusion and remained elevated or even continued to increase further for 60 minutes after infusion. However, in the rats with cervical VGX or with dorsal RZ, the increases in renal SNA were greatly attenuated, and the initial increases in arterial pressure (due in part to simple volume expansion) were not well maintained.](http://hyper.ahajournals.org/content/6/2/671/F3)

Downloaded from http://hyper.ahajournals.org/ by guest on August 29, 2017
We determined empirically that 0.8 mg/kg is the smallest dose of CsA that when administered intravenously causes a reproducible increase in renal sympathetic activity. Thus, the failure of this dose of CsA to increase renal sympathetic activity when administered either into the lateral cerebral ventricles or directly onto the ventrolateral surface of the medulla argues against a central mechanism of action. With both routes of administration, drug access to the medullary cardiovascular centers was documented by eliciting large increases in sympathetic activity and blood pressure when CsA was replaced with capsaicin, which is well known to stimulate excitatory neural circuits in the medulla. We considered the possibility that a metabolite of CsA, which would not be produced during central administration, may be more important than the parent molecule in altering sympathetic outflow. This possibility is unlikely because CsA is metabolized mainly by hepatic cytochrome P-450 hydroxylation producing metabolites that are much more hydrophilic than CsA and therefore do not cross the blood-brain barrier. In addition, intracerebroventricular administration of the parent CsA molecule did exert a statistically significant effect on sympathetic outflow, but one that is directionally opposite that produced by systemic administration.

Because central administration of 0.8 mg CsA per kilogram body weight would represent a very large dose per kilogram brain tissue, the unexpected decrease in sympathetic activity with intracerebroventricular CsA might be a neurotoxic and not a pharmacological effect. This possibility is unlikely because sympathetic activity still decreased when this dose was reduced to 0.05 mg/kg body weight. We therefore suggest that during systemic administration of CsA the observed increase in sympathetic activity is likely to be the net result of a small central inhibitory and a larger peripheral excitatory action of CsA. Because CsA causes sympathetic activity to increase (not decrease) at a time when blood pressure also is increased, we considered the possibility that CsA might impair the sinoaortic baroreflex. CsA, like many other chemical and humoral agents, might...
alter the discharge properties of the baroreceptors or it might cause a central resetting of the baroreflex. However, the observed CsA-induced sympathoexcitation clearly is not critically dependent on intact sinoaortic baroreceptors because this excitation was well preserved after SAD. Furthermore, CsA did not cause a chemical sinoaortic deafferentation because, despite CsA treatment, a brisk baroreflex still could be readily elicited: profound sympathoinhibition during phenylephrine-induced increases in blood pressure. Although we have excluded a primary role for the sinoaortic baroreceptors in this setting, we cannot from these data exclude the possibility that CsA may cause a shift in the set point or gain of the baroreflex. Indeed, this possibility is suggested by the unexpected finding that the CsA-induced increase in sympathetic activity was not augmented after SAD.

The salient feature of this study is the notion that CsA-induced sympathoexcitation is critically dependent on input from afferent nerve fibers traveling in both the vagus nerves and in the low thoracic dorsal spinal roots: surgical interruption of either of these afferent neural pathways alone greatly attenuated the increases in sympathetic activity and blood pressure caused by intravenous CsA. However, these observations do not permit definitive conclusions regarding the precise relative contributions of the vagal and spinal pathways or the possibility that the various afferent inputs traveling in these neural pathways may interact synergistically in producing the integrated reflex response. For example, there is a tendency in the data to suggest that the renal afferents may be of greater importance in the initiation of the sympathoexcitatory response to CsA and vagal afferents of greater importance in its maintenance; however, this tendency for such differential effects did not reach statistical significance.

In the rat, blood pressure decreases with electric stimulation of afferents in the cervical vagi but increases with electric stimulation of afferents in the subdiaphragmatic vagi. Thus, the attenuation of CsA-induced sympathetic activation by subdiaphragmatic as well as by cervical vagotomy suggests that this sympathetic effect is mediated in part by activation of an excitatory reflex involving subdiaphragmatic vagal afferents. This conclusion is strengthened by our additional observation that intravenous CsA increased the discharge of subdiaphragmatic but not of cardiopulmonary vagal afferents in the cervical vagi.

Taken together, these neurophysiological observations may provide a functional correlate for previous neuroanatomic observations in the rat by Gattone et al's suggesting that the majority of renal afferents project to the nodosal ganglion via the vagus nerves with a smaller number projecting to the dorsal root ganglia via the T11 through L2 spinal roots. Stimulation of renal afferents with spinal projections has been implicated in increasing renal sympathetic activity and blood pressure both by causing segmental renal reflexes and by increasing central sympathetic outflow from the brainstem. Previous work by Moss et al19 suggests that CsA causes a rather generalized activation of subdiaphragmatic visceral afferent nerves involving not only renal but also genitofemoral and hypogastric afferents. While there is considerable precedent for the notion that these latter groups of afferents project to the spinal cord, our data raise the possibility that when stimulated by CsA, these afferents may use vagal as well as spinal pathways to reflexively increase sympathetic activity and blood pressure. Because the existence of vagal afferent innervation of the kidney and other subdiaphragmatic viscera may be highly species dependent, the present data cannot be extrapolated to make general statements regarding the mechanism of CsA-induced hypertension in larger animals and humans.

Rodents also differ from many larger animals in that the subdiaphragmatic vagus nerves are associated with large numbers of paraganglia scattered throughout their course. Because these paraganglia contain glomus tissue, which is necessary for chemoreceptor signal transduction in the carotid body, it has been hypothesized that they also may function as chemosensitive receptors. We therefore speculate that the abdominal vagal paraganglia may contain CsA-sensitive receptors that could contribute to the vagally mediated reflex increase in effenter sympathetic activity.

Acknowledgments

This study was supported by funds from the National Heart, Lung, and Blood Institute to Dr Victor (RO-1 HL-44010) and to Dr Morgan (National Research Service award HL-08085) and by institutional resources from the University of Texas Southwestern Medical School Summer Research Fellowship Program for David M. McMullan, Dr Victor is an Established Investigator of the American Heart Association. The authors are indebted to Rich Cooley for research assistance; to Cynthia Lawson and Patricia Powell for secretarial assistance; to Drs Jere H. Mitchell and R. Sanders Williams for their continued support; to Gary Dohanich, PhD, for his scientific advice; and to Lori A. Smith from Dr Ulla Kopp's laboratory at the University of Iowa, College of Medicine for technical assistance in performing dorsal rhizotomy; and to Sandoe Corporation for providing Sandimmune. The authors would like to acknowledge the contribution of the late Dr Michael J. Brody, whose comments led us to perform the crucial experiments.

References


Mechanism of cyclosporine-induced sympathetic activation and acute hypertension in rats.

T Lyson, D M McMullan, L D Ermel, B J Morgan and R G Victor

Hypertension. 1994;23:667-675
doi: 10.1161/01.HYP.23.5.667

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/23/5/667