THE ENIGMA OF CYCLOSPORINE-INDUCED HYPERTENSION

UNIVERSITY OF TEXAS
SOUTHWESTERN MEDICAL CENTER AT DALLAS

INTERNAL MEDICINE GRAND ROUNDS

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TABLE OF CONTENTS

I. INTRODUCTION

In about the year 520 A.D., according to legend, the Saints Cosmas and Damian, one a physician and the other a surgeon, replaced the gangrenous white leg of an aged sacristan with the healthy dark leg of a recently deceased Ethiopian Moor (1,2). This celebrated operation, the first description of an orthotopic allograft, was to have taken place near the edge of the Roman Forum.

In 1981, 14 centuries later, Black and colleagues at the University of California at Irvine successfully replaced the white leg of a Lewis rat with the dark leg of a hybrid brown Norway rat (3).

Figure 1

Saints Cosmas and Damian Performing a Leg Allograft. (Fernando del Rincon, The Prado, Madrid)

Figure 2

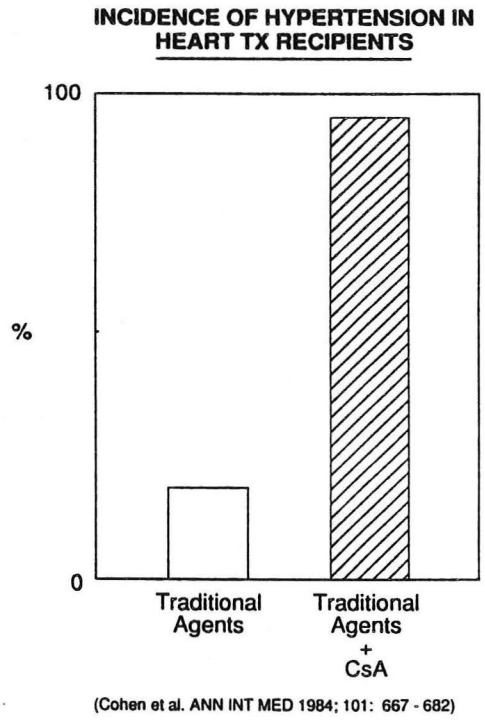
Lewis Rat with an Allografted Hindlimb from a Hybrid Brown Norway Rat. (Black et al., N Engl J Med 1981; 306: 368-9).

The first operation was performed posthumously (Cosmas and Damian died in 287 A.D.) and thus with divine intervention, the second with cyclosporine immunosuppression.

Cyclosporine A (CsA), a fungal metabolite, is a novel immunosuppressive agent with relatively specific action against helper T lymphocytes and remarkably little myelosuppression (4,5). This agent, when added to the traditional immunosuppressive regimen of prednisone and azathioprine, has greatly reduced the incidence of rejection and serious infection following organ transplantation, causing a striking improvement in long-term survival (6). As a result, CsA been a major impetus to transplantation surgery as evidenced by the exponential escalation in the performance of orthotopic cardiac transplantation in the past 6 years (7).

Although transplant recipients now are living longer with the use of CsA, the incidence of hypertension is increasing at an alarming rate (8,9). For example, the incidence of hypertension in heart transplant recipients has increased from 20% with traditional immunosuppressive agents alone to now >90% with the addition of CsA (6,10,11). This hypertension typically is moderate-to-severe and its treatment often requires the use of multiple antihypertensive agents. Thus, hypertension has emerged as one of the most important problems in the medical management of heart transplant recipients. Although there is no question that CsA is an important cause of hypertension not only in heart transplant recipients but also in many other groups of transplant and non-transplant patients, the underlying mechanism of this iatrogenic hypertension has been an enigma.

Figure 3



The aim of this Grand Rounds is to review what is known and not known about CsA-induced hypertension, and in so doing, develop a conceptual framework for understanding the blood pressure-raising effects of this novel immunosuppressive agent. An understanding of the pathogenesis of CsA-induced hypertension should indicate rational approaches to its treatment. In addition, CsA has provided a new experimental model of hypertension to investigate fundamental mechanisms of blood pressure regulation.

Particular emphasis will be placed on heart transplant recipients because these individuals appear to be especially sensitive to the hypertensive effect of CsA (6,8-11). Three major concepts will be presented.

The hypertensive effect of cyclosporine:

1) Is NOT caused either by nephrotoxicity or by activation of the renin/angiotensin system.

2) Is accompanied by excessive activation of the sympathetic nervous system. Cyclosporine appears to potentiate neurogenic vasoconstriction both by stimulating central sympathetic outflow and by enhancing the effects of sympathetic nerve stimulation on vascular smooth muscle.

3) May be explained by increased calcium influx causing stimulation of the calcium messenger system in neural tissue and vascular smooth muscle. In contrast, the immunosuppressive action of cyclosporine may be related to inactivation of calmodulin in helper T lymphocytes.

II. PHARMACOLOGY OF CYCLOSPORINE A

A. Historical developments (5):

1970: Thiele and colleagues isolated two new strains of fungi imperfecti producing antifungal metabolites.

1972: Borel and colleagues at Sandoz Research Institute discovered the immunologic properties of these fungal metabolites in rats.

1973: Ruegger and colleagues purified cyclosporine A.

1978: Calne et al. and Powles et al. performed the first clinical trials of cyclosporine.

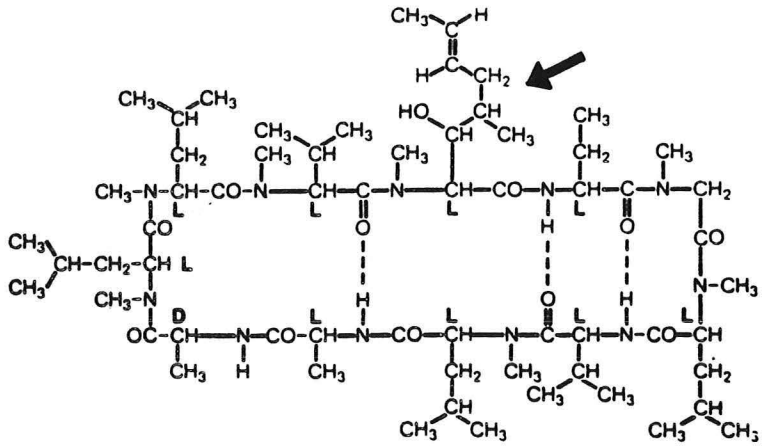
1980: Wenger et al. performed the first total synthesis of cyclosporine.

1983: Sandimmune first registered by Sandoz in Switzerland.

Cyclosporine A (CsA) is one of three polypeptides (Cyclosporine C and G are the other two) isolated from 2 species of soil fungi, Tolypocladium inflatum Gams (formerly Trichoderma polysporum) and Cylindrocarpon lucidum. As determined by x-ray crystallography, the basic structure consists of 11 amino acids

(C62H111O12, MW=1202), the first of which is unique and termed C 9-ene. The beta-hydroxyl group in the novel C 9-ene side chain forms a hydrogen-bond to the carbonyl oxygen atom on the same amino acid and extends outward. This distinctive portion of the molecule may be related to its biologic action (12).

Figure 4



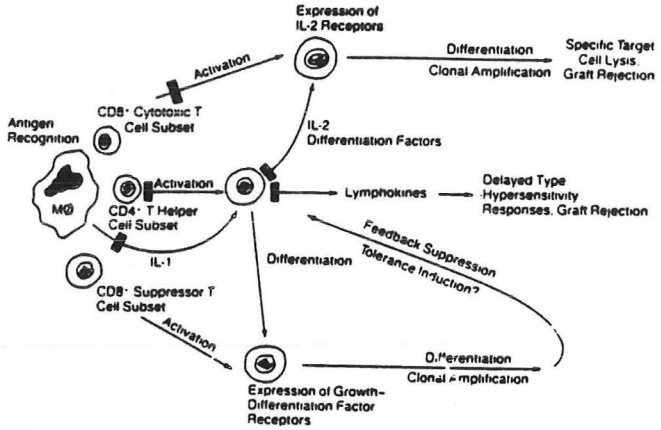
Chemical Structure of CsA

B. Postulated mechanism of immunosuppressive action

Based on studies of the mixed lymphocyte reaction, an in vitro model of an allograft response, a simplistic representation of the steps involved in the activation of cytotoxic T lymphocytes is as follows (13): 1) antigen recognition, 2) presentation of antigen by macrophages causing the production and release of interleuken 1 (IL-1), 3) activation of precursor cytotoxic lymphocytes with acquisition of the receptor for IL-2, 4) activation of T helper cells with the production and release of IL-2, 5) clonal amplification of activated cytotoxic T lymphocytes, and 6) activation of T suppressor cells that modulate these responses.

CsA inhibits proliferation of cytotoxic T lymphocytes mainly by inhibiting transcription of mRNA for IL-2 (6,13-15). In addition, CsA also appears to inhibit the ability of cytotoxic T-lymphocytes to respond to IL-2, presumably by inhibiting the induction of the IL-2 receptor (13).

Figure 5



Effect of CsA on the Immune Response to Allo-antigens in the Mixed Lymphocyte Reaction (Hess et al., Transpl Proc 1988; 20 (Supp 2): 29-40).

CsA stands in marked contrast to the traditional immunosuppressive agents (e.g., azathioprine) in several ways (4,6): CsA

1) has a much greater effect on T cells than on B cells;

2) selectively inhibits T helper cells without inhibiting T suppressor cells;

3) causes minimal bone marrow suppression; and

4) causes minimal reduction in peripheral blood cell counts.

A key concept, therefore, is that the relatively selective action of CsA to inhibit the activation of helper T cells and the lack of myelosuppression and cytotoxicity account for the observation that patients treated with CsA have much fewer opportunistic infections than patients treated with more traditional immunosuppressive regimens (6).

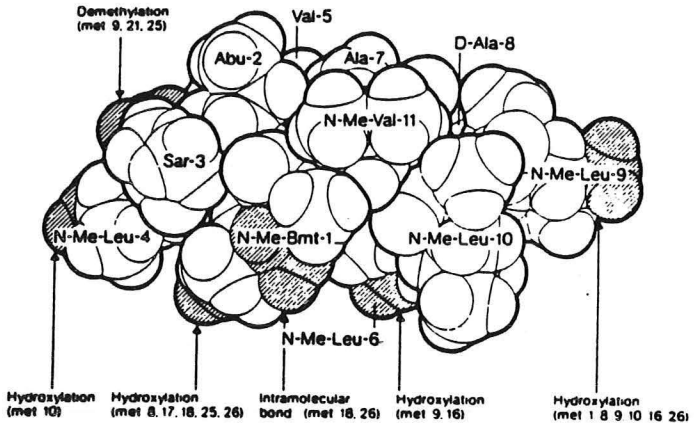
C. Pharmacokinetics and metabolism

CsA is highly lipophilic. The intravenous preparation is dissolved in olive oil and alcohol (cremaphor vehicle), the oral preparation in castor oil. The usual adult dose now is 2.5-10 mg/kg/day in divided doses. This dose range has been found to have a favorable toxic/therapeutic ratio compared with earlier usage of much higher doses that were associated with a much higher incidence of toxic side effects (16,17) (see below). With oral administration, absorption is highly variable. The current practice is to titrate the dosage in order to maintain trough blood levels between 100-200 ng/ml, as determined by HPLC (16).

Due to its lipophilic nature, CsA distributes in body tissues largely according to organ fat content with the extensive deposits in the kidneys and liver (15,18,19). In the circulation, CsA mainly is bound to blood cells. Once the concentration of CsA exceeds the saturation of the cells, the drug spills over into the plasma where it is bound to lipoproteins and serum albumin (20,21).

CsA is metabolized almost exclusively in the liver by cytochrome P 450 microsomal enzymes (22). Four of the 11 amino acids are hydroxylated (phase 1 reactions) to at least 15 compounds that are excreted in bile and urine (22). Several of these hydroxylated metabolites, in particular M-17 and M-1, possess significant immunologic activity.

Figure 6



Metabolism of CsA (Ryffel et al., Transpl Proc 1988; 20 (Supp 2): 575-584).

An understanding of CsA metabolism is important for several reasons:

1. Currently both HPLC and RIA kits are used to monitor CsA blood levels (16). In most clinical labs including our own, the HPLC assay measures only the parent compound whereas the common RIA method measures all CsA-related material: the composite of CsA plus all of its metabolites.

2. The biologic action and tissue distribution of CsA metabolites may be important determinants of immunosuppressive efficacy and organ toxicity, effects that traditionally have been attributed to the parent compound. Although <0.1 % of CsA itself is excreted unchanged in the urine, the M-17 and M-8 metabolites are extensively excreted by the kidney (22). These metabolites therefore are likely to be more important than the parent compound in causing the changes in renal function that accompany chronic CsA therapy.

3. The rate of CsA metabolism is critically dependent upon liver function (22) which becomes a key issue in the appropriate use of CsA in a) children (increased hepatic metabolism), b) liver transplant recipients, c) patients taking drugs that alter hepatic metabolism.

D. Drug interactions (23)

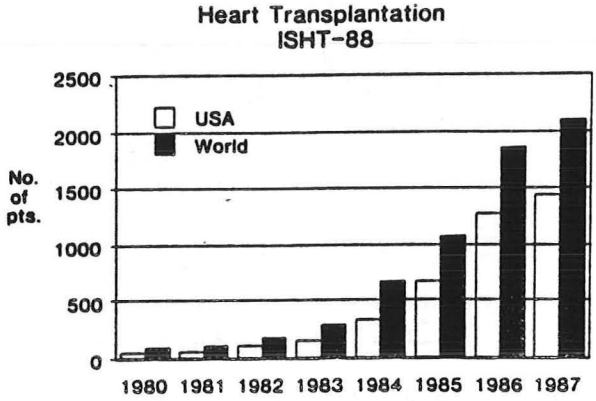
Drugs that inhibit P 450 enzymes increase CsA blood levels. These commonly include: 1) ketaconazole, a commonly used antifungal agent, 2) erythromycin, used to treat Legionella pneumophila and Mycoplasma pneumonia, 3) cimetidine, 4) most of the calcium channel blockers (used to treat hypertension), and 5) corticosteroids.

Drugs that induce P 450 enzymes decrease CsA blood levels. These commonly include: 1) phenytoin, 2) barbiturates, and 3) rifampin.

E. Clinical indications

The improved long-term survival of organ transplant recipients who are treated with cyclosporine has lead to an enormous growth of transplantation programs in North America. In the case of heart transplantation, for example, CsA has been held responsible for improving 5-year survival from 40 to 80% (7). As a result, the number of heart transplantations performed in this country alone has increased from 70 per year in 1980 to 1436 per year in 1987 (7).

Figure 7



Number of Heart Tx Performed Annually: 1980 to 1987 (Registry of Intl Soc Heart Tx, J Heart Transpl 1988; 7: 249-253).

In addition, CsA is rapidly becoming a useful adjunct in the management of an increasing number of autoimmune diseases. Thus, over the next decade, probably millions of patients will be receiving CsA. The major current clinical indications are shown below.

Table 1. Clinical Indications for CsA.

Transplant Operations	Autoimmune Diseases
Kidney (24,25)	Uveitis (32)
Heart (7,26)	Rheumatoid arthritis (33)
Heart-lung (27,28)	Psoriasis (34)
Liver (29)	Primary biliary cirrhosis (35)
Pancreas (30)	Myasthenia gravis (36)
Bone Marrow (31)	Diabetes mellitus (37,38)
	Celiac sprue (39)
	Crohn's disease (40)
	Nephrotic syndrome (41)

F. Side Effects

The side effects which have been encountered to date with CsA are as follows (6,15,42-46):

Table 2. Side Effects of CsA.

Side Effect	Incidence
HYPERTENSION	20-95%
"Nephrotoxicity" (serum Cr >2.0)	<30%
Hepatotoxicity (abnormal liver enzymes)	10%
Tremor	20%
Hirsutism	20%
Gingival hypertrophy	20%
Viral infections	25%
Anorexia	20%
Hyperuricemia	80%
and gout	10%
Paresthesia/hyperesthesia	35%
Hyperkalemia (K+ <5.5 mmoles/l)	95%
Hypomagnesemia (Mg >1.2 n/eq/l)	20%
Lymphoma	??

As will be discussed in detail below, the reported incidence of CsA-induced hypertension varies greatly depending upon the specific patient population under study. With the increasing emphasis on using lower doses of CsA and on careful attention to blood levels, the incidence and severity of renal dysfunction is decreasing. Reports of CsA treatment leading to dialysis are rare and mainly in the earlier series. Hepatotoxicity and electrolyte disturbances are mainly chemical abnormalities without as yet clear-cut clinical importance. Interestingly, hyperkalemia often is related to the development of hyporeninemic hypoaldosteronism (Type IV renal tubular acidosis) presumably due to effects of CsA on the renal tubule. Tremor and paresthesias are the most commonly discussed forms of neurotoxicity. In addition, seizures related to malignant hypertension have been reported in children. CsA-induced hyperuricemia, which was the topic of a recent article in the New England Journal of Medicine (46), leads to gout in <10% of patients. The principal infections seen in CsA-treated patients are caused by Herpes simplex and zoster viruses and cytomegalovirus. Patients treated with CsA also may be at increased risk of developing pneumocystis pneumonia. The degree to which suppression of T helper cells with CsA facilitates the development of lymphomas remains to be determined.

Importantly, the majority of these side effects are generally dose-related and are reversible upon discontinuation of CsA.

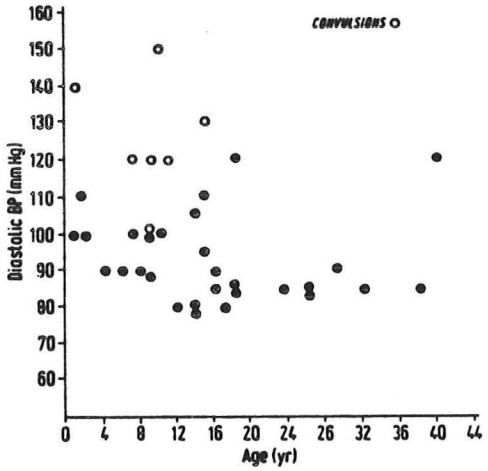
III. CLINICAL SPECTRUM OF CsA-INDUCED HYPERTENSION

A. Pediatric vs. adult patients

In general, children are thought to be more sensitive than adults to the hypertensive effects of CsA (47,48). This statement, however, must be tempered by the fact that in most series the CsA dose used to treat children is 5-to-10 fold greater than that used to treat adults. In children, high doses of CsA are needed to achieve "therapeutic" CsA levels in blood mainly because children have high rates of hepatic metabolism.

Joss et al. (47) studied 36 children and adolescents receiving CsA for bone marrow transplantation. Of note, 25 of 29 children under the age of 17 became hypertensive and 7 developed grand mal seizures. The authors found an inverse relationship between age and diastolic blood pressure; however, the dose of CsA was enormous by adult standards: 25-50 mg/kg/day.

Figure 8



Diastolic BP is Inversely Related to Age in Bone Marrow Tx Recipients Treated with CsA (Joss et al., Lancet 1982; I 906).

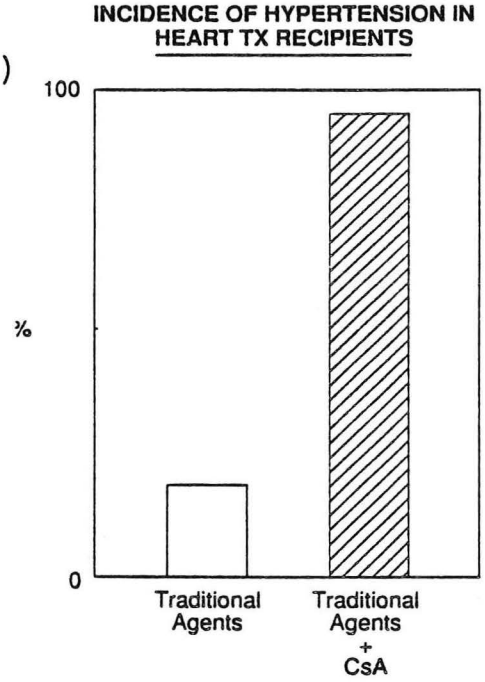
B. Heart transplant recipients vs. other groups of patients receiving CsA

A salient feature of CsA-induced hypertension is that the propensity for CsA to raise blood pressure is much greater in heart transplant recipients than in any other group of patients (6,8-11).

Prior to the introduction of CsA in 1983, the incidence of hypertension in heart transplant recipients being treated with azathioprine and prednisone was about 20% (6), an incidence that is no more than what one would see in a middle-aged population in general. The incorporation of CsA into the immunosuppressive regimen of these patients has caused a 5-fold increase in the incidence of post-transplant hypertension. Now virtually all patients become hypertensive after cardiac transplantation (6,8-11). In most cases, the hypertension is moderately severe, with diastolic pressures averaging 105-115 mm Hg, and often requires the use of multiple antihypertensive agents for management(6,8-11). This degree of hypertension, if untreated, almost certainly would seriously compromise longevity by accelerating coronary atherosclerosis and setting the stage for recurrence of congestive heart failure, which after all was the indication for the operation.

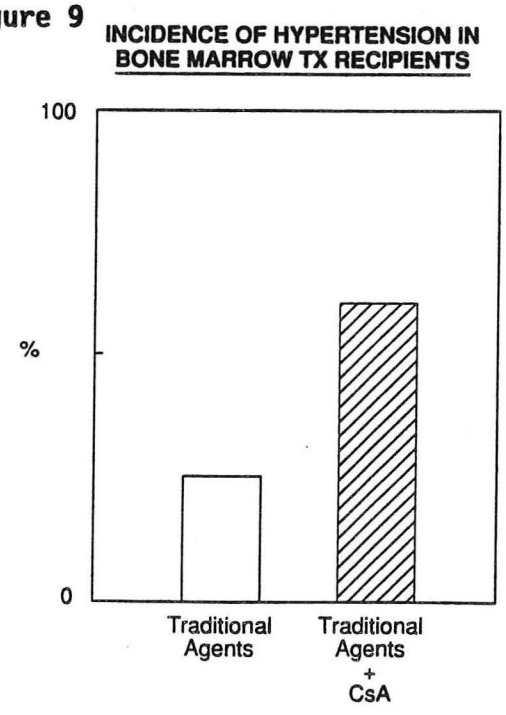
In all other groups of patients treated with CsA -- i.e., patients with kidney, liver, and bone marrow transplants and non-transplant patients with autoimmune diseases -- the incidence and severity of hypertension attributable to CsA clearly is much less than that observed in the heart transplant recipients (6,8,9,24,25,29,31,36,42). Dr. Richard Tindall and colleagues in our Neurology Department, for example, found that the incidence and severity of hypertension in patients with myasthenia gravis was about 20% higher in patients treated with prednisone and CsA than in those treated with prednisone alone (36).

Figure 3
(repeated)



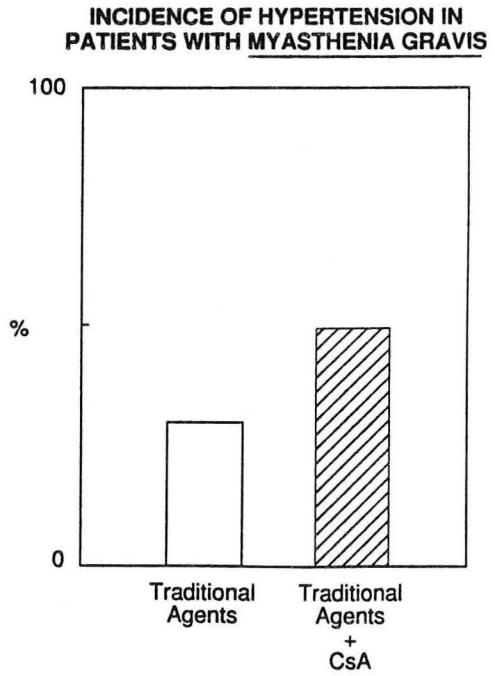
(Cohen et al. ANN INT MED 1984; 101: 667 - 682)

Figure 9



(Laughran et al. BR J HAEMATOL 1985; 59: 547 - 553)

Figure 10



(Tindall et al. N ENGL J MED 1987; 316: 719 - 24)

Any theory that proposes to explain the pathogenesis of CsA-induced hypertension needs to account for the differential blood pressure raising effects of CsA in these different groups of patients.

IV. POTENTIAL PHYSIOLOGIC MECHANISMS CAUSING CsA-INDUCED HYPERTENSION

Increased systemic vascular resistance, with inappropriately "normal" cardiac output, is the basic hemodynamic fault in almost every clinical and experimental form of chronic stable hypertension (49). Not surprisingly, this also is the case with CsA-induced hypertension. Studies in animals and humans consistently have shown that the blood pressure-raising effects of CsA are caused by increased vascular resistance with a normal, or slightly decreased, cardiac output (8-10,50,51). Although a myriad of factors are likely to contribute to sustained elevations in vascular resistance (i.e., the "mosaic theory"), two principal mechanisms seem to be responsible for increased vascular resistance in the majority of hypertensive states (49):

1) **Augmented neurohumoral drive to the peripheral vasculature;** and
2) **Defect(s) in vascular smooth muscle.**

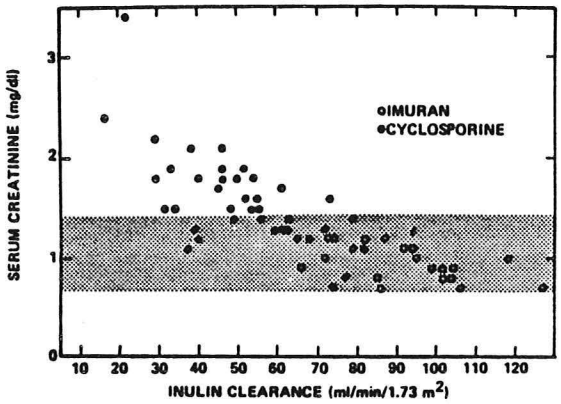
Within this context, the following mechanisms have been implicated in the pathogenesis of CsA-induced hypertension:

A. NEPHROTOXICITY

The initial suspicion was that CsA-induced hypertension is a renal form of high blood pressure caused by direct toxic effects of CsA on the kidney (6,10,52). Dr. Hal Helderman presented a scholarly review of CsA nephrotoxicity in his Grand Rounds on April 16, 1987. This topic will be reviewed here only to examine the role played by the kidney in the pathogenesis of CsA hypertension.

In evaluating the available clinical data, it is important to understand that measurement of either serum creatinine or creatinine clearance underestimates the actual reduction in glomerular filtration rate produced by CsA (53). As renal function declines, creatinine is progressively hypersecreted by the renal tubules thus elevating creatinine clearance. Direct comparison of serum creatinine with inulin clearance in the same patients (heart transplant recipients treated either with CsA or with azathioprine) indicates that GFR frequently can decrease by as much as 50% with little or no rise in serum creatinine. Nevertheless, a serum creatinine of <1.8 mg/dl generally means that the reduction in GFR is moderate rather than severe. Furthermore, this relationship is approximately linear when serum creatinine is between 1.5 to 2.0 mg/dl which is the range of values that most transplant physicians now aim to achieve.

Figure 11



Rise in Serum Cr Underestimates the True Fall in GFR in CsA-Treated Heart Tx Reciepients (Tomlanovich et al., Am J Kid Dis 1986; 8: 332-7).

With these considerations in mind, 3 principal syndromes of CsA nephrotoxicity have been described (52,54,55):

1) Asymptomatic, dose-dependent and reversible decreases in GFR probably occurs to some extent in most CsA-treated patients.

2) Progressive destruction of functioning nephrons leading to end-stage renal failure is very rare. The incidence was about 0.5% in earlier series with CsA doses that were >2 times higher than those used currently.

3) A syndrome resembling hemolytic-uremic syndrome or acute thrombotic thrombocytopenic purpura characterized by acute thrombotic occlusion of the renal microcirculation and multiple organ involvement seems to be an idiosyncratic reaction occurring very rarely.

Earlier anatomic studies have focused on the tubular epithelium as the primary site of CsA-induced renal injury (52,54,55). This concept, however, has been very difficult to establish for several reasons: a) the histologic lesions are rather non-specific; b) most, but not all (52), of the histologic studies in humans have been performed on biopsy specimens from renal transplant recipients in whom it is very difficult to distinguish CsA-toxicity from transplant rejection; and c) it has been extremely difficult, often impossible, to produce renal lesions by giving CsA to experimental animals (56):

> In rhesus monkeys, 13 weeks of CsA at 200 mg/kg/ day (2 orders of magnitude above the clinical dose) produced no nephrotoxic lesions. In most rat species, several weeks-to-months of CsA at 30-90 mg/kg/day produces highly variable degrees of vacuolization, necrosis, and regeneration of tubular cells (57); the immunosuppressive dose in the rat is about 5 mg/kg/day. In dogs, administra-tion of CsA for an entire year at a dose of 45 mg/kg/day produced no renal lesions.

These findings have prompted the conclusion that renal damage per se does not contribute importantly to the development of CsA-induce hypertension. This conclusion is strongly supported by a wealth of clinical data showing that in the vast majority of patients elevation in arterial blood pressure is dissociated from reduction in estimated GFR (8,9,11,42): 1) hypertension often occurs with minimal reductions in GFR; and 2) there is no significant difference

in GFR between patients who become hypertensive and those who remain normotensive during chronic administration of CsA.

Recent studies have provided increasing evidence that CsA "nephrotoxicity" is functional rather than anatomic and caused primarily by preferential constriction of the afferent renal arteriole (57,58). Numerous studies in both patients and rats have shown that CsA-induced reduction in GFR is universally accompanied by decreases in renal blood flow (51,59,60).

Thomson et al. (57) recently evaluated the effects of short-term administration of CsA (30 mg/kg/day for 8 days) on the determinants of single nephron GFR using micropuncture techniques in rats. In this experimental model, single nephron GFR is determined by:

1) **Glomerular Ultrafiltration Coefficient (LpA)**, an index of the intrinsic diffusion properties of the glomerular capillaries;

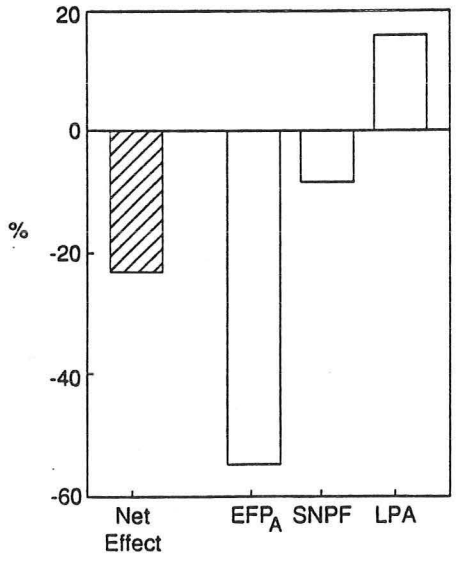
2) **Effective Filtration Pressure (EFP)**, which is determined by the transglomerular hydrostatic pressure gradient (the pressure in the glomerular capillaries minus the pressure in Bowman's space) minus plasma oncotic pressure);

3) **Single Nephron Plasma Flow (SNPF)**; and

4) **Plasma Protein Oncotic Pressure (π)**.

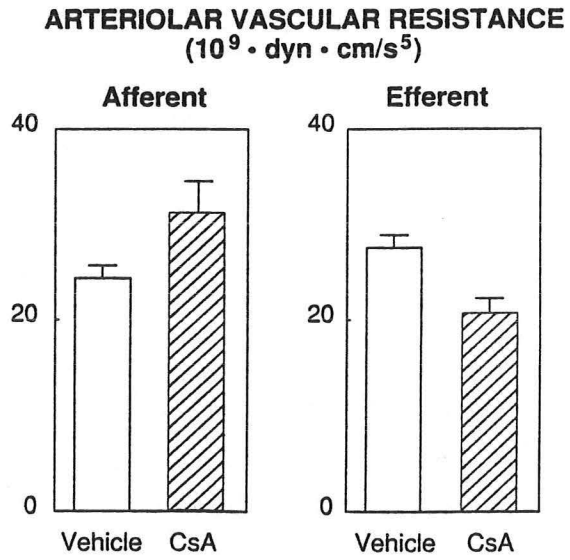
In this study, CsA caused a 30% reduction in single nephron GFR. This net effect was explained almost entirely on the basis of a decrease in effective filtration pressure in the afferent arteriole. Plasma flow and plasma protein concentration in the afferent arteriole did not change significantly. The micropuncture data indicated that the observed decrease in effective filtration pressure was caused mainly by constriction of the afferent arteriole without constriction of the efferent arteriole.

Figure 12



(Effects of CsA on Determinants of Single Nephron GFR (from Thomson et al. J CLIN INVEST 1989; 83: 960 - 9)

Figure 13



(From Thomson et al. J CLIN INVEST 1989; 83: 960-9)

While this is an important study, the results may or may not be applicable to the clinical setting because the rats were given toxic doses of CsA that caused them to lose weight and actually decrease, rather than increase, their blood pressures. This is a common problem in many of the animal studies.

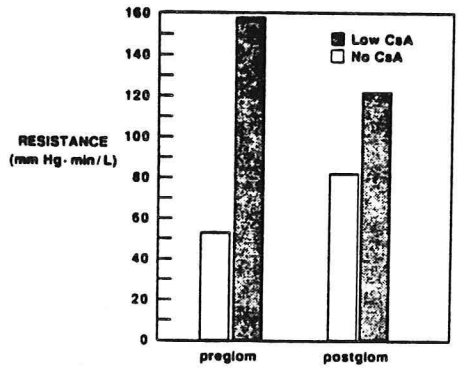
With a technical tour de force, Myers' group at Stanford attempted to provide this same kind of information in heart transplant recipients receiving therapeutic doses of CsA (5 mg/kg/day)(58). Catheters were placed in the femoral artery, the right atrium, the renal vein, and in the arcuate vein where the catheter was wedged to obtain an approximation of peritubular capillary pressure. Renal blood flow was measured by PAH clearance, GFR by inulin clearance. The glomerular transcapillary hydrostatic pressure difference was estimated from the sieving behavior of glomeruli toward uncharged dextrans of various sizes. Assuming that glomerular press = glomerular transcapillary pressure **minus** a proximal tubular pressure of approximately 20 mm Hg, then

$$\text{preglomerular resistance} = \frac{\text{arterial press - glom trans cap press}}{\text{renal blood flow}}$$

$$\text{postglomerular resistance} = \frac{\text{glom trans cap press - renal vein press}}{\text{renal blood flow - GFR}}$$

Compared with a group of heart transplant recipients who never received CsA, the CsA-treated patients showed a 41% reduction in GfR, a 43% reduction in renal blood flow, and an increase in mean arterial pressure of 16 mm Hg. The major new conclusions were two-fold. CsA treatment: 1) increased the fractional glomerular clearance of dextrans (34-52 angstroms) and thus decreased estimated glomerular transcapillary hydrostatic pressure difference; and 2) caused a disproportionate increase in calculated preglomerular over postglomerular vascular resistance.

Figure 14



CsA Preferentially Increases Preglomerular Resistance in Heart Tx Recipients (Myers Transpl Proc 1989; 21: 1430-2).

Although these calculations are based upon a number of assumptions, nevertheless the findings support the conclusions of the micropuncture studies in rats. The notion is that CsA rather selectively increases resistance in afferent arterioles which over time produces an occlusive afferent arteriolopathy and a form of "renovascular hypertension".

A key question, therefore, is what mechanisms cause the increases in renal vascular resistance, particularly preglomerular resistance, evoked by CsA? The answer to this question may provide important clues about the mechanism by which CsA raises blood pressure. The following mechanisms will be considered: a) renin/angiotensin system, b) prostaglandins, c) sympathetic nervous system, and d) end organ response of vascular smooth muscle.

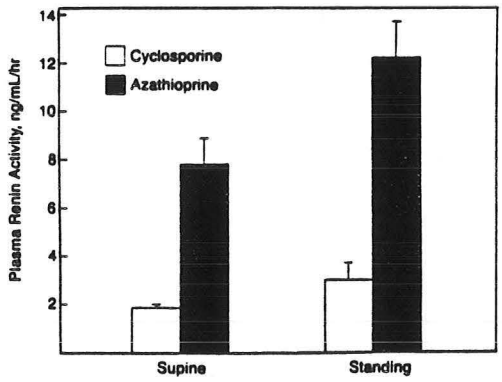
B. Renin/angiotensin system

Numerous investigators have considered the possibility that activation of the renin/angiotensin system may be the cause (as well as the consequence) of the increased renal vascular resistance and hypertension evoked by CsA. This possibility is very unlikely for several reasons:

1. Although plasma renin activity (PRA) clearly increases when CsA is given acutely to rats (61,62), several studies have demonstrated that this response is usually transient. With chronic administration of CsA, PRA often tends to return to, or even fall below, the control value, an effect that is usually explained by a gradual increase in plasma volume (51,63). Again, the interpretation of many of the rat studies is complicated by the use of high doses of CsA which made the rats sick and caused them to lose weight.

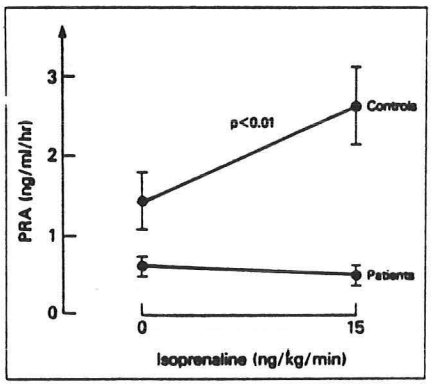
2. Chronic treatment with CsA in humans consistently has been accompanied by diminished PRA (64-67). In CsA-treated patients, PRA fails to increase appropriately either during orthostatic stress (64) or during beta-adrenergic stimulation (65).

Figure 15



CsA Attenuates Orthostatic Stimulation of PRA in Renal Tx Recipients (Bantle et al., Arch Int Med 1985; 145: 505-8).

Figure 16



CsA Abolishes Beta-Adrenergic Stimulation of PRA in Heart Tx Recipients (Held et al., Am J Cardiol 1989; 63: 1142-4).

3. Converting enzyme inhibition has little or NO effect either on the renal hemodynamic responses to chronic administration of CsA in rats (51) and or on blood pressure and PRA in CsA-treated heart transplant recipients (66).

Thus, chronic administration of CsA in humans appears to decrease, not increase the activity of the renin/angiotensin system.

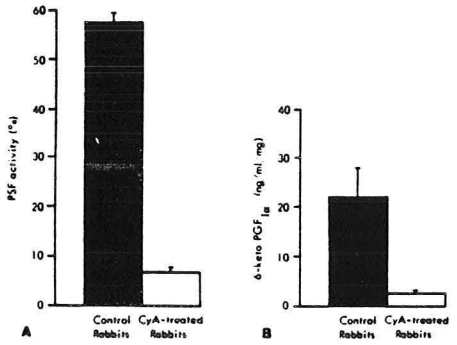
There also is experimental evidence to suggest that CsA a) inhibits the release of renin by juxtaglomerular cells (68), and b) impairs the ability of

angiotensin-II to stimulate the release of aldosterone (69). There is increasing evidence that angiotensin-II has important effects on intrarenal, as well as systemic, hemodynamics (70,71); in particular, angiotensin-II has been shown to cause comparable constriction of afferent and efferent arterioles (71). "Down-regulation" of the renin/angiotensin system, therefore, prompts the hypothesis that CsA-treated patients may lack effective means for maintaining the proper balance between pre- and post-glomerular resistances, a role generally subserved by intrarenal angiotensin-II (72,73).

C. Prostaglandins

In addition to angiotensin-II, prostaglandins also are thought to play an important role in the regulation of renal and systemic vascular resistances. Rabbits treated with CsA 50 mg/kg/day for 6 weeks) had a marked reduction in the plasma concentration of a prostacyclin-stimulating factor (72).

Figure 17



CsA Inhibits Production of Prostacyclin Stimulating Factor in Rabbits (Neild et al., Transpl Proc 1983; 15: 2398-2400).

Addition of CsA to cell cultures has been shown to:

1) inhibit the release of PGE-2 from rat aortic smooth muscle cells (73);

2) inhibit the production of PGE-2 in isolated rat glomeruli and papilla (74); and

3) suppress the production of prostacyclin by endothelial cells harvested from human umbilical vein (75).

There is also some evidence that CsA enhances platelet aggregability by increasing the production of thromboxane relative to prostacyclin (76).

Taken together, these findings have prompted the hypothesis that CsA causes an imbalance between vasoconstrictor and vasodilator prostaglandins. While this is an interesting hypothesis, there is at present no evidence that prostaglandins play an important role in the initiation and maintenance of elevated vascular resistance and arterial pressure during chronic administration of CsA to human patients. However, in conscious rats the cyclooxygenase inhibitor meclofenamate has been shown to potentiate the renal vasoconstrictor response induced by acute administration of CsA (10 mg/kg rapid i.v. infusion) (77). It is interesting, therefore, that prostaglandins normally are thought to modulate the stimulation of renin release and increases in renal vascular

resistance elicited by renal sympathetic nerve stimulation (78). One could hypothesize that the interplay between renin, prostaglandin, and sympathetic systems is important in understanding the effects of CsA on renal vascular resistance and arterial pressure.

D. Sympathetic nervous system

Animal Studies

Renal tissue has a rich sympathetic innervation that when stimulated elicits three distinct end organ responses that all serve to raise blood pressure (79):

1) renal vasoconstriction,

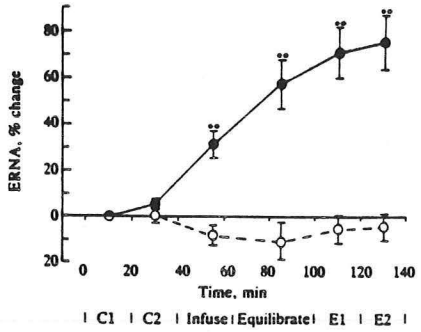
2) release of renin, and

3) retention of salt and water.

Neurogenic renal vasoconstriction is caused mainly by stimulation of alpha adrenergic receptors. Activation of sympathetic neural outflow to the kidney provides an attractive hypothesis to explain the effects of CsA on renal vascular resistance because:

a) When given acutely to anesthetized rats, CsA increases renal sympathetic nerve activity (80,81).

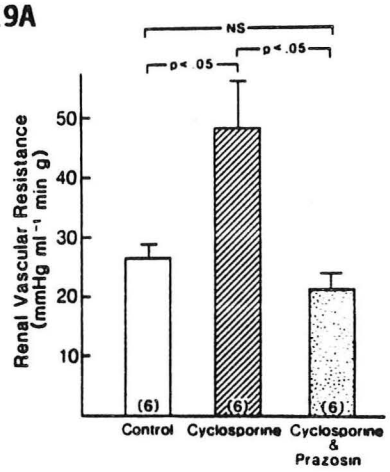
Figure 18



CsA Increases Renal Sympathetic Nerve Activity in Pentobarbital-Anesthetized Rats (Moss et al., Proc Natl Acad Sci 1985; 82: 8222-6).

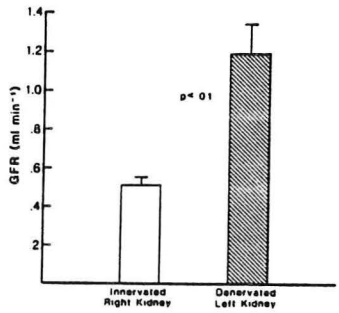
b) In conscious rats, the increases in renal vascular resistance and decreases in GFR evoked by acute administration of CsA were abolished either by administration of an alpha-adrenergic blocker or by denervating the kidney (51).

Figure 19A



Alpha-Adrenergic Blockade Greatly Attenuates Acute Effects of CsA on Renal Hemodynamic Effects in Conscious Rats (Murray et al., Kid Intl 1985; 28: 767-774).

Figure 19B



Renal Denervation Greatly Attenuates Acute Effects of CsA on Renal Hemodynamics in Conscious Rats (Murray et al., Kid Intl 1985; 28: 767-774).

c) Like administration of CsA, stimulation of renal sympathetic nerves characteristically evokes greater increases in pre- than in post-glomerular vascular resistance (79,82). In the micropuncture study by Thomson et al. (57), renal denervation significantly attenuated but did not completely normalize thee renal hemodynamic responses to CsA .

Neurogenic release of renin is caused mainly by stimulation of beta-adrenergic receptors. This effect probably accounts for the increases in PRA associated with acute administration of CsA to rats.

Retention of salt and water, the most potent effector response to renal nerve stimulation, is caused by a direct action of the sympathetic nerves on the renal tubules. Indeed, renal sympathetic nerve activity is one of the most important determinants of sodium and water retention by the distal tubule (79). For example, renal denervation is thought to be the explanation for the obligatory salt-losing nephropathy that occurs in patients with idiopathic autonomic failure (83). Activation of renal sympathetic nerves, therefore, provides a potential explanation for the progressive increases in plasma volume that are associated with the pathogenesis of CsA-induced hypertension in patients (67). Based on experimental data, this secondary expansion of plasma volume would be expected to override the primary effects of renal nerve stimulation on renin release.

Regarding the relationship of sympathetic activation to the development of CsA hypertension, the previous animal studies used barbiturate anesthesia which is known to markedly depress autonomic reflexes and blood pressure reactivity. An unanswered question, therefore, is whether the increases in sympathetic neural outflow induced by CsA are of sufficient magnitude and duration to produce sustained elevations in blood pressure.

To begin to address this question, Dr. Barbara Morgan, a postdoctoral fellow in my lab, has been studying effects of CsA (5 mg/kg i.v., infused over 20 min) on renal sympathetic nerve activity and blood pressure in rats anesthetized with alpha-chloralose, an anesthetic agent that causes much less depression of autonomic reflexes than does pentobarbital. The principal new findings are two-fold (81):

1) CsA consistently evokes large and sustained increases in renal sympathetic outflow and blood pressure in chloralose-anesthetized rats. In contrast to most neural responses which are measured in seconds or minutes, the renal sympathetic response to a single dose of CsA lasts for several hours.

Figure 20A

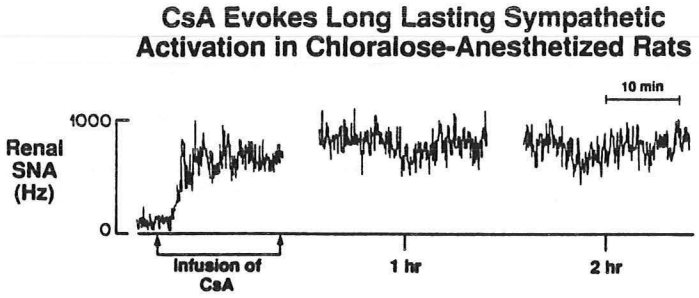
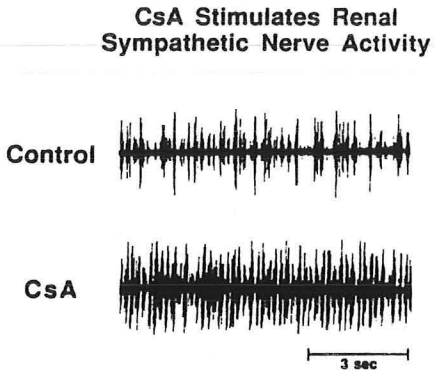
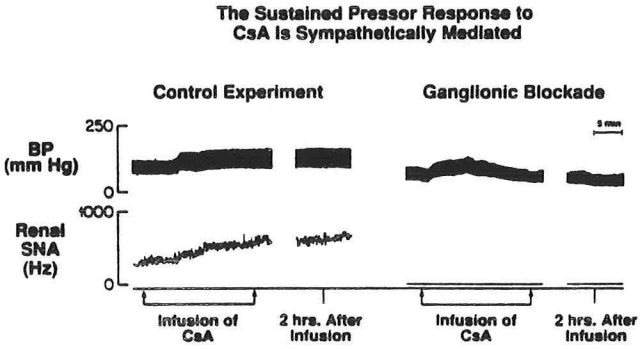


Figure 20B



2) In this model, the long lasting rise in blood pressure induced by CsA is caused by sympathetic activation because this CsA-induced pressor response is blocked by ganglionic blockade.

Figure 21



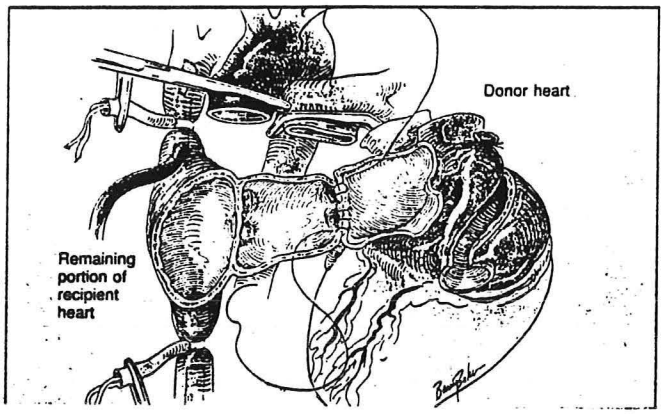
In the rat, the large increases in renal sympathetic activity induced by CsA are accompanied by parallel increases in lumbar and cardiac sympathetic outflow (81). Thus, CsA appears to evoke a diffuse activation of the sympathetic nervous system, at least when administered acutely to the chloralose-anesthetized rat.

Human Studies

Based on these findings in experimental animals, my colleagues and I set out to determine if sympathetic neural activation also plays an important role in the pathogenesis of CsA-induced hypertension in the clinical setting. We began by studying heart transplant recipients because of the extremely high incidence of CsA-induced hypertension in this patient population.

We hypothesized that heart transplant hypertension is particularly dependent upon sympathetic neural activation for 2 reasons: 1) CsA is a potent stimulus to sympathetic nerve activity in anesthetized animal preparations 80,81); and 2) The surgical procedure of orthotopic cardiac transplantation completely disconnects the transplanted heart from the nervous system and, in so doing, removes an inhibitory influence on sympathetic outflow to the peripheral circulation. The latter concept is explained as follows.

Figure 22

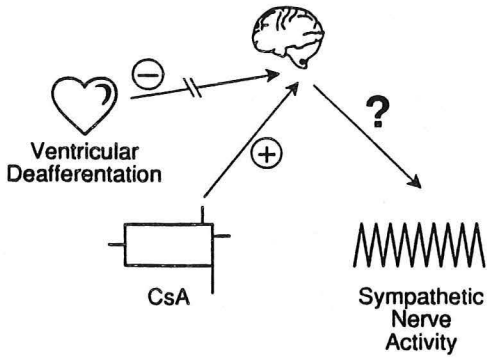


ORTHOTOPIC CARDIAC TRANSPLANTATION (YOUNG, BAYLOR CARDIOLOGY SERIES. 1989. VOL. 12:13)

In the procedure developed by Lower, Stofer, and Shumway (84), the surgeon removes the ventricles of the recipient heart and connects the donor ventricles and atria to the atrial remnant of the recipient heart. Cardiac transplantation not only interrupts the autonomic outflow to the donor heart but also interrupts the sensory, or afferent, neural connections from the donor heart to the central nervous system (85-88). Efferent sympathetic neural activity normally is tightly regulated by a many different inhibitory afferent inputs originating not only in the sinoaortic baroreceptors but also in pressure-sensitive endings that are located throughout the cardiopulmonary region but concentrated most heavily in the inferoposterior wall of the left ventricle. These ventricular baroreceptors are thought to cause the bradycardia and hypotension that accom-pany acute inferior wall myocardial infarction (89) but, more importantly, they also are thought to tonically restrain sympathetic outflow (89). Cardiac transplantation, therefore, theoretically might cause sympathetic excitation by removing ventricular baroreceptor restraint on sympathetic outflow.

Figure 23

HEART TX RECIPIENTS - CsA



Accordingly, we attempted to measure sympathetic neural activity directly in hypertensive and normotensive heart transplant recipients to address the following questions: 1) Is hypertension in heart transplant recipients accompanied by excessive activation of the sympathetic nervous system? and, if so, 2) Is such sympathetic activation caused primarily by CsA or by ventricular deafferentation or by a combination of these factors?

To address these questions we recorded postganglionic sympathetic action potentials with microelectrodes inserted into muscle nerve fascicles of the peroneal nerve in the leg and simultaneously measured plasma norepinephrine (HPLC), calf blood flow (plethysmography), donor and recipient heart rates, and blood pressures in 4 groups of patients: 1) 13 heart transplant recipients who were being treated with azathioprine and prednisone <u>plus</u> CsA (3.8 mg/kg/day); 2) 5 heart transplant recipients who had never been treated with CsA (i.e., with azathioprine and prednisone alone); 3) 5 patients with essential hypertension, and 4) 9 normotensive middle-aged control subjects. In order to obtain the requisite number of CsA and non-CsA treated patients, this truly has been a multicenter study involving patients transplanted by Dr. Steves Ring at U.T. Southwestern, Dr. Herbert Berkoff at the University of Wisconsin at Madison, and Dr. Ronald Lower and associates at the medical College of Virginia in Richmond. These experiments were performed in collaboration with Dr. Urs Scherrer, a visiting assistant professor in my laboratory from the University of Lausanne, Switzerland and the principal investigator on the human side of this project; Dr. Susanne Vissing, visiting research fellow in my laboratory from the University of Copenhagen; Drs. Barbara Morgan and Peter Hanson at the University of Wisconsin; and Dr. P.K. Mohanty at the Medical College of Virginia who arranged for use to study a rare handful of heart transplant recipients who had never received CsA. The characteristics of the patients and the experimental set-up are shown below.

Figure 24

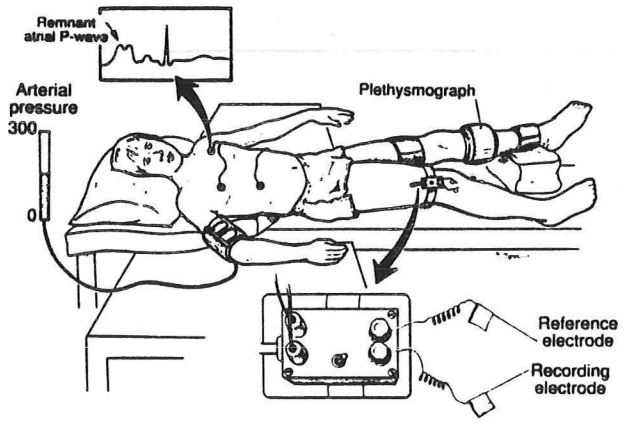


Table 3. <u>Patient Characteristics</u> (90).

	Heart Tx CsA	Heart Tx No CsA	Essential HTN	Normotensive Controls
n	13	5	5	9
Age (yrs)	47 ±3	46 ±4	49 ±2	45 ±3
Mean Arterial Pressure (mm Hg)	113 ±3	96 ±4	110 ±2	85 ±2

The principal new finding is that heart transplant recipients treated with CsA have resting levels of sympathetic traffic that are 3 times greater than normal(90). In the CsA-treated patients, the muscle sympathetic nerves fired 81±3 times/min vs. 28±4 times/min in the normotensive control subjects (mean±SE, $p<0.001$). In contrast, sympathetic activity was normal both in heart transplant recipients who were taking prednisone and azathioprine but not CsA (30±4 sympathetic bursts/min) and in patients with essential hypertension (29±7 bursts/min). Compared with the non-CsA group of heart transplant recipients, the CsA-treated patients had higher levels of plasma norepinephrine (265±29 vs. 207± 50 pg/ml, $p<0.05$), calf vascular resistance (63.9±1.2 vs. 35.1±4.2 resistance units, $p<0.05$), and mean arterial pressure (113± 3 vs. 96±4 mmHg, $p<0.05$).

Figure 25

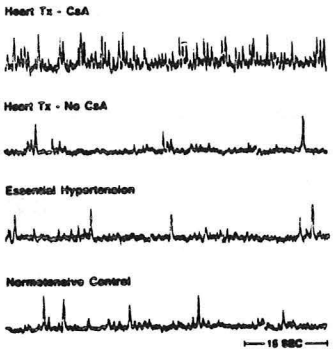
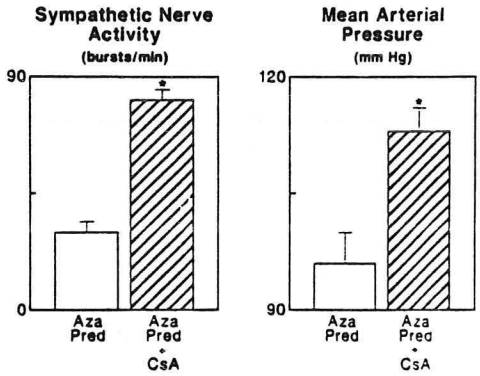


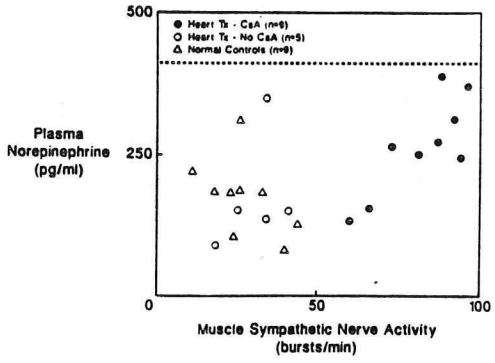
Figure 26



These observations suggest that 1) hypertension after heart transplantation is accompanied by marked activation of the sympathetic nervous system; and 2) this sympathetic effect is caused primarily by CsA.

This conclusion, at first glance, would appear to come into conflict with catecholamine studies showing that plasma and urinary norepinephrine levels, which are markedly elevated in heart failure, are rapidly normalized following cardiac transplantation (91). Although our CsA-treated patients had plasma norepinephrine levels that all fell within the accepted normal range of the assay, these levels were 50% higher than those either of the aged-matched normotensive control subjects or the normotensive heart transplant recipients who were not treated with CsA. The finding that a relatively small increase in plasma norepinephrine concentration is associated with a striking degree of sympathetic neural excitation demonstrates that involvement of the sympathetic system in hypertension, in particular in heart transplant hypertension, cannot be excluded on the basis of "normal" norepinephrine levels.

Figure 27

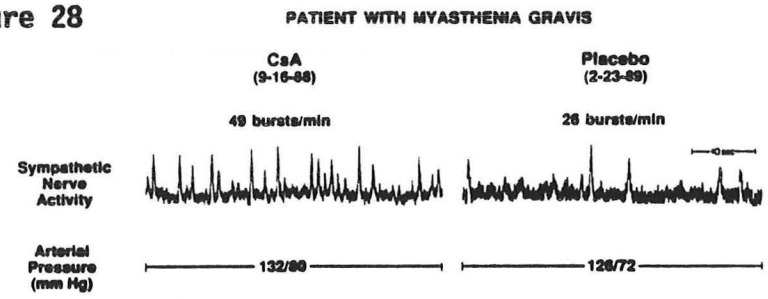


Relationship between muscle sympathetic nerve activity and plasma norepinephrine (90).

The transplanted human heart shows no evidence of efferent or afferent reinnervation for up to 10 years after orthotopic cardiac transplantation (84,87-89). Our findings, therefore, indicate that, without CsA, ventricular deafferentation alone is not sufficient to cause sympathetic activation in heart transplant recipients. The data, however, do not exclude the possibility that ventricular deafferentation might amplify the sympathoexcitatory effects of CsA. To examine this possibility, we turned our attention to individuals who are receiving CsA but who have normal, innervated hearts. In collaboration with Dr. Richard Tindall and Nancy Rollins, RN in our Neurology Department, we have recorded sympathetic nerve activity in 16 patients with myasthenia gravis who are enrolled in a randomized, cross-over trial of CsA (3.3±0.7 mg/kg/day) vs. placebo. This study is on-going and so far we have studied two patients who have crossed over between groups.

Compared with placebo, CsA treatment doubles the frequency of sympathetic nerve discharge (92). Muscle sympathetic nerves fired 46±3 times/min in the CsA group vs. 23±4 times/min in the placebo group ($p<0.01$).

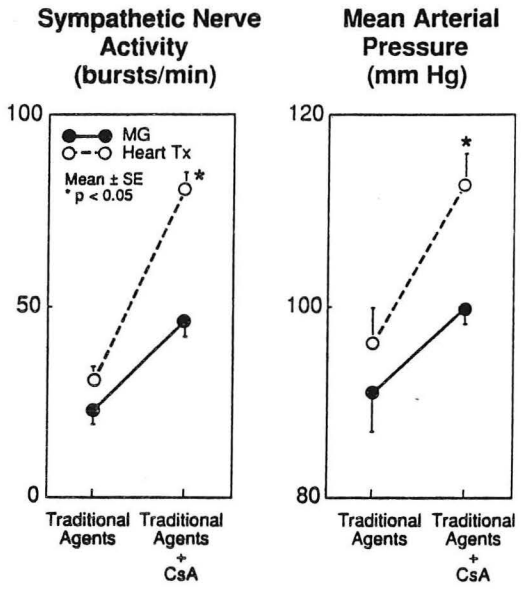
Figure 28



Recordings of sympathetic nerve activity in patients with myasthenia gravis treated wither with CsA or placebo (92).

These findings indicate that, without ventricular deafferentation, CsA alone can cause sustained sympathetic activation in humans. However, the degree of CsA-induced sympathetic excitation is much less in patients with innervated ventricles (patients with myasthenia gravis) than in patients with denervated ventricles (heart transplant recipients). The smaller degree of sympathetic activation in the patients with myasthenia, compared with heart transplant patients taking roughly comparable doses of CsA, was associated with a much smaller degree of hypertension. CsA treatment increased mean arterial pressure by 17 mm Hg in heart transplant recipients but only by 9 mm Hg in patients with myasthenia.

Figure 29



Differential effects of CsA treatment on sympathetic nerve activity and arterial pressure in patients with myasthenia gravis vs. patients with heart transplants (90,92).

Thus, removal of ventricular afferent restraint on the stimulation of sympathetic outflow caused by CsA may help to explain the clinical observation that the hypertensive effect of CsA is much greater in heart transplant recipients than in any other group of patients taking the drug.

Figure 30A

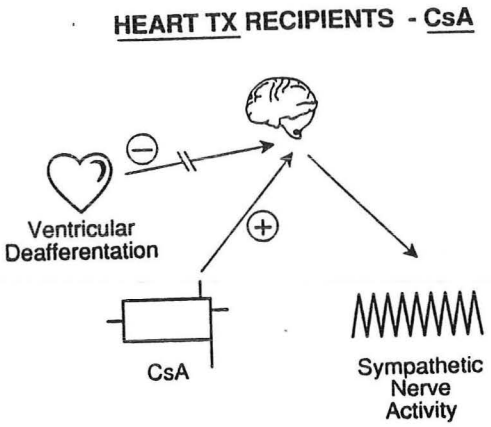


Figure 30B

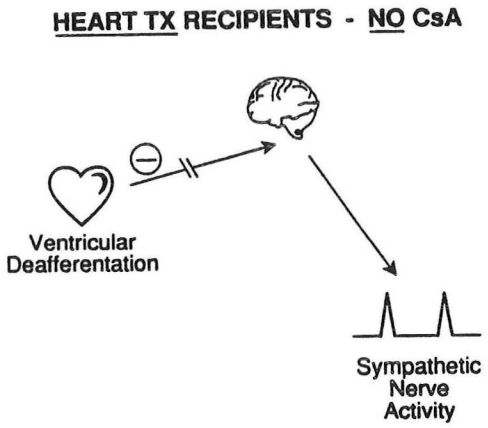
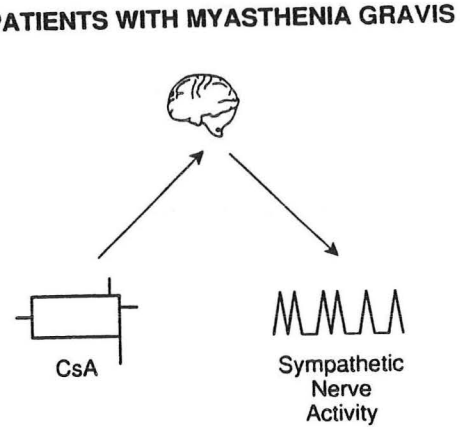
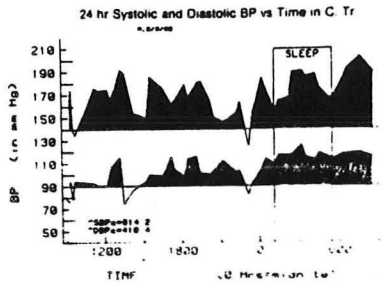


Figure 30C



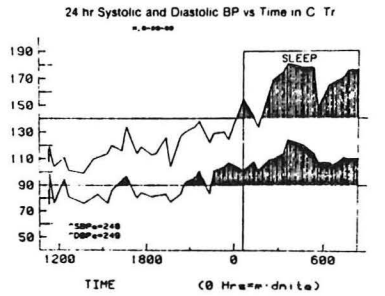
Ventricular deafferentation also may play a role in explaining the reversal of the normal diurnal variability in blood pressure observed in heart transplant recipients with CsA-induced hypertension (93). In normal individual and even patients with essential hypertension, blood pressure consistently decreases during sleep; whereas, in hypertensive heart transplant recipients, blood pressure either does not fall or actually rises during sleep so that night time blood pressures are often much higher than daytime pressure (93).

Figure 31A



Loss of Nocturnal Fall in Blood Pressure in CsA-Treated Hypertensive Heart Tx Recipient (Reeves et al., Hypertension 1986; 73: 401-8).

Figure 31B



Nocturnal Rise in Blood Pressure in CsA-Treated Hypertensive Heart Tx Recipient (Reeves et al., Hypertension 1986; 73: 401-8).

Cardiopulmonary afferents function as volume receptors such that volume expansion causes these receptors to increase their firing and thus their restraint on sympathetic outflow (89). Reduction in cardiopulmonary afferent restraint following cardiac transplantation, together with CsA-induced sympathetic excitation, may allow blood pressure to rise at night as plasma volume expands with prolonged recumbency.

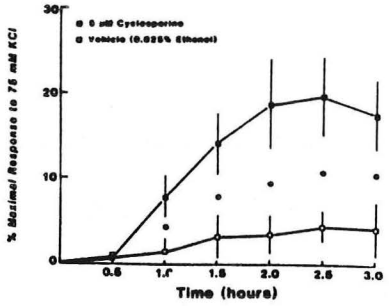
E. Effects on vascular smooth muscle

In addition to stimulating central sympathetic outflow, CsA also has been shown to enhance the effects of norepinephrine and other vasoconstrictor substances on vascular smooth muscle (94-97):

1. In anesthetized rats in which neurogenic vasoconstriction was prevented by renal denervation, CsA (20 mg/kg) doubled or tripled the renal vasoconstrictor responses elicited by progressive renal artery infusion of norepinephrine (94).

2. Unstimulated rat aorta shows a contractile response to prolonged incubation with CsA (95). This effect is markedly attenuated by alpha-adrenergic blockade, suggesting that CsA may release norepinephrine from adrenergic nerve terminals.

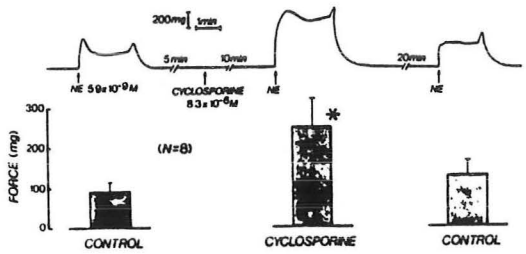
Figure 32



CsA Constricts Rat Aorta (Xue et al., Transplant 1987; 43: 715-8).

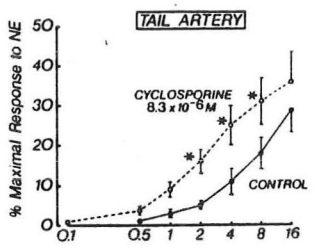
3. Incubation of unstimulated rat tail artery strips with CsA (10 -5 g/ml) did not elicit a contractile response but did augment the contractile response evoked both by a) sympathetic nerve stimulation, and b) exogenous norepinephrine (96). In addition, rats chronically treated with CsA show impairment in vascular relaxation with nitroprusside (97).

Figure 33A



CsA Augments Contractile Response of Rat Tail Artery to Exogenous Norepinephrine (Lamb and Webb, Life Sci 1987; 40: 2571-8).

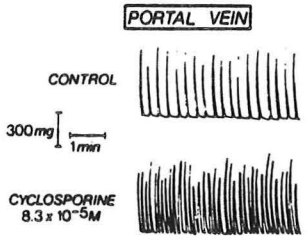
Figure 33B



CsA Augments Contractile Response of Rat Tail Artery to Sympathetic Nerve Stimulation (Lamb and Webb, Life Sci 1987; 40: 2571-8).

4. The rat portal vein normally undergoes spontaneous phasic contractions that are associated with firing of a calcium- driven action potential in the smooth muscle cells (96). Incubation with CsA greatly increases the frequency of these myogenic events, suggesting that CsA may be altering the resting membrane potential and thus bringing the tissue closer to the threshold for contraction (96). This concept is supported by the additional finding that CsA augments the contractile response of the tail artery to extracellular KCl (96).

Figure 34



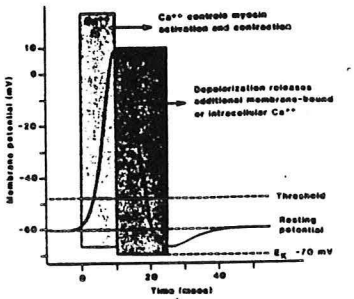
CsA Stimulates Ca++-Dependent Contractions in Rat Portal Vein (Lamb and Webb, Life Sci 1987; 40: 2571-8).

V. POTENTIAL CELLULAR MECHANISMS CAUSING CsA-INDUCED HYPERTENSION

A. Effects of CsA on the calcium messenger system in vascular smooth muscle

Contraction of vascular smooth muscle and depolarization of some neurons (see below) is regulated by the calcium messenger system (98-100). There are two main branches to the calcium messenger: 1) The calmodulin branch generally initiates rapid cellular responses or the initial phase of sustained responses; whereas 2) the protein kinase C branch maintains long lasting cellular responses such as prolonged constriction of vascular smooth muscle (99). Although norepinephrine, angiotensin, and vasopressin -- three important endogenous vasoconstrictor substances -- have different membrane receptors, they all are thought to activate the same calcium-dependent pathways that lead to contraction (99).

Figure 35



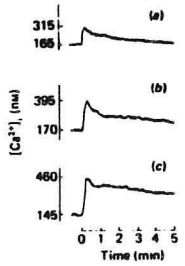
Ca++-Dependent Contraction in Vascular Smooth Muscle (Atwood and MacKay, Essentials of Neurophysiology 1989; p. 119).

Measurements of 45 Ca++ influx in cell culture from rat aortic smooth muscle cells (101,102) and hepatocytes (103) have advanced the concept that CsA increases calcium membrane permeability and thus selectively increases calcium influx. This effect appears to be resistant to verapamil, at least in cell culture (101). Because the resultant influx of intracellular calcium is taken up by intracellular organelles such as the endoplasmic reticulum, basal levels of $[Ca^{++}]_i$ are not increased in the presence of CsA (103). However, Ca^{++} influx would be expected to stimulate both branches of the calcium messenger system in vascular smooth muscle (99):

a) In CsA-treated patients, increased calcium influx should stimulate calmodulin-dependent depolarization by augmenting angiotensin-induced (101) or vasopressin-induced (103) release of Ca^{++} from the endoplasmic reticulum. Although as yet unproven, it is likely that CsA would also facilitate the increases in $[Ca^{++}]_i$ caused by norepinephrine acting on alpha-adrenergic receptors.

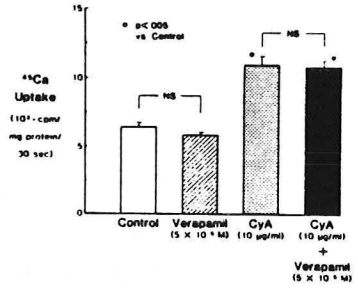
b) Increased cycling of Ca^{++} across the plasma membrane may potentiate sustained vasoconstriction by augmenting the activity of membrane-bound protein kinase C (99).

Figure 36A



CsA Augments Rise in Intracellular Ca++ Evoked by AT-II. a, control b, CsA 1 microgram/ml c, CsA 10 micrograms/ml (Pfeilschifter and Ruegg, Biochem J 1987; 248: 883-7).

Figure 36B

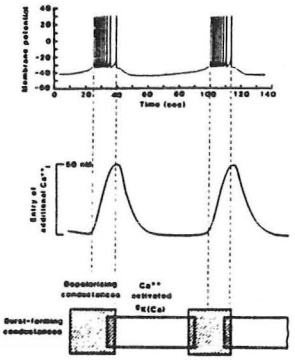


CsA Stimulates 45-Ca++ Uptake Via a Verapamil-Resistant Mechanism (Myer-Lehnert and Schrier, Hypertension 1989; 13: 352-360).

B. Calcium-dependent depolarization of bursting neurons: A possible mechanism for the sympathoexcitatory effect of CsA

Many neurons, in particular sympathetic neurons, do not fire in a regular fashion but rather show explosive "bursts" of activity interspersed between periods of neural silence. In such neurons, a burst of action potentials is triggered by a sudden influx of Ca^{++} which then opens a Ca^{++}-dependent K^+ channel that, in turn, hyperpolarizes the membrane and produces the silent period (98,100).

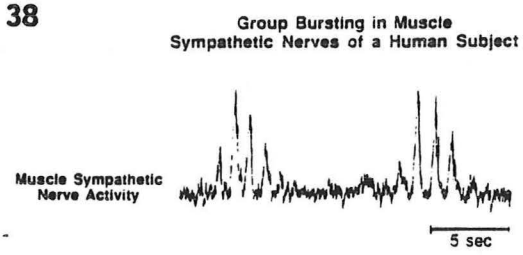
Figure 37



Group Bursting in Ganglion Neurons of the Mollusc (Smith, Doctoral Dissertation, University of Washington, Seattle, 1978).

This Ca^{++}-dependent mechanism has been shown to produce the bursting pattern of neural activity that characterizes sympathetic discharge in both crustaceans and vertebrates (98,100). The similarity between patterns of sympathetic discharge across species suggests that this mechanism also is likely to be operative in humans.

Figure 38



One possibility, therefore, is that CsA elicits long lasting stimulation of sympathetic nerve discharge by enhancing the influx of Ca^{++} either into central sympathetic neurons or into peripheral sensory (afferent) neurons that, in turn, reflexly increase central sympathetic outflow.

In this regard, the kidney contains a rich supply of afferents that, when stimulated by renal ischemia or by chemical substances in urine, reflexly stimulate sympathetic outflow and raise blood pressure (104). We and others (80) have that CsA, when given intravenously to anesthetized rats, stimulates the firing of these chemically sensitive renal afferent nerves. Like sympathetic nerves, these afferent renal nerves also discharge in bursts. In rats in whom the confounding influence of the sinoaortic baroreceptors has been removed, we have found that CsA produces parallel responses in afferent and efferent renal nerve activity, suggesting a possible casual relationship.

Figure 39A

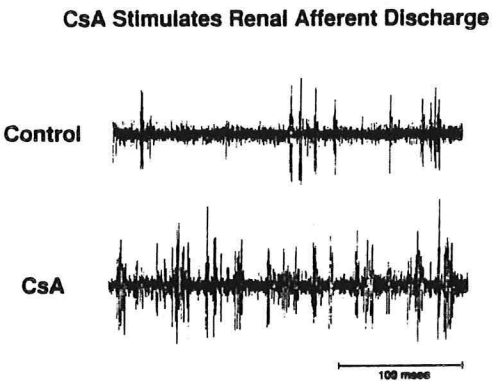
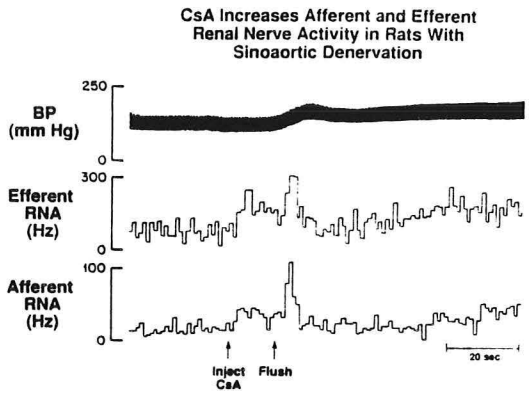


Figure 39B



These observations raise an important question: Is the blood pressure raising effect of CsA related to the immunosuppressive action of the drug?

C. Effects of CsA on T lymphocytes

The detailed molecular mechanism responsible for CsA's suppression of T lymphocytes is only beginning to be elucidated (105). The observation that CsA selectively inhibits helper T cells without inhibiting suppressor T cells suggested that cellular CsA receptor(s) might be involved in conferring CsA sensitivity, or resistance, to specific populations of cells. The current thinking about CsA binding to cellular receptors is very briefly summarized as follows:

1. Lymphocytes, and other cells, probably do not posses membrane surface receptors for CsA (106). Rather, CsA partitions into the membrane phospholipid bilayer and enters the cell largely by virtue of its extremely hydrophobic nature (105).

2. Handschumacher and colleagues at Yale have isolated a specific cytosolic binding protein for CsA, termed **cyclophilin** (107). This protein has a molecular weight of 15,000 with a K_d of 10^{-7} to 10^{-8} M. The principal concept arising from this work is that the immunosuppressive action of CsA is mediated in the cytosol by the binding of CsA to cyclophilin. The evidence is that the binding of various natural and synthetic cyclosporine derivatives to cyclophilin is directly proportional to their immunosuppressive activity in mixed lymphocyte reaction (107).

Table 4. Specificity of Cyclophilin for CsA Analogs (107).

Cyclosporin analog	Immuno-suppressive activity in mixed lymphocyte reaction	Cyclophilin affinity		Retention time of analog relative to CsA
		0.25 μg/ml	1.0 μg/ml	
CsA	+++	0.34	0.90	1.00
CsC	+++	0.31	0.69	0.70
Dihydro-CsC	+++	0.33	0.70	0.79
CsC ester	+++	0.00	0.04	1.40
CsG	++	0.30	0.87	1.14
Dihyro-CsD	+	0.19	0.72	1.28
8-Acido-dihydro-CsA	+	0.12	0.53	1.00
CsD	±	0.21	0.69	1.20
CsH	−	−0.01	0.02	0.98
O-Acetyl-CsA	−	−0.02	−0.03	1.69

Importantly, cyclophilin activity has been detected in non lymphoid tissue with the highest concentrations in brain and kidney, target organs that may be intimately involved in causing the effects of CsA on sympathetic nerve activity and blood pressure (107).

The precise relationship between cyclophilin and biologic activity of CsA and its metabolites, however, remains poorly understood. For example, some cell populations that are resistant to CsA (e.g., T suppressor cells) have high concentrations of cyclophilin, raising the possibility that binding to cyclophilin may actually inactivate CsA (108).

3. Colombani and colleagues at Johns Hopkins have provided evidence that CsA also binds to **calmodulin** and in so doing inhibits calmodulin-dependent activation of key regulatory enzymes (109). Specifically, CsA inhibited calmodulin-dependent activation of phosphodiesterase in cloned T lymphocytes in vitro. These investigators proposed that binding of CsA to calmodulin may prevent calmodulin's role in the activation of second messengers and enzymes that regulate the transcription of IL-2 mRNA. Although the pathway(s) by which the calcium messenger system might regulate the IL-2 gene is at present unknown, the above concept is supported by the observation that CsA inhibits the production of IL-2 only in those cells in which IL-2 production requires an increase in $[Ca^{++}]$ (110). Cells in which IL-2 production does not depend upon the Ca^{++} activation pathway are resistant to the effects of CsA. Furthermore, the inhibitory effect of CsA on T cell proliferation appears to be augmented by Ca^{++} channel blockers (108).

On the other hand, LeGrue and colleagues at U.T. Houston (111) have cast doubt on this hypothesis. They reported that calmodulin-dependent enzyme systems were equally inhibited by CsA as well as by two relatively inactive CsA analogs, and only at concentrations that were 100 times greater than those necessary to block lymphocyte activation. Furthermore, all three CsA derivatives showed comparable in vitro binding to calmodulin, regardless of their immunosuppressive activity.

We, therefore, are left with an apparent paradox. In vascular smooth muscle cells (and possibly in certain neurons), CsA would appear to enhance the cell's normal function by stimulating the influx of Ca^{++}. In T lymphocytes, however, CsA inhibits the cell's normal function perhaps in part by inhibition of Ca^{++}-dependent pathways. A better understanding of these basic mechanisms may suggest ways to uncouple the blood pressure raising effects from the immunotherapeutic effects of CsA.

VI. CONCLUSIONS: THERAPEUTIC APPROACHES

The ideal solution to the problem of CsA-induced hypertension would be the development of a cyclosporine analog, or other T cell specific immunosuppressive agent, with a perfect therapeutic/toxic ratio. This is an important goal for future research.

At present, there are no systematic trials to evaluate the relative efficacy of various antihypertensive agents in the treatment of CsA-induced hypertension. Experimental data, however, can provide some clues about the classes of drugs that are likely to be efficacious and those that are not:

In light of the evidence that the activity of the renin/angiotensin system is decreased, not increased, in patients with CsA-induced hypertension, converting enzyme inhibitors are unlikely to be beneficial. Beta adrenergic blockers also might not be the first agent of choice because of their propensity to augment peripheral vasomotor tone.

Because of the experimental evidence linking CsA hypertension to increased sympathetic outflow and increased calcium-dependent vascular reactivity, particular attention should be paid to **sympatholytic agents** and **Ca^{++} channel blockers**. Dr. Ingemar Dawidson in our Surgery Department recently has found that verapamil greatly attenuates the renal vasoconstriction response to CsA in kidney transplant recipients (112). In collaboration with Dr. Ring, we are beginning to investigate the effects of clonidine on sympathetic outflow and blood pressure in heart transplant recipients.

In conclusion, solving the enigma of CsA-induced hypertension is important for two reasons. First, in the short run, better treatment of this hypertension should reduce cardiovascular morbidity and mortality, particularly in heart transplant recipients. Second, long after CsA has been replaced by newer and better immunosuppressive agents, the lessons learned from the study of CsA-induced hypertension may provide important insights into the pathogenesis and treatment of other forms of hypertension.

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