CYCLOSPORINE-INDUCED HYPERTENSION

The Search for the Billion Dollar Molecule

Figure 1. From (1)

INTERNAL MEDICINE GRAND ROUNDS

November 2 , 1995

Ronald G. Victor, MD

TABLE OF CONTENTS

I. Introduction

In 520 A.D., according to legend, the patron Saints of medicine and surgery, Cosmas and Damian, replaced the gangrenous white leg of an aged sacristan with the healthy dark leg of a recently deceased Ethiopian man (1,2). This celebrated operation, captured in the historic painting by Fernando del Rincon (on the cover of the handout) is the first description in the Western literature of an orthotopic allograft.

In 1981, 14 centuries later, this ancient wish became a modern day reality when 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).

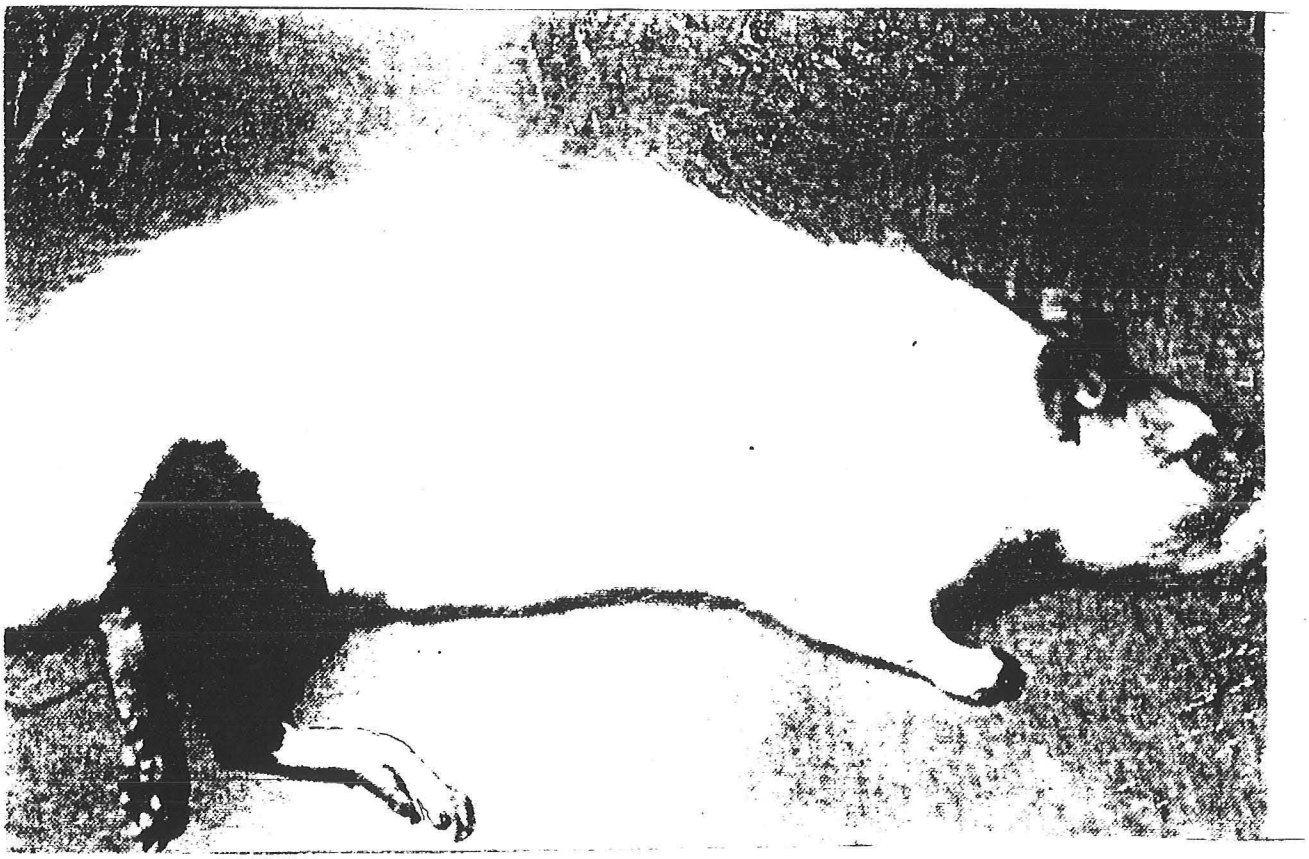


Figure 2. From (3).

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

Cyclosporine A (abbreviated **CsA**) is the first immunosuppressive agent with relative T-cell specificity (4,5). In the past decade, CsA has greatly improved long-term survival after organ transplantation, leading to the exponential growth of organ transplant programs in the 1980's (6).

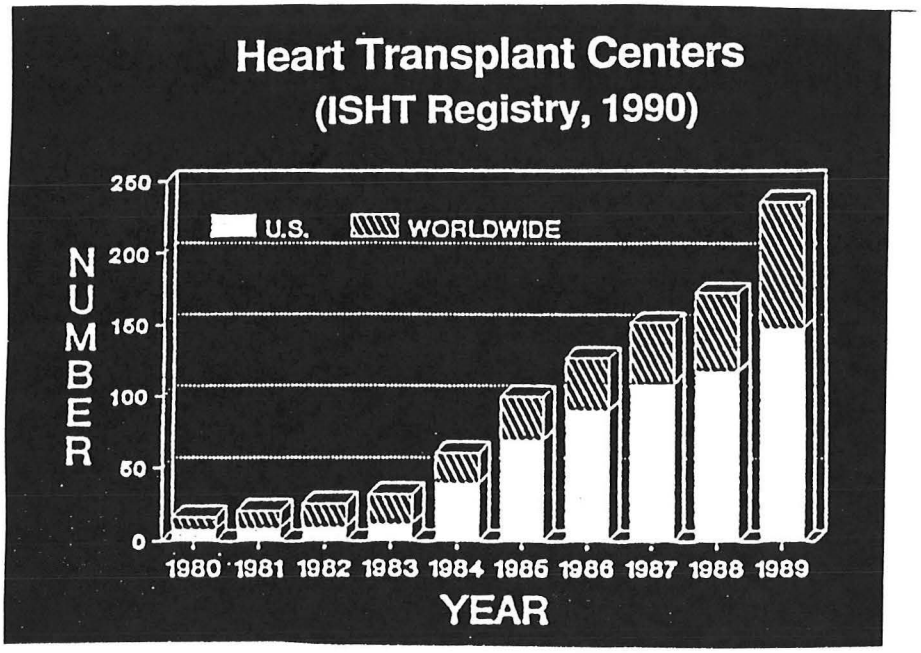


Figure 3 . From (6).

In addition, CsA is rapidly gaining acceptance as being remarkably efficacious for an increasing list of autoimmune diseases, including psoriasis and psoriatic arthritis, rheumatoid arthritis, and Crohn's Disease (7-9).

Despite these beneficial effects, CsA causes considerable toxicity, most notably hypertension (10-17), renal insufficiency (18), and CNS toxicity (19- 23).

Adverse Effects of CsA:

Adverse Effect	Incidence (%)
Hypertension	25-95
Nephrotoxicity (serum Cr > 2.0)	<30
Tremor	50
Paresthesia	35
Seizures / Encephalopathy	<5
Hyperuricemia	80
Hyperkalemia	25
Hyperglycemia	40
Hirsuitism	30
Viral Infections	25

Figure 4.

CsA has emerged as a major new cause of hypertension. Indeed, it now is one of the commonest causes of secondary hypertension. In heart transplant recipients, for example, the incidence of hypertension has increased from 20% in the pre-CsA era to currently 90% (4,10,16).

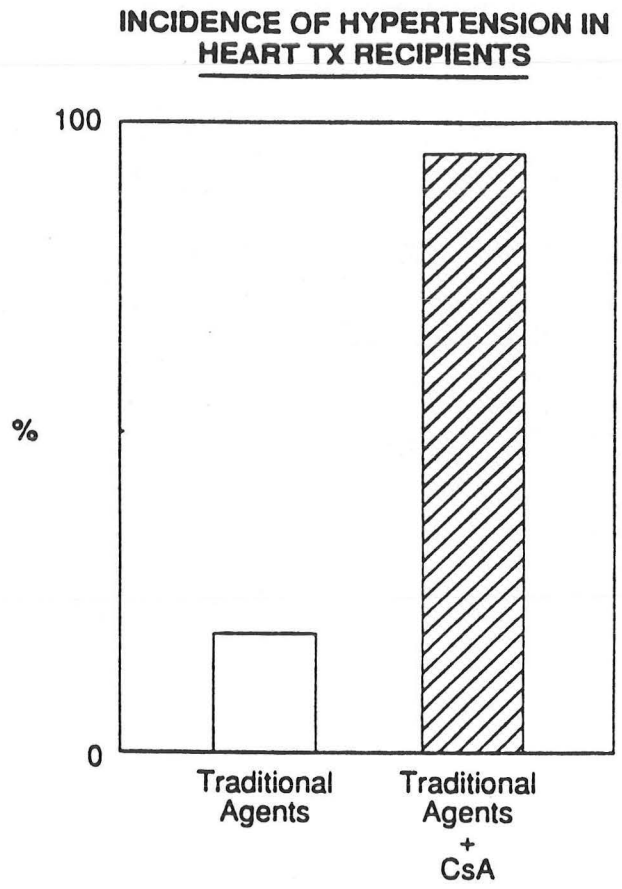


Figure 5. Adapted from (4,10,16).

Two syndromes have been described: (1) Chronic hypertension, which is evident usually by 6 months after transplant, typically is moderate or severe, often requiring treatment with multiple antihypertensive medications. Hypertension after heart transplantation has been implicated as a risk factor for shortened allograft survival (24,25), left ventricular hypertrophy (26-28), and coronary allograft and peripheral vascular angiopathy (29-30). (2) Acute fulminant hypertension with seizures has been described with high dose intravenous CsA after bone marrow transplantation (31,32).

Hypertension also is the main reason why internists have been hesitant to prescribe CsA for their patients with autoimmune diseases for which this drug is remarkably efficacious.

Despite the clinical importance of CsA-induced hypertension, the underlying mechanisms have been an enigma.

The aim of this Grand Rounds is to present a conceptual framework for understanding the pathophysiologic basis of CsA-induced hypertension. This is an excellent example of the syngery between basic research and clinical practice. CsA has not only proven to be a powerful immunosuppressant drug in patients but also to be a powerful new scientific probe to study cellular signal transduction (33), the process by which extracellular molecules alter intracellular function.

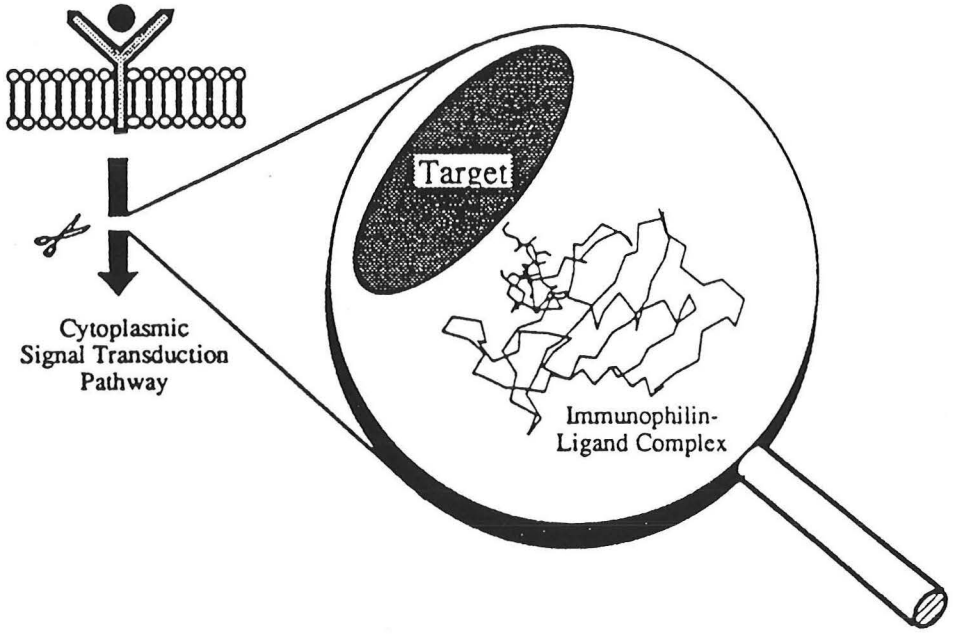


Figure 6. From (33).

The recent elucidation of novel CsA-sensitive cellular signaling pathways has lead to the search for the ideal immunosuppressant drug, which retains CsA's immunosuppressive efficacy without its toxicity, i.e., the "billion dollar molecule (34). "

BILLION DOLLAR MOLECULE

- greater immunosuppressive potentcy than CsA
- no toxicity

Figure 7.

This burgeoning field of basic research also has exciting implications for the treatment of a variety of disease processes far beyond immunosuppressant drug toxicity.

My review is not meant to be encyclopedic [the reader is referred to several recent reviews (35-40)], but rather to address the following question:

QUESTION

Does a common molecular mechanism mediate

the immunosuppressant and the hypertensive

effects of CsA ?

Figure 8.

II. Mechanism of CsA-Induced Immunosuppression

A. CsA, the first immunosuppressant drug with relative T cell specificity

CsA was the first immunosuppressive agent with relative T cell specificity (4,5) This means that, in contrast to more the traditional agents prednisone and azathioprine, CsA causes much fewer opportunistic infections.

CsA: T CELL SPECIFICITY

- greater effect on T than B cells
- inhibits T helper but not suppressor cells
- minimal bone marrow suppression
- minimal reduction in peripheral blood counts

= fewer opportunistic infections

Figure 9.

CsA: HISTORICAL DEVELOPMENTS

1970	CsA discovered from soil fungi
1972	immunosuppressant effect discovered by serendipity
1978	first clinical trials with CsA
1980	CsA synthezied de novo
1983	CsA registered by Sandoz
1992	molecular mechanism of immunosuprression

Figure 10.

The drug was used clinically for almost a decade before the molecular mechanism of action was elucidated. When I first reviewed this topic at Grand Rounds in 1989 (41), I concluded, "The detailed molecular mechanism of CsA's suppression of T lymphocytes is only beginning to be elucidated. "

In the past 6 years, the scientific progress in this field has been remarkable (33, 42-44 for excellent brief reviews).

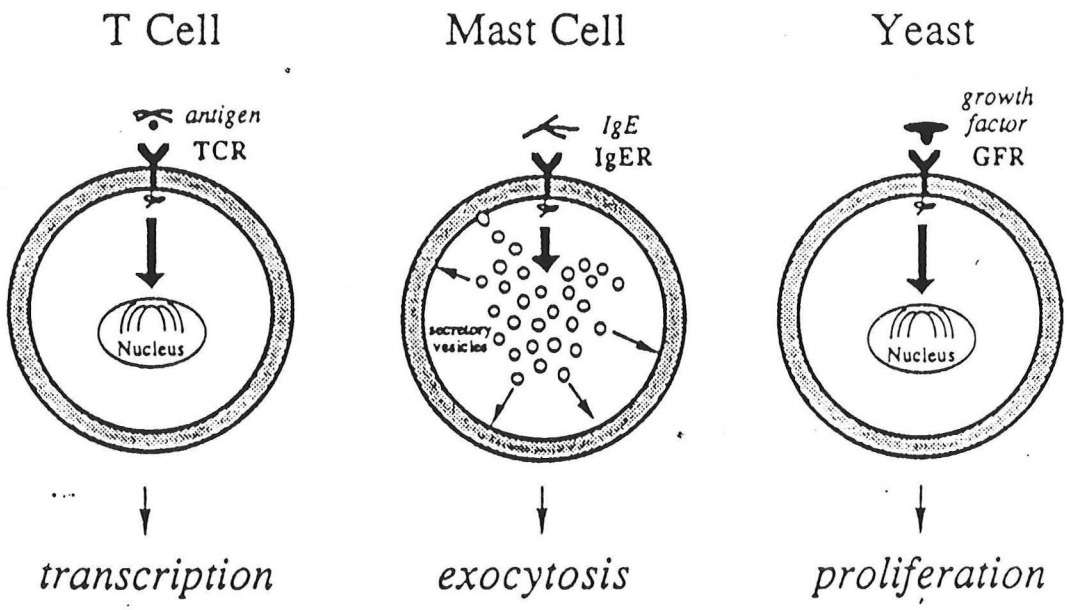


Figure 11 From (33).

These are 3 processes which are sensitive to CsA. In T cells it inhibits transcriptional activation of the interleukin 2 (IL-2) gene and this is thought to be the primary immunosuppressive action. The effect is not at the level of the T cell receptor but somewhere between the cytoplasm and nucleus. CsA also inhibits exocytotic release of histamine from mast cells and again the site of action is not the cell surface receptor but inside the cell. The yeast that produce CsA are resistant to its antifungal action against other species and this is accomplished again by interfering with proliferation not at the cell surface but at the intracellular level.

I want to emphasize that most drugs and hormones work by acting on cell surface receptors, a classic example being the interaction of epinephrine with the beta-adrenergic receptor. In contrast, CsA does not work on cell surface receptors. It is so lipophilic that is readily passes through the cell membrane and interacts with a newly discovered family of intracellular receptors.

B. Cyclophilin, the CsA receptor.

The cytoplasmic receptor for CsA is a soluble protein termed *cyclophilin*. (42-47). Actually, there are a dozen or so cyclophilins. They are uniformly expressed in yeast, in human T cells and mast cells, and in all mammalian cells. Handschumacker et al. (45) found that the uptake and concentration of [^{3}H] CsA in cultured cells occurs in the cytosol and not the cell membrane. They then purified the cytosolic receptor (15,000 kD protein) by chromatography. They hypothesized that the immunosuppressive action of CsA is mediated in the cytosol by binding to cyclophilin, since they could rank order the binding of various natural and synthetic CsA analogues to cyclophilin with their immunosuppressive potency in cell culture.

Interestingly, in various strains of yeast, sensitivity to antifungal action of CsA correlates with cyclophilin binding. In CsA-resistant mutants, the cyclophilin protein is either lost or has lost its ability to bind CsA (48).

Then it was discovered that the cyclophilins possess intrinsic enzymatic activity: they catalyze folding of proteins, a property termed cis-trans peptidyl-prolyl isomerase (46,47). This led to the "Isomerase Model:" CsA binding was postulated to result in a loss of function of cyclophilin (33).

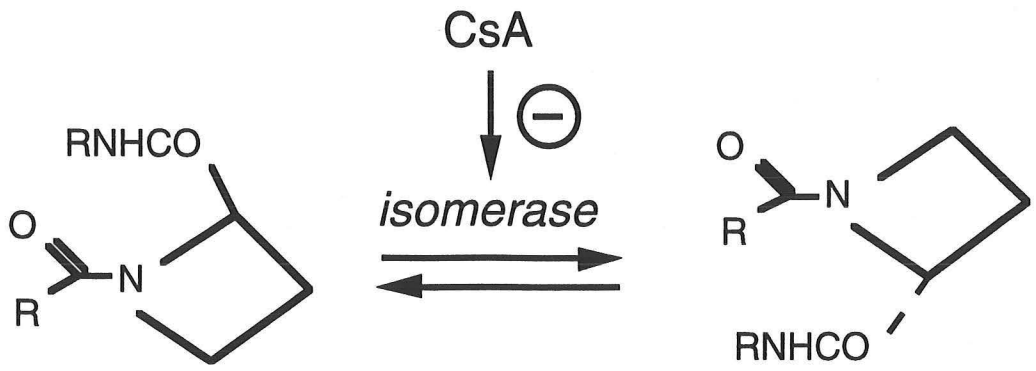


Figure 12. Adapted from (33).

Thus, prevention of the folding (cis-trans isomeration of a prolyl bond) of some unknown target protein was assumed to be the CsA-sensitive step in T cell activation

The simplicity of this model was so exciting that one of the senior scientists at Merck named Jeffrey Boger started his own drug company (Vertex) on the premise that one could design from basic principles a second generation CsA-like molecule which retained the key ingredient in immunosuppressive potency --inhibition of the isomerase--but removed whatever portion of the molecule was responsible for causing drug toxicity, the mechanism of which remained to be determined. This story recently captured the interest of the lay press (34).

T H E

BILLION - DOLLAR
M O L E C U L E

ONE COMPANY'S QUEST
FOR THE PERFECT DRUG

BARRY WERTH

SIMON & SCHUSTER, 1994

Figure 13. Adapted from (34)

Unfortunately for Boger and his financial associates, the isomerase model was completely refuted soon after the discovery of FK506 and rapamycin, two more immunosuppressants with relative T cell specificity (42-44).

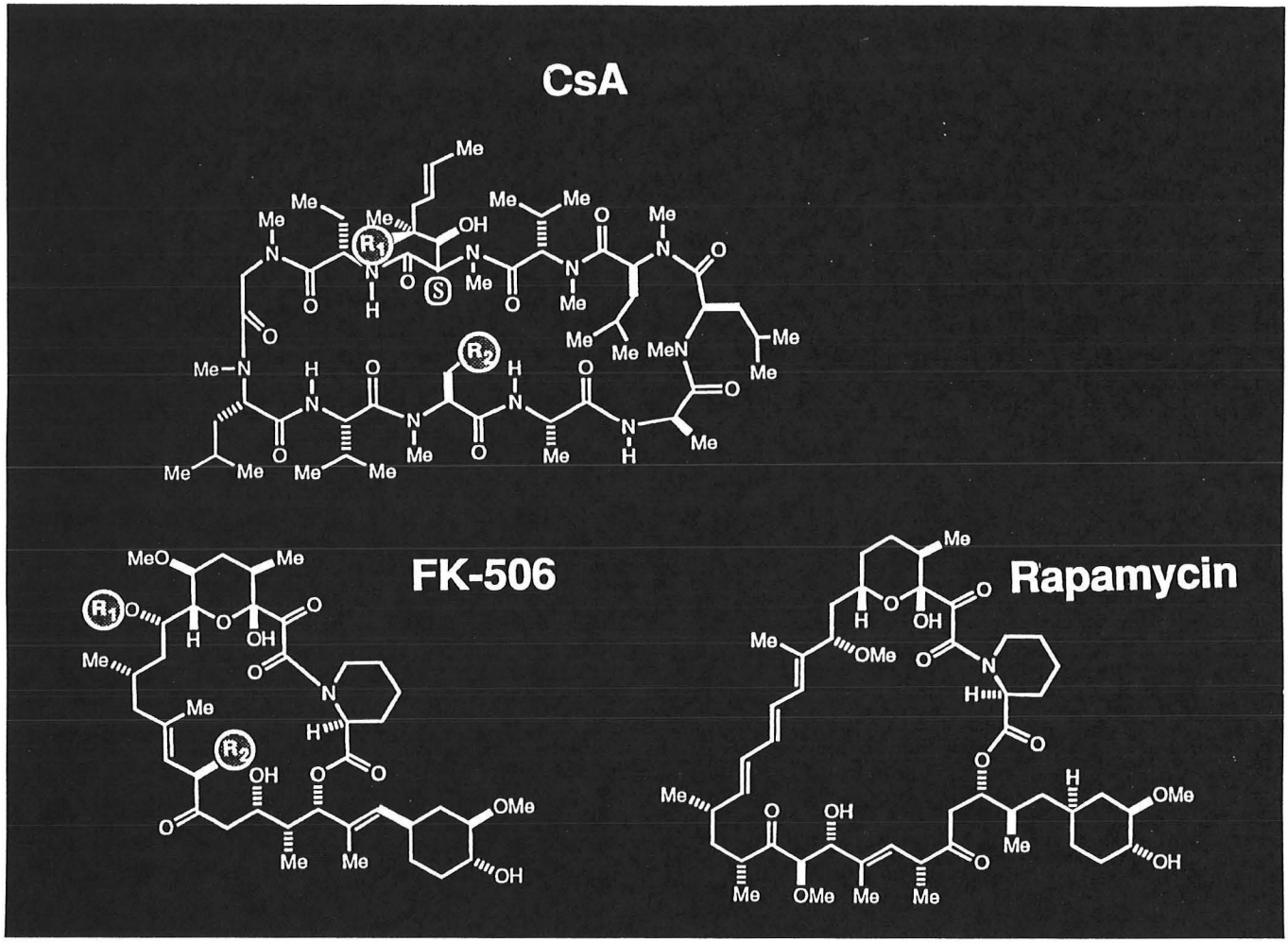


Figure 14 .

Whereas CsA is a cyclic polypeptide, FK506 and its structural analogue rapamycin are macrolides. Like CsA, FK506 is another fungal product (this time discovered in Japan) and it has the identical action on T cells and mast cells. Furthermore, FK506 binds to and inhibits the activity of another isomerase, termed *FK binding protein* (FKBP) (49,50). What is remarkable is that the two drugs...and their receptors (termed ***immunophilins***)... have absolutely no structural features in common.

CsA binds cyclophilin whereas FK506 and rapamycin bind FKBP. However, there is no cross-reactivity: CsA does not bind to FKBP and FK506 and rapamycin do not bind to cyclophilin (42-44, 49,50).

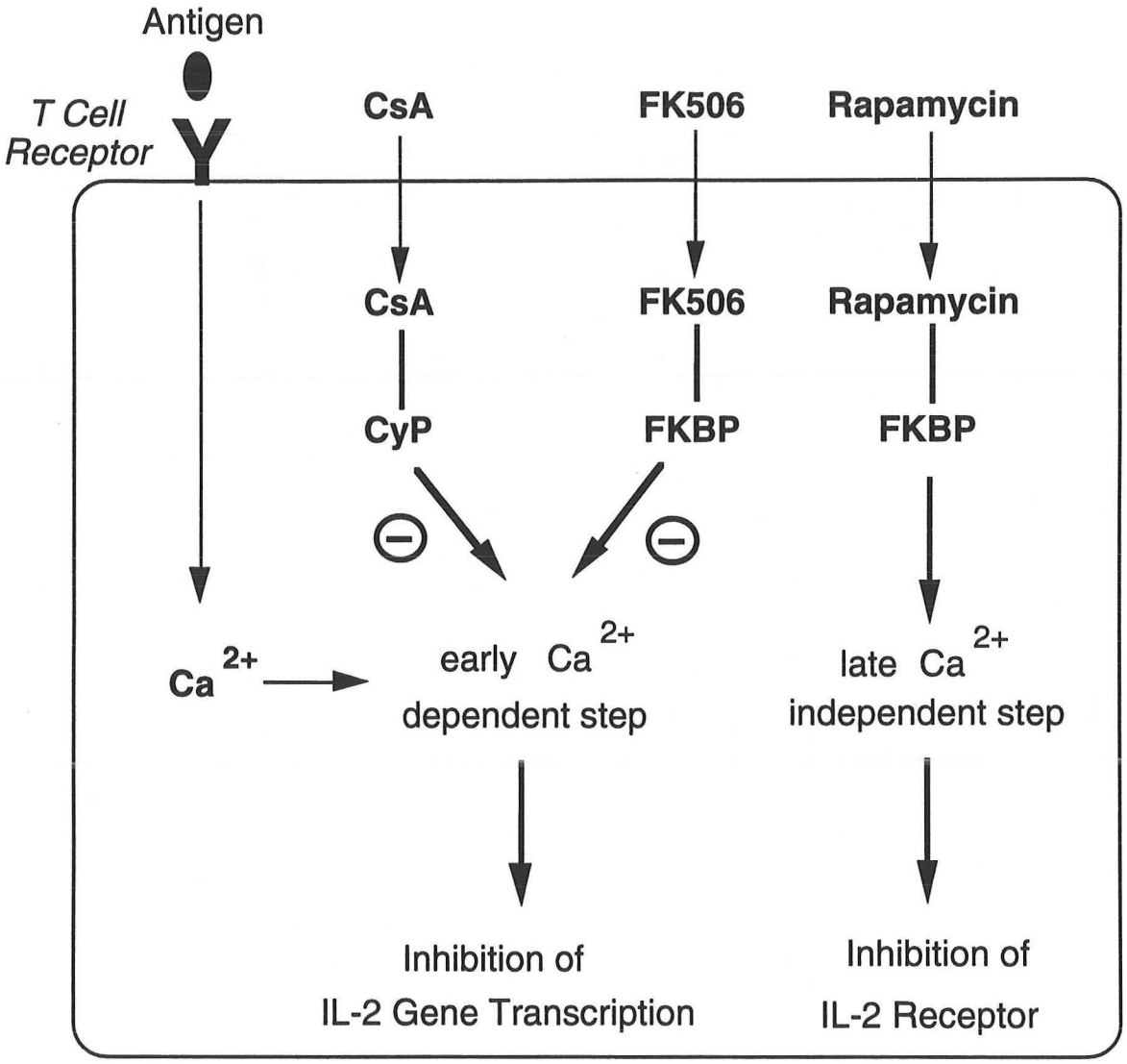


Figure 15.

Two observations dissociated T cell inhibition from isomerase inhibition. First, although FK506 and rapamycin are equally effective in inhibiting the isomerase activity of FKBP, the two drug /immunophilin complexes inhibit entirely different steps in T cell activation (42-44, 51). Second, Stuart Schreiber's laboratory synthesized de novo an unnatural immunophilin ligand termed 506 BD (FK506-like binding drug) which contains only the common structural elements of FK506 and rapamycin (52). The 506 BD binds FKBP with high affinity and inhibits its isomerase activity but has absolutely no effect on T cell activation in cell culture.

That CsA and FK506 inhibit the identical Ca^{2+} dependent step in T cell activation suggested that they have a common molecular mechanism of action.

C. Calcineurin, the cellular target of the CsA / cylophilin complex.

In 1991-92, the story broke that the common target of the two drugs (as their immunophilin complexes) is a Ca2+ binding protein termed ***calcineurin*** *(53-58).*

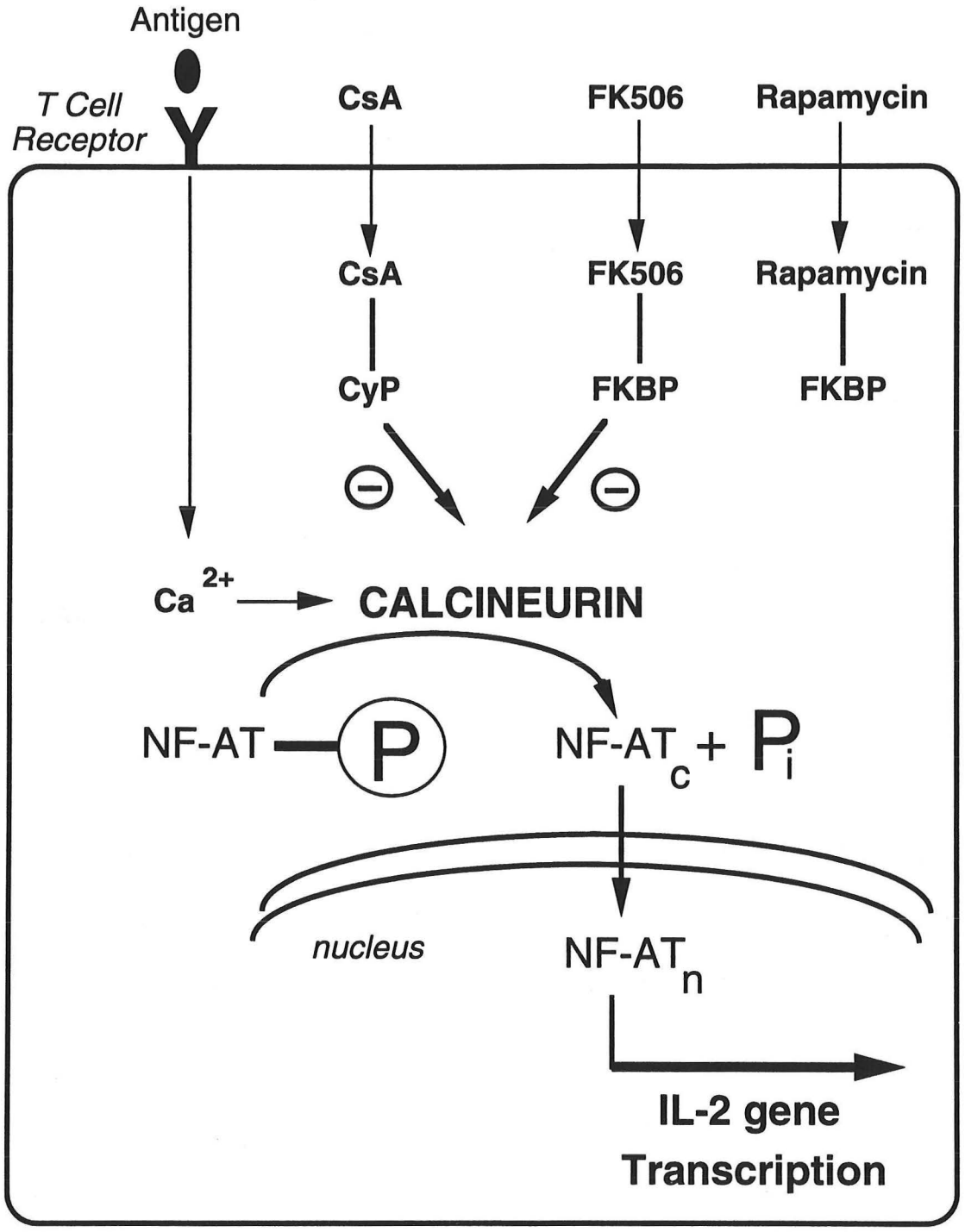


Figure 16.

This cartoon depicts the current thinking about the molecular mechanism of CsA's immunosuppressive action. This was elucidated by comparison of CsA's effects with those of two investigational immunosuppressant drugs, FK506 and rapamycin. Whereas CsA is a cyclic polypeptide, FK506 and rapamycin are macrolides. All three drugs are membrane permeant but they are biologically inert until they bind to their respective cytoplasmic receptors, termed *immunophilins. Cyclophilin* binds CsA while *FK-binding protein* (FKBP) binds either FK506 or rapamycin. The cellular target of both the CsA-cyclophilin and FK506-FKBP complexes is ***calcineurin***, the Ca^{2+}/calmodulin dependent phosphatase (53), which is inhibited by these immunophilin-ligand complexes (54-58). In the T cell, the relevant substrates for calcineurin are phosphorylated transcriptional factors on the interleukin-2 gene such as the nuclear factor of activated T cells (NF-AT). When dephosphorylated in the cytoplasm by calcineurin, a subunit of NF-AT enters the nucleus where it initiates transcriptional activation of the IL-2 gene. Inhibition of calcineurin plays a pivotal role in preventing T cell activation (i.e., graft rejection, automimmunity) by preventing dephosphorylation of NF-AT (33,43,54-58).

Calcineurin also mediates effects of CsA and FK506 on post-transcriptional immune events including exocytotic release of histamine from mast cells and of cytokines from cytotoxic T cells (59-62).

Recent evidence suggests that clinical doses of CsA lead to marked decreases calcineurin activity in circulating mononuclear cells in patients (63).

Although rapamycin, a structural analogue of FK506, is a high affinity ligand for FKBP, this immunophilin complex has no effect on calcineurin but inhibits T cell activation by a completely different (Ca^{2+} independent) mechanism (51, 64).

The evidence that calcineurin is the common cellular target for both CsA and FK506 is:

1. Calcineurin's phosphatase activity in vitro is inhibited by CsA-bound to cyclophilin or FK506 bound to FKBP, but not by rapamycin bound to FKBP (54,55).

2. The Schreiber laboratory identified the immunophilin receptor binding site and the calcineurin binding site on the CsA and FK506 molecules. By making minor alterations in the calcineurin binding site, they created a series of structural analogues with progression reduction in their ability to bind to calcineurin. The ability of these analogues to inhibit calcineurin's phosphatase activity in vitro correlated well with their ability to inhibit NF-AT activity in cultured human T cells (56).

3. Over-expression of calcineurin in T cells rendered their NF-AT activity less sensitive to inhibition by CsA or FK506 (57,58).

This work led to the following new concepts:

CsA and FK506 are **prodrugs.**

Figure 17.

When they circulate in the bloodstream, CsA and FK506 are biologically inert until they enter the cell and bind to their respective immunophilin receptors. When unbound, neither CsA nor FK506 have any effect on calcineurin.

CsA and FK506 are **molecular match makers.**

Figure 18.

They bring together two molecules -- in this case, the immunophilins and calcineurin --which normally do not interact.

GLOSSARY

Chaperone prevents undesirable associations

Schatchen promotes desirable associations
(Yiddish word for
marriage broker)

Figure 19. Adapted from (65,66)

CsA and FK506 act by forming a new molecular surface, composed of parts of the immunophilins and part of themselves. This new surface is selectively sticky for calcineurin. NMR studies show that when bound to cyclophilin CsA is literally turned inside out (66). The hydrophobic side chains of the ring structure normally are tuned inward. When bound to cyclophilin some of these side chains are turned outward to interact with the immunophilin whereas others are directed away from the immunophilin receptor site to help form a calcineurin binding site.

In summary, there is compelling evidence that calcineurin inhibition mediates CsA- and FK506-induced immunosuppression at the cellular level. A calcineurin-knock out mouse has just been made (67), allowing investigators to determine soon whether these results can be extrapolated to the intact animal.

Lets return to the question of whether the CsA-induced immunosuppression and toxicity share a common molecular mechanism.

III. Mechanisms of CsA-Induced Hypertension

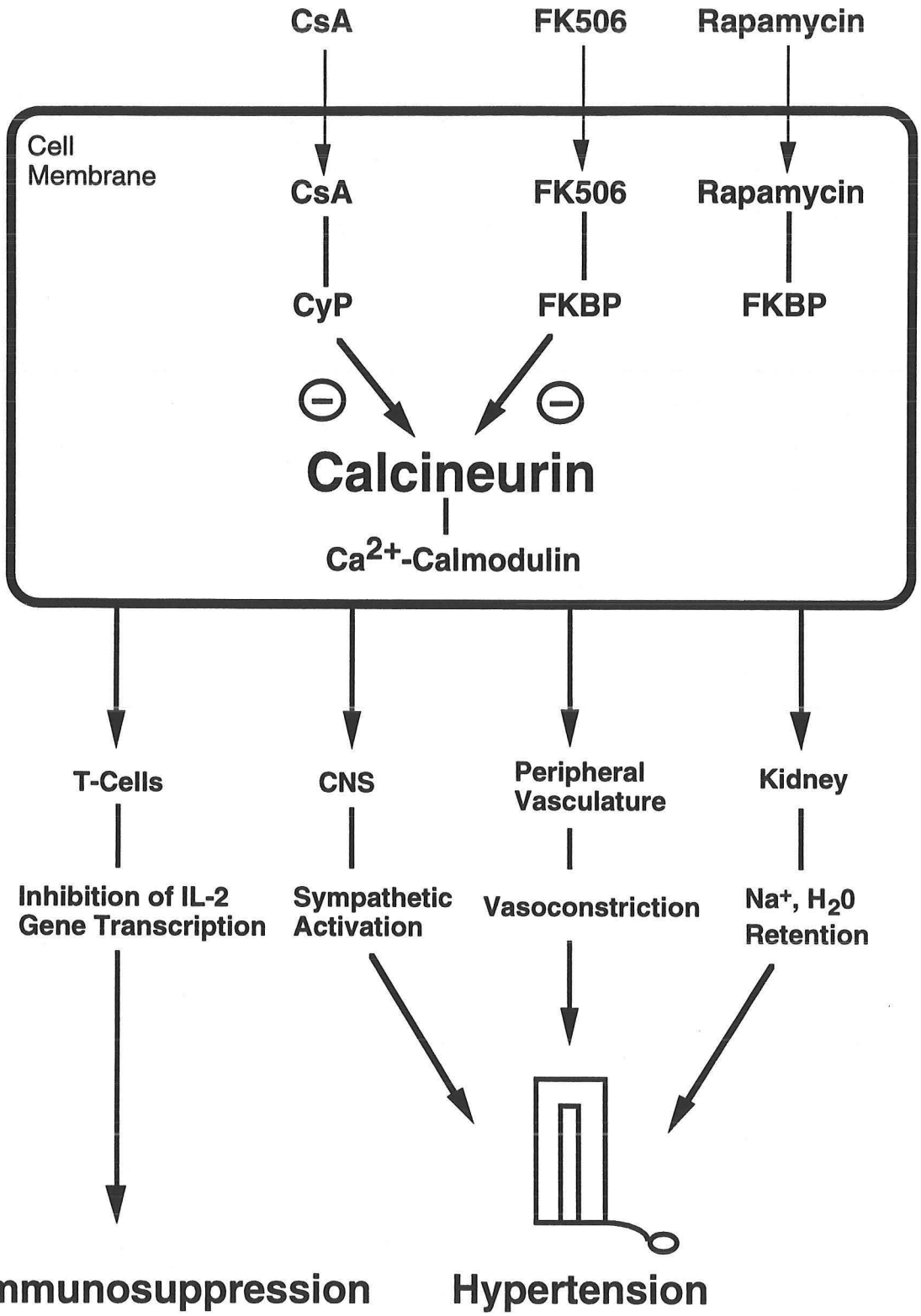


Figure 20. From (35).

Calcineurin and the immunophilins are even more plentiful in non-lymphoid tissues such as the nervous system (53, 68,69), vascular smooth muscle (70), and kidney (71). Because these are the main target sites for CsA-induced toxicity -- especially, hypertension -- a key question is whether inhibition of calcineurin in these different tissues mediates CsA-induced hypertension. If so, one would predict that in both the experimental and clinical settings CsA's toxicity profile would be duplicated by FK506 but not by rapamycin.

There is debate as to the relative contribution of various target tissues in the pathogenesis of CsA-induced hypertension. Renal (72-83), vascular (84-104), and neural (105-112) mechanisms all have been implicated and are not mutually exclusive. For example, CsA-induced hypertension is a low renin / salt-sensitive form of hypertension, due to an expanded plasma volume. In addition, CsA appears to augment the vascular reactivity of blood vessels either by impairing vasodilator mechanisms (e.g., nitric oxide) or enhancing vasoconstrictor mechanisms (e.g., endothelin). This review will focus only on the neural mechanisms, which is an on-going project in my laboratory. We have the opporutnity to take this project from the clinical setting to the experimental animal laboratory, to the cell and molecular level.

As suggested by the name, calcineurin was first discovered in the brain where it accounts for > 1% of total protein (53). Despite this abundance, until very recently almost nothing was known about its neuronal function. In 1992, Solomon Snyder's laboratory at Johns Hopkins demonstrated the colocalization of calcineurin with extraordinarily high concentrations of immunophilins in rat brain and brainstem (69), which plays a key role in the sympathetic neural control of blood pressure. This led us to hypothesize that inhibition of calcineurin by CsA leads to an increase in the activity of sympathetic neurons which contributes to hypertension (111). This was a reasonable hypothesis, since CsA clearly crosses the blood brain barrier (19-23).

A. Clinical Investigation

Using intraneural microelectrodes to record sympathetic nerve discharge targeted to the skeletal muscle circulation. Scherrer et al. (107) provided evidence of sympathetic overactivity in patients with CsA-induced hypertension.

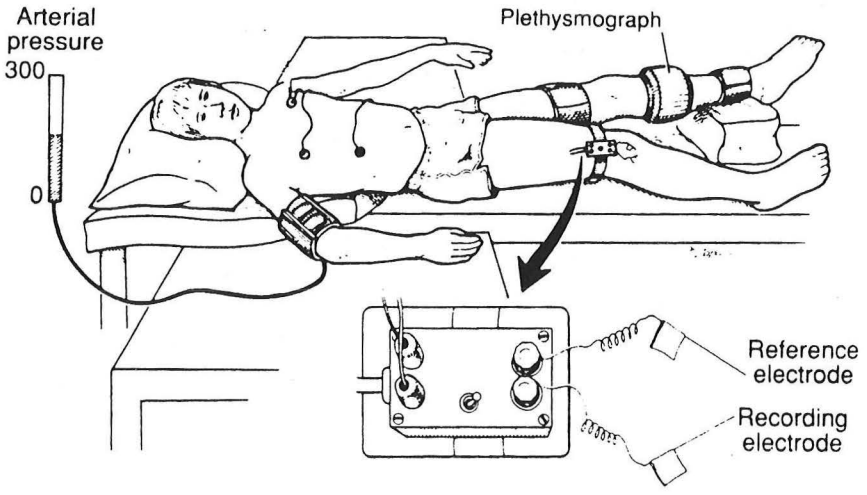


Figure 21.

We measured arterial pressure and sympathetic nerve activity in 5 heart transplant recipients treated with azathioprine and prednisone alone and in 16 heart transplant recipients treated with azathioprine and prednisone plus CsA.

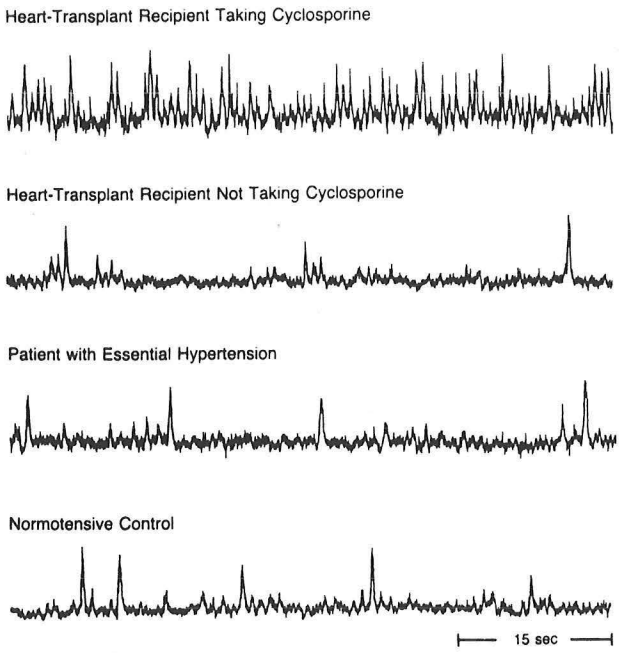


Figure 22 . (From 107).

The higher blood pressures in the CsA group were accompanied by higher sympathetic nerve activity.

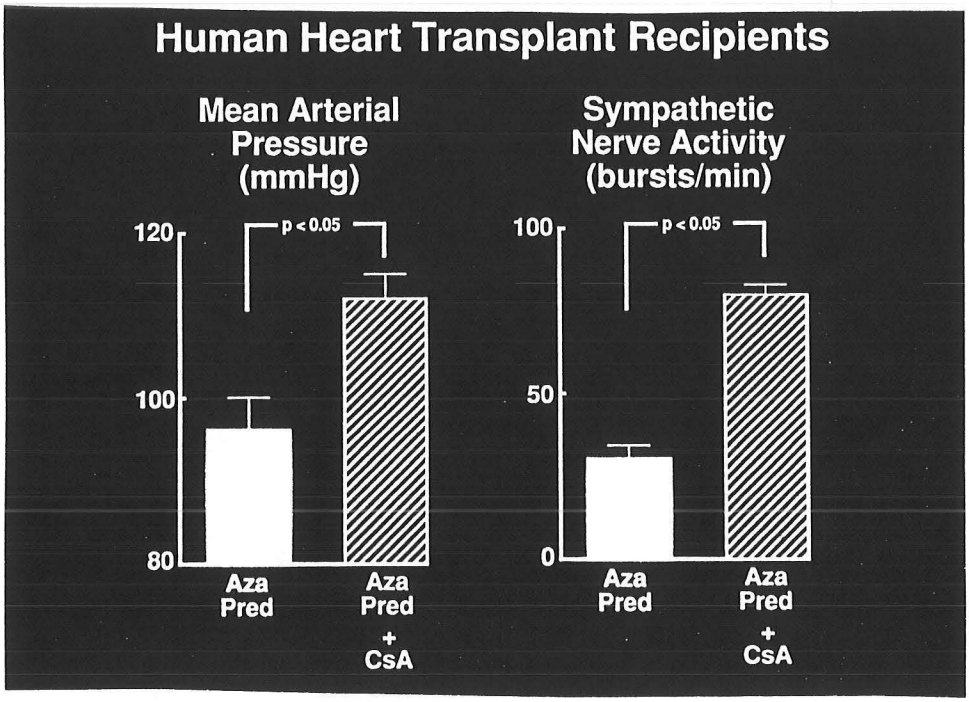


Figure 23. Adapted from (107).

We (113) and others (114-116) have confirmed the finding of sympathetic overactivity in heart transplant recipients, although this has not been a universal finding (100,116), and we have extended our findings to patients receiving CsA for autoimmune disease (107).

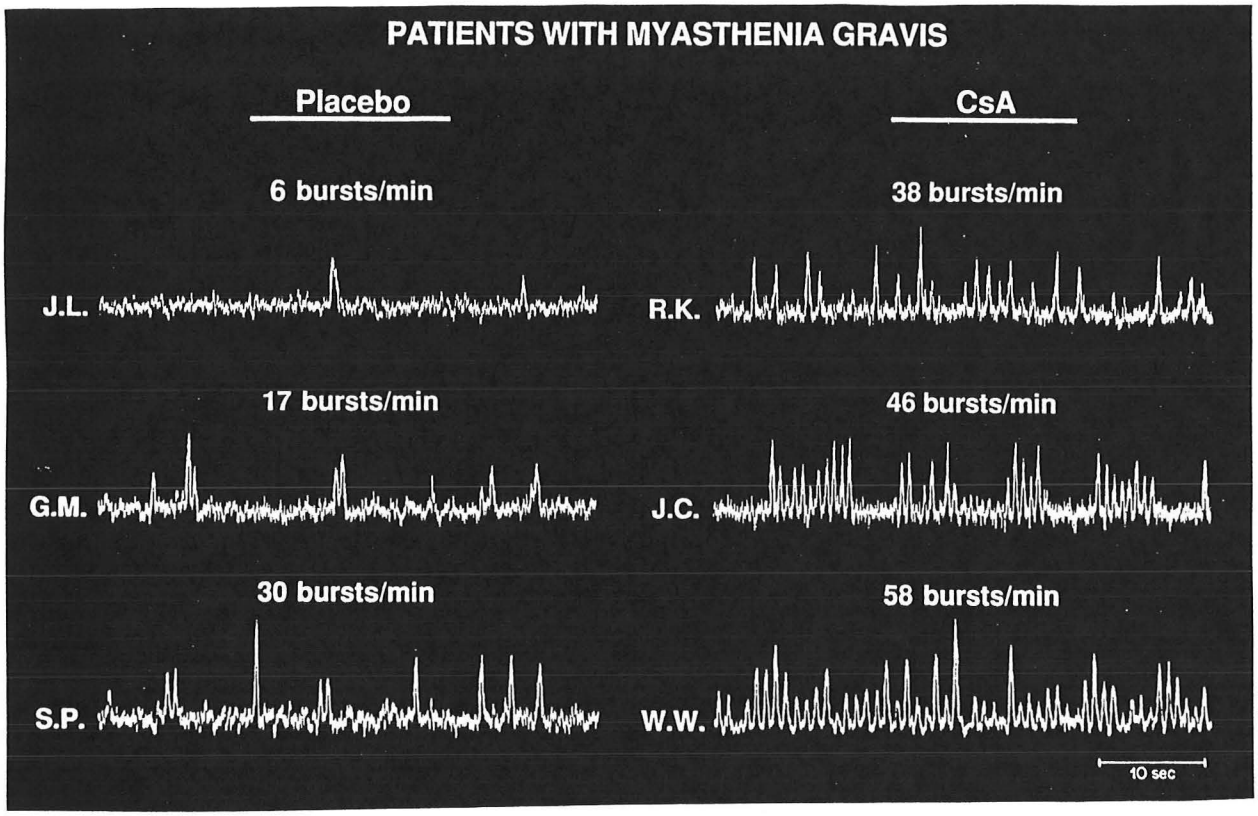


Figure 24. Adapted from (107).

In a double-blind randomized cross-over trial of CsA vs. placebo, we found that CsA-treatment also was accompanied by sympathetic overactivity in patients with myasthenia gravis (107).

It has been more difficult to establish sympathetic overactivity in CsA-treated patients using measurements of norepinephrine spillover, which are influenced not only by sympathetic nerve traffic but also by plasma clearance and presynaptic modulation. One study found increased norepinephrine spillover in CsA treated patients (117) whereas others have not (100, 104).

Because of the difficulty in controlling a number of potentially confounding variables in clinical studies (e.g., antihypertensive medications, recent transplant surgery), we soon will begin to record sympathetic traffic during i.v. CsA (and eventually i.v. FK506 and rapamycin) in normal human subjects.

In the meantime, we have made those comparisons in rodent models.

B. Animal Investigation

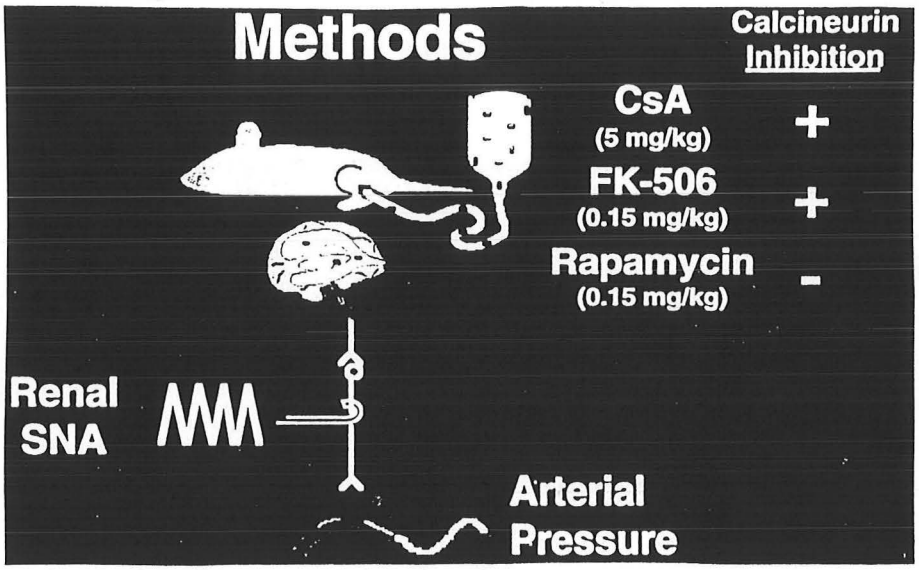


Figure 25.

In anesthetized rats, we recorded renal sympathetic nerve activity and arterial pressure during i.v. CsA, FK506, or rapamycin (35, 108,111,112).

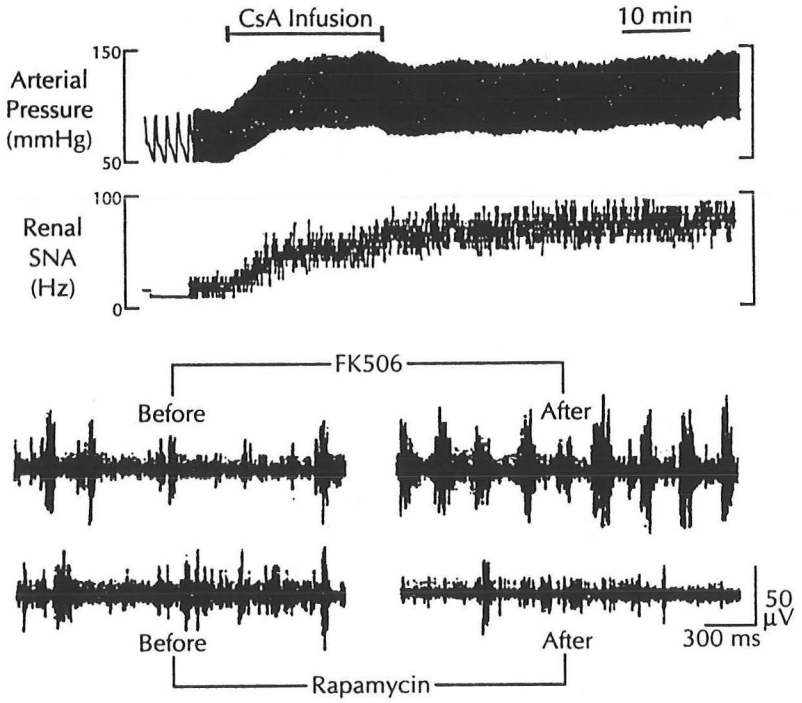


Figure 26. Adapted from (35,108,111).

So, in rats, renal SNA and blood pressure are increased by FK506 as well as CsA but not by rapamycin, suggesting calcineurin mediation. The increase in blood pressure is sympathetically-mediated because it is eliminated by chemical or surgical sympathectomy 108,109). To further pursue the calcineurin hypothesis, we performed the following structure - function studies in collaboration with the Schreiber laboratory

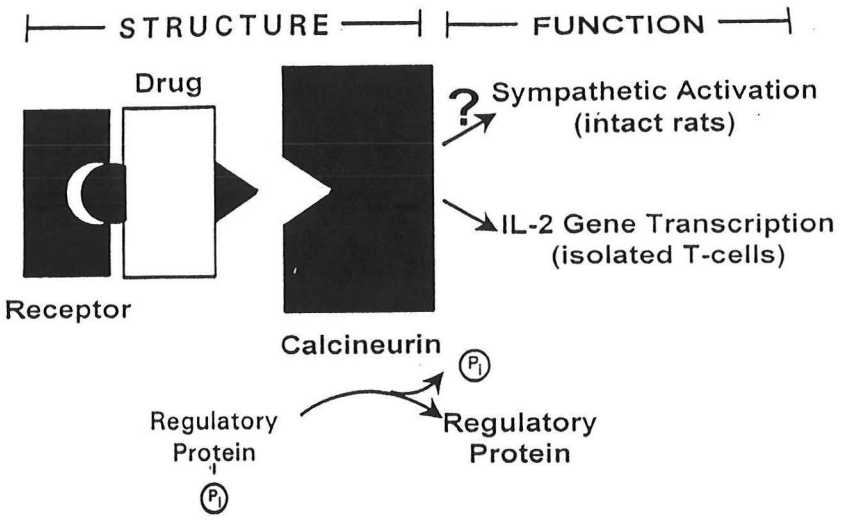


Figure 27.

The immunosuppressant drugs CsA and FK506 have an immunophilin receptor binding site and a distinctly different calcineurin binding site. By making minor structural alterations in only the calcineurin biding site, the Schreiber laboratory produced a series of drug analogues with progressive reductions in the ability of the parent molecules to bind to and inhibit calcineurin (56).

When injected i.v. in our rats, these analogues produced attenuated increases in renal SNA and blood pressure in such a way that closely paralleled their attenuated ability to inhibit calcineurin-mediated signalling in isolated human T cells (111).

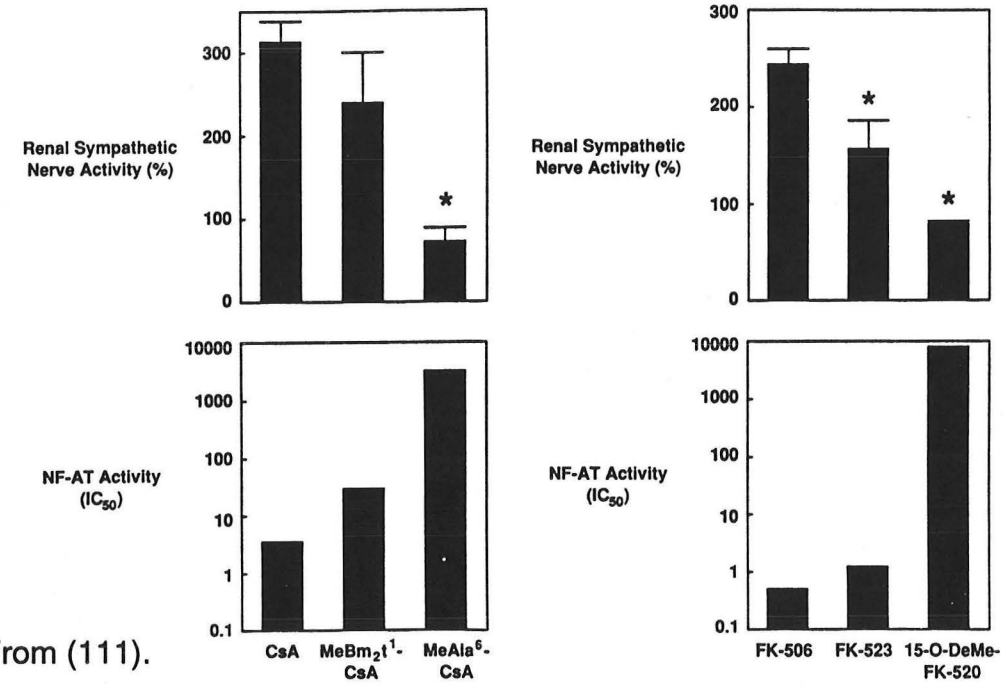


Figure 28 From (111).

A similar correlation has been established between calcineurin inhibition and nephrotoxicity in rodent models (82,83). The strength of these correlation's in intact animals indicated that this project was ready for pursuit at the cell and molecular level.

C. Cellular and Molecular Investigation

We recently have begun to use patch clamp techniques (118) (for which Neher and Sackmann won the Nobel Prize in 1990) to probe calcineurin's role in electrical signalling between neurons.

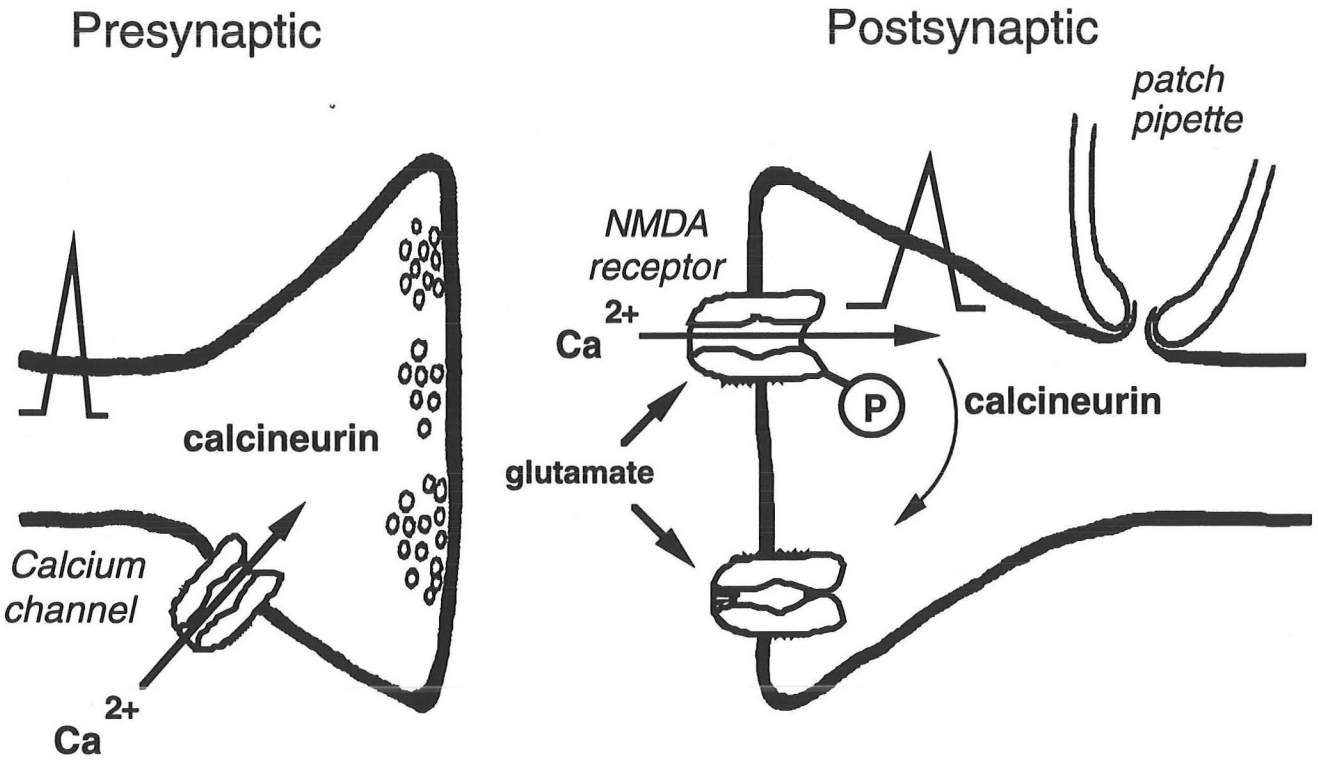


Figure 29.

Glutamate is the primary excitatory neurotransmitter in the brain and it plays a major role in sympathetic neurotransmission at the level of the spinal cord, brainstem, and higher brain centers (119-124). When an action potential invades a presynaptic nerve terminal, neurosecretory vesicles containing glutamate are released into the synaptic cleft. Glutamate activates a postsynaptic receptor (termed *NMDA* because it also can be activated by a synthetic compound N-methyl-D-aspartate), an ion channel which controls the influx of Ca^{2+} into the postsynaptic neuron. The resultant Ca^{2+} triggered intracellular action potential can be recorded with a patch pipette electrode.

It has been hypothesized recently that calcineurin modulates this complex process of glutamatergic neurotransmission via both pre- and post-synaptic sites of action.

For example, calcineurin has been shown to cause the dephosphorylation of postsynaptic glutamate receptors in excised membrane patches (125) and it has been shown to dephosphorylate certain presynaptic vesicle proteins in vitro (126,127).

We tested these hypotheses using cultured rat cortical neurons, a reductionist model of glutamatergic neurotransmission. We recorded spontaneous glutamate-driven action potentials from the neurons and found that the action potential firing rate is increased by CsA or FK506 but not rapamycin, indicating calcineurin mediation (128).

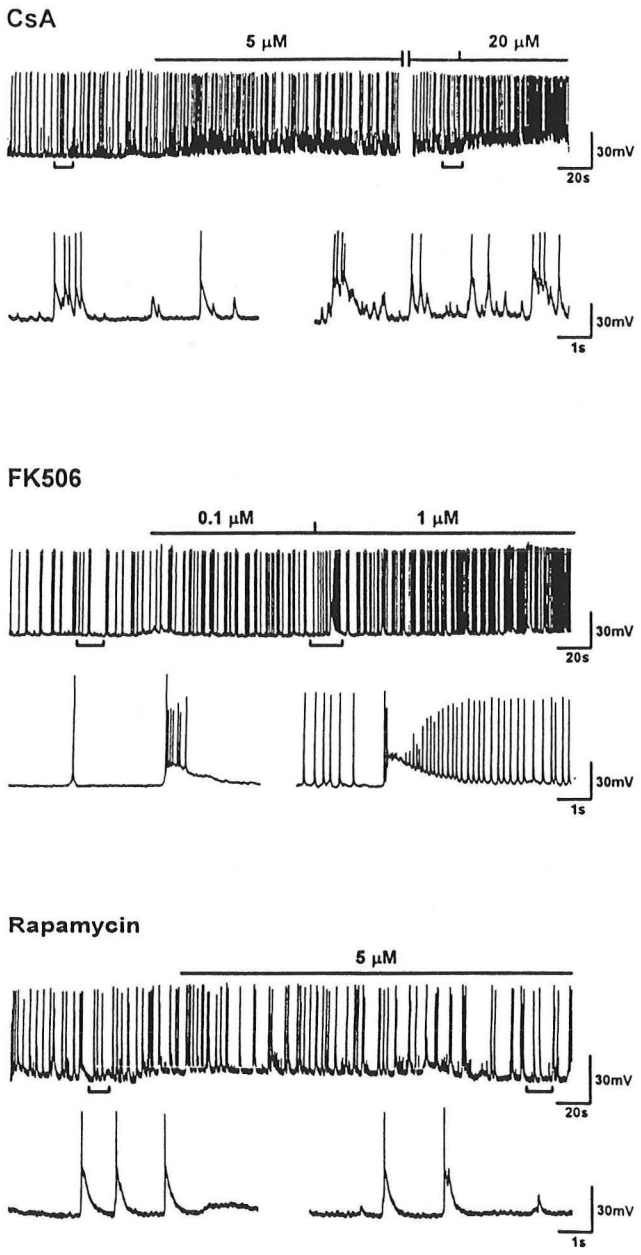


Figure 30. From (128).

We went on to show that in this cell culture model calcineurin modulates glutamatergic neurotransmission via a presynaptic site of action: it appears to increase the frequency of glutamate release from presynaptic nerve terminals.

Calcineurin could regulate any one of multiple steps involved in the release of glutamate beginning with entry of calcium into the presynaptic nerve terminal and ending with exocytotic release of neurosecretory vesicles.
Our working hypothesis is that calcineurin is a key component of a negative feedback mechanism which prevents excessive release of neurotransmitter.

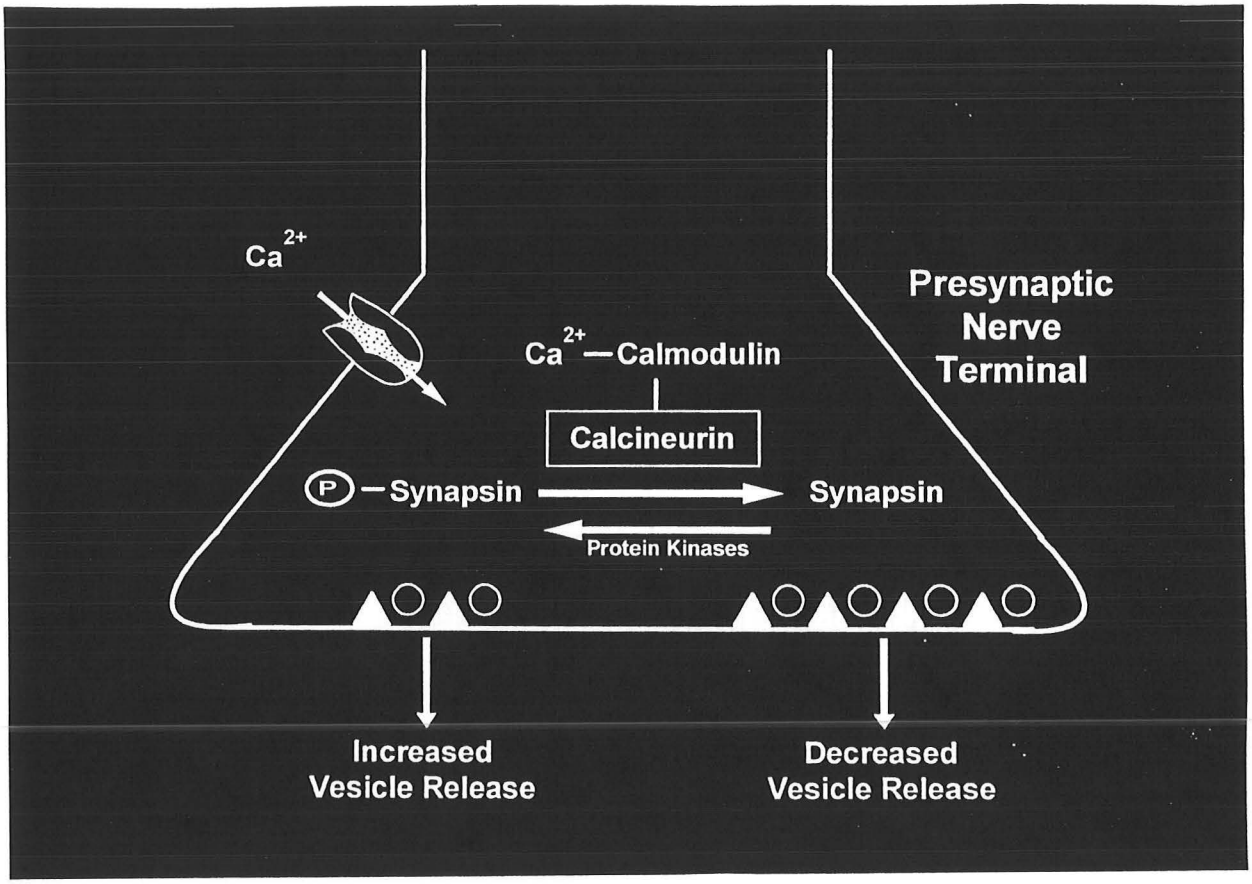


Figure 31.

We hypothesize that, during depolarization, entry of Ca^{2+} into the presynaptic neuron activates calcineurin which then dephosphorylates a family of vesicle-associated proteins termed ***synapsins***, which are known calcineurin substrates in vitro. There is evidece to suggest that dephospho-synapsin exerts an inhibitory effect on vesicle release. We hypothesize that CsA and FK506 block this dephosphorylation, thereby removing an inhibitory influence on vesicle release (129).

Dr. Thomas Rosahl in Dr. Tom Sudof 's laboratory here at UT Southwestern produced a knock-out mouse lacking the synapsin I gene (130). They also have produced knock out mice lacking synapsin II and double knock-out mice lacking both synapsin I and II (131). These transgenic mice provide a unique opportunity to test out hypothesis not only in cell culture but also in the intact organism. To accomplish the latter, we have miniaturized our techniques to record blood pressure and sympathetic nerve activity in mice weighing 30 grams.

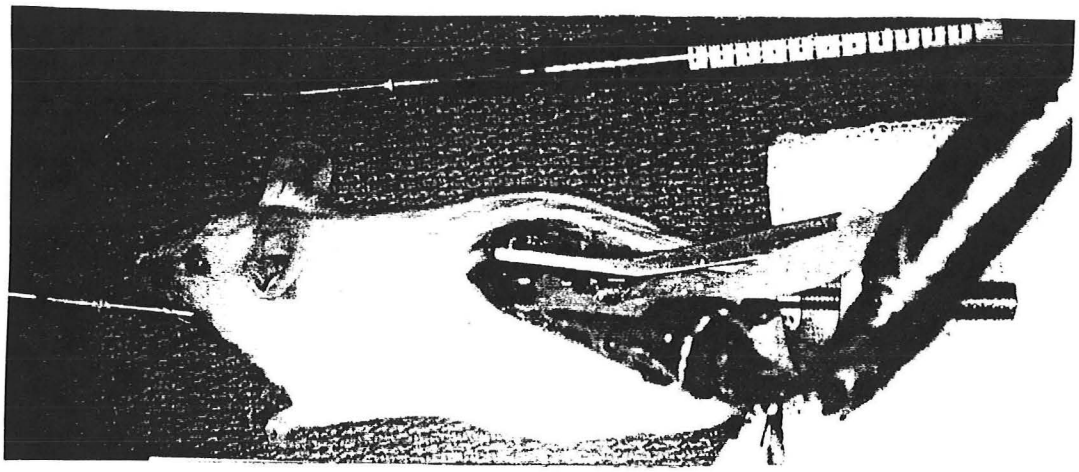


Figure 32. (Actual size).

In the wild -type mouse, i.v. CsA causes large increases in blood pressure, heart rate, and sympathetic nerve activity similar to those previously observed in rats.

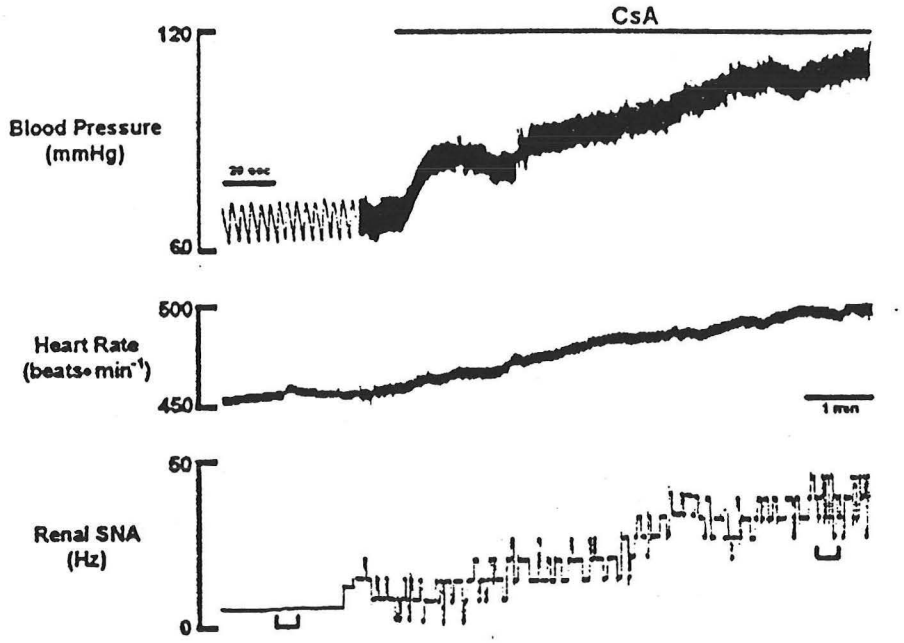


Figure 34.

A key unanswered question is whether the synapsin deficient genotype will produce a phenotype which is resistant to these effects of CsA.

Another key question is whether these experimental studies suggest new approaches to the management of CsA-induced hypertension in patients.

IV. Management and Prevention of CsA-Induced Hypertension

Calcium channel blockers seem to have become the initial drug of choice for the treatment of CsA-induced hypertension, despite a paucity of controlled studies (35,132,133). If indeed there is an important sympathetic neural component to CsA-induced hypertension, sympatholytic agents would be a rationale choice but no firm recommendations can be made in the absence of large randomized trials.

However, the ultimate goal is to eliminate CsA-induced hypertension by replacing CsA with a better agent with equal or greater immunosuppressive efficacy without the toxicity (34) . FK506, which was approved for clinical use in 1994, was touted at first as having greater immunosuppressive potency than CsA with much less toxicity (34,134-136). However, these initial studies were not randomized and involved rather small numbers of patients. In the past year, several studies including two large multicenter controlled randomized clinical trials (137,138) have demonstrated convincingly that FK506 causes as much hypertension as does CsA and even more nephrotoxicity and central neural toxicity, at least in liver transplant recipients.

In the U.S. Multicenter FK506 Liver Study Group (137) of 478 adult and 51 pediatric patients, the incidence of hypertension was 47% with FK506 vs. 56% with CsA. The main reasons for withdrawal from the latter study were nephrotoxicity and neurotoxicity, which were more frequent with FK506 than with CsA.

Multicenter FK506 Liver Study Group :
Reasons for Withdrawal from the Study

	FK506 (N = 263)	**CsA** (N = 266)
Reason for Withdrawal	no. of patients	
Total withdrawn	83	102
Adverse event	37	13
Nephrotoxicity	14	5
Neurotoxicity	12	4
Lack of efficacy	6	32

Figure 35. Adapted from (137).

Multicenter FK506 Liver Study Group :
Incidence of Adverse Events

	FK506 (N = 263)	**CsA** (N = 266)
	% of patients	
Adverse Event		
Hypertension	**47**	**56**
Headache	64	60
Tremor	56	46
Paresthesia	40	30
Hyperkalemia	45*	26
Diarrhea	72*	47

* P<0.05 vs. CsA. *Figure 36.* Adapted from (137).

Similarly, in the European FK506 Multicenter Liver Study Group Trial (138) of 545 liver transplant recipients, the incidence of hypertension at one year was 35% with FK506 vs. 42% with CsA .

The toxicity of rapamycin is as yet unknown as Phase I and II clinical trials are just underway. If the calcineurin hypothesis is correct, rapamycin will not cause hypertension (or renal or neural toxicity) in patients.

Furthermore, because rapamycin and CsA bind to different classes of immunophilins, one would predict immunosuppressive synergy. This would be very important, because it may be possible with combined CsA-rapamycin therapy to decrease the dose of both drugs to maintain immunosuppression while greatly decreasing toxicity, possibly even eliminating hypertension as a side-effect of clinical immunosuppressive therapy (139). In contrast, rapamycin cannot be combined with FK506, which would compete for the same immunophilin.

Is rapamycin the "billion dollar molecule?" The cellular target of rapamycin (i.e., rapamycin's "calcineurin") recently has been identified. It is termed the FK.BP-Rapamycin Associated Protein (*FRAP*) (64). FRAP is a member of a newly discovered family of kinases which transfer phosphate groups not to a protein but rather to phosphatidylinositol, which is a phospholipid. Members of this new class of phosphatases normally participate in the cell cycle and their dysfunction has been implicated (but as yet unproven) in leading to medical disorders ranging from Alzheimer's Disease to cancer (140). FRAP is most abundant in the testis, raising the possibility that rapamycin could be a male contraceptive.

FK - Rapamycin Binding Protein (*FRAP*)

- cellular target of rapamicn / FKBP complex
- a phosphatidylinositol kinase
- essential role in cell cycle
- consequences of its inhibition ??

Figure 37.

So, in the clinical setting the long-term toxicity of rapamycin, which works by inhibiting FRAP, needs to be determined.

V. Novel Indications for Immunophilin Ligands

The elucidation of CsA and FK506 sensitive signalling pathways provides a conceptual framework for suggesting revolutionary approches to the treatment of important medical disorders other than immunosuppressive drug toxicity.

A. FK506 as a Treatment for Parkinson's Disease?

One of the best natural substrates for calcineurin is a dopamine receptor related protein (termed *DARPP*) found in the substantia nigra (126).

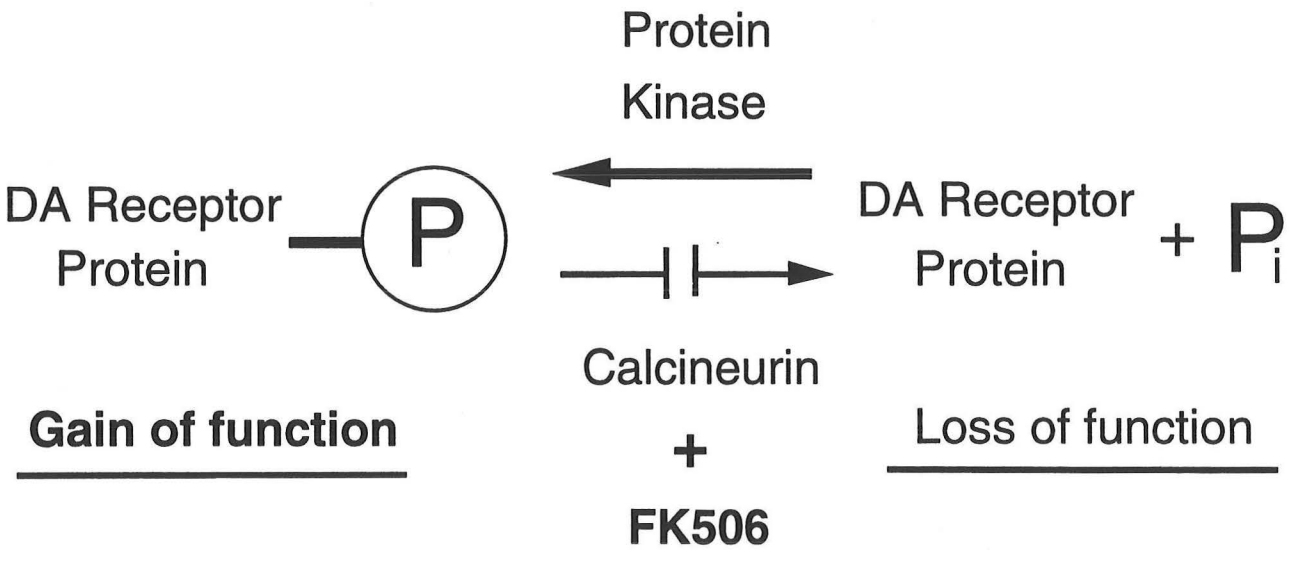
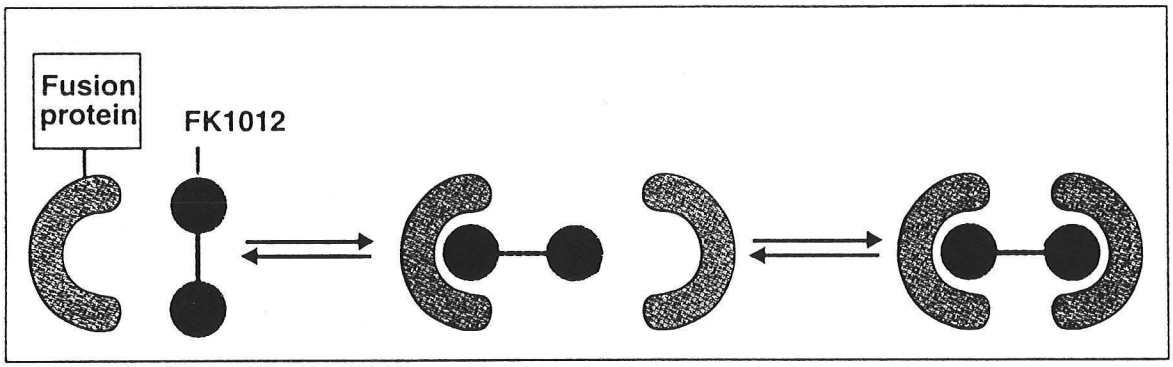


Figure 38.

When phosphorylated, DARPP increases the sensitivity of postsynaptic dopamine receptors. When DARPP is dephosphorylated by calcineurin, dopamine receptor sensitivity decreases. Thus, inhibition of calcineurin by FK506 theoretically would shift this reaction in favor of the phosphorylated form, thereby increasing dopaminergic neurotransmission which is the desired therapeutic goal. Because the enzymatic efficiency of calcineurin is much greater in dephosphorylating DARPP than NF-AT and other substrates in T cells, it may be possible to enhance dopaminergic neurotransmission in patients using low doses of FK506 which do not cause immune suppression (141). This hypothesis should be tested, since the current treatments of Parkinson's Disease are far from ideal.

B. Synthetic Immunophilin Ligands As Gene Therapy Switches?

Building on the concept of CsA and FK506 as molecular match makers, the Schreiber and Crabtree laboratories recently have embarked on a series of experiments demonstrating that they can exploit the "schatchen" function of FK506 to gain control of signal transduction (142,143). The idea is that dimerization of certain intracellular molecules can result in a huge gain in their function. Specifically, they dimerized the FK506 molecule to create a two-headed "FK1012 " molecule. The dimerization (a) removes the calcineurin binding site so FK1012 does not cause immune suppression and (b) exposes 2 FKBP binding sites, 1 on either side.



A marriage made in cells. The two-headed FK1012 molecule brings two fusion proteins together by binding to their immunophilin domains.

Figure 39. From (142).

They then spliced DNA to made a "fusion protein", which is the essential part of the T cell receptor attached to FKBP. The 2-headed FK1012 molecule brings 2 fusion proteins together by binding their immunophilin domains.

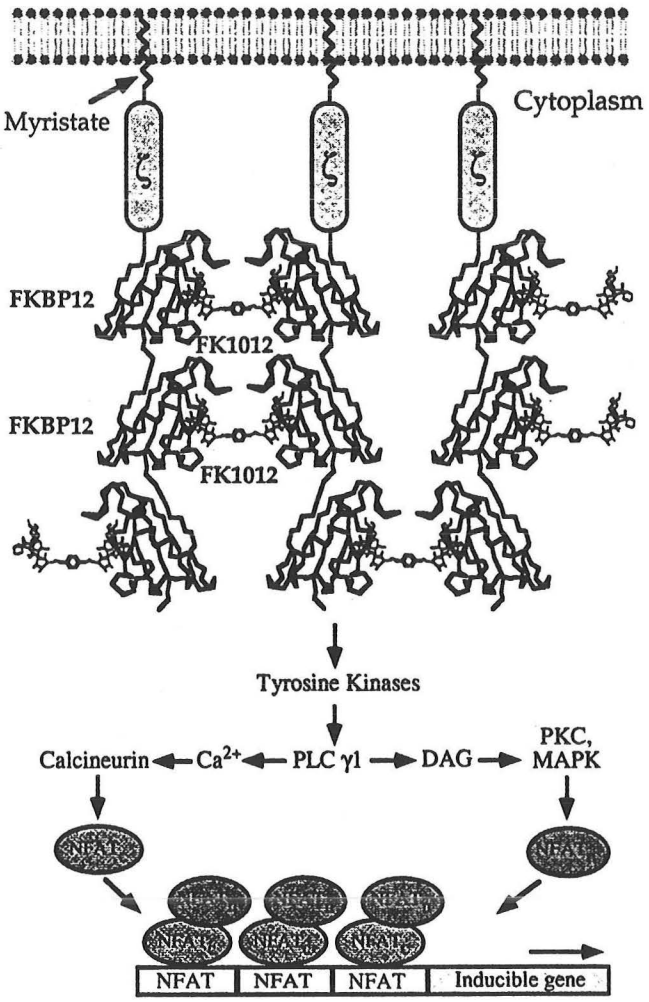


Figure 40 From (143).

A myristal group was then hooked to the fusion protein so that, when T cells were transfected with it, the fusion protein would become anchored to the inner surface of the cell membrane. The transfection had absolutely no effect on cell function until the T cells were exposed to FK1012, which crosses the cell membrane and dimerized the fusion proteins. Such dimerization mimicked the normal effect of antigen presentation: it induced IL-2 gene transcription. This dramatic effect was blocked by native FK506.

This research may have profound implications for human gene therapy. It may be possible in the future for patients to turn on their body's synthesis of a needed protein simply by taking a pill. For example, rather than a Type I diabetic patient injecting insulin, it may be possible to turn on modified insulin genes by swallowing a drug such as FK1012. In addition, this strategy may be useful in turning off undesirable genes, such as oncogenes and those leading to amyloidosis.

C. CsA as a Treatment for AIDS?

Like the immunosuppressant drugs CsA, FK506, and rapamycin, human immunodeficiency virus (HIV) interferes with T cell activation. Could there be a common mechanism?

HIV consists of a viral capsid which encompasses the RNA genome. Once inside the host cell, the capsid formation of the progeny viruses is initiated by the assembly of a protein termed *Gag.* (144). The precursor polypeptide, termed *Pr55gag*, is sufficient for the formation and release from host cells of virion particles (144).

Pr55gag turns out to be a high affinity ligand for cyclophilin, which is highly expressed in HIV (144,145).

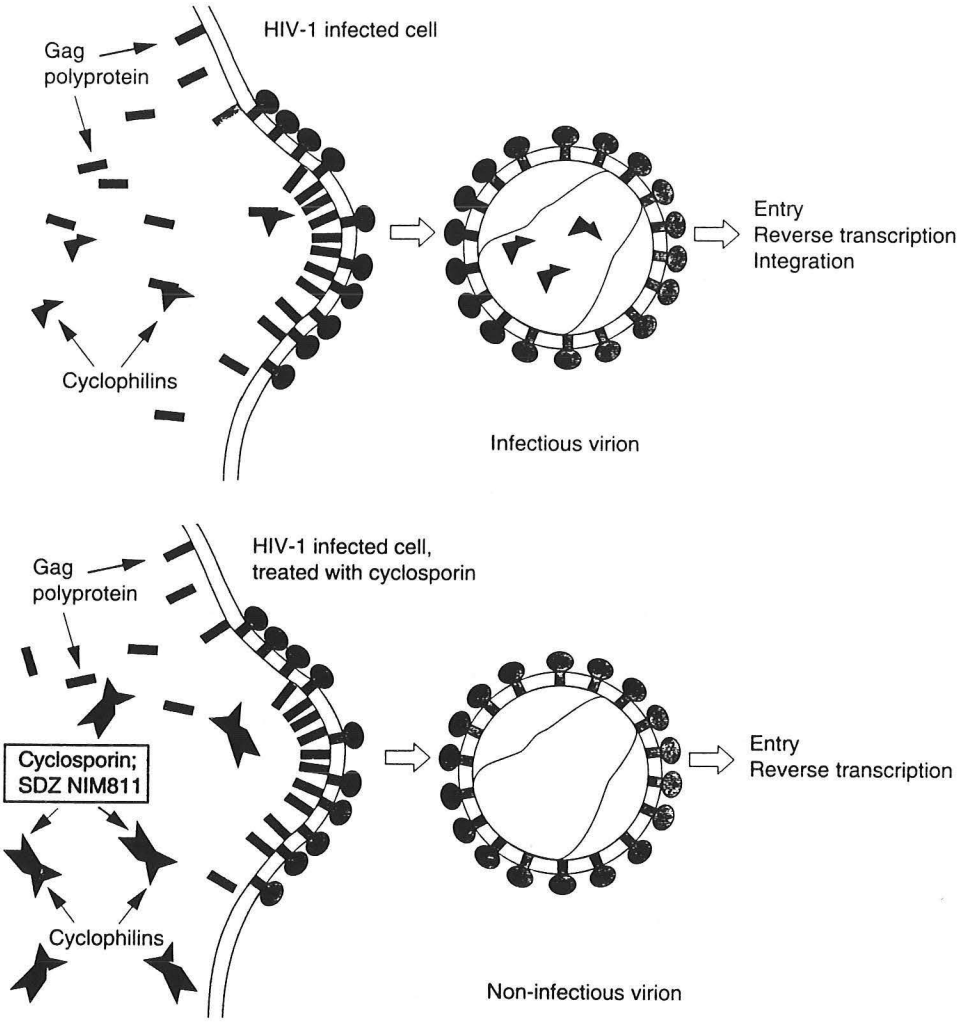


Figure 41. From (144).

Indeed, Pr55gag is similar to CsA in its affinity to bind cyclophilin. Unlike the CsA / cylophilin complex, however, the Pr55gag / cyclophilin complex has no effect on calcineurin (142). In contrast, the isomerase activity of the cyclophilin unfolds the Gag protein, a key step in the assembly of infectious virions. This interaction between

cyclophilin and Gag is blocked by CsA or a non-immunosuppressive ligand, SDZ NIM811. In the CsA-treated virion, the encorporatation of cyclophilin (which normally is substantial) is blocked, rendering the virion much less infectious than normal.

In addition to inhibiting HIV replication in vitro (146), CsA may even inhibit HIV replication in organ transplant recipients who are inadvertently infected with HIV at the time of transplantation (147) .

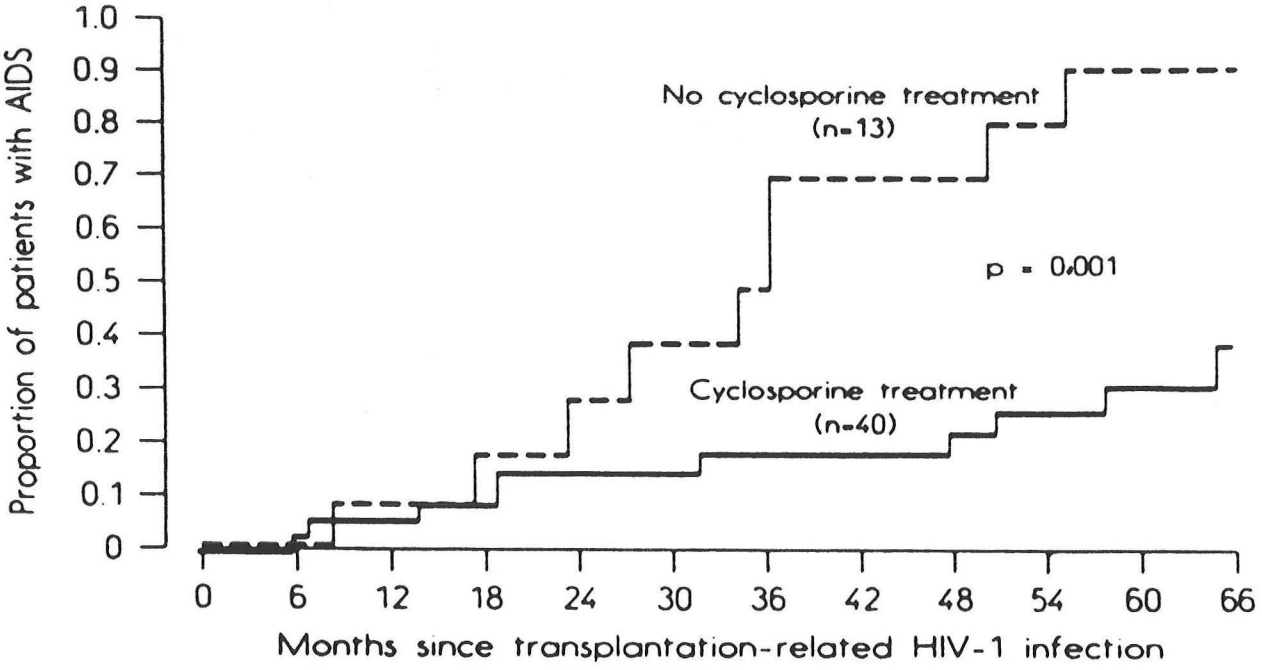


Figure 42. From (147).

In a review of 53 such cases, the 5-year cumulative incidence of AIDS was significantly lower in 40 transplant recipients treated with azathioprine and prednisone plus CsA than 13 transplant recipients treated with azathioprine and prednisone but not CsA: 31 vs. 90% ($P<0.001$). The delayed onset of AIDS in the CsA-treated patients previously has been assumed to an indirect effect of CsA decreasing the reservoir of T cells that could possibly be infected. However, the new basic research raises the alternative possibility that CsA inhibits HIV replication directly.

VI. Conclusions

In closing, I have attempted to synthesize a large amount of data which leads to a new conceptual framework for understanding the pathophysiologic basis of CsA-induced hypertension. This research has raised more questions than it has answered and the search for the perfect immunosuppressant drug continues. However, the recent scientific progress in this field is an excellent example of how clinical observations can foster basic research and the power of basic research to impact medical practice. The elucidation of CsA-senstive signalling pathways holds exciting promise for improving the treatment of a variety of disease processes far beyond immunosuppressant drug toxicity.

Acknowledgments: I am indebted to my postdoctoral fellows (Teresa Lyson, MD PhD, Gail Thomas, PhD, Mikael Sander, MD, Barbara Morgan, PhD, and Urs Scherrer, MD) who have been the principal investigators on this work; to our collaborators (Eduardo Marban, MD PhD, Brian O'Rourke, PhD, Thomas Sudhof, MD, Thomas Rosahl, PhD, and Stuart Schreiber, PhD) without whom most of this work could not have been accomplished; to Drs. Jere H. Mitchell, R. Sanders Williams, and Eduardo Marban for their continued advice and critical review of my work; and to Ms. Sybil Wallace for superb secretarial support.

References:

1. Danilevicius Z: SS. Cosmas and Damian. The patron saints of medicine in art. *JAMA* 201: 145-149, 1967.

2. Kahan BD: Cosmas and Damian in the 20th century? *N Engl J Med* 305: 280-281, 1981.

3. Black KS, Hewitt CW, Fraser LA, Osborne JG, Aghauer BM, Martin DC, and Furnas DW: Cosmas and Damian in the laboratory. *N Engl J Med* 306: 368-369, 1982.

4. Cohen DJ, Loertscher R, Rubin MF, Tilney NL, Carpenter CB, Strom TB. Cyclosporine. A new immunosuppressive agent for organ transplantation. *Ann Intern Med* 101:667-682, 1984

5. Kahan BD, ed. Complications of cyclosporine therapy. In: Cyclosporine: Therapeutic Use in Transplantation. Philadelphia, PA: Grune & Stratton, Inc. pp 519-623, 1988.

6. Kaye MP. Registry report: International Society for Heart and Lung Transplantation.Clinical Transplants. 39-44, 1991.

7. Kahan BD, ed. In: Cyclosporine: Application in autoimmune diseases. Philadelphia, PA: Grune & Stratton, pp 3-383, 1988.

8. Ellis CN, Fradin MS, Brown MD et al.: Cyclosporine for plaque-type psoriasis. Resullts of a mulitdose, double blind tiral. *N Engl J Med* 324: 277-284, 1991.

9. Tugwell P, Pincus T, Yocum D et al.: Combination therapy with cyclosporine and methotrexate in severe rheumatoid arthritis. The Methotrexate-Cyclosporine Combination Study Group. *N Engl J Med* 333: 137-141, 1995.

10. Thompson ME, Shapiro AP, Johnsen AM et al. New onset of hypertension following cardiac transplantation: A preliminary report and analysis. *Transplant Proc* 15 (Suppl 1):2573-2577, 1983.

11. Schachter M. Cyclosporine A and hypertension. *J Hypertens* 6:511-516, 1988

12. Bennett WM, Porter GA. Cyclosporine-associated hypertension. *Am J Med* 85:131-133, 1988.

13. Curtis JJ. Cyclosporine and posttransplant hypertension. *J Am Soc Nephrol* 2(Suppl 12):S243-S245, 1992.

14. Loughran T Jr., Deeg HJ, Dahlberg S, Kennedy MS, Storb R, Thomas ED. Incidence of hypertension after marrow transplantation among 112 patients

randomized to either cyclosporine or methotrexate as graft-versus-host disease prophylaxis. *Br J Haematol* 59 :547-53, 1985.

15. Kirk AJ, Omar I, Bateman DN, Dark JH. Cyclosporine-associated hypertension in cardiopulmonary transplantation. The beneficial effect of nifedipine on renal function. *Transplantation* 48:428-30, 1989.

16. Olivari MT, Antolick A, Ring WS. Arterial hypertension in heart transplant recipients treated with triple-drug immunosuppressive therapy. *J Heart Transplant* 8:34-39, 1989.

17. Morrison RJ, Short HD, Noon GP, Frost AE. Hypertension after lung transplantation. *J Heart Lung Transplant* 12: 928-31, 1993.

18. Myers BD, Ross J, Newton L, Luetscher J, Perlroth M. Cyclosporine-associated chronic nephropathy. *N Engl J Med* 1984;311 699-705.

19. Hughes RL: Cyclospoirne related central nervous system toxicity in cardiac transplantation. *N Engl J Med* 323: 420-421, 1990.

20. Rubin AM and Kang H: Cerebral blindness and encephalophathy with cyclosporine A toxicity. *Neurology* 1072-1076, 1987.

21. Lane RJM, Roche SE, Leung AAW, Greco A, and Lange LS: Cyclosporine neurotoxicity in cardiac transplant recipients. *J Neurol Neuosurg Psychiatry* 51: 1434-1437, 1988.

22. Atkinson K, Biggs J, Darveniz P, Boland J et al, Cyclosporine -associated central nervous system toxicity afeter allogenic bone marrow transplantation. *N Engl J Med* 310: 527, 1984.

23. Gross MLP, Pearson RM, Sweny P, Moorhead JF: Convulsions associated with cyclosporine A in renal transplant recipients. *Br Med J* 290: 555, 1985.

24. Vianello A, Mastrosimone S, Calconi G, Gatti PL, Calzavara P, Maresca MC. The role of hypertension as a damaging factor for kidney grafts under cyclosporine therapy. *Am J Kidney Dis* 21(5 Suppl 2):79-83, 1993.

25. Cosio FG, Dillon JJ, Falkenhein Me et al.: Racial differences in renal allograft survival: the role of systemic hypertension. *Kidney Int* 47:1136-41, 1995.

26. Frist WH, Stinson EB, Oyer PE, Baldwin JC, Shumway NE. Long-term hemodynamic results after cardiac transplantation. *J Thorac Cardiovasc Surg* 94(5):685-93, 1987.

27. Angermann CE, Spes CH, Willems S, Dominiak P, Kemkes BM, Theisen K. Regression of left ventricular hypertrophy in hypertensive heart transplant recipients treated with enalapril, furosemide, and verapamil. *Circulation* 84(2):583-93, 1991.

28. Ventura HO, Lavie CJ, Messerli FH, et al. Cardiovascular adaptation to cyclosporine-induced hypertension. *J Hum Hypertens* 8(4):233-7, 1994.

29. Bull DA, Hunter GC, Copeland JG, et al. Peripheral vascular disease in heart transplant recipients. *J Vasc Surg* 16(4):546-53, 1992.

30. Randall C, Starling MD, Cody RJ. Cardiac transplant hypertension. *Am J Cardiol* 65:106-111, 1990.

31. Joss DV, Barret AJ, Kedra JR et al., Hypertension and convulsions in chidren receiving cyclospoirne A. *Lancet* 1:906, 1982.

32. Reece DE, Frei-Lahr DA, Shepherd JD et al.: Neurologic complications in allogenic bone marrow tranasplant patients receiving cyclosporin. *Bone Marrow Transplant* : 393-401, 1991.

33. Schreiber S.L. Liu J, Albers MW et al: Immunophilin-ligand comp[lexes as probes of intracellulare signalling pathways. *Transplant Proc* 23: 2839-2844, 1991.

34. Werth B. The Billion-Dollar Molecule, Simon and Schuster: New York, pp 1-445, 1994.

35. Sander M and Victor RG: Hypertension after cardic transplantation: pathophysiology and management. *Current Opinion Nephrol Hypertension* 4: 443-451, 1995.

36. Mimran A, Mourad G, Ribstein J, Halimi J-M. Cyclosporine-associated hypertension. In: Hypertension: patophysiology, diagnosis and management, 2nd Ed., JH Laragh and BM Brenner (eds.). Raven Press, Ltd., New York: pp 2459-69, 1995.

37. Luke RG, Curtis JJ; Biology and treatment of tranplant hypertension. In: Hypertension: patophysiology, diagnosis and management, 2nd Ed., JH Laragh and BM Brenner (eds.). Raven Press, Ltd., New York: pp 2471-83, 1995.

38. Textor SC. De novo hypertension after liver transplantation [clinical conference]. *Hypertension* 22(2):257-67, 1993.

39. Weir MR. Therapeutic benefits of calcium channel blockers in cyclosporine-treated organ transplant recipients: blood pressure control and immunosuppression. *Am J Med* 90(5A):32S-36S, 1991.

40. First MR, Neylan JF, Rocher LL, Tejani A. Hypertension after renal transplantation. *J Am Soc Nephrol* 4(8 Suppl):S30-6, 1994.

41. Victor RG: The enigma of cyclosporine-induced hypertension. UT Southwestern Internal Medicine Grand Rounds, October 26, 1989.

42. Schreiber SL: Chemistry and biology of the immunophilins and their immunosuppressive ligands. *Science*. 251: 283-287, 1991.

43. Schreiber SL and GR Crabtree: The mechanism of action of cyclosporine A and FK506. *Immuol Today* 13: 136-142, 1992.

44. Kunz J, Hall MN. Cyclosporine A, FK506 and rapamycin: more than just immunosuppression. *TIBS* 18:334-338, 1993.

45. Handschumacker RE, Harding MW, Rice J, Drugge RJ, Speicher DW. Cyclophilin: A specific cytosolic binding protein for cyclosporine A. *Science* 226:544-547, 1984.

46. Walsh CT, Zydowsky LD, McKeon FD. Cyclosporin A, the cyclophilin class of peptidylprolyl isomerases, and blockade of T cell signal transduction. *J Biol Chem* 267: 13115-13118, 1992.

47. Takahashi N, Hayano T, Suzuki M. Peptidyl-prolyl cis-trans isomerase is the cyclosporine A binding protein cyclophilin. *Nature* 337:473-475, 1989.

48. Tropschug M, Bartelmess IB, adn Neupert W: Sensitivity to cyclosporine "A si mediated by cyclophilin in Neurospora crassa and Saccharomyces cerevisiae. Nature 342: 953-955, 1989.

49. Harding MW, Galat A, Uehling DE, Schreiber SL. A receptor for the immunosuppressant FK506 is a cis-trans peptidyl prolyl isomerase. *Nature* 341:758-760, 1989.

50. Siekierka JJ, Hung SHY, Poe M et al.: A cytosolic binding protein for the immunosuppressant FK506 has peptidyl-prolyl isomerase activity but it is distinct from cyclophilin. Nature 341: 755-757, 1989.

51. Bierer BE, Mattila PS, Standaert RF et al.: Two distinct signal transmission pathways in T lymphocytes are inhibited by complexes formed between an immunophilin and either FK506 or rapamycin. Proc Natl Acad Sci (USA) 87: 9231-9235, 1990.

52. Bierer BE, Somers PK, Wandless TJ, Burakoff SJ, Schreiber SL. Probing immunosuppressant action with a nonnatural immunophilin ligand. *Science*. 250:556-559, 1990.

53. Klee CB, Crouch TH, Krinks MH. Calcineurin: A calcium- and calmodulin-binding protein of the nervous system. *Proc Natl Acad Sci USA* 76:6270-6273, 1979.

54. Liu J, Farmer JD, Lane WS, Friedman J, Weissman I, Schreiber SL. Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. *Cell* 66:807-815, 1991.

55. Swanson SK-H, Born T, Zydowsky LD et al. : Cyclosporin-mediated inhibition of bovine calcineurin by cyclophilin A and B. *Proc Natl Acad Sci USA* 89:3741-3745, 1992.

56. Liu J, Albers MW, Wandless TJ et al. Inhibition of T cell signalling by immunophilin-ligand complex correlates with loss of calcineurin phosphatase activity. *Biochemistry* 31:3896-3901, 1992.

57. Clipstone NA, Crabtree GR. Identification of calcineurin as a key signalling enzyme in T-lymphocyte activation. *Nature* 357:695-697, 1992.

58. O'Keefe SJ, Tamura J, Kincaid RL, Tocci MJ, O'Neill EA. FK506- and CsA-sensitive activation of the interleukin-2 promoter by calcineurin. *Nature* 357:692-694, 1992.

59. Dutz JP, Fruman DA, Burakoff SJ, Bierer BE. A role for calcineurin in degranulation of murine cytotoxic T lymphocytes. *J Immunol* 150:2591-2598, 1993.

60. Hultsch T, Albers MW, Schreiber SL, Hohman RJ. Immunophillin ligands demonstrate common features of signal transduction leading to exocytosis or transcription. *Proc Natl Acad Sci USA* 88:6229-6233, 1991.

61. Trenn G, Taffs R, Hohman R, Kincaid R, Shevach EM, Sitkovsky M. Biochemical Characterization of the inhibitory effect of CsA on cytolytic T lymphocyte effector functions. *J Immunol* 142:3796-3802, 1989.

62. Triggiani M, Cirillo R, Lichtenstein LM, Marone G. Inhibition of histamine and prostaglandin D_2 release from human lung mast cells by CsA. *Int Arch Allergy Immunol* 88:253-255, 1989.

63. Pai SY, Furman DA, Leong T et al.: Inhibition of calcineurin phophatase activity in adult bone marrow transplant patients treated with cyclosporien A. *Blood* 84: 3974-3979, 1994.

64. Brown EJ, Albers MW, Shin TB et al., A mammalian protein target by G1-arresting rapamycin-receptor complex. *Nature* 369: 756-758, 1994.

65. Shenolikar S: Opening a window on immunosuppression. *Current Biology* 2: 549-551, 1992.

66. Ringe D: Molecular matchmakers. *Current Biology* 2: 545-547, 1992.

67. Kayyali US, Zhang W, Yee AG, Seidman J, and Potter H: Accumulation of hyperphosphorylated tau in knock-out mice lacking calcineurin. *Soc Neurosci Abstr* 21 (part 1) 742, 1995.

68. Koletsky AJ, Hrding MW, and Handschumacher RE: Cyclophilin: distribution and variant properties in normal and neoplastic tissues. *J Immunol* 137: 1054-1059, 1986.

69. Steiner JP, Dawson TM, Fotuhi M et al.: High brain densities of the immunophilin FKBP colocalized with calcineurin. *Nature* 358:584, 1992.

70. Dawson JF, Boland MP, Holmes CF. A capillary electrophoresis-based assay for protein kinases and protein phosphatases using peptide substrates. *Anal Biochem* 220(2):340-5, 1994.

71. Otsuka M, Terada Y, Yang T, Nonoguchi H, Tomita K, Marumo F. Localization of cyclophilin A and cyclophilin C mRNA in murine kidney using RT-PCR. *Kidney Int* 45(5):1340-5, 1994.

72. Bellet M, Cabrol C, Sassano P, Lëger P, Corvol P, Mënard J. Systemic hypertension after cardiac transplantation: Effect of cyclosporine on the renin-angiotensin-aldosterone system. *Am J Cardiol* 56:927-931, 1985.

73. Feutren G, Mihatsch MJ. Risk factors for cyclosporine-induced nephropathy in patients with autoimmune diseases. International Kidney Biopsy Registry of Cyclosporine in Autoimmune Diseases. *N Engl J Med* 326(25):1654-60, 1992.

74. Zietse R, Balk AH, v, et al. Time course of the decline in renal function in cyclosporine-treated heart transplant recipients. *Am J Nephrol* 14(1):1-5, 1994.

75. Bantle JP, Boudreau RJ, Ferris TF. Suppression of plasma renin activity by cyclosporine. Am J Med 83(1):59-64, 1987.

76. Singer DR, Markandu ND, Buckley MG, et al. Blood pressure and endocrine responses to changes in dietary sodium intake in cardiac transplant recipients. Implications for the control of sodium balance. *Circulation* 89(3):1153-9, 1994.

77. Thomson SC, Tucker BJ, Gabbai F, Blantz RC. Functional effects on glomerular hemodynamics of short-term chronic cyclosporine in male rats. *J Clin Invest* 83:960-969, 1989.

78. Lanese DM, Conger JD. Effects of endothelin receptor antagonist on cyclosporine-induced vasoconstriction in isolated rat renal arterioles. *J Clin Invest* 91(5):2144-9, 1993.

79. Myers BD, Newton L, Boshkos C, et al. Chronic injury of human renal microvessels with low-dose cyclosporine therapy. *Transplantation* ;46(5):694-703, 1988.

80. Curtis JJ, Luke RG, Dubovsky E, Diethelm AG, Whelchel JD, Jones P. Cyclosporin in therapeutic doses increases renal allograft vascular resistance. *Lancet* 2(8505):477-9, 1986.

81. Sigal NH, Dumont FJ, Siekierka JJ, Wiederrecht G, Parent SA, Brizuela L. Is FKBP involved in the immunosuppressive and/or toxic mechanism of action of FK 506? *Transplant Proc* 23(6):2846-9, 1991.

82. Sigal NH, Dumont F, Durette P, et al. Is cyclophilin involved in the immunosuppressive and nephrotoxic mechanism of action of cyclosporin A? *J Exp Med* 173(3):619-28, 1991.

83. Dumont FJ, Staruch MJ, Koprak SL, et al. The immunosuppressive and toxic effects of FK-506 are mechanistically related: pharmacology of a novel antagonist of FK-506 and rapamycin. *J Exp Med* 176(3):751-60, 1992.

84. Textor SC, Burnett JC, Jr., Romero JC et al. Urinary endothelin and renal vasoconstriction with cyclosporine or FK506 after liver transplantation. *Kidney Int* 47:1426-33, 1995.

85. Richards NT, Poston L, Hilton PJ. Cyclosporine A inhibits endothelium-dependent, prostanoid-induced relaxation in human subcutaneous resistance vessels. *J Hypertension* 8: 159-163, 1990.

86. Roullet JB, Xue H, McCarron DA, Holcomb S, Bennett WM. Vascular mechanisms of cyclosporin-induced hypertension in the rat. *J Clin Invest* 93(5):2244-50, 1994.

87. Amore A, Gianoglio B, Ghigo D et al. A possible role for nitric oxide in modulating the functional cyclosporine toxicity by arginine. *Kidney Int* 47:1507-14, 1995.

88. Marumo T, Nakaki T, Hishikawa K, Suzuki H, Kato R, Saruta T. Cyclosporin A inhibits nitric oxide synthase induction in vascular smooth muscle cells. *Hypertension* 25(4 Pt 2):764-8, 1995.

89. Meyer-Lehnert H, Schrier RW. Potential mechanism for cyclosporine-induced vascular smooth muscle contraction. *Hypertension* 13: 352-360, 1989.

90. Bokemeyer D, Kramer HJ, Meyer-Lehnert H. Atrial natriuretic peptide blunts the cellular effects of cyclosporine in smooth muscle. *Hypertension* 21(2):166-72, 1993.

91. Bokemeyer D, Friedrichs U, Backer A, Kramer HJ, Meyer-Lehnert H. Cyclosporine A enhances total cell calcium independent of Na-K-ATPase in vascular smooth muscle cells. *Clin Investig* 72(12):992-5, 1994.

92. Lamb FS, Webb RC. Cyclosporine augments reactivity of isolated blood vessels. *Life Sciences* 40(26): 2571-2578, 1987.

93. Xue H, Bukoski RD, McCarron DA, Bennett WM. Induction of contraction in isolated rat aorta by cyclosporine. *Transplantation* 43: 715-718, 1987.

94. Rego A, Vargas R, Suarez KR, Foegh ML, Ramwell PW. Mechanism of cyclosporin potentiation of vasoconstriction of the isolated rat mesenteric arterial bed: role of extracellular calcium. *J Pharmacol Exper Ther* 254:799-808, 1990.

95. Brillantes AB, Ondrias K, Scott A, et al. Stabilization of calcium release channel (ryanodine receptor) function by FK506-binding protein. *Cell* 77(4):513-23, 1994.

96. Cameron AM, Steiner JP, Sabatini DM, Kaplin AI, Walensky LD, Snyder SH. Immunophilin FK506 binding protein associated with inositol 1,4,5-triphosphate receptor modulates calcium flux. *Proc Natl Acad Sci USA* 92(5):1784-8, 1995.

97. Garr MD, Paller MS. Cyclosporine augments renal but not systemic vascular reactivity. *Am J Physiol* 258: F211-F217, 1990.

98. Haas GJ, Wooding-Scott M, Binkley PF, Myerowitz PD, Kelley R, Cody RJ. Effects of successful cardiac transplantation on plasma endothelin. *Am J Cardiol* 71(2):237-40, 1993.

99. Textor SC, Powell-Smith L, Telles T. Altered pressor responses to NE and ANG II during cyclosporin A administration to conscious rats. *Am J Physiol* 258: H854-860, 1990.

100. Kaye D, Thompson J, Jennings G, Esler M. Cyclosporine therapy after cardiac transplantation causes hypertension and renal vasoconstriction without sympathetic activation. *Circulation* 88:1101-1109, 1993.

101. Takeda Y, Miyamori I, Yoneda T, Takeda R. Increased concentration of endothelin messenger RNA in the mesenteric arteries of cyclosporine-induced hypertensive rats. *Am J Hypertens* 6 :427-30, 1993.

102. Bunchman TE, Brookshire CA. Cyclosporine-induced synthesis of endothelin by cultured human endothelial cells. *J Clin Invest* 88(1):310-4, 1991.

103. Grieff M, Loertscher R, Shohaib SA, Stewart DJ. Cyclosporine-induced elevation in circulating endothelin-1 in patients with solid-organ transplants. *Transplantation* 56(4):880-4, 1993.

104. Stein CM, He H, Pincus T, and Wood AJJ: Cyclosporine impairs vasodilation without increased sympathetic activity in humans. *Hypertension* 26: 705-710, 1995.

105. Murray BM, Paller MS, Ferris TF. Effects of cyclosporine administration on renal hemodynamics in conscious rats. *Kidney Int* 28:767-774, 1985.

106. Moss NG, Powell SL, Falk RJ. Intravenous cyclosporine activates afferent and efferent renal nerves and causes sodium retention in innervated kidneys in rats. *Proc Natl Acad Sci USA* 82:8222-8226, 1985.

107. Scherrer U, Vissing SF, Morgan BJ, Rollins JA, Tindall RSA, Ring S, Hanson P, Mohanty PK, Victor RG. Cyclosporine-induced sympathetic activation and hypertension after heart transplantation. *N Engl J Med* 323:693-699, 1990.

108. Morgan BJ, Lyson T, Scherrer U, Victor RG. Cyclosporine causes sympathetically mediated elevations in arterial pressure in rats. *Hypertension* 18:458-466, 1991.

109. Chiu PJ, Vemulapalli S, Sabin C, Rivelli M, Bernardino V, Sybertz EJ. Sympathoadrenal stimulation, not endothelin, plays a role in acute pressor response to cyclosporine in anesthetized rats. *J Pharmacol Exp Ther* 261:994-999, 1992.

110. Tronc F, Carrier M, Pelletier CL. Mechanism of hind limb vasoconstriction due to cyclosporine A in the dog. *Circ Res* 71:1159-1164.1992.

111. Lyson T, Ermel LD, Belshaw PJ, Alberg DG, Schreiber SL, Victor RG. Cyclosporine and FK-506-induced sympathetic activation correlates with calcineurin-mediated inhibition of T-cell signalling. *Circ Res* 73:596-602, 1993.

112. Lyson T, McMullan DM, Ermel LD, Morgan BJ, Victor RG. Mechanism of cyclosporine-induced sympathetic activation and acute hypertension in rats. *Hypertension* 23: 667-675, 1994

113. Jacobsen TN, Morgan BJ, Scherrer U et al.: Relative contributions of cardopulmonaryu and sinoaortic baroreflexes in causing symapthetic activaiton in the human skeletal muscle circulation during orhtostatic stress. *Circ Res* 73: 367-378, 1993.

114. Cavero PG, Sudhir K, Galli F et al.: Effect of orthotopic cardiac transplantation on peripheral vascular function in congestive heart failure: influence of cyclosporine therapy. *Am Heart J* 127: 1581-1587, 1994.

115. Seals DR, Suwarno NO, Joyner MJ et al.: Respiratory modulation of muscle sympathetic nerve activity in intact and lung denervated humans. *Circ Res* 72: 440-54, 1993.

116. Elam M, Casale R, La Rovere M-T, Mortara A, Tavazzi L. Is sympathetic neural hyperactivity in chronic heart failure affected by heart transplantation? *Eur Heart J* 14:521-525, 1993.

117. Baily RG, Davis D, Clemson B, Zelis R. Normalization of the norepinephrine kinetic response to orthostatic stress after cardiac transplantation. *Am J Cardiol* 74:624-5, 1994.

118. Hamill OP, Marty A, Neher E, Sakmann B, and Sigworth FJ: Improved patch-clamp techniques for high-resolution current recrding from cells and cell-free membrane patches. *Pflugers Arch* 391: 85-100, 1981.

119. Andresen MC, Kunze DL. Nucleus Tractus solitarius - gateway to neural circulatory control. *Ann Rev Physiol* 56:93-116, 1994.

120. Mayer ML, Westbrook GL. The physiology of excitatory amino acids in the vertebrate central nervous system. *Prog Neurobiol* 28:197-276, 1987.

121. Sved AF, Gordon FJ. Amino acids as central neurotransmitters in the baroreceptor reflex pathway. *News Physiol Sci* 9:243-246, 1994.

122. Guyenet PG, Filtz TM, Donaldson SR. Role of excitatory amino acids in rat vagal and sympathetic baroreflexes. *Brain Res* 407:272-284, 1987.

123. Radhakrishnan, Henry JL. Excitatory amino acid receptor mediation of sensory inputs to functionally identified dorsal horn neurons in cat spinal cord. *Science* 55:521-544, 1993.

124. Yoshimura M, Jessell T. Amino acid-mediated EPSPs at primary afferent synapses with substantia gelatinosa neurones in the rat spinal cord. *J Physiol Lond* 430:315-335, 1990.

125. Lieberman DN, Mody I. Regulation of NMDA channel function by endogenous Ca^{2+}-dependent and phosphatase. *Nature* 369:235-239, 1994.

126. King MM, Huang CY, Chock PB et al.: Mammalian brain phosphoproteins as substrates for calcineurin. J *Biol Chem* 259: 8080-8083, 1984.

127. Nichols RA, Suplick GR, and Brown JM: Calcineurin-mediated protein dephosphorylation in brain nerve terminals regulates the release of glutamate. *J Biol Chem* 269: 23817-23823, 1994.

128. Victor RG, GD Thomas, E Marban, B. O'Rourke: Presynaptic modulation of cortical synaptic activity by calcineurin. *Proc Natl Acad Sci USA* 92: 6269-6273, 1995.

129. Greengard P, Valtorta F, Czernik AJ et al.: Synaptic vesicle phosphoproteins and regulaiton of synaptic function. *Science* 259: 780-785, 1993.

130. Roshal TW, Geppert M, Spillane D et al.: Short-term synaptic plasticity is altrered in mice lacking syanpsin I. *Cell* 75: 661-670, 1993.

131. Rosahl TW, Spillane D, Missler M et al.: Essential functions of syanpsisn I and II in synaptic vesicle regulation. *Nature* 375:488-493, 1995.

132. van der Schaaf MR, Hene RJ, Floor M, Blankestijn PJ, Koomans HA. Hypertension after renal transplantation. Calcium channel or converting enzyme blockade? *Hypertension* 25(1):77-81, 1995.

133. Textor SC, Schwartz L, Wilson DJ, et al. Systemic and renal effects of nifedipine in cyclosporine-associated hypertension. *Hypertension* 23(1 Suppl):I220-4, 1994.

134. Armitage JM, Kormos RL, Fung J, Starzl TE. The clinical trial of FK506 as primary and rescue immunosuppression in adult cardiac transplantation. *Transplant Proc.* 23:3054-3057, 1991.

135. Armitage JM, Kormos RL, Morita S, et al. Clinical trial of FK 506 immunosuppression in adult cardiac transplantation. *Ann Thorac Surg* 54(2):205-10, 1992.

136. Armitage JM, Fricker FJ, del Nido P, Starzl TE, Hardesty RL, Griffith BP. A decade (1982 to 1992) of pediatric cardiac transplantation and the impact of FK 506 immunosuppression. *J Thorac Cardiovasc Surg* 05(3):464-72, 1993.

137. The US Multicenter FK506 Liver Study Group. A comparison of tacrolimus (FK506) and cyclosporine for immunosuppression in liver transplantation. *New Engl J Med* 331(17):1110-1115, 1994.

138. The European FK506 Multicenter Study Group. Randomized trial comparing tacrolimus (FK506) and cyclosporine in prevention of liver allograft rejection. *Lancet* 344:423-8, 1994.

139. Tu Y, Stepkowski SM, Chou T-C, Kahan BD: The synegistic effects of cyclosporine, sirolimus, and brequinar on heart allograft survival in mice. *Transplantation* 59:177-83, 1995.

140. Keith CT and Schreiber SL: PIK-related knasses: DNA repair, recombination, and cell cycle check points. *Science* 270: 50-51, 1995.

141. Wera S and Neyts J: Calcineurin as a possible new target for treatment of Parkinson's Disease. *Medical Hyoptheses* 43: 132-134, 1993.

142. Travis J: Making molecular matches in the cell. *Science* 262: 989, 1993.

143. Spencer DM, Wandless TJ, Schreiber SL, and Crabtree GR: Controlling signal transduction with synthetic ligands. Science 262: 1019- 1024,1994.

144. Thali M: Cyclosporins: immunosuppressive drugs with anti-HIV activity. Molecular *Medicine Today* 1: 287-291, 1995.

145. Thali M, Bukovsky A, Kondo E et al.: Functional association of cyclophilin A with HIV-1 virions *Nature* 372 :319-20, 1994

146. Karpas A, Lowdell M, Jacobsen SK et al.: Inhibition of human immunodeficiency virus and growth of infected T cells by the immunosuppressive drugs cyclosporin A and FK506. *Proc Natl Acad Sci USA* 89: 8351-8355, 1992.

147. Schwarz A, Offermann G, Keller F et al.: The effect of cyclosporine on the progression of human immunodeficiency virus 1 infection transmitted by transplantation--data on four cases and review of the literature. *Transplantation* 55: 95-103, 1993.