Sympathetic Neural Mechanisms of Cyclosporine-Induced Hypertension

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The immunosuppressant drug cyclosporine A (CsA) has emerged as an important new cause of hypertension in both organ transplant recipients and patients with autoimmune diseases. Despite the clinical importance of this hypertension, the underlying mechanisms have been enigmatic. This article presents a conceptual framework for understanding the pathophysiologic basis of CsA-induced hypertension and focuses on the hypothesis that a common molecular mechanism is involved in mediating the immunosuppressive and the hypertensive effects of CsA. This mechanism involves the binding of CsA to a newly discovered class of cytoplasmic receptors (termed "immunophilins") not only in T lymphocytes but also in the kidney, vascular smooth muscle, and central nervous system, which are the main target tissues mediating CsA-induced hypertension. Binding of CsA to its receptor leads to inhibition of calcineurin, the Ca²⁺/calmodulin-dependent protein phosphatase. Evidence is reviewed to support the hypothesis that calcineurin inhibition plays a pivotal role in mediating both CsA-induced immunosuppression and hypertension, the latter being produced at least in part by sympathetic neural activation. The elucidation of novel CsA-sensitive cellular signaling pathways has lead to the search for the ideal immunosuppressant drug, one which retains CsA's immunosuppressive efficacy but without its toxicity. Am J Hypertens 1996; 9:121S-138S

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Cyclosporine A (CsA) was the first immunosuppressive agent with relative T cell specificity. 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 1980s.1

In addition, CsA is proving to be remarkably efficacious for an increasing list of diseases with definite or presumed autoimmune etiology,2 including psoriasis and atopic dermatitis,3-8 uveitis,9 rheumatoid arthritis,10-14 ulcerative colitis,15 and Crohn's disease.16 Despite these beneficial effects, CsA causes considerable toxicity, most notably renal insufficiency,6,17,16 central nervous system toxicity,19-24 and hypertension.25-32

Indeed, CsA has emerged as a major new cause of hypertension; and it has become one of the most common causes of secondary hypertension. Two forms of CsA-induced hypertension have been described in humans: 1) an acute hypertensive effect developing with the first dose of CsA,33-34 which has been linked to the onset of seizures seen in young bone marrow recipients after the first large dose of CsA,35,36 and 2) chronic hypertension developing after weeks or months of...
CsA treatment and requiring antihypertensive medication. In heart transplant recipients, for example, the incidence of hypertension has increased from ~20% in the pre-CsA era to ~90% currently.30,31,37-40

Figure 1 summarizes data from several randomized clinical trials comparing the incidence of chronic hypertension in patients treated with traditional immunosuppressive agents (eg, azathioprine and prednisone) alone or in combination with cyclosporine.4,8,10,12,13,29,39,41,42 The results from these relatively small prospective studies have been corroborated by a wealth of retrospective data.30,31,38,40 CsA clearly is a proximate cause of this new form of hypertension, which resolves when the drug is discontinued.4,8,43-45 However, the incidence and severity of CsA-induced hypertension are modulated by several factors including the dose5 and duration9 of CsA as well as the patient population being treated. Although CsA causes chronic hypertension in all patient groups, the incidence and severity of the hypertension appears to be consistently greater in heart transplant recipients than in patients receiving CsA for other organ transplants or for autoimmune diseases. In heart transplant recipients, CsA-induced hypertension typically is moderate to severe and often requires combination antihypertensive therapy.31,37,40

Hypertension after transplantation has been implicated as a major reason for the shift in primary cause of death from primarily related to infectious disease in the 1970s to cardiovascular disease related in the 1980s.47 Hypertension is a risk factor for shortened allograft survival,38,49 progressive renal failure,17,50 left ventricular hypertrophy,51-53 coronary allograft and peripheral vascular angiopathy,4,45 and encephalopathy.54

In addition, hypertension is one of the main reasons why internists are hesitant to prescribe and continue CsA for patients with autoimmune diseases, for which this drug can be remarkably efficacious. Thus, hypertension is an important reason for withdrawal of patients from the CsA arm of placebo-controlled studies of CsA for autoimmune diseases.6,56

Despite the clinical importance of CsA-induced hypertension, the underlying mechanisms remain enigmatic. The aim of this review is to present a conceptual framework for understanding the pathophysiologic basis of CsA-induced hypertension. This review is not meant to be an encyclopedia, rather it focuses on the hypothesis that a common molecular mechanism is involved in mediating the immunosuppressive and hypertensive effects of CsA. In this regard, CsA has proven to be not only a powerful immunosuppressant drug in patients, but also a powerful new scientific probe to study cellular signal transduction,57 the process by which extracellular molecules alter intracellular function.

MECHANISM OF CsA-INDUCED IMMUNOSUPPRESSION

CsA was the first immunosuppressive agent with relative T cell specificity.58,59 It exerts a greater effect on T than B cells and it causes minimal bone marrow

FIGURE 1. Graphic representation of data compiled from a total of nine prospective, randomized trials comparing the incidence of chronic hypertension (defined as need to treat) in patients treated with immunosuppressive regimens with or without cyclosporine A. Numbers in parentheses indicate the studies referenced. Abbreviations: CsA, cyclosporine A; Pred, prednisone; Aza, azathioprine; Cpm, cyclophosphamide; Mtx, methotrexate; Plac, placebo.
suppression. This means that, compared to the more traditional agents prednisone and azathioprine, CsA causes much fewer opportunistic infections.\textsuperscript{39,47,60} The drug was used clinically for almost a decade before the molecular mechanism of action was elucidated. During the past 6 years, the scientific progress in this field has been remarkable\textsuperscript{57,61–63} and is summarized as follows.

In T cells, CsA inhibits transcriptional activation of the interleukin 2 (IL-2) gene and this is thought to be the primary immunosuppressive action.\textsuperscript{62,64–66} The effect is not at the level of the T cell receptor located on the cell surface but somewhere between the cytoplasm and nucleus. CsA is lipophilic and passes readily through cell membranes and interacts with a newly discovered family of intracellular receptors. The cytoplasmic receptor for CsA is a soluble 15 kD protein called cyclophilin.\textsuperscript{51–53,67–69} Actually, there are a dozen or so cyclophilins, among which cyclophilin A is expressed ubiquitously in mammalian cells, and is considered the most important for CsA’s immunosuppressive actions.\textsuperscript{70} Handschumacker et al\textsuperscript{70} hypothesized that the immunosuppressive action of CsA is dependent upon its binding to cyclophilin, since one could rank order the binding of various natural and synthetic CsA analogs to cyclophilin with their immunosuppressive potency in cell culture.

The cyclophilins possess intrinsic enzymatic activity: they catalyze folding of proteins, a property termed \textit{cis-trans} peptidyl-prolyl isomerase.\textsuperscript{66,69} CsA binding was postulated to result in a loss of function of cyclophilin.\textsuperscript{57} Thus, prevention of the folding of some unknown target protein was assumed to be the CsA-sensitive step in T cell activation. This assumption, however, was refuted soon after the discovery of FK506 and rapamycin, two more immunosuppressants with relative T cell specificity.\textsuperscript{61–63}

Whereas CsA is a cyclic polypeptide, FK506 and its structural analog rapamycin are macrolides (Figure 2). Like CsA, FK506 is a fungal product and the two drugs share the identical mechanism of immunosuppressive action. FK506 binds to and inhibits the activity of another isomerase called FK binding protein (FKBP).\textsuperscript{71,72} FKBPs and cyclophilins collectively are called “immunophilins.” However, the two drugs and their respective immunophilins have no structural features in common. Like FK 506, rapamycin is a high affinity ligand for FKBP and drug binding completely inhibits its isomerase activity. Despite this inhibition and the considerable structural homology between rapamycin and FK506, their immunophilin complexes produce immunosuppression by completely disparate mechanisms of action.\textsuperscript{61–63,73} This dissociates isomerase inhibition from T cell inhibition. Instead, as described below, immunophilin-ligand interactions alter the three-dimensional structure of the immunosuppressive drugs in such a way as to allow them to bind to key “downstream” target proteins.

Figure 3A depicts schematically the current thinking about the primary molecular mechanism of CsA- and FK506-induced versus rapamycin-induced inhibition of T cell activation. All three drugs are lipophilic and thus membrane permeant, but they are biologically inert until they bind to their respective cytosol immunophilin receptors, cyclophilin, and FK binding protein. The common target of CsA and FK506, as their immunophilin complexes, is the \textit{Ca}^{2+}/calmodulin dependent phosphatase calcineurin,\textsuperscript{74–76} which is inhibited by both immunophilin-ligand complexes. In
FIGURE 3. Schematic representation of (A) calcineurin’s pivotal role in mediating CsA- and FK506-induced immunosuppression, and (B) its putative role in mediating CsA- and FK506-induced hypertension. Abbreviations: CsA, cyclosporine A; CyP, cyclophilin; FKBP, FK506 binding protein; FRAP, FK binding protein/rapamycin associated protein; NF-AT, nuclear factor of activated T cells (NF-ATc, catalytic subunit); IL-2, interleukin 2. See text for explanation.

The T cell, the relevant substrates for calcineurin are phosphorylated transcriptional factors, such as the nuclear factor of activated T cells (NF-AT) \(^87-88\). When dephosphorylated in the cytoplasm by calcineurin, the catalytic subunit enters the nucleus and initiates transcriptional activation of the IL-2 gene. \(^82-85\) CsA- or FK506-induced inhibition of calcineurin prevents the dephosphorylation necessary for nuclear translocation and thereby prevents IL-2 gene transcription. \(^82-84,86\) Thus, inhibition of calcineurin plays a pivotal role in preventing T cell activation (e.g. graft rejection, autoimmunity) by preventing dephosphorylation of NF-AT. \(^62,75-79\)

Calcineurin also is involved in several other steps in T cell activation. \(^87-90\) In the clinical setting, CsA treatment is accompanied by decreased calcineurin activity in circulating mononuclear cells. \(^91-93\)

Although rapamycin binds FK binding protein with high affinity, this immunophilin complex, while highly immunosuppressive, has absolutely no effect on calcineurin and it causes immunosuppression by inhibiting a completely different Ca\(^{2+}\)-independent step in T cell activation. \(^74\) The cellular target of the rapamycin-FKBP complex is termed the FKBP-Rapamycin Associated Protein (FRAP). \(^94\) Rapamycin binding to FRAP in turn leads to inhibition of kinase activity, and this inhibition is considered responsible for rapamycin’s immunosuppressive action. \(^73,94-98\)

MECHANISMS OF CsA-INDUCED HYPERTENSION

Calcineurin and the immunophilins are even more plentiful in nonlymphoid tissues, such as the nervous system, vascular smooth muscle, and kidney. \(^102,105\) Because these are the main target sites for CsA-induced toxicity—especially hypertension—inhbition of calcineurin in these different tissues could mediate 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. This hypothesis is illustrated schematically in Figure 3B.

There is debate as to the relative contribution of various target tissues to the pathogenesis of CsA-induced hypertension. Renal, vascular, and neural mechanisms all have been impli-
cated and are not mutually exclusive. The relative contributions may vary considerably depending on a number of factors including species, dose, and duration of CsA treatment. This review focuses on the potential role of neural mechanisms, which reflects the work from this laboratory. Because renal and vascular mechanisms are reviewed only briefly, the reader is referred to several excellent reviews.

RENAL MECHANISMS

The first thought was that CsA-induced hypertension is a volume-dependent form of hypertension due to direct nephrotoxic effects of CsA on the renal tubules. In this regard, plasma renin activity generally is found to be normal or low in patients with CsA-induced hypertension, providing indirect evidence for an expanded plasma volume. A recent study by Singer et al. indicates that CsA-induced hypertension after heart transplantation is partially sensitive to dietary sodium intake: blood pressures were 148/97 vs. 137/94 mm Hg on a high (350 mmol/day) versus low sodium diet (10 mmol/day). By comparison, though, these salt-induced changes in blood pressure were less than those observed in patients with essential hypertension.

Several clinical studies demonstrate convincingly that CsA-induced hypertension is dissociated both from histologic changes on renal biopsy and from increases in serum creatinine. The prevailing view is that CsA-induced decreases in renal function are due mainly to preferential pregglomerular constriction of the afferent renal arteriole rather than to direct poisoning of nephrons. For example, after administration of CsA (10 mg/kg/day) for 9 days to healthy volunteers, a small but significant increase in blood pressure was accompanied by decreased renal plasma flow but unchanged urinary sodium excretion, suggesting renal vasoconstriction in the absence of an expanded plasma volume.

Although there are large gaps in our understanding of the underlying mechanisms mediating CsA-induced renal vasoconstriction, there is increasing evidence to suggest that inhibition of calcineurin is involved. In dogs, rats, and mice, CsA, FK506, and their structural analogs caused decreases in renal function in a way that closely paralleled their ability to cause calcineurin-mediated immunosuppression. Rapamycin, for example, had no effect on renal function in these models.

In human liver transplant recipients, FK506 and CsA (in doses that produce comparable clinical immunosuppression) recently were found to produce comparable increases in vascular resistance and decreases in glomerular filtration rate. Of note, calcineurin is involved in mediating the effects of α-adrenergic stimulation on Na-K-ATPase in the distal nephron. Both vascular and sympathetic neural mechanisms have been implicated in causing CsA- and FK-506-induced vasoconstriction in the renal and other regional vascular beds.

VASCULAR MECHANISMS

CsA could inhibit vasodilator pathways or activate vasoconstrictor pathways. Increasing evidence suggests that nitric oxide synthesis produces tonic endothelial dependent vasodilatation in the peripheral circulation; inhibition of this tonic vasodilatation could contribute to hypertension. In this regard, endothelium dependent vasodilatation was found to be attenuated in subcutaneous arteries from CsA-treated patients and in the mesenteric arteries from CsA-treated rats. Administration of L-arginine, the substrate for nitric oxide synthase, conferred protection against CsA-induced renal vasoconstriction in rats. Nitric oxide synthase is a calcineurin substrate in vitro, suggesting a potential mechanism to explain an inhibitory effect of CsA on nitric oxide-induced vasodilatation. However, there are conflicting data as to whether calcineurin inhibition decreases, or has any effect on the activity of nitric oxide synthase in vitro, and the physiologic significance in vivo remains to be determined.

CsA has been reported to enhance the transient increase in intracellular Ca²⁺ during agonist stimulation, thereby augmenting the contractile response of isolated blood vessels to a variety of vasoconstrictor agents, such as norepinephrine or angiotensin II. CsA could either enhance Ca²⁺ influx from the extracellular compartment or it could enhance Ca²⁺ release from intracellular stores. There is increasing evidence, for example, that the FKBP modulates the ryanodine and the inositol-1,4,5-triphosphate (IP₃) receptor, the Ca²⁺ release channels.

There is some evidence to suggest that CsA causes a generalized augmentation of vascular reactivity in vivo, but it is unclear how much this contributes to hypertension. In human heart transplant recipients undergoing maintenance CsA therapy, a standard dose of CsA was found to cause renal vasoconstriction without evidence for sympathetic activation, suggesting a vascular mechanism of action. However, the contribution of such vasoconstriction to CsA-induced hypertension is unknown, since blood pressure remained unchanged during acute CsA dosing despite acute renal vasoconstriction. In patients with rheumatoid arthritis receiving short-term treatment with CsA versus placebo, CsA was accompanied by attenuated venodilator responses of hand veins to isoproterenol or prostaglandin E₂ but had no effect on venoconstrictor responses to the α-adrenergic agonist phenylephrine or on norepinephrine spillover.

Endothelin, the most potent endogenous vasocon-
of CsA (10 mg/kg/day) to healthy volunteers. The importance, if any, of increased endothelin in CsA-induced hypertension is unknown. Studies of rodents have indicated that chronic CsA-induced hypertension is partially attenuated by an endothelin receptor antagonist, but that acute CsA-induced hypertension is unaffected by endothelin inhibition. In contrast, acute CsA-induced hypertension is consistently abolished by chemical or surgical sympathectomy, indicating sympathetic neural mediation.

NEURAL MECHANISMS

As suggested by its name, calcineurin was first discovered in the brain where it accounts for >1% of total protein. Despite this abundance, until recently almost nothing was known about its neuronal function. In 1992, Steiner et al. demonstrated the colocalization of calcineurin with high densities of immunophilins in rat brain and brainstem. This colocalization prompted the hypothesis that during systemic administration of CSA, which crosses the blood-brain barrier, immunophilin-ligand inhibition of calcineurin leads to increased activity of central sympathetic neurons, which contributes to CsA-induced hypertension.

Clinical Studies Using intraneural microelectrodes (microneurography) to record sympathetic nerve discharge targeted to the skeletal muscle circulation, Scherrer et al. provided evidence of sympathetic overactivity in patients with CsA-induced hypertension. Arterial pressure and sympathetic nerve activity were measured in five heart transplant recipients treated with azathioprine and prednisone alone and in 16 heart.
transplant recipients treated with azathioprine and prednisone plus CsA. The higher blood pressures in the CsA group were accompanied by higher sympathetic nerve activity.

Importantly, the control group for CsA-treated heart transplant recipients consisted of heart transplant recipients not receiving CsA. In contrast, both Elam et al. and Kaye et al. compared CsA-treated heart transplant recipients to healthy age-matched control subjects. Muscle sympathetic nerve activity (SNA) was comparable to normal in the study by Kaye et al. and was marginally higher than normal in the CsA-treated heart transplant recipients studied by Elam et al. In our laboratory, however, the higher levels of muscle SNA in heart transplant recipients with CsA-induced hypertension compared to levels in normotensive age-matched healthy controls have been a consistent and reproducible finding, as evidenced by the new data shown below (Figure 4). Elevated levels of muscle SNA also have been reported in patients receiving CsA after combined heart-lung transplantation. In a double blind, randomized trial of CsA versus placebo for the treatment of myasthenia gravis, CsA was also accompanied by increased muscle sympathetic nerve activity (Figure 5). However, the degree of elevation in blood pressure and in sympathetic activity associated with CsA treatment was considerably less in the patients with myasthenia than in those with heart transplants, even though CsA doses were similar. One potential explanation for this finding is that the cardiac denervation that results from heart transplantation removes inhibitory ventricular afferent restraint on sympathetic

FIGURE 6. Comparative effects of CsA, FK506, and rapamycin on renal sympathetic nerve activity in the rat. A. Segment of an illustrative experiment from one rat showing continuous recordings of intranephric pressure and sympathetic nerve activity (displayed as a time frequency histogram) in response to CsA (5 mg/kg, intravenously). CsA evokes prompt and sustained increases in both sympathetic nerve activity and arterial pressure. Adapted from Lyson T et al. with permission. B. Illustrative multifiber recordings of renal sympathetic nerve activity from two rats, one treated with FK506 and the other with rapamycin (each 0.15 mg/kg, intravenously). The sympathetic nerve activity (and arterial pressure) increased after FK506, but not after rapamycin. Adapted from Lyson T et al. with permission. Abbreviations: SNA, sympathetic nerve activity; CsA, cyclosporine A.

FIGURE 7. Cartoon illustrating the rationale for structure-function studies. The immunosuppressant drugs CsA and FK506 possess both immunophilin receptor binding sites and calcineurin binding sites. By making minor structural alterations in only the calcineurin binding site(s), a series of drug analogs were produced with progressive reductions in the ability of the molecules to bind to calcineurin and inhibit its phosphatase activity. These analogs were injected intravenously into rats to determine if these drug analogs would produce attenuated sympathetic activation.
FIGURE 8. Correlation between peak increases in renal sympathetic nerve activity and inhibition of calcineurin-mediated T cell signalling (activity of the nuclear factor of activated T cells) caused by analogs of CsA and FK506. Asterisks refer to increases in sympathetic activity that are significantly smaller than those evoked by the parent drug (P < .05). Abbreviations: NF-AT, nuclear factor of activated T cells; IC50, drug concentration needed to inhibit 50% of NF-AT activity in Jurkat T cells. Reprinted with permission from Lyson T et al.¹³⁰

outflow, thereby amplifying the hypertensive effects of CsA.¹⁴³

There also are conflicting results regarding effects of CsA treatment on 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,¹⁷⁰ whereas others have not.¹³⁷,¹⁴¹ In the most recent of these negative studies in which norepinephrine spillover was measured in 12 patients with rheumatoid arthritis, patients received rather small doses of CsA (averaging 2.8 mg/kg/day) that did not increase their blood pressures.¹⁴¹

FIGURE 9. Cartoon illustrating our hypothesis. 1, CsA stimulates renal and other excitatory subdiaphragmatic visceral afferents. 2, CsA could enhance synaptic neurotransmission at one or more synapses in the reflex pathway by blocking an inhibitory effect of calcineurin on neurotransmitter release. This excitatory reflex may interact with the inhibitory arterial baroreflex. Solid lines represent glutamatergic synapses, the dashed line represents a GABA-ergic synapse. Abbreviations: DRG, dorsal root ganglia; NTS, nucleus tractus solitarius; RVLM, rostral ventro lateral medulla; IML, intermediolateral column; symp., sympathetic; Postgang, postganglionic; Vasc, vascular; GABA, γ-aminobutyric acid.
Studies using animal (mainly rodent) models have progressed more rapidly than the clinical (mainly cross-sectional) studies in this field, which are limited by a number of potentially confounding variables (eg, antihypertensive medications, recent transplant surgery, dose and length of CsA treatment).

**Animal Studies**

Animal models have proved conclusively that acute CsA-induced hypertension is mediated by activation of sympathetic vasoconstrictor nerves. Infusion of CsA into the dog hindlimb produces local vasoconstriction that is abolished by lumbar sympathectomy or α-adrenergic blockade. In anesthetized rats, CsA causes a robust and widespread increase in SNA targeted to the kidney, genitofemoral region, and hindlimb. This sympathetic activation causes a corresponding regional vasoconstriction with acute hypertension that is abolished by a variety of interventions that eliminate SNA, including ganglionic blockade, clonidine, reserpine, or the combination of cervical spinal cord transection plus adrenalectomy.

The first evidence that calcineurin is a relevant cellular target mediating CsA-induced sympathetic activation was provided by Lyson et al. in anesthetized rats, CsA causes a robust and widespread increase in SNA targeted to the kidney, genitofemoral region, and hindlimb. This sympathetic activation causes a corresponding regional vasoconstriction with acute hypertension that is abolished by a variety of interventions that eliminate SNA, including ganglionic blockade, clonidine, reserpine, or the combination of cervical spinal cord transection plus adrenalectomy.

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 binding site, a series of drug analogs were produced with progressive reductions in the ability of the parent molecules to bind to and inhibit calcineurin. When injected intravenously into intact rats, these analogs produced attenuated increases in renal SNA and blood pressure in such a way that closely paralleled their attenuated ability to inhibit calcineurin-mediated signaling in isolated human T cells.

Although the studies in rats have not yet localized precisely the site of CsA’s excitatory action in the sympathetic nervous system, both central neural and peripheral reflex mechanisms are likely to be involved.

Our working hypothesis is that stimulation of chemosensitive renal and other subdiaphragmatic excitatory afferents initiates the CsA-induced increase in renal SNA, which then is amplified and sustained by central synaptic potentiation.

There is considerable evidence that a peripheral reflex mechanism plays an important role in triggering CsA-induced sympathetic activation and acute hypertension in rats. CsA stimulates excitatory chemically-sensitive renal and other subdiaphragmatic visceral afferents that project centrally via both the vagal nodosal ganglia and the low thoracic dorsal spinal root ganglia. Surgical interruption of these afferent neural pathways partially attenuates the sympathoexcitatory response to intravenous CsA. However, the remarkable feature of the sympathoexcitatory response to CsA is that the SNA remains elevated and actually continues to increase for hours after a single intravenous dose of CsA. This suggests potentiation at one or more synapses within this neural reflex pathway.

**Cellular and Molecular Investigation**

Glutamate is the primary excitatory neurotransmitter in the brain.
FIGURE 12. Comparative effects of CsA (a), FK506 (b), and rapamycin (c) on spontaneous intracellular action potential discharge measured in cultured fetal rat cortical neurons using patch electrodes. Bracketed segments of the original records are reproduced below at an expanded time scale to better illustrate the excitatory postsynaptic potentials, the largest of which give rise to action potential spikes. Summary data are displayed to the right. Action potential discharge increased with CsA or FK506, but not with rapamycin, indicating calcineurin mediation. Abbreviations: BL, baseline; RPM, rapamycin. Reproduced from Victor RG et al with permission.
and it plays a major role in sympathetic neurotransmission at the level of the spinal cord, brainstem, and higher brain centers.\textsuperscript{171–176} When an action potential invades a presynaptic nerve terminal, neurosecretory vesicles containing glutamate are released into the synaptic cleft. Glutamate activates postsynaptic receptors (both NMDA and non-NMDA receptor subtypes). Flux of cations (Na\textsuperscript{+}, K\textsuperscript{+}, Ca\textsuperscript{2+}) through these receptor-operated ion channels generates intracellular action potentials which can be recorded with patch pipette electrodes (Figure 11).

It has been hypothesized recently that calcineurin modulates this complex process of glutamatergic neurotransmission via both pre- and postsynaptic sites of action. For example, calcineurin has been shown to cause the dephosphorylation of postsynaptic NMDA receptors in excised membrane patches\textsuperscript{178} and it has been shown to dephosphorylate certain presynaptic vesicle proteins in vitro.\textsuperscript{179,180} Victor et al tested these hypotheses using cultured rat cortical neurons, a reductionist model of glutamatergic neurotransmission. Spontaneous glutamate-driven action potentials could be recorded from the majority of neurons and the action potential firing rate was increased by CsA or FK506, but not by rapamycin (Figure 12), indicating calcineurin mediation.\textsuperscript{181}

In this cell culture model, the calcineurin-sensitive modulation of glutamatergic neurotransmission is achieved via a presynaptic site of action: calcineurin inhibition appears to increase the frequency of glutamate release from presynaptic nerve terminals. This interpretation has been confirmed by direct measurements of glutamate release from presynaptic fractions studied in vitro.\textsuperscript{180,182} Calcineurin could regulate anyone of multiple steps involved in the release of glutamate, beginning with entry of calcium into the presynaptic nerve terminal\textsuperscript{182} and ending with exocytotic release of neurosecretory vesicles. Our working hypothesis is that calcineurin is a key component of a negative feedback mechanism that prevents excessive release of neurotransmitter; CsA or FK506 (but not rapamycin) appear to interrupt this negative feedback mechanism, thereby accelerating neurotransmitter release.

An important question is the extent to which conclusions derived from these reductionist and experimental animal studies can be extrapolated to suggest new approaches to the management of CsA-induced hypertension in patients.

**MANAGEMENT AND PREVENTION OF CsA-INDUCED HYPERTENSION**

Despite a paucity of controlled long-term studies comparing the efficacy of different classes of antihypertensive agents in the treatment of CsA-induced hypertension, calcium channel blockers are rapidly gaining acceptance as the initial drugs of choice in this setting.\textsuperscript{37,46} There are a number of theoretical arguments, but only incomplete experimental data, to recommend calcium antagonists rather than angiotensin-converting enzyme (ACE) inhibitors in this setting.\textsuperscript{37,46} For example, CsA-induced hypertension is considered to be low renin hypertension and ACE inhibitors might exacerbate CsA-induced decreases in glomerular filtration rate by causing postglomerular vasodilation. In contrast, acute studies in renal transplant recipients strongly suggest that calcium channel blockers exert a beneficial effect on renal blood flow and glomerular filtration rate compared with ACE inhibitors.\textsuperscript{183} Textor et al\textsuperscript{184} recently found that nifedipine monotherapy achieved control of blood pressure in 64\% of liver transplant recipients with CsA-induced hypertension. However, this beneficial effect on blood pressure was accompanied by no change in glomerular filtration rate. In a randomized prospective crossover comparison of the ACE inhibitor lisinopril and the calcium channel antagonist nitrendipine, Schwietzer et al\textsuperscript{185} found these two drugs to be equally efficacious in controlling hypertension in eight heart transplant recipients. The same conclusion was reached by Sennesael et al\textsuperscript{186} who, in a similarly designed trial, found no difference between perindopril and amlodipine with regard to 24 h blood pressure control and graft function in 10 CsA-treated kidney recipients. Furthermore, in a large randomized multicenter trial with graft function as the primary end-point, nitrendipine had no beneficial effect over placebo in normotensives and no beneficial effect over other antihypertensive drugs (primarily ACE inhibitors) in hypertensive CsA-treated kidney recipients.\textsuperscript{187} If indeed there is an important sympathetic neural component to CsA-induced hypertension after heart transplantation, clonidine or other sympatholytic agents would be a rational choice, but no firm recommendations can be made in the absence of large randomized prospective trials.

The ultimate goal should be to eliminate (rather than treat) post-transplant hypertension by replacing CsA with a better agent with equal or greater immunosuppressive efficacy without the toxicity. FK506, which was approved for clinical use in 1994, at first was touted as having greater immunosuppressive potency than CsA with much less toxicity.\textsuperscript{188–191} 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, have demonstrated convincingly that FK506 causes as much hypertension (and nephrotoxicity) as does CsA and even more central neural toxicity, at least in liver transplant recipients.\textsuperscript{192–196} In the European FK506 Multicenter Liver Study Group Trial of 545 liver transplant...
plant recipients, the incidence of hypertension at 1 year was 35% with FK506 v 42% with CsA. In the US Multicenter FK506 Liver Study Group of 478 adult and 51 pediatric patients, the incidence of hypertension was 47% with FK506 v 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.

The toxicity of rapamycin is as yet unknown as Phase I and II clinical trials are just getting underway. The results will be very important, since the cellular reasons for withdrawal from the latter study were nephrotoxicity and neurotoxicity, which were more frequent with FK506 than with CsA. The toxicity of rapamycin is as yet unknown as Phase I and II clinical trials are just getting underway. The results will be very important, since the cellular reasons for withdrawal from the latter study were nephrotoxicity and neurotoxicity, which were more frequent with FK506 than with CsA.

CONCLUSIONS

In closing, we have attempted to synthesize a large amount of data that leads to a new conceptual framework for understanding the pathophysiologic basis of CsA-induced hypertension. The elucidation of CsA-sensitive signalling pathways holds exciting promise for reducing immunosuppressant drug toxicity and it may have broader implications for the pathophysiology and treatment of other forms of hypertension.

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