Failure of Calcineurin Inhibitors to Prevent Pressure-Overload Left Ventricular Hypertrophy in Rats

Weiguo Zhang, Robert C. Kowal, Frank Rusnak, Robert A. Sikkink, Eric N. Olson, Ronald G. Victor

Abstract—A rapidly emerging body of literature implicates a pivotal role for the Ca\textsuperscript{2+}-calmodulin–dependent phosphatase calcineurin as a cellular target for a variety of Ca\textsuperscript{2+}-dependent signaling pathways culminating in left ventricular hypertrophy (LVH). Most of the recent experimental support for this hypothesis is derived from in vitro studies or in vivo studies in transgenic mice expressing activated calcineurin or mutant sarcomeric proteins. The aim of the present study was to test whether calcineurin inhibitors, cyclosporin A (CsA) and FK 506, prevent pressure-overload LVH using 2 standard rat models: (1) the spontaneously hypertensive rat (SHR) and (2) aortic banding. The major new findings are 2-fold. First, in SHR, LVH (left ventricular weight to body weight ratio) was unaffected by a dose of CsA (5 mg·kg\textsuperscript{-1}·d\textsuperscript{-1}) that was sufficient to raise blood pressure and to inhibit calcineurin-mediated transcriptional activation in skeletal muscle. Second, in rats with aortic banding, LVH was unaffected by FK 506 (0.3 mg·kg\textsuperscript{-1}·d\textsuperscript{-1}) or even higher doses of CsA (10 and 20 mg·kg\textsuperscript{-1}·d\textsuperscript{-1}) that were sufficient to inhibit 90% of total calcineurin phosphatase activity in the hypertrophied myocardium. In the latter experiments, CsA blocked neither the elevated left ventricular end-diastolic pressures, a measure of diastolic function, nor the induction in atrial natriuretic peptide mRNA in the hypertrophic ventricles. Thus, in numerous experiments, systemic administration of potent calcineurin inhibitors did not prevent the development of LVH in 2 classic models of pressure-overload hypertrophy. These results demonstrate that pressure-overload hypertrophy can arise through calcineurin-independent pathways. (Circ Res. 1999;84:722-728.)

Key Words: left ventricular hypertrophy • calcineurin • cyclosporin A • spontaneously hypertensive rat • aortic banding

In patients with many forms of heart disease, the development of left ventricular hypertrophy (LVH) constitutes one of the most powerful predictors of subsequent cardiovascular morbidity and mortality.\textsuperscript{1-4} Increased left ventricular mass strongly predicts an increased incidence of cardiovascular death, myocardial infarction, angina requiring revascularization, stroke, and congestive heart failure. Despite the clinical importance of LVH, the underlying mechanisms mediating the hypertrophic process have been enigmatic.

A variety of intrinsic and extrinsic pathological stimuli can initiate a hypertrophic response in the left ventricle. Extrinsic stimuli include pressure overload, volume overload, and neurohumoral factors. Intrinsic stimuli include contractile abnormalities resulting from altered expression or mutations of sarcomeric proteins. Although these diverse stimuli are known to activate diverse intracellular signal transduction pathways,\textsuperscript{5-6} it is unknown whether these various pathways ultimately converge on a final common signaling pathway culminating in cardiomyocyte hypertrophy.\textsuperscript{5-6}

In this regard, changes in [Ca\textsuperscript{2+}], have been proposed to play a pivotal role in the hypertrophic response.\textsuperscript{4-6} Consistent with this notion, Molkentin et al\textsuperscript{8} advanced the hypothesis that calcineurin, the Ca\textsuperscript{2+}-calmodulin–dependent protein phosphatase, can act as a transducer of hypertrophic signals in vitro and in vivo. This is an attractive hypothesis, because calcineurin is activated in response to sustained low-amplitude changes in [Ca\textsuperscript{2+}], levels.\textsuperscript{6,10} In cultured cardiac myocytes, the immunophilin ligands cyclosporin A (CsA) and FK 506, which form cytoplasmic immunophilin complexes that are potent calcineurin inhibitors, prevented hypertrophy induced by either angiotensin II or the \textalpha-\textadrenergic agonist phenylephrine.\textsuperscript{8} In transgenic mice, cardiac-specific expression of constitutively active calcineurin induced a severe form of hypertrophy, which was prevented by systemic CsA or FK 506.\textsuperscript{8} Moreover, systemic CsA or FK 506 recently was found to rescue cardiac hypertrophy in 3 different transgenic mouse models of cardiac hypertrophy due to inappropriate expression of a variety of sarcomeric proteins.\textsuperscript{11} These results suggest that calcineurin activation mediates hypertrophic growth in response to various intrinsic, or genetically determined, forms of heart disease. On the strength of these experimental findings, it was suggested that

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calcineurin inhibitors merit investigation as potential therapeutic agents for some forms of human heart disease.11

Indeed, in the study of Sussman et al.,11 systemic CsA also was found to prevent the development of cardiac hypertrophy in rats with aortic banding, a classic model of pressure-overload hypertrophy. This experimental observation, however, is difficult to reconcile with the repeated clinical observation that LVH is prevalent in patients with CsA-induced hypertension.12–18

The goal of the present study, therefore, was to further test the hypothesis that systemic administration of calcineurin inhibitors constitutes an effective novel strategy to prevent hypertensive, or pressure-overload, LVH using 2 different rat models: (1) the spontaneously hypertensive rat (SHR), and (2) aortic banding. In the present study, we show that blocking cardiac calcineurin with systemic CsA is not sufficient to prevent the hypertrophic process in either model. The results demonstrate that pressure-overload hypertrophy can arise through calcineurin-independent pathways.

Materials and Methods

Male SHR (Harlan Inc, Indianapolis, Ind) at 5 weeks of age and male Sprague-Dawley rats (Harlan Inc) weighing 170 to 200 g were studied in accordance with protocols approved by the Institutional Animal Care and Research Advisory Committee of the University of Texas Southwestern Medical Center. Rats were fed standard rat chow and tap water ad libitum and kept in a controlled environment with 12-hour dark and light cycles. Calcineurin Inhibitors: CsA (Sandimmune) used in these experiments was from Sandoz (Novartis). FK 506 (tacrolimus) was kindly provided by Fujisawa (Deerfield, Ill). Both agents were dissolved in the same vehicle (Cremophor EL, Sigma). In all experiments, rats were weighed weekly to calculate the doses of CsA and FK 506 on the basis of body weight.

Specific Protocols

Protocol 1: Effects of CsA on LVH in SHR

To determine whether CsA attenuates the development of LVH in SHR, 5-week-old SHR were randomized to 6 weeks of treatment with (1) CsA 5 mg \( \cdot \) kg \(^{-1} \cdot \) d \(^{-1} \) injected subcutaneously (n = 12) or (2) an equal volume of vehicle (n = 12). The age of 5 weeks was chosen because this predates the onset of hypertension and LVH in SHR.19,20

At the 6-week end point, rats were anesthetized with methohexital sodium (Eli Lilly; 50 mg/kg IP). A catheter was inserted into the left carotid artery and tunneled subcutaneously to exit the nape for subsequent measurements of arterial pressure in the conscious state. After a 24-hour recovery period, rats were placed in individual cages in a quiet room. Carotid artery catheters were placed on a counterweight lever system, allowing the rats to move freely. Catheters were connected to a pressure transducer (P23ID; Gould Inc) for intra-arterial pressure recordings (RS3400, Gould Inc). At least 30 minutes were allowed for acclimation. Arterial pressure was then being obtained during periods in which the rats were not moving. Mean arterial pressure was calculated from the phasic arterial pressure tracing as one third of the pulse pressure plus the diastolic pressure. Heart rate was measured directly from the arterial pressure tracing.

Euthanasia was performed with sodium pentobarbital (3-0 mg/kg IP), a laparotomy was performed to expose the descending aorta near the origin of left renal artery and a curved needle (21-gauge). The needle was removed, leaving the vessel constricted to the diameter of the needle. Sham-operated rats underwent the identical manipulation, but no ligature was placed. The abdomen was closed after antibiotic prophylaxis using procaine penicillin G (10 000 U).

Protocol 2A: 4-Week End Point

After surgery, rats were randomly assigned to one of seven separate groups: (1) sham-operated rats treated with CsA 10 mg \( \cdot \) kg \(^{-1} \cdot \) d \(^{-1} \) (SHAM-CsA, n = 7), (2) sham-operated rats treated with vehicle (SHAM-Vehicle, n = 11), (3) aortic banded rats treated with CsA 10 mg \( \cdot \) kg \(^{-1} \cdot \) d \(^{-1} \) IP (AB-CsA 10, n = 12), (4) aortic banded rats treated with CsA 20 mg \( \cdot \) kg \(^{-1} \cdot \) d \(^{-1} \) IP (AB-CsA 20, n = 10), (5) aortic banded rats treated with FK 506 0.3 mg \( \cdot \) kg \(^{-1} \cdot \) d \(^{-1} \) IP (AB-FK 506, n = 8), (6) aortic banded rats treated with vehicle (AB-Vehicle, n = 9), and (7) aortic banded rats treated with vehicle but also pair-fed (AB-PairFed) to match the caloric intake of the AB-CsA 10 group. Treatment with CsA, FK 506, or vehicle was initiated immediately after surgery. The doses of CsA and FK 506 used were 2 to 4 times higher than the highest clinical doses.

After 4 weeks of treatment, all rats were anesthetized (sodium pentobarbital 50 mg/kg IP) for hemodynamic measurements. Pressure gradients were measured with catheters placed in the carotid artery (above the stenosis) and the femoral artery (below the stenosis). Then, the heart was excised for measurement of left ventricle and right ventricle weight.

Protocol 2B: 2-Week End Point

Before surgery, rats were randomly assigned to one of three groups: (1) sham-operated rats treated with vehicle (SHAM-Vehicle, n = 8), (2) aortic banded rats treated with vehicle (AB-Vehicle, n = 6), or (3) aortic banded rats treated with CsA 10 mg \( \cdot \) kg \(^{-1} \cdot \) d \(^{-1} \) IP (AB-CsA 10, n = 8). In this protocol, treatment with CsA (or vehicle) was initiated 2 days (3 injections) before surgery.

After 2 weeks of treatment, rats were anesthetized (sodium pentobarbital 50 mg/kg IP) for measurements of carotid and femoral arterial pressures and of left ventricular end-diastolic pressure (LVEDP) using a catheter placed into the right carotid artery and advanced into the left ventricle. Whole-blood CsA levels were measured by a fluorescence polarization immunoassay (Abbott Laboratories).23 With a median thoracotomy, the heart was vented via the right atrium, and 30 mL of a 1.5% solution of KCl in PBS was infused through the carotid artery to arrest the heart. After the heart was excised and weighed, left ventricular tissue was processed as follows.

Calcineurin Phosphatase Assay

Left ventricular samples were immediately placed in liquid nitrogen and stored at –80°C for analysis of phosphatase activity. The frozen rat heart left ventricles were weighed and freeze-fracture pulverized to a powder while frozen in liquid nitrogen. In a 1.5-mL microfuge tube, the frozen powdered tissue was homogenized in 2 volumes (vol/wt) of 100 mmol/L MOPS (pH 7.0), 2 mmol/L EDTA, and protease inhibitors (75 μg/mL PMSF, 10 μg/mL trypsin inhibitor, 10 μg/mL TPCK, and 1 μg/mL leupeptin) by douncing twice with a small glass rod with one freeze/thaw cycle in between. The samples were microfuged at 16 000g for 15 minutes at 4°C, and the supernatants were saved as the tissue extracts. Resulting extract samples were either assayed immediately or stored at –70°C. Typically, 20 to 30 μg of protein was used for each assay. The phosphatase assay buffer consisted of 20 mmol/L Tris (pH 7.5), 6 mmol/L MgCl\(_2\), 0.1 mol/L KCl, 0.5 mmol/L CaCl\(_2\), 1 mmol/L DTT, and 1 μmol/L bovine calmodulin.

Phosphatase activity was evaluated by measuring the rate of dephosphorylation of a synthetic [\(^{32}P\)]-ATP–labeled phosphopeptide substrate (R-Il peptide) in the presence of 0.5 mmol/L CaCl\(_2\), 1.0 μmol/L calmodulin, and 0.5 μmol/L okadaic acid, where necessary, as described in our previous publications.24–28 Because the samples contain the serine/threonine protein phosphatases 1, 2A, 2B
Calcineurin Inhibition and LVH

### TABLE 1. Effects of Vehicle Versus CsA in SHR

<table>
<thead>
<tr>
<th></th>
<th>Vehicle (n=12)</th>
<th>CsA (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>236±4</td>
<td>216±4*</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>154±5</td>
<td>168±4*</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>392±7</td>
<td>402±6</td>
</tr>
<tr>
<td>LVW, mg</td>
<td>669±13</td>
<td>635±19</td>
</tr>
<tr>
<td>LVW/BW, mg/g</td>
<td>2.84±0.06</td>
<td>2.92±0.04</td>
</tr>
</tbody>
</table>

SHR were treated with vehicle or CsA (5 mg · kg⁻¹ · d⁻¹) for 6 weeks as described in Materials and Methods. Body weight (BW), mean arterial pressure (MAP), heart rate (HR), LVW, and LVW/BW ratio were then determined. *P<0.05 vehicle vs CsA.

### Measurement of Atrial Natriuretic Peptide (ANP) mRNA

Rat left ventricular myocardium (50 to 100 mg) was homogenized in 1.5 mL Trizol reagent (Gibco, Life Sciences), and total RNA was isolated according to the manufacturer’s protocol. The indicated amount of RNA was denatured in 67% formaldehyde, 2.5 mol/L formaldehyde, and 50 mmol/L MOPS (pH 7.0) at 65°C for 15 minutes. Samples were fractionated with formaldehyde/agarose electrophoresis followed by transfer to nylon membranes according to standard techniques.26 The blot was hybridized with 32P-labeled probes derived from the ANP cDNAs prepared with the RadPrime kit (Gibco, Life Sciences) by standard techniques. A probe recognizing the ribosomal L7 mRNA was used as a loading control, and data were normalized as the ratio of ANP mRNA to L7 mRNA.

### Statistical Analysis

Statistical analysis was performed using 2-tailed Student t test and 1-way ANOVA. Values are expressed as mean±SEM. Statistical significance was set at P<0.05.

### Results

#### Postoperative Mortality Rates

Our goal was to thoroughly test the effects of calcineurin inhibition in SHR and aortic banded rats, 2 well-characterized rodent models of pressure-overload LVH. In all, we obtained data on a total of 110 rats (24 in protocol 1, 64 in protocol 2A, and 22 in protocol 2B). In all protocols, CsA caused a dose-dependent retardation in weight gain (Tables 1 and 2), which was accompanied by increased early postoperative mortality after aortic banding. In protocol 2A (aortic banding for 4 weeks), early postoperative mortality was 33% in the first 48 hours after aortic banding but 45% in banded rats treated with CsA at 10 mg · kg⁻¹ · d⁻¹ and 56% in those treated with CsA at 20 mg · kg⁻¹ · d⁻¹. In protocol 2B (aortic banding for 2 weeks), early postoperative mortality in the first 24 hours increased from 45% after aortic banding alone to 67% when rats were pretreated with CsA for >2 days before banding. In both protocols 2A and 2B, organs that survived the first 24 to 48 hours after banding, the subsequent 2- and 4-week survival was >90%.

### Effects of CsA in SHR

Because LVH is prevalent in patients with CsA-induced hypertension, we explored the relation between blood pressures and LVH in SHR, a rodent model of hypertension that is exacerbated by CsA administration. In SHR, CsA (5 mg/kg), as expected, was sufficient to raise blood pressure by 14±5 mm Hg (P<0.05 versus vehicle), but it had no effect on the development of LVH, as measured by left ventricular weight to body weight (LVW/BW) ratios (Table 1). Five milligrams per kilogram was chosen because this systemic dose of CsA is clinically relevant, produces high concentrations of CsA in blood and heart,20 and was shown to effectively inhibit calcineurin-mediated signaling in skeletal muscle of SHR.21

### Effects of CsA and FK 506 in Rats With Aortic Banding

Because the genetic abnormalities in SHR are not precisely defined, we next tested effects of CsA on the development of LVH in response to a well-defined pressure overload superimposed on rats with a normal genetic background. In the initial series of experiments, rats were subjected to aortic banding for 4 weeks, which is considered as a minimum time required to observe the full effect of other strategies (eg, angiotensin-converting enzyme inhibition) to attenuate the development of LVH in this model.22 In Sprague-Dawley rats, 4 weeks of aortic banding alone caused a 47% increase in the LVW/BW ratio (Table 2). This increase was not significantly attenuated by either 10 or 20 mg · kg⁻¹ · d⁻¹ of CsA (Table 1). Five milligrams per kilogram was chosen because this systemic dose of CsA is clinically relevant, produces high concentrations of CsA in blood and heart,20 and was shown to effectively inhibit calcineurin-mediated signaling in skeletal muscle of SHR.21

### TABLE 2. Effects of Vehicle Versus CsA or FK 506 in Rats With Aortic Banding: 4-Week Protocol

<table>
<thead>
<tr>
<th></th>
<th>SHAM-Vehicle (n=11)</th>
<th>SHAM-CsA 10 (n=7)</th>
<th>AB-Vehicle (n=9)</th>
<th>AB-PairFed (n=7)</th>
<th>AB-CsA 10 (n=12)</th>
<th>AB-CsA 20 (n=10)</th>
<th>AB-FK 506 (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>321±5</td>
<td>262±2*</td>
<td>299±10*§</td>
<td>269±2*</td>
<td>249±6†</td>
<td>231±8†</td>
<td>245±6†</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>389±13</td>
<td>394±9</td>
<td>385±14</td>
<td>386±13</td>
<td>390±14</td>
<td>415±10</td>
<td>415±19</td>
</tr>
<tr>
<td>Pressure gradient, mm Hg</td>
<td>4.1±0.9</td>
<td>5.8±0.9</td>
<td>38.1±3.3§</td>
<td>31.4±5.2§</td>
<td>24.9±2.5§</td>
<td>25.4±3.5§</td>
<td>26.3±3.5§</td>
</tr>
<tr>
<td>LVW/BW, mg/g</td>
<td>2.15±0.03</td>
<td>2.08±0.14</td>
<td>3.17±0.11*§</td>
<td>2.99±0.19*§</td>
<td>2.73±0.12§</td>
<td>2.62±0.12§</td>
<td>2.85±0.11*§</td>
</tr>
</tbody>
</table>

Rats were subjected to aortic banding for 4 weeks. BW, HR, pressure gradient between the ascending aorta and femoral artery, and LVW/BW ratio were then determined in the following experimental groups: sham-operated rats treated with vehicle (SHAM-Vehicle); sham-operated rats treated with CsA 10 mg · kg⁻¹ · d⁻¹ (SHAM-CsA 10); aortic banded rats treated with vehicle (AB-Vehicle); aortic banded rats treated with vehicle and provided with the same amount of food as consumed by aortic banded rats treated with CsA 10 mg · kg⁻¹ · d⁻¹ (AB-Vehicle+PairFed); aortic banded rats treated with CsA 10 mg · kg⁻¹ · d⁻¹ (AB-CsA 10); aortic banded rats treated with CsA 20 mg · kg⁻¹ · d⁻¹ (AB-CsA 20); and aortic banded rats treated with FK 506 0.3 mg · kg⁻¹ · d⁻¹ (AB-FK 506). *P<0.05 compared with SHAM-Vehicle; §P<0.05 compared with SHAM-CsA; and †P<0.05 compared with AB-Vehicle.
Table 3. Effects of Vehicle Versus CsA in Rats With Aortic Banding: 2-Week Protocol

<table>
<thead>
<tr>
<th></th>
<th>SHAM-Vehicle (n=8)</th>
<th>AB-Vehicle (n=6)</th>
<th>AB-CsA 10 (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>268±3</td>
<td>265±9</td>
<td>173±10</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>368±12</td>
<td>388±18</td>
<td>375±9</td>
</tr>
<tr>
<td>Pressure gradient, mm Hg</td>
<td>5.0±0.3</td>
<td>21.5±1.2</td>
<td>23.9±1.5</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>4.8±0.6</td>
<td>12.6±0.6</td>
<td>12.2±0.4</td>
</tr>
<tr>
<td>LVW/BW, mg/g</td>
<td>1.95±0.03</td>
<td>2.69±0.15</td>
<td>2.50±0.06</td>
</tr>
<tr>
<td>CsA blood level, μg/L</td>
<td>...</td>
<td>...</td>
<td>&gt;4500</td>
</tr>
</tbody>
</table>

After pretreatment with vehicle or CsA for 2 days, rats were subjected to aortic banding (or sham operation) for 2 weeks. BW, HR, mean pressure gradient between the ascending aorta and femoral artery, LVEDP, LVW/BW ratio, and CsA blood levels were then determined in the following experimental groups: sham-operated rats treated with vehicle (SHAM-Vehicle); aortic banded rats treated with vehicle (AB-Vehicle); and aortic banded rats treated with CsA 10 mg·kg⁻¹·d⁻¹ (AB-CsA 10). *P<0.05 compared with SHAM-Vehicle; †P<0.05 compared with AB-Vehicle.

Effects of CsA on Cardiac Calcineurin Activity in Rats With Aortic Banding

To document the extent to which systemic CsA inhibited cardiac calcineurin activity, we measured calcineurin phosphatase activity in left ventricular samples from sham-operated rats and banded rats treated with and without CsA (10 mg·kg⁻¹·d⁻¹). Two points are made by the data shown in Figure 3. First, banding alone did not increase cardiac calcineurin activity. Because this assay is performed in the presence of exogenous Ca²⁺ and calmodulin, it measures maximum possible calcineurin activity. Thus, we did not test in the hypertrophied hearts whether calcineurin activity may have been enhanced because of increased cytosolic Ca²⁺ levels in vivo. Second, in the hypertrophied hearts, our systemic CsA regimen inhibited maximal Ca²⁺-dependent molecular, morphological, nor hemodynamic responses to pressure overload were prevented by CsA.
activation of calcineurin by 90%. Thus, CsA should have blocked virtually all Ca^{2+}-dependent activation of calcineurin in vivo.

Discussion

A rapidly emerging body of literature implicates a pivotal role for calcineurin as an important target for a variety of Ca^{2+}-dependent signaling pathways culminating in LVH.8,11 To date, most of the experimental support for this hypothesis is derived from in vitro studies or in vivo studies in transgenic mice. The data herein evaluate the extent to which these mechanistic conclusions can be translated to the integrated regulation of the hypertrophic process in the intact organism. The major new findings are 2-fold. First, in SHR, LVH was unaffected by a dose of CsA that was sufficient to raise blood pressure and to inhibit calcineurin-mediated transcriptional activation in skeletal muscle. Second, in rats with aortic banding, LVH was unaffected by FK 506 or even higher doses of CsA that were sufficient to inhibit 90% of calcineurin phosphatase activity in the hypertrophied myocardium. Thus, in numerous experiments, systemic administration of calcineurin inhibitors did not prevent the development of LVH in 2 classic models of pressure-overload hypertrophy.

Because these conclusions are drawn on the basis of the systemic administration of CsA or FK 506, it is important that in aortic banded rats our drug regimens produced nearly complete inhibition of cardiac calcineurin activity. The doses of CsA used in these experiments produced enormous trough CsA blood levels that were 10-fold higher than therapeutic blood levels needed to inhibit calcineurin signaling in T cells.28 Although we did not measure cardiac calcineurin activity in the SHR experiments, CsA raised blood pressure, which has been linked to sympathetic activation caused by the inhibition of neuronal calcineurin,29 and it was shown to inhibit calcineurin-mediated transcriptional activation in the skeletal muscle of the same rats.21 In SHR, this exact CsA regimen produces a final tissue concentration of CsA in the heart that is almost 4 times higher than in the skeletal muscle.30

Our results differ from those of Sussman et al11 who reported that systemic CsA virtually eliminated LVH not only in several transgenic mouse models of sarcomeric disruption but also in rats with aortic banding. Whereas the study of Sussman et al11 focused mainly on transgenic mouse models of dilated cardiomyopathy, our study provides a more comprehensive examination of pressure-overload hypertrophy in rats. Although both studies examined LVW/BW ratios in rats with aortic banding, the distinctive features of our study include the following: (1) the failure of CsA to prevent LVH in 2 complementary models, SHR and aortic banding; (2) hemodynamic measurements to document the magnitude of the pressure stimulus to hypertrophy; and (3) the demonstration that CsA almost completely blocks cardiac calcineurin activity but exerts no major effect on either the initiation or the maintenance of pressure-overload hypertrophy.

Our finding that CsA augmented the hypertension but did not prevent the cardiac hypertrophy in SHR is consistent with the clinical observation that LVH is prevalent in patients with CsA-induced hypertension.12–18 Echocardiographic LVH was found to be even greater in heart transplant recipients with CsA-induced hypertension than in patients with primary hypertension matched for age, body weight, and severity of hypertension.14 Because CsA treatment in SHR was accompanied by an increase in blood pressure without a statistically significant increase in LVW/BW ratio, we cannot exclude the possibility that calcineurin inhibition caused a minor attenuation in the hypertrophic process.

We therefore turned to aortic banding in normotensive rats as a complementary model to test our hypothesis. Sprague-Dawley rats, unlike SHR, do not develop chronic hypertension from CsA29; therefore, the CsA treatment per se should not alter the hemodynamic stimulus to hypertrophy. The confounding genetic abnormalities in SHR are avoided by studying normal rats, and the aortic banding model of pressure-overload hypertrophy involves the GATA4 transcription factor,35,34 which Molkenstet al35 recently indicated to be a cognate downstream target for cardiac calcineurin.

In our initial series of experiments, LVH was assessed 4 weeks after aortic banding, the minimum time required to observe the full effect of other strategies (eg, angiotensin-converting enzyme inhibition) to attenuate the development of LVH in this model.30 The failure of CsA and FK 506 to attenuate LVH is particularly telling because treatment with CsA or FK 506 was accompanied by a reduction in the pressure gradient, the hemodynamic stimulus to LVH. The latter was an unexpected finding, which is best explained by reduced weight gain in the CsA- or FK 506–treated animals. When young rats undergo aortic banding, the magnitude of the pressure gradient normally increases over time (between the second to the fourth week in our experiments), as the aorta grows around a fixed extrinsic stenosis.

It is unclear why our results differ from those of Sussman et al,11 but there are differences in the experimental procedures that could have affected outcomes. In the study by
Sussman et al., experiments could be carried out only over a 6-day time period because of lethality in banded/CsA-treated rats. Lethality was attributed to the lack of an adequate hypertrophic response in the banded rats treated with CsA. We also found that CsA increased the operative mortality associated with aortic banding. However, we attribute this lethality to the generalized toxicity of systemic CsA rather than an inadequate hypertrophic response to aortic banding, because (1) almost all the deaths occurred in the early postoperative period when body weights already were less in the CsA-treated than in the vehicle-treated rats, and (2) cardiac hypertrophy was evident in the survivors, which were viable for at least 4 weeks after banding.

We considered the possibility that calcineurin activation might play a greater role in the initiation than in the maintenance of pressure-overload hypertrophy. However, this possibility seems unlikely, because in our additional experiments, CsA also failed to attenuate the hypertrophic response during the first 2 weeks of aortic banding, although CsA was initiated before activation of the hypertrophic signal. Because there was a tendency for LVW/BW ratios to be slightly (7%) lower in banded rats treated with CsA than in rats treated with vehicle, despite similar pressure gradients, we cannot exclude the possibility that CsA treatment did prevent a certain percentage of the total hypertrophic response. However, that CsA also had no effect on either LVEDP, a measure of diastolic function, or activation of a hypertrophic fetal gene strengthens the conclusion that under these experimental conditions, pressure-overload hypertrophy was not substantially affected by systemic administration of this potent calcineurin inhibitor.

The inability of the systemic calcineurin inhibitors to prevent hypertensive or pressure-overload hypertrophy by no means refutes the hypothesis that calcineurin activation plays an important role in the hypertrophic process. Rather, the results strongly suggest that additional signaling pathways are involved in pressure-overload hypertrophy that, in the intact animal, can compensate for reduced calcineurin activity in the presence of CsA. No attempt was made to directly compare the relative importance of calcineurin-mediated signaling with other pathways. A likely possibility is that pressure overload activates signals that are involved in the complex process of cardiomyocyte hypertrophy. The data of Sussman et al. document how many switches, how many wires. J Mol Endocr. 1993;11:439–442.


In conclusion, our data call attention to 2 animal models of pressure-overload hypertrophy that are unaffected by systemic administration of potent calcineurin inhibitors. While our manuscript was in review, 2 brief communications have been published from other laboratories that support our conclusion that calcineurin inhibitors do not prevent pressure-overload hypertrophy in rodent models. Defining the calcineurin-dependent and calcineurin-independent forms of cardiac hypertrophy is likely to provide some important clues about the relative contributions of the various intracellular signals that are involved in the complex process of cardiomyocyte hypertrophy. The data of Sussman et al. document a certain degree of specificity, because CsA failed to prevent
dilated cardiomyopathy in a transgenic mouse model in which hypertrophy is not directly related to sarcomeric dysfunction. In contrast, our data indicate that pressure-overload LVH arises through calcineurin-independent pathways. A likely possibility is that pressure overload activates multiple, possibly redundant, or interdependent signal transduction systems leading to hypertrophy.

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References


Failure of Calcineurin Inhibitors to Prevent Pressure-Overload Left Ventricular Hypertrophy in Rats
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