Calcineurin-Mediated Hypertrophy Protects Cardiomyocytes From Apoptosis In Vitro and In Vivo

An Apoptosis-Independent Model of Dilated Heart Failure

Leon J. De Windt, Hae W. Lim, Tyler Taigen, Detlef Wencker, Gianluigi Condorelli, Gerald W. Dorn II, Richard N. Kitsis, Jeffery D. Molkentin

Abstract—We have previously shown that the calcium-calmodulin–regulated phosphatase calcineurin (PP2B) is sufficient to induce cardiac hypertrophy that transitions to heart failure in transgenic mice. Given the rapid onset of heart failure in these mice, we hypothesized that calcineurin signaling would stimulate myocardial cell apoptosis. However, utilizing multiple approaches, we determined that calcineurin-mediated hypertrophy protected cardiac myocytes from apoptosis, suggesting a model of heart failure that is independent of apoptosis. Adenovirally mediated gene transfer of a constitutively active calcineurin cDNA (AdCnA) was performed in cultured neonatal rat cardiomyocytes to elucidate the mechanism whereby calcineurin affected myocardial cell viability. AdCnA infection, which induced myocyte hypertrophy and atrial natriuretic factor expression, protected against apoptosis induced by 2-deoxyglucose or staurosporine, as assessed by terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL) labeling, caspase-3 activation, DNA laddering, and cellular morphology. The level of protection conferred by AdCnA was similar to that of adenoviral Bcl-xL gene transfer or hypertrophy induced by phenylephrine. In vivo, failing hearts from calcineurin-transgenic mice did not demonstrate increased TUNEL labeling and, in fact, demonstrated a resistance to ischemia/reperfusion–induced apoptosis. We determined that the mechanism whereby calcineurin afforded protection from apoptosis was partially mediated by nuclear factor of activated T cells (NFAT3) signaling and partially by Akt/protein kinase B (PKB) signaling. Although calcineurin activation protected myocytes from apoptosis, inhibition of calcineurin with cyclosporine was not sufficient to induce TUNEL labeling in Gqα-transgenic mice or in cultured cardiomyocytes. Collectively, these data identify a calcineurin-dependent mouse model of dilated heart failure that is independent of apoptosis. (Circ Res. 2000;86:255-263.)

Key Words: calcineurin • apoptosis • cardiac hypertrophy • phenylephrine • caspase-3

Heart failure can result from a wide range of pathological conditions such as infarction, chronic left ventricular hypertrophy, ischemic cardiomyopathy, idiopathic dilated cardiomyopathy, hypertrophic cardiomyopathy, valvular heart disease, and viral infection. In response to these pathologic conditions, cardiomyocytes may undergo apoptosis, or programmed cell death, which has been postulated to contribute to the progressive pathology associated with heart failure.1–4 Apoptosis is an energy-requiring process that transitions to heart failure in transgenic mice. Given the rapid onset of heart failure in these mice, we hypothesized that calcineurin signaling would stimulate myocardial cell apoptosis. However, utilizing multiple approaches, we determined that calcineurin-mediated hypertrophy protected cardiac myocytes from apoptosis, suggesting a model of heart failure that is independent of apoptosis. Adenovirally mediated gene transfer of a constitutively active calcineurin cDNA (AdCnA) was performed in cultured neonatal rat cardiomyocytes to elucidate the mechanism whereby calcineurin affected myocardial cell viability. AdCnA infection, which induced myocyte hypertrophy and atrial natriuretic factor expression, protected against apoptosis induced by 2-deoxyglucose or staurosporine, as assessed by terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL) labeling, caspase-3 activation, DNA laddering, and cellular morphology. The level of protection conferred by AdCnA was similar to that of adenoviral Bcl-xL gene transfer or hypertrophy induced by phenylephrine. In vivo, failing hearts from calcineurin-transgenic mice did not demonstrate increased TUNEL labeling and, in fact, demonstrated a resistance to ischemia/reperfusion–induced apoptosis. We determined that the mechanism whereby calcineurin afforded protection from apoptosis was partially mediated by nuclear factor of activated T cells (NFAT3) signaling and partially by Akt/protein kinase B (PKB) signaling. Although calcineurin activation protected myocytes from apoptosis, inhibition of calcineurin with cyclosporine was not sufficient to induce TUNEL labeling in Gqα-transgenic mice or in cultured cardiomyocytes. Collectively, these data identify a calcineurin-dependent mouse model of dilated heart failure that is independent of apoptosis. (Circ Res. 2000;86:255-263.)

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signaling pathways. Cardiotrophin-1 signaling through the gp130 receptor was shown to protect cardiomyocytes from apoptosis. Induction of cardiac hypertrophy through MKK6 and P38β has also been associated with protection from apoptosis. Insulin-like growth factor-1 (IGF-1) stimulation protects cardiomyocytes from apoptosis through phosphatidylinositol 3-kinase and MAPK-dependent signaling pathways. Hypertrophic agonists such as phenylephrine (PE) and endothelin-1 are also associated with protection from apoptosis, suggesting that hypertrophy itself may confer protection. 

We recently described a novel mediator of cardiac hypertrophy through the calcium-calmodulin–regulated intracellular phosphatase calcineurin (PP2B) and the transcriptional regulator nuclear factor of activated T cells (NFAT3). Expression of a constitutively active form of calcineurin in transgenic mouse hearts resulted in profound cardiac hypertrophy that progressed to dilated heart failure within 8 to 12 weeks. It was of interest to determine whether hypertrophic signaling mediated by calcineurin induced apoptosis, which might explain the rapid progression to heart failure in these mice. However, we determined that cardiomyocyte terminal deoxynucleotidyltransferase–mediated dUTP nick end labeling (TUNEL) in calcineurin-transgenic hearts is not significantly different from that in wild-type hearts. In fact, calcineurin-transgenic hearts were significantly less susceptible to ischemia/reperfusion–induced apoptosis than were wild-type hearts. To define the mechanism whereby calcineurin signaling might protect cardiac myocytes from apoptosis, we generated a calcineurin adenovirus (AdCnA) to infect cultured rat neonatal cardiomyocytes. AdCnA infection induced a hypertrophic response that was protective against apoptosis induced by 2 different pharmacological agents. Cells infected with AdCnA, and 54% (±2.7%) of those treated with PE were positive (P < 0.05). These data indicate that expression of activated calcineurin is sufficient to drive the hypertrophic program in cultured neonatal cardiomyocytes.

**Materials and Methods**

The mouse model of ischemia/reperfusion by ligation of the left descending coronary artery was performed as described previously. Primary cultures of neonatal rat cardiomyocytes were maintained as described previously. E1a-deleted, replication-deficient adenovirus expressing a constitutively active form of mouse calcineurin Aα amino acids 1 to 398 (AdCnA), full-length human Bcl-xL (AdBcl-xL), or a constitutively nuclear form of NFAT3 (AdNFAT3) was initially constructed in pACCMV-pLpA and cotransfected into HEK293 cells with pJM17 as described before. For the generation of the adenovirus expressing a calcineurin-inhibitory peptide (Adcain), a 582-bp fragment corresponding to amino acids 1989 to 2182 of calcineurin was generated by polymerase chain reaction and subcloned with a flag epitope as a HindIII fragment into pACCMV-pLpA. All initial recombinants were plaque-purified, expanded, and titered by duplicate plaque assays in monolayers of HEK293 using the agarose gel overlay method. Adenoviral infection of cardiomyocytes was performed at a multiplicity of infection of 100 plaque-forming units in 2 mL (6-cm culture dishes) of DMEM supplemented with 2% FBS for 2 hours at 37°C in a humidified, 5% CO2 incubator. Under these infection conditions, ~99% of the cells were positive for protein expression by immunocytochemistry or stained β-galactosidase positive after 24 hours. TUNEL labeling of cultured cardiomyocytes or tissue sections was performed with the CardiotACS kit (Trevena) as recommended by the manufacturer. Cardiomyocytes were prepared for immunocytochemistry as described previously. Data are expressed as mean ± SEM, and differences were evaluated for significance using the Student t test for unpaired data or 1-way ANOVA followed by the Bonferroni post test when appropriate.

An expanded Materials and Methods section is available online at http://www.circresaha.org.

**Results**

**Calcineurin Adenovirus Drives Neonatal Cardiomyocyte Hypertrophy In Vitro**

To investigate the importance of calcineurin signaling in vitro, we generated a replication-deficient adenovirus expressing the constitutively active form of mouse calcineurin Aα (AdCnA) (amino acids 1 to 398) to perform gene transfer in cultured cardiac myocytes. Consistent with the effect of activated calcineurin expression in vivo, AdCnA induced morphological hypertrophy, whereas control infection with a β-galactosidase adenovirus (Adβgal) had no effect (Figure 1A through 1H). The hypertrophic response was independent of cellular density and produced a quantitative increase in cell surface area 96 hours after infection (Figure 1I). AdCnA-infected cardiomyocytes also demonstrated greater sarcomeric organization (Figure 1E and 1F), rhythmic beating 24 hours after infection, and ANF protein expression (Figure 1K). Quantification of ANF expression in a representative experiment revealed that among cardiomyocytes, 9.2% (±2.4%) of those infected with Adβgal, 39% (±2.5%) of those infected with AdCnA, and 54% (±2.7%) of those treated with PE were positive (P < 0.05). These data indicate that expression of activated calcineurin is sufficient to drive the hypertrophic program in cultured neonatal cardiomyocytes.

**Calcineurin Adenoviral Infection Protects Cardiomyocytes From Apoptosis**

Activation of certain intracellular signaling pathways in cardiomyocytes has been shown to profoundly affect myocardial cell viability. It was of interest to determine whether constitutive calcineurin activation would promote or protect cardiomyocytes from cell death. To this end, we determined that AdCnA infection of cultured neonatal cardiomyocytes did not induce apoptosis over any time period compared with Adβgal infection or uninfected cells (data not shown). In contrast, AdCnA infection was found to protect cultured cardiomyocytes from apoptosis induced by 2 different pharmacological agents. Cells were cultured for 24 hours after adenoviral infection and treated with either staurosporine (1.0 μmol/L) or 2-deoxyglucose (2 mmol/L) in glucose- and serum-free medium. Staurosporine was previously shown to induce cardiomyocyte apoptosis in a caspase-dependent manner. Similarly, metabolic inhibition with 2-deoxyglucose was also shown to induce apoptosis in cultured cardiomyocytes. The data show that AdCnA infection protected cardiomyocytes from morphological features associated with cell death, whereas Adβgal infection had no protective effect (Figure 2C, 2D, 2G, and 2H). As a positive control for these assays, we generated an adenovirus expressing the antiapoptotic gene Bcl-xL. AdBcl-xL infection also protected cardiomyocytes from both staurosporine- and 2-deoxyglucose–mediated effects (Figure 2E and 2F). As a further control, the calcineurin-inhibitory drug cyclosporine was added to AdCnA-infected cardiomyocytes, resulting in a reversal of protection.
However, cyclosporine alone did not induce apoptosis in untreated cardiomyocytes in serum-free medium (data not shown).

TUNEL assays were performed to quantify the induction of cell death by staurosporine and 2-deoxyglucose. Only 2% to 4% of nontreated cardiomyocytes were TUNEL-positive, whereas staurosporine or 2-deoxyglucose treatment induced 17% and 26% TUNEL-positive staining, respectively ($P<0.05$) (Figure 3). Consistent with cellular morphology shown above, AdCnA and AdBcl-xL infection significantly reduced staurosporine- and 2-deoxyglucose–induced cell death, whereas control Adβgal infection had no protective effect ($P<0.05$) (Figure 3). Adenoviral delivery routinely resulted in 98% to 99% cardiomyocyte infection, so that uninfected cells did not appreciably influence quantification. The protective effect of AdCnA was only partially reversed by cyclosporine ($P<0.05$), likely because this agent is not a complete inhibitor of calcineurin. This interpretation is consistent with the known profile of only partial immune suppression in vivo, even at a high dosage of cyclosporine.31

AdCnA infection also significantly protected cardiomyocytes from staurosporine- or 2-deoxyglucose–induced caspase-3 activation, poly(ADP-ribose) polymerase and protein kinase Cδ degradation, and DNA laddering compared with Adβgal infection (data not shown). We and others have previously characterized that 2-deoxyglucose or staurosporine treatment of cultured cardiomyocytes results in caspase activation and cytochrome c release.28–30

### Activation of Endogenous Calcineurin Protects Myocytes From Apoptosis

It was previously reported that α-adrenergic stimulation (PE) protected cultured cardiomyocytes from apoptosis.20 We have determined that PE stimulation leads to calcineurin activation in cultured cardiomyocytes.31a To investigate the potential
involvement of calcineurin in PE-mediated protection from apoptosis, we treated cardiomyocytes with cyclosporine or a calcineurin-inhibitory adenovirus. As previously reported, we also found that PE significantly protected cardiomyocytes from staurosporine- and 2-deoxyglucose–induced cell death (Figure 3). Remarkably, inhibition of calcineurin with cyclosporine partially reversed the antiapoptotic effects of PE to 2-deoxyglucose (Figure 4). Because cyclosporine is known to affect proteins other than calcineurin, we also specifically targeted calcineurin by adenoviral expression of a 194–amino acid inhibitor peptide from the cain protein.25,26 Adcain infection significantly reversed the antiapoptotic effects of PE (Figure 4), whereas Adβgal had no effect. Adcain infection alone in serum-free medium did not induce cell death (data not shown). These data indicate that calcineurin is a necessary component of PE-mediated protection from cardiomyocyte cell death in vitro.

These data also indicate that physiological activation of endogenous calcineurin affords protection from cell death. It was also of interest to determine whether MAPK signaling factors played a role in PE-mediated protection from apoptosis. Inhibition of p38-reactive signaling with SB202190 (20 μmol/L) did not reverse the protective effect of PE, whereas inhibition of extracellular signal–regulated kinase (ERK) signaling with the mitogen-activated protein/ERK (MEK)–1 inhibitor PD98059 (25 μmol/L) partially reversed the protective effect of PE (Figure 4). These data indicate that ERKs, but not p38, are components of PE-mediated protection from cardiomyocyte apoptosis. However, the hierarchy between calcineurin and MAPK signaling pathways in regulating the hypertrophic program or protection from apoptosis is currently unknown.

Because NFAT3 acts downstream of calcineurin in mediating cardiac hypertrophy,21 we investigated the ability of NFAT3 to protect cardiomyocytes from apoptosis induced by 2-deoxyglucose using an adenovirus expressing the same truncated NFAT3 cDNA that promoted cardiac hypertrophy in transgenic mice.21 AdNFAT3 infection partially protected cardiomyocytes from 2-deoxyglucose–induced apoptosis (Figure 5). These data suggest that NFAT3 is partially responsible for mediating the antiapoptotic effects of cal-
cineurin activation in cardiomyocytes. However, AdNFAT3 infection was not as protective as AdCnA infection, suggesting that calcineurin provides protection by additional mechanisms.

To this end, we examined the levels or activation state of multiple proteins known to promote cell survival. Although the phosphorylation state of BAD and the protein amounts of Bcl-2, Bcl-xL, and Bax proteins were unchanged, the Akt/PKB phosphorylation state was found to be enhanced. Akt was previously shown to directly promote cell viability through multiple mechanisms.32–34 We observed that PE stimulation for 3 hours was sufficient to induce Akt phosphorylation, but that inhibition of calcineurin with cyclosporine blocked Akt phosphorylation (Figure 6A). Augmentation of intracellular calcium levels with the ionophore A23187, a known inducer of calcineurin, stimulated Akt phosphorylation after 6 hours, which is sensitive to cyclosporine. C, Western blot of protein extracts from 8-week-old, failing calcineurin-transgenic hearts shows increased Akt phosphorylation, without a change in total Akt protein. CsA indicates cyclosporine; TG, transgenic; NTG, nontransgenic.

significant increase in Akt phosphorylation in calcineurin-transgenic hearts at 8 weeks, but not 8 days (Figure 6C). The increase in Akt phosphorylation at 8 weeks is associated with the onset of heart failure in these mice, whereas day 8 is prehypertrophic. Collectively, these data indicate that Akt is partially responsible for mediating the antiapoptotic effects of calcineurin in vitro and in vivo.

Calcineurin-Transgenic Mice Show Dilated Heart Failure

We have previously reported that transgenic mice expressing the constitutively active calcineurin cDNA in the heart develop profound hypertrophy characterized by a 2- to 3-fold increase in heart-to-body-weight ratio, which rapidly progresses to dilated heart failure.21 To analyze this effect in more detail, we performed echocardiography on 8-week-old transgenic mice (mouse line 37). A representative M-mode tracing demonstrates the severity of heart failure in these mice (Figure 7). Fractional shortening was consistently reduced by 70% to 80% by 8 weeks of age compared with nontransgenic littermate controls (S.A. Witt, T.R. Kimball, and J.D. Molkentin, unpublished results, 1998).

Absence of Increased Cardiomyocyte TUNEL Staining in Calcineurin-Transgenic Hearts

Because dilated heart failure is associated with increased cardiomyocyte apoptosis, we reasoned that failing calcineurin-transgenic hearts would show enhanced cell death. However, an exhaustive histologic analysis of
TUNEL-stained 8-week-old hearts failed to reveal a significant difference in cardiomyocyte TUNEL reactivity between transgenic and wild-type mice (Figure 8A). In contrast, calcineurin-transgenic hearts showed a statistically significant increase in nonmyocyte TUNEL staining, likely because of the increase in interstitial space associated with mild edema, immune cell infiltration, and heart failure ($P < 0.05$) (Figure 8B). A typical TUNEL-stained histological section from a calcineurin-transgenic and a nontransgenic heart demonstrates predominantly interstitial cell reactivity (blue) (Figures 8C and 8D).

**Calcineurin Protects Against Apoptosis Occurring After Ischemia/Reperfusion**

To test the hypothesis that activated calcineurin might protect cardiomyocytes from apoptosis in vivo, calcineurin- and wild-type hearts were subjected to ischemia/reperfusion–induced cell death. Temporary occlusion of the left coronary artery followed by reperfusion is known to potently induce cardiomyocyte apoptosis. Cardiac ischemia followed by reperfusion in nontransgenic control mice produced substantial DNA laddering, indicating activation of apoptosis (Figure 9). However, ischemia/reperfusion of calcineurin-transgenic hearts demonstrated substantial protection against typical DNA fragmentation, indicating that calcineurin-mediated hypertrophy protects cardiomyocytes from apoptotic stimuli in vivo.

**Cyclosporine Administration Does Not Induce Apoptosis in Gqα-transgenic Mice**

The observation that calcineurin protects cardiomyocytes from apoptosis in vivo might suggest that cyclosporine would be proapoptotic. To investigate this possibility in vivo, we...
Cyclosporine administration to Gqα-transgenic mice does not induce TUNEL labeling. Gqα-transgenic mice were treated with cyclosporine at 20 mg/kg per day for 8 weeks, after which the hearts were collected for histologic TUNEL labeling. Cyclosporine did not induce TUNEL labeling in transgenic or wild-type hearts, whereas aortic banding (positive control) induced abundant labeling. Three animals were characterized in each treatment group, whereas only 1 aortic-banded control was examined. NTG indicates nontransgenic. *P<0.05 vs Gqα saline.

Figure 10. Cyclosporine administration to Gqα-transgenic mice does not induce TUNEL labeling. Gqα-transgenic mice were treated with cyclosporine at 20 mg/kg per day for 8 weeks, after which the hearts were collected for histologic TUNEL labeling. Cyclosporine did not induce TUNEL labeling in transgenic or wild-type hearts, whereas aortic banding (positive control) induced abundant labeling. Three animals were characterized in each treatment group, whereas only 1 aortic-banded control was examined. NTG indicates nontransgenic. *P<0.05 vs Gqα saline.

Discussion

In this study, we provide multiple lines of evidence that calcineurin protects cardiomyocytes from apoptosis both in vivo and in vitro. First, apoptosis induced by ischemia/reperfusion injury was largely rescued in calcineurin-transgenic hearts compared with nontransgenic hearts. Second, AdCnA infection protected cardiomyocytes in culture from TUNEL labeling, caspase activation, and DNA laddering. Third, direct inhibition of endogenous calcineurin either with cyclosporine or with a noncompetitive peptide inhibitor reversed the antiapoptotic effects of PE stimulation. We demonstrate calcineurin-dependent protection from apoptosis both in vivo and in vitro through expression of an activated calcineurin mutant or by activation of endogenous calcineurin. Taken together, these data establish a role for calcineurin signaling in protecting myocardial cells from apoptotic insults. These results also establish a mouse model of dilated heart failure that is independent of apoptosis.

Inspection of the literature suggests that humans in dilated heart failure and many animal models that mimic human disease have increased numbers of apoptotic myocytes (reviewed in Reference 36). These cumulative studies have suggested a causal relationship between the onset of apoptosis and progression of heart failure. Calcineurin-transgenic mice develop dramatic concentric hypertrophy (3-fold increase in heart size) that transitions to dilated heart failure by 8 weeks of life. Despite this phenotype, dilated and failing calcineurin-transgenic hearts do not show increased TUNEL labeling, but instead show resistance to ischemia/reperfusion-induced DNA laddering. This predicts that calcineurin-transgenic mice are a nonapoptotic model of heart failure. However, the causality of heart failure in calcineurin-transgenic mice is likely linked to other pathologies. First, the extreme degree of cardiac hypertrophy in calcineurin-transgenic mice is predicted to negatively impact function based on geometric constraints. Second, the profound hypertrophy in calcineurin mice is also associated with bradycardia and arrhythmia (Figure 7, data not shown). Third, calcineurin-transgenic hearts show considerable interstitial cell fibrosis. Finally, the default response of the myocardium when unable to hypertrophy further may simply be to dilate. It is likely that these factors, in the absence of apoptosis, are sufficient to induce heart failure.

The relationship between hypertrophic signaling pathways and the regulation of apoptosis is rather complex. Whereas some studies have shown antiapoptotic effects associated with various hypertrophic stimuli, other studies have shown induction of apoptosis by certain reactive signaling pathways. Among these proapoptotic pathways are the heterotrimeric GTP-binding proteins Gqα and Gsα.7,8 Similarly, cardiomyocyte apoptosis has been shown to be augmented by p38α, β-adrenergic receptors, NO synthase activity, atrial natriuretic peptide, tumor necrosis factor-α, angiotensin II, overexpression of the transcription factor Id, and p53.9–13,15,37–39

Taken together, it appears that the regulation of cardiac myocyte apoptosis in the hypertrophied myocardium is complex and depends on the relative activation pattern of distinct signaling pathways, with Gqα, Gsα, p53, and p38α stimulation promoting cardiomyocyte death and calcineurin, NFAT3, p38β, IGF-1/phosphatidylinositol 3-kinase/Akt, and ERK activation promoting cardiomyocyte survival. It is difficult to dissect the direct mechanisms whereby calcineurin protects myocytes from apoptosis, because calcineurin activation also induces significant hypertrophy, which itself may afford protection. Previous studies have demonstrated that certain hypertrophic agonists or activation of certain reactive signaling pathways provides protection from apoptosis.14–20 Although PE, endothelin-1, cardiotoxin-1 (gp130), IGF-1, ERK activation, and p38β activation have all been shown to be protective, a correlation with cell death–regulatory pathways (caspases or death receptors) or with proteins that regulate mitochondrial integrity (Bcl-2) has not been described.14–20 The direct mechanisms whereby certain reactive signaling pathways protect cardiomyocytes from apoptosis are largely uncharacterized. Simi-
larly, calcineurin-transgenic hearts and AdCnA-infected cardiomyocytes did not demonstrate a perturbation in the Bcl-2-to–Bax ratio or the Bcl-xL-to–Bax ratio. However, one mechanism whereby calcineurin signaling directly protects cardiac myocytes is through activation of PKB/Akt. Akt is known to downregulate caspase-9 activation, phosphorylate BAD, and directly activate nuclear factor-κB to promote cell survival.32–34 Although we have demonstrated that Akt activation is associated with calcineurin-induced hypertrophy, it is not clear how this activation occurs. However, it is likely that calcineurin signaling first activates the hypertrophic program, which then indirectly leads to Akt activation, providing protection from cell death. NFAT3 adenosinergic gene transfer also provided partial protection from 2-deoxyglucose–induced TUNEL labeling, suggesting an additional mechanism whereby calcineurin provides protection. However, the direct mechanism whereby NFAT3 provides protection from cell death is unknown, but it can be speculated that protection is linked to the hypertrophic response itself.

The observation that calcineurin protects cardiomyocytes from apoptosis might also suggest that cyclosporine treatment could promote myocardial cell apoptosis in vivo. However, patients on chronic cyclosporine therapy have not been reported to be at increased risk for heart failure, although an increased propensity toward hypertrophy has been described.40 We directly addressed this issue experimentally by treating a mouse model that is prone to apoptosis (Gqα) with cyclosporine at 20 mg/kg per day for 8 weeks. Characterization of TUNEL labeling did not reveal an induction of cell death in wild-type or Gqα-transgenic mice with cyclosporine, yet aortic banding induced a profound increase (Figure 10, J. Ross and G.W. Dorn, unpublished data, 1999). In other cell types, cyclosporine has been shown to inhibit apoptosis by preventing mitochondrial pore transition, which is regulated by cyclophilin-D (reviewed in Reference 41). These data suggest that cyclosporine has diverse intracellular effects, some of which protect cells and others of which may be proapoptotic. Indeed, calcineurin was recently shown to either protect cells from apoptosis or to directly induce apoptosis depending on cross talk between other intracellular signaling pathways.42

Calcineurin has been shown to promote viability in multiple cell types by suppressing apoptosis,33–46 whereas other studies have shown that calcineurin can activate apoptosis in disparate cell types.47–50 These differing accounts suggest that the role of calcineurin in controlling cell viability is complex and integrated with other signaling pathways. In cardiomyocytes, it is likely that calcineurin functions in concert with other reactive signaling pathways to achieve a balanced activation of cellular hypertrophy, which is beneficial to cellular viability.

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References


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