Functional Role of Phosphodiesterase 3 in Cardiomyocyte Apoptosis
Implication in Heart Failure

Bo Ding, MD*; Jun-ichi Abe, MD, PhD*; Heng Wei, MS*; Qunhua Huang, PhD; Richard A. Walsh, MD; Carlos A. Molina, MD; Allan Zhao, PhD; Junichi Sadoshima, PhD; Burns C. Blaxall, PhD; Bradford C. Berk, MD, PhD; Chen Yan, PhD

**Background**—Myocyte apoptosis plays an important role in pathological cardiac remodeling and the progression of heart failure. cAMP signaling is crucial in the regulation of myocyte apoptosis and cardiac remodeling. Multiple cAMP-hydrolyzing phosphodiesterases (PDEs), such as PDE3 and PDE4, coexist in cardiomyocytes and elicit differential temporal/spatial regulation of cAMP signaling. However, the role of PDE3 and PDE4 in the regulation of cardiomyocyte apoptosis remains unclear. Although chronic treatment with PDE3 inhibitors increases mortality in patients with heart failure, the contribution of PDE3 expression/activity in heart failure is not well known.

**Methods and Results**—In this study we report that PDE3A expression and activity were significantly reduced in human failing hearts as well as mouse hearts with chronic pressure overload. In primary cultured cardiomyocytes, chronic inhibition of PDE3 but not PDE4 activity by pharmacological agents or adenovirus-delivered antisense PDE3A promoted cardiomyocyte apoptosis. Both angiotensin II (Ang II) and the β-adrenergic receptor agonist isoproterenol selectively induced a sustained downregulation of PDE3A expression and induced cardiomyocyte apoptosis. Restoring PDE3A via adenovirus-delivered expression of wild-type PDE3A1 completely blocked Ang II– and isoproterenol-induced cardiomyocyte apoptosis, suggesting the critical role of PDE3A reduction in cardiomyocyte apoptosis. Moreover, we defined a crucial role for inducible cAMP early repressor expression in PDE3A reduction–mediated cardiomyocyte apoptosis.

**Conclusions**—Our results suggest that PDE3A reduction and consequent inducible cAMP early repressor induction are critical events in Ang II– and isoproterenol-induced cardiomyocyte apoptosis and may contribute to the development of heart failure. Drugs that maintain PDE3A function may represent an attractive therapeutic approach to treat heart failure. (Circulation. 2005;111:2469-2476.)

**Key Words:** phosphodiesterases ▪ apoptosis ▪ heart failure

The progressive loss of cardiomyocytes due to apoptosis is considered to play a critical role in pathological cardiac remodeling and heart failure because the myocardium has limited regenerative capacity.1,2 Neurohormonal activation of the angiotensin and adrenergic systems contributes to progressive ventricular remodeling and worsening clinical heart failure.3,4 cAMP signaling in cardiomyocytes may contribute to both normal physiological adaptation and pathological remodeling and may be associated with cellular hypertrophy, apoptosis, and alterations in contractile function. However, animal models with perturbations of cAMP-mediated signaling have yielded inconsistent results, making it difficult to conclude whether enhancing cAMP signaling is beneficial or deleterious for failing hearts. For example, results from a panel of transgenic animals with cardiac-specific overexpression of β1-adrenergic receptor (β1-AR),5 Gs,6 and protein kinase A (PKA)7 point to a harmful role for persistent activation of the β-AR/cAMP axis. However, results from cardiac-specific overexpression of adenylyl cyclases type 6 and 8 (AC6 and AC8)8–10 or β2-AR11 suggest a beneficial effect of increased β-AR responsiveness and cAMP signaling on heart failure. One potential explanation for these differences may be that the temporal/spatial changes of cAMP generation and subsequent PKA-dependent phosphorylation

Received September 27, 2004; revision received December 14, 2004; accepted January 10, 2005.
From the Center for Cardiovascular Research, University of Rochester School of Medicine and Dentistry, Aab Institute of Biomedical Science, Rochester, NY (B.D., J.A., H.W., Q.H., B.C. Berk, C.Y.); Case Western Reserve University, Cleveland, Ohio (R.A.W.); University of Medicine and Dentistry of New Jersey, Newark (C.A.M., J.S.); University of Pittsburgh, Pittsburgh, Pa (A.Z.); and Center for Cellular and Molecular Cardiology, University of Rochester, Rochester, NY (B.C. Blaxall).
*These authors contributed equally to this work.
The online-only Data Supplement can be found with this article at http://circ.ahajournals.org/cgi/content/full/01.CIR.0000165128.39715.87/DC1.
Correspondence to Chen Yan, PhD, and Jun-ichi Abe, MD, PhD, University of Rochester, 601 Elmwood Ave, Box 679, Rochester, NY 14642. E-mail Chen_Yan@urmc.rochester.edu and Jun-ichi_Abe@urmc.rochester.edu
© 2005 American Heart Association, Inc.
Circulation is available at http://www.circulationaha.org DOI: 10.1161/01.CIR.0000165128.39715.87

2469
are divergent among various genetically manipulated animals. This concept is supported by the findings of multiple different cAMP pools in cardiomyocytes.12,13

Phosphodiesterases (PDEs) play important roles in regulating not only the amplitude/duration but also the compartmentalization of cyclic nucleotides. PDEs belong to a complex and diverse superfamily of at least 11 structurally related gene families (PDE1 to PDE11).14 At least 22 genes encoding >50 different PDE isoforms have been identified, and these PDE variants are selectively expressed in different tissue, cell, and subcellular compartments.15 In cardiomyocytes, the cAMP-hydrolyzing PDE activity contributed by PDE3 and PDE4 families represents ≥90% of total cAMP PDE activities, although their relative contributions may differ among species.16 The PDE3 gene family contains 2 subfamilies (PDE3A and PDE3B). Although it has been long believed that the major PDE3 isoform in cardiomyocytes is PDE3A,14 a recent study showed that PDE3B is also expressed in mouse heart (accounting for ≈30% of the total PDE3 activity in mouse heart) and may also be important in the regulation of cardiac function.16 The PDE4 gene family contains 4 subfamilies (PDE4A, PDE4B, PDE4C, and PDE4D), and PDE4B and PDE4D are the major PDE4 subfamilies expressed in rat neonatal cardiomyocytes.13 PDE3 and PDE4 have been shown to be localized to distinct compartments and regulate distinct pools of cAMP in cardiomyocytes.15 However, the functional role of PDE3 and PDE4 in regulation of cardiomyocyte apoptosis has not been studied.

In the present study we report that PDE3A expression/activity was significantly reduced in left ventricles of human failing hearts as well as in mouse hypertrophic and failing hearts induced by chronic pressure overload. Reduction of PDE3A but not PDE4 expression/activity in vitro upregulated the proapoptotic transcriptional repressor inducible cAMP early repressor (ICER) and enhanced cardiomyocyte apoptosis. Interestingly, we found that cardiomyocyte apoptosis induced by proapoptotic stimuli such as angiotensin II (Ang II) and isoproterenol was mediated by selective downregulation of PDE3A expression and subsequent induction of ICER. These data suggest that the downregulation of PDE3A observed in failing hearts may play a causative role in the progression of heart failure, in part by inducing ICER and promoting cardiomyocyte apoptosis.

Methods
Expanded methods are available in Data Supplement File I.

Reagents and Adenovirus Vectors
See Data Supplement File I.

Rat Neonatal Cardiomyocytes
Primary cultures of neonatal rat cardiomyocytes were performed as described previously.17 More than 90% of cells were cardiomyocytes (positive for α-actinin). Adenovirus-mediated transfection efficiency in cardiomyocytes is 90% to 95%. See Data Supplement File I for details.

Western Blot Analysis
See Data Supplement File I.

Relative Quantitative Reverse Transcription–Polymerase Chain Reaction
See Data Supplement File I.

cAMP and PDE Assays
See Data Supplement File I.

Analysis of Apoptosis
See Data Supplement File I.

Patient Population
Left ventricular (LV) myocardium samples were from explanted patient hearts with end-stage heart failure due to either dilated cardiomyopathy (DCM) or ischemic heart disease (IHD) as well as from nonfailing donor hearts as normal controls as described previously.18 All tissues were harvested from LV free wall within 2 inches of LV apex and excised cautiously to avoid the fibrotic scar area. See Data Supplement File I for patient information.

Mouse Model of Thoracic Aorta Constriction and Hemodynamic Measurements
Thoracic aorta constriction (TAC) was performed in male FVB mice at 10 to 12 week of age as described previously.19 Age-matched animals underwent the same procedure without placement of the aortic ligature to serve as controls. At 8 weeks after surgery, in vivo LV hemodynamic measurements were performed before euthanasia by methods described previously.19 See Data Supplement File I for details.

Statistical Analysis
Data are reported as mean±SD. Statistical analysis was performed with StatView 4.0 package (ABACUS Concepts). Differences were analyzed with 1-way or 2-way repeated-measures ANOVA as appropriate, followed by Scheffé correction for multiple comparisons. A value of P<0.05 was considered statistically significant.

Results
Reduction of PDE3 Activity and PDE3A Expression in LV of Human Failing Hearts
Decreased PDE3A mRNA levels and PDE3 activity have been reported in pacing-induced failing dog hearts.20,21 However, changes of PDE3 protein and activity in human heart failure are still not well characterized. Therefore, we examined PDE3 activity and protein expression in LV of end-stage failing human hearts. As shown in Figure 1A, PDE3 activity was significantly decreased in patients with DCM and IHD. Total PDE4 activity was low and not significantly altered (data not shown). Western blot analysis revealed that the protein level of a PDE3A isoform (≈120 kDa and corresponding to the myocardial PDE3A isoform) was significantly decreased in DCM and IHD (Figure 1B, 1C, 1D). The magnitude of PDE3 activity reduction was smaller than that of PDE3A protein reduction (Figure 1A, 1D), which is probably due to the fact that PDE3 activity reflects a mixture of multiple PDE3 isoforms present in different cell types in the heart.

Reduction of PDE3A Expression in Mouse Hearts After Chronic Pressure Overload
We also examined PDE3A expression in a mouse model of chronic pressure overload via TAC. As shown in the supplementary table (Data Supplement File II), LV weight was increased by 89±10.5% in 8-week TAC mice compared with age-matched control mice. The LV systolic developed pressure per gram of LV mass (a measure of force development...
per unit of myocardium) was significantly depressed in 8-week TAC mice compared with the control group, indicating the presence of depressed systolic performance. Apoptotic myocytes were also significantly increased. Interestingly, PDE3A protein levels were significantly decreased in 8-week TAC hearts (Figure 2A, 2B). These data support the possible involvement of PDE3A reduction in the progression of heart failure.

Reduction of PDE3-Induced Cardiomyocyte Apoptosis

Previous data have shown that an increase in cAMP levels via chronic stimulation of β-AR signaling or cAMP analogues leads to cardiomyocyte apoptosis, and PDE3 is one of the major cAMP-hydrolyzing PDE families in cardiomyocytes. Therefore, to determine the pathological role of PDE3A downregulation in cardiomyocytes, we examined the effects of reducing PDE3 activity or protein expression on cardiomyocyte apoptosis using cultured rat neonatal cardiomyocytes. Apoptosis was measured by 2 different methodologies: terminal deoxynucleotidyl transferase–mediated nick-end labeling (TUNEL) staining and enzyme-linked immunosorbent assay (ELISA) for detecting DNA fragments. Ang II and isoproterenol were used as positive controls because they are known to stimulate cardiomyocyte apoptosis and play important roles in cardiac remodeling and the progression of heart failure. As shown in Figure 3A, inhibition of PDE3 activity with two structurally different PDE3-selective inhibitors, milrinone and cilostamide, significantly increased apoptosis in cardiomyocytes, equivalent to that induced by Ang II or isoproterenol (Figure 3A). However, inhibition of PDE4 by the PDE4-selective inhibitor rolipram, another major cAMP-PDE in rat neonatal cardiomyocytes, had no significant effect on cardiomyocyte apoptosis (Figure 3A). The proapoptotic effects of PDE3 inhibitors very likely result from PDE3 inhibition because the doses of PDE3 inhibitors had no significant cross-inhibitory effects on other cAMP-hydrolyzing PDEs expressed in cardiomyocytes (Data Supplement File III), and 2 structurally different PDE3 inhibitors (milrinone and cilostamide) elicited similar effects on cardiomyocyte apoptosis.

To verify the effects of PDE3 and PDE4 inhibition on global intracellular cAMP, we measured cAMP levels under various doses of the PDE3 inhibitor milrinone or the PDE4 inhibitor rolipram in the total cell lysates using a conventional enzymatic immunoassay method. In contrast to the effects on cardiomyocyte apoptosis, PDE4 inhibition elicited a much higher cAMP elevation than PDE3 inhibition at both the basal and β-AR–stimulated states (Figure 3B). This observation is consistent with the previous finding that PDE3 and PDE4 inhibitors elicited strikingly different cAMP dynamics when measured in live cardiomyocytes by real-time imaging of cAMP in situ via fluorescence resonance energy transfer. PDE3 inhibition elicited a transient and moderate increase of intracellular cAMP, whereas PDE4 inhibition elicited a sustained large increase of intracellular cAMP.

To further confirm the specific effect of reduction of PDE3 activity on cardiomyocyte apoptosis, PDE3A expression was reduced by the expression of antisense PDE3A via adenovirus (Ad-PDE3A-AS), which selectively reduced PDE3A but not PDE4D protein levels (Figure 3C). We found that expression of PDE3A-AS induced cardiomyocyte apoptosis similar to the PDE3 inhibitors milrinone and cilostamide.
These data indicate that reduction of PDE3A, but not PDE4, promotes cardiomyocyte apoptosis, although the amplitude of cAMP increase via PDE4 inhibition is even greater than that via PDE3 inhibition (Figure 3B).13

Ang II and Isoproterenol Induced a Sustained Reduction of PDE3A Expression

Because reduction of PDE3A expression and activity induced cardiomyocyte apoptosis to an extent similar to that elicited by Ang II or isoproterenol, we explored the possibility that Ang II and isoproterenol stimulation downregulated PDE3 expression. As shown in Figure 4, Ang II decreased PDE3A protein levels after 12 hours, and this reduction was sustained for \( \geq 72 \) hours (Figure 4A). Ang II–mediated reduction of PDE3A proteins was dose dependent, with an EC\(_{50} \) at \( \approx 40 \) nmol/L. We found that decreased PDE3A mRNA correlated with the decreased PDE3A protein with the use of relative quantitative reverse transcription–polymerase chain reaction (Figure 4B). However, there was no downregulation of PDE3B, PDE4B, or PDE4D mRNA levels (data not shown). Similarly, isoproterenol also induced sustained reduction of PDE3A (Figure 4C). The Ang II– and isoproterenol-mediated PDE3A reduction was completely blocked by losartan (Ang II type 1 receptor [AT1R] selective antagonist) and metoprolol (\( \beta_1 \)-AR selective antagonist), respectively (Figure 4D), indicating that Ang II and isoproterenol elicit reduction of PDE3A expression directly through AT1R and \( \beta_1 \)-AR, respectively.

Expression of Exogenous PDE3A1 Prevented Ang II– or Isoproterenol-Induced Cardiomyocyte Apoptosis

To further determine the role of PDE3A downregulation in mediating Ang II– and isoproterenol-induced cardiomyocyte...
apoptosis, we examined whether persistent high levels of PDE3A could prevent cardiomyocyte apoptosis via exogenous PDE3A expression. We found that expression of exogenous PDE3A1 via adenovirus completely blocked Ang II– and isoproterenol-induced cardiomyocyte apoptosis measured by both TUNEL staining (Figure 5A) and the ELISA method (Figure 5B). However, expression of exogenous PDE4D3 had no effect on Ang II– and isoproterenol-induced cardiomyocyte apoptosis (Figure 5A and 5B) despite much higher cAMP-hydrolyzing PDE activity achieved on exogenous PDE4D3 expression (Figure 5C). In addition, cardiomyocyte apoptosis induced by Ad-PDE3A-AS–mediated reduction of endogenous PDE3A was not further increased by addition of Ang II or isoproterenol (Figure 5D), suggesting that downregulation of PDE3A is likely a critical step for Ang II– and isoproterenol-induced cardiomyocyte apoptosis.

**PDE3A-Mediated Regulation of CREB, ICER, and Bcl-2**

Previous data have shown that chronic β-AR stimulation leads to induction of the proapoptotic transcriptional repressor ICER in a CREB-dependent manner and that the induction of ICER is critical for β-AR–induced cardiomyocyte apoptosis. CREB activation can be induced via phosphorylation of serine 133 by several serine/threonine kinases, including cAMP-dependent PKA. Thus, it is likely that reduction of PDE3 induces ICER and promotes apoptosis by activation of CREB in a cAMP/PKA-dependent manner. We found that inhibition of PDE3 by milrinone increased CREB activation and ICER protein levels in a dose-dependent manner (Figure 6A). However, inhibition of PDE4 by rolipram did not activate CREB and did not induce ICER (Figure 6A), despite the fact that PDE4 inhibition largely increased intracellular cAMP levels (Figure 3B). Bcl-2 is a well-known antiapoptotic molecule, and its expression can be repressed by ICER. We observed decreased Bcl-2 protein levels by PDE3 inhibition but not PDE4 inhibition (Figure 6C). These data suggest that PDE3 and PDE4 may regulate different pools of cAMP, which have distinct effects on CREB activation, ICER induction, and Bcl-2 expression.

We also determined whether PDE3 reduction by Ang II or isoproterenol activates CREB and induces ICER. As shown in Figure 6B and 6C, CREB activation, ICER induction, and Bcl-2 reduction were observed by Ang II stimulation for 48 hours, which paralleled the PDE3A reduction. Expression of exogenous PDE3A1 but not PDE4D3 via adenovirus blocked Ang II–induced CREB activation, ICER induction, and Bcl-2 protein levels were detected by Western blotting.

---

**Figure 5.** Role of expression of exogenous PDE3A1 in prevention of Ang II– or isoproterenol (ISO)-induced cardiomyocyte apoptosis. Cardiomyocytes were transduced with Ad-LacZ, Ad-PDE3A1, Ad-PDE4D3, or Ad-PDE3A-AS. The dose of each adenovirus is 30 MOI. Twenty-four hours after treatment or transduction, cardiomyocytes were stimulated with or without Ang II (200 nmol/L) or isoproterenol (10 μmol/L) for an additional 48 hours. Apoptosis was examined by either TUNEL staining (A) or ELISA method (B and D). Data represent mean±SD of 4 culture preparations. C, cAMP PDE activity was measured with the use of 1 μmol/L cAMP as substrate. Data represent mean±SD of 3 assays.

**Figure 6.** Role of PDE3 and PDE4 in regulation of CREB, ICER, and Bcl-2. Cardiomyocytes were treated with milrinone or rolipram at the doses indicated for 48 hours (A, C) or transduced with either Ad-LacZ, Ad-PDE3A1, or Ad-PDE4D3 at 30 MOI for 24 hours, followed by treatment with or without Ang II (200 nmol/L) for 48 hours (B, C). Phosphorylated CREB, total CREB, ICER, or Bcl-2 protein levels were detected by Western blotting.
reduction (Figure 6B and 6C). Similarly, exogenous PDE3A1 but not PDE4D3 prevented isoproterenol-induced CREB activation and ICER induction (data not shown). These data suggest a critical role of PDE3A in the regulation of CREB, ICER, and Bcl-2 by chronic Ang II and isoproterenol stimulation.

Critical Role of ICER in PDE3A Reduction–Mediated Cardiomyocyte Apoptosis

To determine whether ICER induction is critical in PDE3A-regulated cardiomyocyte apoptosis, we examined the effect of antisense ICER (ICER-AS) on Ang II–, isoproterenol–, and PDE3 reduction–induced cardiomyocyte apoptosis. Similar to previous observations, this Ad-ICER-AS can efficiently block ICER induction by isoproterenol and Ang II (data not shown). We found that expression of ICER-AS via adenovirus (Ad-ICER-AS) completely blocked Ang II– and isoproterenol-induced cardiomyocyte apoptosis, analyzed by both TUNEL (Figure 7A) and ELISA methods (Figure 7C). Furthermore, cardiomyocyte apoptosis, induced by PDE3 reduction due to either milrinone or PDE3A-AS, was also diminished by Ad-ICER-AS (Figure 7B and 7C). These data together strongly suggest that the reduction of PDE3A plays a critical role in Ang II– or isoproterenol-induced apoptosis through activating CREB and inducing ICER expression. In contrast, PDE4 does not appear to be critical in the regulation of cardiomyocyte apoptosis because it lacks the ability to stimulate CREB and ICER.

Induction of ICER Expression in Human Failing Hearts and in Mouse Hearts With Chronic Pressure Overload

Because we found that reduction of PDE3A leads to the induction of ICER in cultured cardiomyocytes, we investigated whether an upregulation of ICER correlates with an observed downregulation of PDE3A in human and mouse failing hearts. Interestingly, ICER expression was significantly increased in human failing hearts (Figure 8A, 8B, and 8C) as well as mouse 8-week TAC mouse hearts (Figure 8D and 8E), in which PDE3A expression was significantly decreased. These results suggest that concomitant reduction of PDE3A and induction of ICER may be a cause of myocyte apoptosis and cardiac dysfunction in human heart failure.

Discussion

The major novel findings of the present study include the following: (1) there was concomitant PDE3A reduction and ICER induction in human failing hearts and mouse failing hearts induced by chronic pressure overload (A and B). Western blots show ICER and β-actin protein levels in LV of nonfailing (control) and DCM (A) or IHD (B) hearts. C, Relative ICER expression level. The intensity of the band representing ICER was normalized to the intensity of 1 control sample that was designated as 1. D, Western blots show ICER and β-actin protein levels in control mouse hearts and 8-week TAC mouse hearts. E, Relative ICER expression level. The intensity of the band representing ICER was normalized to the intensity of 1 control sample that was designated as 1. Data represent mean±SD of 4 samples. *P<0.01 vs control mice.
pressure. However, in long-term clinical trials, the hemodynamic improvements seen early in therapy were typically not sustained, and a 40% increase in mortality was reported after several months of treatment, primarily as a result of arrhythmias and sudden death.

The mechanisms for loss of therapeutic benefit and increased mortality observed with chronic PDE3 inhibitor treatment are not well understood. Our finding that PDE3 inhibitors induced cardiomyocyte apoptosis may provide an explanation for the increased arrhythmias and sudden death in chronic PDE3 inhibitor therapy.

Both PDE3 and PDE4 are present in myocardium from various species, although their relative ratio might be different among species. PDE4D has been shown to play an important role in the regulation of β2-AR signaling in cardiomyocytes via controlling PKA activity at the membrane because phosphorylation of the β2-AR by cAMP-activated PKA switches its predominant coupling from stimulatory guanine nucleotide regulatory protein (G_s) to inhibitory guanine nucleotide regulatory protein (G_i). It also has been reported recently that PDE4D associates with the cardiac calcium release channel (ryanodine receptor) and regulates Ca^{2+} mobilization. We found in this study that inhibition of PDE3 but not PDE4 activity stimulates ICER induction and promotes cardiomyocyte apoptosis, further supporting the notion that PDE3 and PDE4 regulate specific signaling pathways and cellular functions in cardiomyocytes. The functional differences of PDE3 and PDE4 on cardiomyocyte apoptosis may reflect the differential effect of distinct pools of cAMP regulated by PDE3 versus PDE4. A recent study by Mongillo et al demonstrated that PDE3 and PDE4 regulate different compartmentalized cAMP in rat neonatal cardiomyocytes. For example, PDE3A was mainly localized in internal membrane, whereas PDE4B and PDE4D are localized in M/Z line and Z line, respectively. The cAMP response via inhibition of PDE3 or PDE4 was also strikingly different. Therefore, it is likely that PDE3 and PDE4 reside in distinct subcellular compartments, couple to distinct AC signaling complexes, differentially regulate compartmentalized cAMP signaling, and have distinctive functions in cardiomyocytes. Of course, the involvement of other cAMP-independent mechanisms cannot be ruled out.

Recently, Masciarelli et al reported that mice lacking PDE3A (PDE3A^−/−) are viable, and there are no other obvious deficiencies except that female PDE3A^−/− mice are completely sterile, which is due to cAMP/PKA-dependent meiotic arrest of the oocyte. Of note, there is no cardiac abnormality in PDE3A^−/− mice, although PDE3A is the major PDE3 isoform in cardiac muscle and PDE3 inhibitors show great effects on cardiac contractility. It is possible that depletion of PDE3A alone may not be sufficient to induce cardiac injury in vivo, although PDE3A is essential in cardiomyocyte survival. Thus, additional challenge of PDE3A^−/− mice with “stress” such as chronic pressure overload by TAC might be required to induce cardiac dysfunction. The effects of CREB activation on cardiomyocyte survival and heart failure are controversial, which is probably due to the dual effects of CREB on cell survival. For example, transgenic mice with cardiac-specific overexpression of DN-CREB through the predevelopment and postdevelopment stages of hearts developed dilated cardiomyopathy, likely because of impairment of the effects of CREB on normal cell growth and survival. However, our findings and those of others have shown that under elevated neurohormonal stimulation such as Ang II or isoproterenol in vitro or in vivo, CREB becomes proapoptotic via induction of ICER. The prosurvival effect of CREB is mediated by upregulation of antiapoptotic molecules such as Bcl-2 and inhibitor of apoptosis protein-2. The proapoptotic effects of CREB can be mediated by induction of ICER, which antagonizes antiapoptotic molecule expression. CREB-dependent induction of ICER may be critical for maintaining the balance of cell survival and death.

In vitro and in vivo studies have demonstrated that Ang II induces cardiomyocyte apoptosis primarily via the type 1 (AT1) receptor; however, the mechanisms by which Ang II induced cardiomyocyte apoptosis remain largely unknown. Regardless of the effect of Ang II treatment on cAMP levels, we demonstrated that AT1R stimulation leads to PDE3A reduction, CREB activation, ICER induction, and cardiomyocyte apoptosis similar to β-AR stimulation with isoproterenol. The previous evidence for a functional and physiological interaction between AT1R and β-AR may provide an explanation for the similar effects of AT1R and β-AR stimulation on the regulation of PDE3A, ICER, and cardiomyocyte apoptosis. The precise mechanism by which Ang II and isoproterenol downregulate PDE3A expression will be determined in the future.

β_1-AR and β_2-AR are 2 major types of β-ARs in cardiomyocytes. β_1-AR and β_2-AR signaling have markedly different roles in apoptosis, with β_2-AR proapoptotic and β_1-AR antiapoptotic. Isoproterenol, with equal affinity for both β_1-AR and β_2-AR, stimulates cardiomyocyte apoptosis, which is mainly mediated by the stimulation of β_2-AR. Similarly, we found that isoproterenol-stimulated downregulation of PDE3A is dependent on β_2-AR activation. However, selective stimulation of β_1-AR protected myocytes from apoptosis. It will be interesting in the future to determine whether selective activation of β_2-AR upregulates PDE3A expression. Nevertheless, these observations suggest that the proapoptotic effect via β_1-AR appears to predominate.

Acknowledgments

This work was supported by American Heart Association (grant 0455847T to Dr Yan) and in part by the National Institutes of Health (grants HL66919 to Dr Abe and HL63462 to Dr Berk). We thank Dr Jane Sottile for her helpful comments on the manuscript.

References


Functional Role of Phosphodiesterase 3 in Cardiomyocyte Apoptosis: Implication in Heart Failure
Bo Ding, Jun-ichi Abe, Heng Wei, Qunhua Huang, Richard A. Walsh, Carlos A. Molina, Allan Zhao, Junichi Sadoshima, Burns C. Blaxall, Bradford C. Berk and Chen Yan

_Circulation._ 2005;111:2469-2476; originally published online May 2, 2005; doi: 10.1161/01.CIR.0000165128.39715.87
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/111/19/2469

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2005/04/16/01.CIR.0000165128.39715.87.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org/subscriptions/