Morphological and Molecular Characterization of Adult Cardiomyocyte Apoptosis During Hypoxia and Reoxygenation

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Abstract—Apoptosis has been implicated in ischemic heart disease, but its mechanism in cardiomyocytes has not been elucidated. In this study, we investigate the effects of hypoxia and reoxygenation in adult cardiomyocytes and the molecular mechanism involved in cardiomyocyte apoptosis. Morphologically, reoxygenation induced rounding up of the cells, appearance of membrane blebs that were filled with margined mitochondria, and ultrastructural findings characteristic of apoptosis. Reoxygenation (18 hours of reoxygenation after 6 hours of hypoxia) and prolonged hypoxia (24 hours of hypoxia) resulted in a 59% and 51% decrease in cellular viability, respectively. During reoxygenation, cell death occurred predominantly via apoptosis associated with appearance of cytosolic cytochrome c and activation of caspase-3 and -9. However, nonapoptotic cell death predominated during prolonged hypoxia. Both caspase inhibition and Bcl-2 overexpression during reoxygenation significantly improved cellular viability through inhibition of apoptosis but had minimal effect on hypoxia-induced cell death. Bcl-2 overexpression blocked reoxygenation-induced cytochrome c release and activation of caspase -3 and -9, but caspase inhibition alone did not block cytochrome c release. These results suggest that apoptosis predominates in cardiomyocytes after reoxygenation through a mitochondrion-dependent apoptotic pathway, and Bcl-2 prevents reoxygenation-induced apoptosis by inhibiting cytochrome c release from the mitochondria and prevents activation of caspase-3 and -9. (Circ Res. 2000;87:118-125.)

Key Words: caspases • Bcl-2 • cytochrome c • adenovirus • mitochondria

Apoptosis is a highly regulated cell deletion process that is characterized by nuclear and cellular fragmentations. Apoptosis induced by staurosporine and UV irradiation in cell-free extracts and proliferating cells has been shown to be associated with the translocation of cytochrome c from the mitochondria to the cytosol.1–3 Released cytosolic cytochrome c, in the presence of dATP, then forms an activation complex with caspase-9 and apoptotic protein activating factor-1 (apaf-1), a novel 130-kDa molecule that shares sequence similarity with the Caenorhabditis elegans cell death regulator CED-4.4,5 This activation complex results in autoprocessing of caspase-9 as well as further activation of downstream caspases, such as caspase-3, to execute the final morphological and biochemical alterations that are characteristic of apoptosis.4

In the heart, there is accumulating evidence from both animal and human studies strongly suggesting that apoptosis occurs in various cardiovascular diseases.6 Especially, the effects of hypoxia and reoxygenation, which result in extremes of altered redox states, have been implicated as the primary causes of oxidative stress and tissue damage in ischemic heart disease.7,8 During hypoxia, there is cessation of mitochondrial oxidative phosphorylation, which normally fulfills the high metabolic needs of cardiomyocytes, and ATP is produced by much less efficient anaerobic glycolysis.7,8 Immediate resumption of oxidative phosphorylation by reoxygenation, therefore, is critical for restoring adequate ATP production and cell survival. However, an abrupt rise in reactive oxygen species in mitochondria during reoxygenation has been associated with a deleterious effect on cardiomyocytes.9 In fact, an increased rate of apoptosis has been observed in cerebral and cardiac reperfusion in animal models, suggesting that the deleterious effect during reperfusion is, at least in part, due to apoptosis.10–12 Because the amount of myocardial damage in patients with ischemic heart disease is the most important determinant of morbidity and mortality, limiting the loss of cardiomyocytes during oxidative stress will have important therapeutic implications.

Bcl-2, a mammalian homologue of the antiapoptotic gene ced-9 in C. elegans, is localized mainly to the mitochondrial membrane13 and is a prototypical member of the Bcl-2 family of proteins that modulates apoptotic responses in various cell types.14,15 Bcl-2 mRNA and proteins are expressed in developing and adult heart,16,17 and the protein is upregulated after...
coronary occlusions.\textsuperscript{17,18} However, the effects of Bcl-2 in heart have not been well characterized, and little is known about the effects of Bcl-2 in heart.

In this study, we used primary adult rat ventricular cardiomyocyte (ARVC) culture to investigate the effects of prolonged hypoxia and reoxygenation on cardiomyocyte apoptosis. ARVCs have an advantage over neonatal cardiomyocytes in that ischemic heart disease is present almost exclusively in the adult population and neonatal cardiomyocytes are relatively resistant to hypoxia\textsuperscript{19,20} (unpublished observation, 1999). We studied the morphological alterations of ARVCs during reoxygenation-induced apoptosis and examined the temporal and spatial localization of cytochrome c and caspase-3 and -9 activities during cardiomyocyte apoptosis. In addition, we studied the effect of caspase inhibition and Bcl-2 overexpression on apoptosis, cytochrome c release, and caspase-3 and -9 activities in cardiomyocyte.

### Materials and Methods

#### Adult Rat Cardiomyocyte Culture and Hypoxia/Reoxygenation Experiments

Adult rat cardiomyocytes were isolated from the hearts of female Sprague-Dawley rats (Charles River Laboratories) by enzymatic dissociation using 0.3\% collagenase, according to a previously published protocol with minor modifications.\textsuperscript{21} Each heart yielded \( \approx 3 \) to \( 4 \times 10^6 \) viable ventricular cardiomyocytes. Caspase inhibition was achieved by adding 25 \( \mu \)mol/L zVAD.fmk (Calbiochem) to the medium, which was the lowest concentration of zVAD.fmk that resulted in significant inhibition of apoptosis (data presented online; available at http://www.circresaha.org).

A hypoxic condition was created by incubating the cells in an airtight Plexiglas chamber with an atmosphere of 3\% CO\textsubscript{2}, 95\% N\textsubscript{2}, at 37\°C for the periods specified in each experiment. Experimental groups were divided into 3 major groups, as follows: (1) normoxic control (C), (2) hypoxia alone (H), and (3) reoxygenation after 6 hours of hypoxia (6H/18R).

#### Morphological Analysis

Immunofluorescent staining was performed according to published methods.\textsuperscript{22} Mitochondria staining using MitoTracker (Molecular Probe) and terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL) assay using dUTP-FITC (Boehringer Mannheim) were performed according to the manufacturers’ protocols. Immunofluorescent images were obtained using a Bio-Rad MRC-1024 laser scanning confocal microscope. Electron microscopic analysis was performed as published previously, with minor modifications.\textsuperscript{23}

#### Biochemical Analysis

Apoptosis was quantified by annexin V staining. Cellular viability was determined using a propidium iodide exclusion assay. Approximately 1000 cells were counted per dish (10 random microscopic fields at \( \times 250 \) magnification). A DNA fragmentation assay was performed on an equal number of cells (\( \approx 1 \times 10^6 \) cells/group) using low molecular weight DNA extraction, as published previously.\textsuperscript{23} Preparation on cytosolic extracts and immunoblotting were performed according to published methods.\textsuperscript{22} Caspase-3 and caspase-9 activities were measured using synthetic caspase substrates AcDEVD-pNa and AcLEHD-pNa, respectively, according to the published protocol.\textsuperscript{24}

#### Generation of Recombinant Adenovirus and Infection

Recombinant adenoviruses that express full-length human Bcl-2 cDNA (AdBcl-2) and nuclear lacZ (AdlacZ) were generated using the Cre-lox recombination system. This system uses the recombination in CRE8 cells, which produce a high amount of Cre recombinase, as described previously.\textsuperscript{25} Subsequent titration of the virus was done in ARVC culture. One hundred multiplicity of infection (MOI), which did not have an effect on cellular viability (data presented online; available at http://www.circresaha.org) and achieved \( >70\% \) to 80\% infectivity, was used for the experiments.

#### Statistics

All data are expressed as mean \( \pm \) SEM. Statistical analyses between 2 groups and among the groups were performed with an unpaired Student \( t \) test and ANOVA with the Bonferroni method, respectively.\textsuperscript{26} Probability (\( P \)) values of \( <0.05 \) were considered to be significant.

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

### Results

Reoxygenation Induces Specific Morphological Changes in ARVCs

Dissociated cardiomyocytes contained \( >90\% \) rod-shaped myocytes and \(<1\% \) nonmyocytes (Figure 1A). Rounding up of initially rod-shaped cells, especially the number of rounded-up cells with plasma membrane blebs, became more evident in cells exposed to 18 hours of reoxygenation after 6 hours of hypoxia (6H/18R), as compared with 24 hours of normoxic control (24C) and 24 hours of hypoxia alone (24H) (Figures 1B and 1C). Inhibition of caspase using zVAD.fmk in the 6H/18R group resulted in the preservation of rod-shaped morphology (Figure 1D).

In rod-shaped cardiomyocytes, immunofluorescent staining for sarcomeric actinin (green) and mitochondria (red) showed well-organized perpendicular and longitudinal staining patterns, respectively (Figure 1E). When rod-shaped cells became rounded up, sarcomere and mitochondria became disorganized and dispersed randomly throughout the cell (Figure 1F). In contrast, most of the rounded-up cells with membrane blebs (irregular membrane) had mitochondria that marginated to the periphery of cell and sarcomeric actinin that displaced centrally (Figure 1G).

The ultrastructural examination by electron microscopy demonstrated characteristic smooth sarcolemma, highly organized myofilbrils (Figure 2A; MF) arranged into sarcomeres, abundant mitochondria (M) packed between sarcomeres, and a normal-appearing nucleus (N) in rod-shaped ARVCs. In comparison, cardiomyocytes after 6H/18R demonstrated disassembly of myofilbrils and nuclear chromatin condensation and margination, which are typical features of apoptosis (Figure 2B, arrow). They also displayed plasma membrane blebs that contained, predominantly, mitochondria (Figure 2C, arrowheads). The cells treated with prolonged hypoxia exhibited findings that are typical of “necrotic” cell death, such as cell membrane disruption, extensive vacuolation, “contraction band,” and swollen nucleus with diffuse nuclear chromatin condensation (Figure 2D).

These results demonstrate that reoxygenation induces specific morphological changes in ARVCs, such as disassembly of sarcomeric and mitochondrial organizations, membrane blebs containing marginated mitochondria, and typical electron microscopic findings consistent with apoptosis. In contrast, cell death after hypoxia alone is characterized by...
ultrastructural changes more typical of nonapoptotic (eg, necrotic) cell death.

Reoxygenation Induces More Apoptotic Cell Death Than Hypoxia Alone

To determine the amount of cell death during various oxidative stresses, cellular viability at specified time points were determined during hypoxia and reoxygenation. In 24H and 6H/18R, the percentage of the viable cells decreased dramatically, reaching only 51% and 59% of the initial control, respectively (24C, 86.9±2.7%; 24H, 53.5±4.3%; and 6H/18R, 59.0±3.9%; n=6 [24C versus 24H and 6H/18R, P<0.01]) (Figure 3A). Of note, although the rate of cell death slowed initially just after reoxygenation, significant cell death occurred after 18 hours of reoxygenation. Characterization of the distinct mode of cell death revealed significant activation of apoptosis during reoxygenation, which was demonstrated by multiple specific biochemical markers of apoptosis. A DNA fragmentation assay demonstrated a significant increase in DNA laddering after 6H/18R as compared with both the normoxic control and the 24H group (Figure 3B). In the TUNEL assay, the rod-shaped and the rounded-up cardiomyocytes without membrane blebs (smooth surface) were TUNEL-negative (Figure 3C). In contrast, most of the rounded-up cells with membrane blebbing were TUNEL-positive (Figure 3C). The number of TUNEL-positive cells correlated with the duration of reoxygenation (24C, 7±1.4%; 24H, 13±1.9%; and 6H/18R, 26±3.8%; n=3 [24C versus 6H/18R, P<0.01]).

In 6H/18R, there was a significant increase in annexin V-positive cells compared with both 24C and 24H (Figure 4A). There was also a significant increase in the rate of apoptosis in 24H, although significantly less than in 6H/18R (24C, 9±1.1%; 24H, 16±2.9%; and 6H/18R, 24±1.5%; n=4 [24C versus 24H, P<0.05; 24C versus 6H/18R, P<0.01]) (Figure 4A). Caspase inhibition using zVAD.fmk significantly attenuated reoxygenation-induced cell death and apoptosis, but did not affect cell death and apoptosis during hypoxia alone (Figures 4B and 4C).

In addition, we measured lactate dehydrogenase (LDH) levels in the media to determine the extent of the leakage of the cytosolic component during oxidative insults. Only hypoxia, but not reoxygenation, resulted in a significant increase in LDH level in the media compared with the control (24C=59.4±6.1 U/L, 6H/18R=60.7±16.9 U/L,

Figure 1. Hypoxia and reoxygenation induce significant morphological changes in ARVCs. Shown are photographs of ARVCs after 24 hours of normoxia (A), 24 hours of hypoxia (B, arrows), and 6 hours of hypoxia/18 hours of reoxygenation without (C, arrows) and with (D) zVAD.fmk. E through G, Immunofluorescent staining of ARVCs at various stages of morphological changes. Rod-shaped cells display well-organized perpendicular striation of sarcomeric actinin (green) and longitudinally organized mitochondria (red). Nuclei are represented in blue (E). Rounded-up cells with smooth sarcolemma demonstrate disassembly of sarcomere and mitochondrial organizations (F). Cells with membrane blebs demonstrate peripheral margination of mitochondria and central displacement of sarcomeric actinin (G).

Figure 2. Reoxygenation induces ultrastructural changes in ARVCs. Electron microscope photographs of cardiomyocytes undergoing distinct mode of cell death. A, Normal cardiomyocyte shows highly organized myofibril (MF), abundant mitochondria (M), and normal-appearing nucleus (N). B, Apoptotic nucleus shows typical characteristics of apoptosis, such as chromatin condensation and margination (arrows). C, Apoptotic cardiomyocyte is characterized by myofibrillar disarray, peripheral displacement of mitochondria, and appearance of membrane blebs that contain numerous mitochondria (arrowheads). D, Cardiomyocyte undergoing characteristic morphological features of ‘‘contraction-band necrosis’’ showing diffuse chromatin condensation and disruption of membrane integrity. Magnification ×10,000.
Taken together, these findings demonstrate that reoxygenation is a more potent inducer of apoptotic cell death than hypoxia alone. In addition, the fact that caspase inhibition restored the cellular viability in 6H/18R almost to the control level suggests that the principal mode of cell death during reoxygenation is most likely through apoptosis.

Reoxygenation Causes Increase in Cytosolic Cytochrome c and Activation of Caspase-3 and -9

To determine whether the mitochondrion-mediated apoptosis pathway is active during reoxygenation-induced cardiomyocytes apoptosis, the temporal and spatial localization of cytochrome c was studied during cardiomyocyte apoptosis. During normoxia, viable rod-shaped cells (TUNEL-negative) showed organized speckled patterns of cytochrome c (blue) that colocalized with mitochondria (red) (Figure 5A, left). In contrast, during reoxygenation, apoptotic cells (TUNEL-positive) demonstrated mitochondria that were mainly localized to the periphery, but diffuse cytochrome c staining throughout the cells, that no longer colocalized to the mitochondria (Figure 5A, right).

There was a significant increase in cytosolic cytochrome c, which peaked at 6 hours of reoxygenation (in arbitrary units) (12C, 0.9±0.2; 6H/18R, 2.5±0.5; n=4 [P<0.05]) and remained elevated even after 18 hours of reoxygenation (Figure 5B). To exclude the possibility of contamination or breakage of mitochondria during the preparation of cytosolic fractions, we checked the cytosolic and mitochondrial fractions for cytochrome oxidase subunit IV, which is exclusively localized to the mitochondria. Strong cytochrome oxidase IV staining was evident only in the mitochondrial fraction even at a very low protein concentration (Figure 5C), confirming
that no significant mitochondrial contamination occurred during the preparation of the cytosolic fraction. Hypoxia alone did not cause significant cell death involving the release of cytochrome c (Figure 5D). In addition, caspase inhibition did not significantly block a reoxygenation-induced increase in cytosolic cytochrome c (Figure 5D). To determine the time-dependent activation of caspase during reoxygenation, caspase-3 and caspase-9 activities were measured using synthetic caspase substrates AcDEVD-pNa and AcLEHD-pNa, respectively. There were significant increases in both caspase-3 and -9 activities after 6 hours of reoxygenation that peaked at 9 hours, as follows: in milli–optical density (mOD)/hour, for caspase-3 activity, control, 5.7±1.5, and 6H/9R, 15.3±1.8 (n=4; P<0.01), and for caspase-9 activity, control, 11.3±1.8, and 6H/9R, 29.0±2.5 (n=4; P<0.01). Significant activation of both caspases persisted even after 18 hours of reoxygenation (Figure 6A).

These data suggest that the mitochondrion-mediated apoptosis pathway that involves cytochrome c and caspase-3 and -9 is operative in reoxygenation-induced cardiomyocyte apoptosis. Also, given that caspase inhibition attenuated apoptosis without blocking cytochrome c release, cytochrome c release is an upstream event of caspase activation and is not a terminal step in the apoptosis cascade.

Bcl-2 Overexpression Prevents Reoxygenation-Induced Apoptosis, Cytochrome c Release, and Caspase Activation

To study the effect of Bcl-2 during hypoxia and reoxygenation, we overexpressed Bcl-2 in ARVCs using recombinant adenovirus. Adenovirus expressing nuclear lacZ was used as the control infection. Both recombinant adenoviruses infected ARVCs in a titer-dependent manner (Figure 7). The titer of 100 MOI, which resulted in >70% to 80% infection efficiency (Figure 7A) and significant increase in Bcl-2 protein expression (Figure 7B), was used for the experiments.

Bcl-2 overexpression did not significantly affect the cellular viability during normoxia and hypoxia alone (Figure 7C). However, during 6H/18R, there was significant improvement in cellular viability with Bcl-2 overexpression (control, 59±3.9%; AdLacZ, 63±1.7%; and AdBcl-2, 84±5.3%; n=4 [control and AdlacZ versus AdBcl-2, P<0.05]) (Figure 7C).
This was mainly due to the inhibition of apoptosis, because Bcl-2 overexpression significantly inhibited reoxygenation-induced apoptosis (control, 23.5 ± 0.8%; AdLacZ, 26.3 ± 3.1%; and AdBcl-2, 12.5 ± 0.5%; n = 4 [control and AdlacZ versus AdBcl-2, P < 0.05]) (Figure 7D).

To elucidate the molecular mechanisms involved in the antiapoptotic effect of Bcl-2, we measured cytosolic cytochrome c and the activities of caspase-3 and -9. Reoxygenation-induced cytochrome c release was inhibited by Bcl-2 overexpression, as follows (in arbitrary units): control, 2.5 ± 0.5; AdLacZ, 2.4 ± 0.3; and AdBcl-2, 1.6 ± 0.3; n = 4 [control and AdlacZ versus AdBcl-2, P < 0.05]) (Figure 8C). Bcl-2 overexpression also resulted in significant inhibition of reoxygenation-induced caspase-3 and -9 activation, as follows (in mOD/hour): for caspase-3 activity, control, 15.3 ± 1.8; AdLacZ, 15.0 ± 1.4; and AdBcl-2, 7.7 ± 1.3; n = 4 [control and AdlacZ versus AdBcl-2, P < 0.05]), and for caspase-9 activity, control, 30.3 ± 2.3; AdLacZ, 27.8 ± 5.2; and AdBcl-2 = 8.3 ± 2.5; n = 4 [control and AdlacZ versus AdBcl-2, P < 0.01]) (Figures 8A and 8B). However, during hypoxia alone, Bcl-2 overexpression did not affect cytosolic cytochrome c level and caspase-3 and -9 activities (Figure 8).

Discussion

In this model of adult cardiomyocyte apoptosis, we demonstrated that different types of oxidative stress are associated with distinct modes of cell death. Our data suggest that apoptosis is a predominant mode of cell death during reoxygenation, but nonapoptotic cell death predominates during prolonged hypoxia alone. Reoxygenation, although associated with both apoptotic and nonapoptotic cell deaths, induced significantly greater apoptosis than hypoxia alone, despite the fact that hypoxia alone induced more overall cell death. This conclusion is further supported by activation of the mitochondrion-mediated apoptosis signaling pathway during reoxygenation. In contrast, hypoxia alone was characterized by membrane disruption, as evidenced by spillage of cytosolic components in the media (eg, LDH). Because therapeutic reperfusion is presently performed without any measures to protect myocardium from apoptosis during the treatment of myocardial infarction, it may be possible to further limit the infarct size by inhibition of apoptosis. In fact, the possible role of caspase inhibition in the heart has been supported by an experimental myocardial infarction model in which inhibition of caspase using zVAD.fmk decreased the infarct size acutely in rats.27,28

One of the main mechanisms of caspase activation has been shown to involve the release of cytochrome c from the mitochondria. In this study, we found that Bcl-2 overexpression significantly inhibited reoxygenation-induced cytochrome c release, consistent with the antiapoptotic effect of Bcl-2. This suggests that Bcl-2 may function by preventing the release of cytochrome c from the mitochondria, thereby inhibiting apoptosis.

Figure 7. Effect of Bcl-2 overexpression in cardiomyocyte apoptosis during reoxygenation and hypoxia. A, AdLacZ infection in ARVC cultures. Titer-dependent expressions of nuclear LacZ staining is demonstrated. B, AdBcl-2 infection in ARVC cultures. Bcl-2 protein overexpression in cardiomyocytes infected with AdBcl-2 is evident at 10 MOI and increased significantly as the titer increased. We used 100 MOI for the experiments. C and D, Quantitative analysis of cellular viability (C) and apoptosis (D) after no infection (open bars), AdLacZ infection (shaded bars), and AdBcl-2 infection (solid bars) under various conditions; n = 4. *P < 0.05, †P < 0.01.

Figure 8. Effect of Bcl-2 overexpression on cytochrome c release, and caspase-3 and -9 activities during hypoxia and reoxygenation. Quantitative analysis of cytosolic cytochrome c (A), caspase-3–like activity (B), and caspase-9 activity (C) after no infection (open bar), AdLacZ infection (shaded bars), and AdBcl-2 infection (solid bars) under various conditions; n = 4. *P < 0.05, †P < 0.01.
mitochondria to the cytosol. In this study, consistent with previous studies in neonatal cardiomyocytes by glucose deprivation, there was activation of a mitochondrial pathway characterized by the increase in cytosolic cytochrome c and the activation of caspase-3 and -9. The increase in cytosolic cytochrome c and the activities of caspases were transient, probably reflecting the transient nature of reoxygenation. Caspase inhibition prevented apoptosis without blocking cytochrome c release, which suggests that caspase inhibition occurs downstream of cytochrome c release. In contrast, Bcl-2 overexpression blocked cytochrome c release and both caspase-3 and -9 activation in adult cardiomyocytes. These findings are consistent with the previous findings in cell lines, in which Bcl-2 acts at the level of mitochondria to prevent cytochrome c release. Of note, inhibition of apoptosis by Bcl-2 was not complete in our study. This could be explained either by incomplete infection of AdBcl-2 titer in the experiment (70% to 80%) or by the contribution of the apoptotic pathway that may be independent of the Bcl-2 effect.

Another important pathway of caspase activation is through a mitochondrial-independent mechanism that uses cell death receptors (eg, Fas and tumor necrosis factor receptor). Expression of the death receptor Fas has been shown to be upregulated on cardiomyocytes during myocardial ischemia, and increased levels of soluble Fas ligand and tumor necrosis factor-α have been reported in patients with end-stage heart failure. However, in order for Fas to induce apoptosis, prior binding of its ligand, FasL, is required. FasL expression is limited primarily to cells of hematopoietic origin, and it is not known whether FasL is comitantly expressed with Fas in the acutely ischemic myocardium. Of note, a recent report suggests that heart and skeletal muscles contain strong inhibitors of Fas-mediated apoptosis, which raises the possibility that the death receptor-mediated pathway may not be the main apoptotic pathway in cardiomyocyte.

Although rounding up of ARVCs with membrane blebbing has been observed in culture, we observed peripheral displacement of mitochondria that localize predominately to the membrane blebs in these cells, which has not been reported. We demonstrated that these cells with membrane blebs represent cells that are undergoing apoptosis. The cause-effect relationship between peripheral margination of mitochondria and apoptosis is unclear at this time. However, recent studies suggest that organization of the cytoskeleton network together with associated protein(s) may be essential in regulating the mitochondrial function and, particularly, the permeability of the mitochondrial outer membrane. Furthermore, recent studies have identified dynamin-related proteins that may control the distribution and function of mitochondria. Disruption of these genes causes morphological defects in mitochondria affecting the distribution as well as the function of mitochondria in mammalian cells and yeast. Further studies are needed to determine whether caspase is involved in the cleavage of these mitochondrial anchoring proteins, thus affecting their function and outer membrane permeability.

It is clear that apoptosis plays an important role in a variety of physiological and pathological states. However, in the cardiovascular system, we have only begun to clarify the role of apoptosis and the therapeutic potentials associated with its inhibition. Reoxygenation or reperfusion may emerge as a clinically important area in which inhibition of apoptosis may prove to be of clinical benefit. However, still more work is necessary to understand the significance of apoptosis and the molecular mechanisms that govern these processes in ischemic heart disease.

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References


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