Possible Involvement of Stress-Activated Protein Kinase Signaling Pathway and Fas Receptor Expression in Prevention of Ischemia/Reperfusion-Induced Cardiomyocyte Apoptosis by Carvedilol

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Abstract—Carvedilol, a new vasodilating β-adrenoceptor antagonist and a potent antioxidant, produces a high degree of cardioprotection in a variety of experimental models of ischemic cardiac injury. Recent clinical studies in patients with heart failure have demonstrated that carvedilol reduces morbidity and mortality and inhibits cardiac remodeling. The present study was designed to explore whether the protective effects of carvedilol on the ischemic myocardium include inhibition of apoptosis of cardiomyocytes and, if so, to determine its mechanism of action. Anesthetized rabbits were subjected to 30 minutes of coronary artery occlusion followed by 4 hours of reperfusion. Detection of apoptosis of cardiomyocytes was based on the presence of nucleosomal DNA fragments on agarose gels (DNA ladder) and in situ nick end labeling. Carvedilol (1 mg/kg IV), administered 5 minutes before reperfusion, reduced the number of apoptotic myocytes in the ischemic area from 14.7 ± 0.4% to 3.4 ± 1.8% (77% reduction, *P* < .001). Propranolol, administered at equipotent β-blocking dosage, reduced the number of apoptotic myocytes to 8.9 ± 2.1% (39% reduction, *P* < .05). DNA ladders were observed in the hearts of all six vehicle-treated rabbits but only one of six carvedilol-treated rabbits (*P* < .01). Immunocytochemical analysis of rabbit hearts demonstrated an upregulation of Fas protein in ischemic cardiomyocytes, and treatment with carvedilol reduced both the intensity of staining as well as the area stained. Myocardial ischemia/reperfusion led to a rapid activation of stress-activated protein kinase (SAPK) in the ischemic area but not in nonischemic regions. SAPK activity was increased from 2.1 ± 0.3 mU/mg (basal) to 8.9 ± 0.8 mU/mg after 30 minutes of ischemia followed by 20 minutes of reperfusion. Carvedilol inhibited the activation of SAPK by 53.4 ± 6.5% (*P* < .05). Under the same conditions, propranolol (1 mg/kg) had no effect on SAPK activation. Taken together, these results suggest that carvedilol prevents myocardial ischemia/reperfusion–induced apoptosis in cardiomyocytes possibly by downregulation of the SAPK signaling pathway, by inhibition of Fas receptor expression, and by β-adrenergic blockade. The former two actions represent novel and important mechanisms that may contribute to the cardioprotective effects of carvedilol. (Circ Res. 1998;82:166-174.)

Key Words: apoptosis ■ cardiomyocyte ■ ischemia ■ reperfusion ■ stress-activated protein kinase ■ Fas

A poptosis (programmed cell death) is a process for disposing of senescent, injured, or redundant cells through self-destruction. Apoptosis is an active gene-directed process of cell death, characterized by fragmentation of chromosomal DNA that can be recognized by electrophoresis on an agarose gel as a characteristic pattern of DNA-ladder formation. Apoptosis has been detected in cultured cardiomyocytes subjected to hypoxia or ischemia followed by reperfusion and in heart papillary muscle exposed to overstretching. Ischemic injury–induced apoptosis in the myocardium has recently been demonstrated in rabbit and rat models. The results confirmed that cardiomyocytes are the major source of nucleosomal DNA degradation when apoptosis occurs in the heart. Apoptosis in human cardiomyocytes has also been demonstrated in patients with end-stage congestive heart failure resulting from idiopathic dilated cardiomyopathy and in patients with arrhythmogenic right ventricular dysplasia. Apoptotic myocytes have also been detected in myocardial samples obtained from patients who died from acute myocardial infarction. On the basis of these observations, it has been proposed that apoptosis represents an important process in the cardiac remodeling and progression of myocardial dysfunction that occurs in patients with congestive heart failure. Accordingly, inhibition of apoptosis in the myocardium may represent...
Selected Abbreviations and Acronyms

AAR = area(s) at risk
ABC = avidin-biotin complex
ANAR = area(s) not at risk
GST = glutathione S-transferase
SAPK = stress-activated protein kinase
TLV = total left ventricle
TUNEL = in situ nick end labeling

a new therapeutic approach to inhibit the relentless progression of left ventricular function that occurs in congestive heart failure.12

Carvedilol is a new vasodilating β-adrenoceptor antagonist with potent antioxidant activity.13,14 Carvedilol has been shown to reduce infarct size in a variety of experimental models of acute myocardial infarction in several species.5 The degree of infarct size reduction produced by carvedilol was significantly greater than that produced by other β-blockers at doses that produce equivalent degrees of β-adrenoceptor blockade, even when vasodilators are administered with the other β-blockers to mimic the hemodynamic effects of carvedilol.15 Recent clinical studies in patients with congestive heart failure have demonstrated that carvedilol significantly reduces morbidity and hospitalization and, more important, reduces mortality by 65% and delays the progression of heart failure when administered in addition to conventional therapy.16 The mechanisms responsible for the high degree of cardiac protection produced by carvedilol in animals and in humans have not been elucidated.

The objective of the present study was to determine whether the cardioprotective effects of carvedilol include inhibition of apoptosis in cardiomyocytes and, if so, to identify the underlying mechanism(s). To examine these possibilities, a standard rabbit cardiac ischemia/reperfusion model was used in which ischemia/reperfusion-induced apoptosis of cardiomyocytes has been shown.17 The effects of carvedilol on the expression of Fas and Bcl-2 in the myocardium were determined because of the established death-promoting effect of Fas17 and the antipapoptotic effect of Bcl-2.18 Moreover, the effect of carvedilol on the SAPK signaling pathway in myocardium was studied because the SAPKs, which represent a family of novel kinases that activate the transcriptional activity of c-Jun,19,20 have recently been suggested to be involved in the signaling pathway that leads to apoptosis.21

Materials and Methods

Animal Model

Male New Zealand White rabbits (2.2 to 3.1 kg) were used for the present study, and a detailed description of the surgical procedure has been previously described.17 Briefly, myocardial ischemia was produced using a 3–0 silk suture placed around the major marginal branch of the left circumflex coronary artery located 10 to 12 mm from its origin. Arterial blood pressure and the ECG were continuously recorded. After thoracotomy and a 30-minute stabilization period, arterial blood pressure and the ECG were continuously recorded. After thoracotomy and a 30-minute stabilization period, myocardial ischemia was initiated (time 0) and maintained for 30 minutes. After this period of ischemia, the ligature was removed, and the ischemic myocardium was reperfused for 4 hours. Vehicle or test drugs (carvedilol or propranolol, 1 mg/kg) were given as single intravenous bolus injections 5 minutes before the initiation of reperfusion. Samples of the myocardium from the normal area (ANAR) and the ischemic area (AAR) were isolated using dye perfusion (Monastral dye). In part of the present study, the unstained portion of myocardium (ie, the AAR) was further separated into the necrotic and nonnecrotic areas using the method of nitroblue tetrazolium staining as described previously.22

DNA Fragmentation (DNA Ladder)

Hearts were removed from the perfusion device, and transmural myocardial samples were isolated as described above. Tissues were frozen in liquid nitrogen and stored at −70°C for up to 1 week. Tissues were minced while thawing in lysis buffer (50 mmol/L Tris-HCl, pH 8.0, 20 mmol/L EDTA, and 1% SDS) on ice for 5 minutes, and proteinase K (100 μg/mL) was then added. After incubation at 55°C with shaking for 18 hours, DNA was extracted with phenol/chloroform three times, precipitated in ethanol, treated with DNA-free RNase, reextracted, and precipitated again. DNA concentration was determined, and 5 μg of DNA was used for electrophoresis on a 1.8% agarose gel.23

TUNEL

After ischemic intervention, the hearts were removed, mounted in a Langendorff perfusion apparatus, and perfused in a retrograde manner with lactated Ringer’s solution, which was changed to 4% paraformaldehyde in PBS (pH 7.4, 4°C). Full-thickness slices of the nonischemic and ischemic left ventricular wall that includes the nonnecrotic and necrotic areas were cut into four pieces each, fixed, dehydrated, and embedded. Paraaffin-embedded myocardial sections (3 to 5 μm) were mounted on silanized slides and dried at 37°C overnight. Immunohistochemical procedures for detecting apoptotic cardiomyocytes were performed by direct immunoperoxidase detection of digoxigenin-labeled genomic DNA using ApopTag (Oncor) according to the manufacturer’s instructions. Cardiomyocytes from four separate sections that were picked randomly from each of the four pieces of the tissue were analyzed per animal. The percentage of positively stained immunolabeled nuclei of myocytes was determined by random counting 10 fields per section, except at the areas where typical signs of necrosis occurred, and the index of apoptosis was determined (ie, number of apoptotic myocytes/total number of myocytes counted×100). Areas of extensive myocardial necrosis were avoided and not included in the evaluation and analysis for apoptosis. As a positive control, sections of heart tissue were exposed to DNase I for 20 minutes before nick end labeling. The numbers of myocyte nuclei stained were easily identified (data not shown).

Immunohistochemical Analysis of Fas and Bcl-2 Expression

Representative sections of heart tissue were fixed in 10% neutral buffered formalin for 24 to 48 hours at 4°C and cut longitudinally into sections 2 to 3 mm thick. After standard histological processing and embedding in paraaffin, 5-μm-thick sections were prepared for immunoperoxidase staining using the Vectastain ABC kit (Vector) according to the manufacturer’s instructions. Briefly, endogenous peroxidase was quenched with 0.3% H2O2 in methanol for 30 minutes. Nonspecific immunoglobulin binding sites were blocked with normal goat serum for 1 hour, and then the sections were incubated with the primary antibody, mouse anti-human Fas (Upstate Biotechnology) or mouse anti-human Bcl-2 (DAKO) antibody, for 1 hour at room temperature. The sections were then incubated for 30 minutes with a biotinylated goat anti-mouse IgM (Fas) secondary antibody (Vector) or a biotinylated goat anti-mouse IgG (Bcl-2) secondary antibody (Vector), followed by 30 minutes of incubation with ABC (Vector). Immunoglobulin complexes were visualized on incubation with 3,3’- diaminobenzidine (Vector), then washed, counterstained with
Gill’s hematoxylin, cleared, mounted, and examined by light microscopy.”

**SAPK Assay**

Rabbit hearts were quickly removed after 30 minutes of ischemia followed by a period of reperfusion. Full-thickness sides of the ventricular wall were cut from the ischemic/reperfused left ventricles and the nonischemic right ventricles (as a control) and frozen in liquid nitrogen. The ventricles were “freeze-clamped” using precooled aluminum tongs and pulverized under liquid nitrogen. The powders were resuspended in ice-cold lysis buffer, and the protein content in the detergent-soluble supernatant fraction was measured as described previously.

A fusion vector for GST–c-Jun(1–81) was constructed by cloning the c-Jun gene fragment (corresponding to the amino acid codons 1 to 81) into a pGEX 4T-3 vector, which contains a DNA sequence encoding GST. The fusion protein was expressed in *Escherichia coli* and purified with glutathione-Sepharose chromatography. SAPK activity was measured using GST–c-Jun(1–81) bound to glutathione-Sepharose 4B as described by Verheij et al. Briefly, 100 µg of tissue protein extract was incubated with anti-SAPK antibody–conjugated Sepharose beads at 4°C for 3 hours. The immunoprecipitates were washed extensively and assayed for kinase activity at 30°C for 20 minutes using 4 µg GST–c-Jun(1–81) fusion protein as a specific substrate. Phosphorylated proteins were resolved by 10% SDS–polyacrylamide gel electrophoresis followed by autoradiography. The incorporation of 32P into GST–c-Jun(1–81) was determined by cutting the bands corresponding to GST–c-Jun(1–81) from the gel, and radioactivity was determined by scintillation counting. One unit of SAPK activity was defined as the incorporation of 1 pmol phosphate from ATP per minute into the respective substrate.

**Statistical Analysis**

All values in the text and figures are presented as mean±SEM of n independent experiments. Statistical evaluation was performed by using one-way ANOVA with subsequent post hoc paired comparisons. The proportions of “DNA ladders” were assessed by χ² analysis. A value of P<.05 was accepted as statistically significant.

**Results**

**Hemodynamic Changes**

In sham-operated rabbits, there were no significant changes in heart rates, left ventricular systolic pressure, and left ventricular end-diastolic pressure during the entire experimental proceeding. There was no significant difference among the three groups (Control, CV, Pro) for any of the hemodynamic parameters measured (HR, LVSP, LVEDP).

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**Hemodynamic Effects of Carvedilol and Propranolol in Rabbits Subjected to Ischemia and Reperfusion**

<table>
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MI indicates myocardial infarction; CV, carvedilol; Pro, propranolol; CV or Pro was administrated intravenously (over a period of 1 minute) at a dose of 1 mg/kg 5 minutes before reperfusion (55 minutes after ischemia). Values are mean±SEM.

I-30 and I-60, 30- and 60-minute ischemia; and R-30, R-60, R-120, and R-180, 30-, 60-, 120-, and 180-minute reperfusion.

HR, heart rate; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure.”

*P<.01 and †P<.05 vs control; †P<.05 vs MI+vehicle group.
ischemia/reperfusion groups in hemodynamic parameters before coronary occlusion. After coronary ligature, all animals showed a comparable blood pressure decrease. Administration of carvedilol or propranolol 5 minutes before reperfusion significantly decreased heart rate and further decreased left ventricular systolic pressure. However, there was no significant difference between carvedilol- and propranolol-treated rabbits in heart rate, left ventricular systolic pressure, and left ventricular end-diastolic pressure either before coronary occlusion, before drug administration, after drug administration, or after reperfusion (Table).

**Protection of Myocardial Injury After Ischemia and Reperfusion**

There was no significant difference in the AAR, expressed as a percentage of TLV among the three groups of animals, indicating that a comparable degree of ischemic risk existed in all three experimental groups. After 30 minutes of coronary artery occlusion followed by 4 hours of reperfusion, the necrotic area, expressed as a percentage of the AAR or a percentage of TLV, was 37±6% and 13.5±3.0%, respectively. Both carvedilol and propranolol reduced the necrotic area, expressed as either a percentage of AAR or TLV, and the protective effects of carvedilol treatment were more profound than those achieved with propranolol (Fig 1). Furthermore, there was a pronounced decrease in the severity of myocardial degeneration and the heterophilic response in the drug-treated rabbits that was consistent with previous observations in other species.15

**Detection of DNA Fragmentation (DNA Ladder) in Hearts Subjected to Ischemia and Reperfusion**

Of six vehicle-treated rabbits subjected to 30 minutes of ischemia and 4 hours of reperfusion, all showed a typical nucleosomal ladder on DNA electrophoresis of tissue obtained from the ischemic/reperfused left ventricles, indicating the occurrence of apoptosis (Fig 2, V1 to V6, lanes marked with R). Nucleosomal DNA ladders were not detected in nonischemic left ventricles of either vehicle- or carvedilol-treated animals (lanes marked with N). In the six animals treated with carvedilol (1 mg/kg IV 5 minutes before reperfusion), DNA ladder formation was observed in only one ischemic left ventricle (Fig 2, last lane) (P<.01 versus vehicle).

**In Situ Determination of Apoptosis in Ischemic/Reperfused Myocardium**

Heart tissue from sham-operated rabbits and from nonischemic regions (ANAR) exhibited very low levels of staining for TUNEL (1.60±0.47%, n=6) (Figs 3A and 4). Because DNA degradation can also occur nonspecifically in necrotic myocardium and because this might also be stained by TUNEL, apoptotic myocytes in areas where typical signs of necrosis occurred (ie, loss of membrane integrity, cell lysis, or swelling) were not assessed. Apoptotic myocytes (stained positively) were localized to a greater degree in salvaged areas surrounding the infarcted tissues than in other parts of the ischemic area, and the apoptotic myocytes were individually dispersed among otherwise normal myocytes, as is characteristic of apoptosis. Significant numbers of myocyte nuclei from ischemic/reperfused left ventricle were stained positively for TUNEL (14.7±1.4%, n=6; Fig 3B, Fig 4) in vehicle-treated rabbits. In contrast, there was a significant reduction in the numbers of myocyte nuclei staining positively in ischemic/reperfused myocardial tissue from rabbits treated with carvedilol (3.4±1.8%, n=6, P<.001 versus vehicle; Fig 3C and Fig 4). The number of positively stained myocytes in the ischemic left ventricles was also reduced, but to a lesser degree, in animals treated with propranolol (8.9±2.1%, n=6, P<.05 versus vehicle; Fig 4).

**Downregulation of Fas Expression in Ischemic Injured Myocardium by Carvedilol**

In nonischemic rabbit hearts, the basal level of Fas receptor expression was not detectable by immunohistochemistry (Fig 5A). However, Fas expression was significantly upregulated in vehicle-treated ischemic/reperfused hearts (AAR) (Fig 5B). Immunostaining for Fas protein was more intense in the nonnecrotic zones compared with the necrotic zones. When mouse IgM was substituted for the primary antibody, positive Fas immunoreactivity was not detected (data not shown). Carvedilol treatment was associated with diminished Fas ex-
expression in ischemic/reperfused hearts (Fig 5C). In contrast, Bcl-2 expression was detected only in intramyocardial arterioles, and no significant change was demonstrated after the ischemia/reperfusion insult (Fig 5, inserts in bottom panels).

Inhibition by Carvedilol of Ischemia/Reperfusion-Induced Activation of SAPK in Myocardium

The basal SAPK activity in right ventricles (2.0 ± 0.5 mU/mg, n = 6) was similar to the basal level of SAPK in nonischemic left ventricles (2.1 ± 0.3 mU/mg, n = 6) and was not activated when the left ventricles were subjected to ischemia/reperfusion (2.0 ± 0.3 mU/mg, n = 6) (Figs 6 and 7). On the basis of this fact, the SAPK activity of the right ventricle was used as the control, and the ratio of SAPK activity in the ischemic left ventricle to SAPK activity in the nonischemic right ventricle was determined (IR/C in Figs 6 and 7) to assess the fold increase in SAPK activity induced by ischemia and reperfusion. Ischemia alone for 30 minutes had no effect on SAPK activity in rabbit myocardium (Fig 6). However, SAPK was remarkably activated by ischemia/reperfusion. A significant increase in SAPK activity was detected by 10 minutes, peaked at 20 minutes, and then returned to basal levels 60 minutes after reperfusion. Administration of carvedilol (1 mg/kg IV) 5 minutes before reperfusion significantly diminished ischemia/reperfusion-induced activation of SAPK in myocardium from 8.9 ± 0.8 to 4.1 ± 0.6 mU/mg (53.4 ± 6.5% reduction, P < .05). Accordingly, the fold change of SAPK activity (IR/C in Figs 6 and 7) induced by ischemia/reperfusion was reduced from 5.3 ± 0.6 to 2.3 ± 0.4 (56.6 ± 6.2% reduction, P < .05; Fig 7). Under the same condition, propranolol had no effect on ischemia/reperfusion-induced activation of SAPK (Fig 7).

Discussion

The two most widely used techniques to identify apoptosis, DNA ladder formation on agarose gel and histochemical visualization of nuclear DNA fragments by TUNEL, were used in the present study. It is widely assumed that an oligonucleosomal ladder-type fragmentation into monomers and oligomers of 180 bp affecting nuclear but not mitochondrial DNA is indicative of apoptotic cell death, and accordingly, DNA ladder formation is one of the hallmarks of apoptosis. The TUNEL method was also used to identify the cell type undergoing DNA fragmentation in which the free 3'-OH end of DNA can be labeled with modified nucleotides in an enzymatic reaction. The specificity of TUNEL to distinguish between apoptotic versus necrotic cells has been controversial. In order to avoid possible errors caused by counting necrotic myocytes that localize in necrotic zones as apoptotic cells, we counted only those apoptotic myocytes that occurred in the AAR but not within the infarcted tissue. Apoptotic myocytes having an intact plasma membrane were localized primarily within salvaged areas of myocardium surrounding the infarcted tissues in the AAR (in the border zone of histologically infarcted myocardium), and the apoptotic...
myocytes were detected as individual cells dispersed among otherwise normal myocytes. Apoptotic myocytes were rarely detected in ANAR. DNA ladder formation was detected in all left ventricles from six rabbits subjected to ischemia/reperfusion. Carvedilol, when administered immediately before reperfusion, significantly reduced the number of apoptotic myocytes by 77% (P < .001) as well as the occurrence of DNA ladders in ischemic/reperfused hearts (P < .01). Propranolol, at equipotent β-blocking dosage, also reduced, but to a lesser degree, the number of apoptotic myocytes in ischemic/reperfused left ventricles. To our knowledge, this represents the first study to demonstrate the effectiveness of a cardiovascular drug approved for congestive heart failure to protect against acute ischemia/reperfusion injury–induced cardiomyocyte apoptosis in vivo.

Apoptosis is an active gene-directed process of cell suicide controlled by proapoptotic and antiapoptotic genes. Apoptosis-related genes in cardiomyocytes have been the subject of intense investigation, yet limited information is currently available.26,27 Because carvedilol significantly inhibited the occurrence of apoptosis induced by ischemia/reperfusion when assessed by two different techniques, we investigated the effects of carvedilol on the expression of Fas (proapoptotic)17 and Bcl-2 (antiapoptotic)18 proteins in the rabbit heart. Fas is a member of the tumor necrosis factor receptor family, Fas-related apoptosis has been demonstrated in a variety of cell

![Figure 5](https://example.com/figure5.png)

**Figure 5.** Immunocytochemical detection of Fas (top panels) and Bcl-2 (bottom panels) protein in ischemic left ventricles from rabbits exposed to 30 minutes of ischemia followed by 4 hours of reperfusion and treated with vehicle (B and E) or carvedilol (1 mg/kg IV) 5 minutes before reperfusion (C and F). Panels A and D were from nonischemic left ventricles. Inserts in the bottom panels are intramyocardial arterioles.
types, and tumor necrosis factor-α–induced cardiomyocyte apoptosis has been demonstrated in vitro. The basal level of Fas in nonischemic ventricular tissue was low or below a detectable level, which is consistent with observations in other species. However, Fas expression was markedly upregulated in areas at risk in left ventricles of the rabbits subjected to ischemia followed by reperfusion. In carvedilol-treated animals, the expression of Fas was reduced significantly, both in terms of staining intensity and the size of area stained positively for Fas. Conversely, expression of Bcl-2 was not detected in cardiomyocytes either within the AAR or ANAR, and was only detected in the intramyocardial arterioles; this expression was not affected by either ischemia/reperfusion or by carvedilol. This finding is in accord with a recent report by Ohno et al.

Our data suggest, therefore, that Fas, but not Bcl-2, is involved in ischemia/reperfusion–induced apoptosis in rabbit cardiomyocytes. The results of the present study also indicate that overexpression of Fas in rabbit cardiomyocytes plays an important role in the acceleration of cellular damage after ischemic injury and that downregulation of Fas expression by carvedilol may be critically involved in the protection of cardiomyocytes against apoptosis.

Recent evidence has suggested that the induction of apoptosis involves activation of a signaling system. However, many elements of the signaling pathway leading to apoptosis, especially in cardiomyocytes, remain unknown. SAPK has recently been implicated as an important signaling pathway mediating programmed cell death. In contrast to mitogen–activated protein kinase, SAPK is weakly activated by growth factors but is strongly activated by cellular stresses. Overexpression of SAPK, or activation of its upstream kinases, induces apoptosis in cultured PC-12, U937, and bovine aortic endothelial cells. In contrast, blockade of its downstream effect by expression of a dominant-negative c-Jun mutant prevented cell death. The apparent relationship between the blockade of the SAPK signaling pathway and the resistance to cell death has suggested that SAPK may be a mediator of cell death. These findings are of particular relevance to hearts that have been exposed to pathological stress. Recently, activation of SAPK has been observed in vitro in perfused rat hearts exposed to ischemia/reperfusion injury, and it has been proposed that SAPK may modulate an apoptotic response in cardiomyocytes. However, activation of the SAPK pathway in the heart in vivo has not yet been demonstrated. Reperfusion is associated with a dramatic increase in SAPK activity, which was significantly inhibited by carvedilol administered before reperfusion. The rapid activation of SAPK is consistent with a role for this kinase in the activation of transcription factors and the stress–activated signaling cascades that follow cellular stress. This represents the first in vivo study to demonstrate quantitatively the activation of SAPK in the heart by ischemia/reperfusion injury and the ability to inhibit this activation by a clinically relevant therapeutic agent.

Carvedilol is a multiple action cardiovascular agent with β-adrenergic blocking, vasodilating, and antioxidant properties. The former two actions can produce a significant reduction in myocardial oxygen demand and therefore reduce ischemic tissue injury; this mechanism of action could well account for its protection of cardiomyocytes from apoptosis. The effectiveness of propranolol, a nonselective β-blocker, in reduction of myocyte apoptosis observed in the present study supports this hypothesis. However, at equipotent β-blocking dosage, propranolol showed significantly less cardiac protection and antiapoptotic activ-
ity, indicating the involvement of other mechanisms. There was no significant difference in hemodynamic changes between carvedilol- and propranolol-treated rabbits, suggesting that the vasodilating activity of carvedilol may not be the mechanism that makes the difference in the cardioprotection. Carvedilol has been demonstrated to be a potent antioxidant in a variety of experimental animal models (for review see Reference 15) and in humans.\(^\text{13}\) The higher degree of cardioprotection produced by carvedilol compared with other \(\beta\)-blockers has been attributed, at least in part, to the antioxidant activity of the drug.\(^\text{15}\) Accumulating data have now provided strong evidence that oxygen-derived free radicals play an important role in cell apoptosis (for review see Reference 35). Addition of oxygen-derivided free radicals, the induction of free radical formation, and the depletion of cellular antioxidants (such as glutathione) all have the capacity to induce apoptosis.\(^\text{36,37}\) Moreover, Fas-related apoptosis can be blocked by antioxidants.\(^\text{38}\) Furthermore, oxidative stress has been demonstrated to activate SAPK, and an increase in intracellular antioxidants prevents the activation of SAPK in cultured cells.\(^\text{39,40}\) The activation of SAPK, which was observed only during reperfusion in the present study, strongly suggests an involvement of free radicals in ischemia/reperfusion-induced SAPK activation. The absence of effect by propranolol on SAPK activity further supported the role of free radicals in activation of SAPK since propranolol has no antioxidant activity.\(^\text{14}\) Inasmuch as oxygen-derived free radicals are potent inducers of SAPK as well as apoptosis and the growing body of evidence indicates that the SAPK signaling pathway may play an important role in apoptosis, it is conceivable that the superior antiapoptotic effect of carvedilol demonstrated in the present study may be, at least partially, attributed to its unique antioxidant activity. Carvedilol, by virtue of its antioxidant activity, may directly reduce oxygen-derived free radical–induced apoptosis in myocytes and/or inhibit free radical–induced SAPK activation and Fas upregulation, thereby indirectly reducing cardiomyocyte apoptosis. Moreover, we cannot rule out the possibility that a synergistic interaction between \(\beta\)-blocking activity and antioxidant potential is operating as well. Because the real role of SAPK signaling pathway in cell apoptosis is still under investigation, the mechanisms of carvedilol for inhibition of apoptosis in cardiomyocytes remain to be further clarified.

In summary, we have demonstrated that cardiac ischemia followed by reperfusion results in cardiomyocyte apoptosis in the rabbit heart in vivo. Yin et al recently reported the activation of JNK2 (SAPK2, 55 kD) in canine heart in vivo after ischemia/reperfusion.\(^\text{41}\) They observed a strong correlation between stress kinase activation and initiation of apoptotic cell death and provided evidence to support a role for JNK in apoptosis in vivo. The SAPK signaling pathway is activated in the heart during this process, and expression of Fas in myocytes is significantly upregulated. Carvedilol, when administered before reperfusion, inhibits SAPK activation, attenuates Fas expression, and reduces apoptosis in cardiomyocytes. Our results suggest that inhibition of cardiomyocyte apoptosis, possibly through suppression of the SAPK signaling pathway, downregulation of Fas expression, and \(\beta\)-adrenergic blockade, may represent an important mechanism for therapeutic cardioprotection.

### References

Effect of Carvedilol on Cardiomyocyte Apoptosis


Possible Involvement of Stress-Activated Protein Kinase Signaling Pathway and Fas Receptor Expression in Prevention of Ischemia/Reperfusion-Induced Cardiomyocyte Apoptosis by Carvedilol

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