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Short communication

Carvedilol inhibits activation of stress-activated protein kinase and reduces reperfusion injury in perfused rabbit heart

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Abstract

Stress-activated protein kinase (SAPK/JNK) has been implicated in the signaling pathway that leads to cell death. Carvedilol, a new vasodilating β-adrenoceptor antagonist with potent antioxidant activity, has been shown to convey a high degree of cardioprotection in a variety of experimental models of myocardial ischemia as well as in patients with congestive heart failure. The present study was designed to explore whether the cardioprotective effects of carvedilol involve inhibition of SAPK activation. Ex vivo ischemia (30 min)–reperfusion (60–120 min) of the rabbit heart resulted in 67% reduction of pressure–rate product, 45% necrosis of left ventricular tissue and 62% loss of myocardial creatine kinase ($P < 0.01$ vs. basal). SAPK levels in the perfused hearts increased markedly following reperfusion (5.6-fold increase, $P < 0.01$ vs. basal). Carvedilol, at 10 μM, administered at time of reperfusion, enhanced recovery of pressure–rate product by 61%, reduced necrotic size by 65% and decreased myocardial creatine kinase loss by 62% ($P < 0.01$ vs. vehicle). Carvedilol also inhibited reperfusion-induced activation of SAPK by 61% ($P < 0.01$ vs. vehicle). Carvedilol, at 1 μM, displayed a trend of cardioprotection and inhibition of SAPK activation. Our results suggest that SAPK may play a role in ischemia/reperfusion-induced cardiac injury and inhibition of SAPK activation by carvedilol may contribute to its cardioprotective effects.

Keywords: Carvedilol; β-adrenoceptor antagonist; Cardiac; Ischemia/reperfusion; SAPK (stress-activated protein kinase); JNK (c-Jun *N*-terminal kinase)

1. Introduction

Stress-activated protein kinase (SAPK/JNK) is a family of novel kinases that bind to the c-Jun transactivation domain and phosphorylate Ser^{63} and Ser^{73} of c-Jun *N*-terminal (Hibi et al., 1993; Kyriakis et al., 1994). SAPK has recently been defined as being involved in the signaling pathways that lead to cell death (Derijard et al., 1994; Xia et al., 1995). SAPK transduced signals to nucleus is primarily in response to cellular stresses such as ischemia, endotoxin and inflammatory cytokines rather than growth factors or other mitogens (Force et al., 1996). Activation of SAPK has been demonstrated in cultured cardiomyocytes (Komuro et al., 1996), perfused rat hearts (Knight and Buxton, 1996; Bogoyevitch et al., 1996) and canine heart subjected to ischemia/reperfusion (Yin et al., 1997). Therefore, it has been suggested that SAPK may be implicated in cardiovascular diseases. However, direct evidence regarding SAPK activation and cardiac injury is still scarce as there is no specific inhibitor for SAPK signaling pathway available at the present time.

Carvedilol is a new vasodilating β-adrenoceptor antagonist with potent antioxidant activity (Ruffolo et al., 1991; Yue et al., 1992). Carvedilol has been shown to convey substantial cardioprotection as reflected by reduced infarct size in a variety of in vivo experimental models of acute myocardial infarction (Feuerstein et al., 1997). Recent clinical studies in patients with heart failure have demonstrated that carvedilol significantly reduces morbidity and hospitalization and, more importantly, reduces mortality by 65%. Moreover, carvedilol has also demonstrated capacity to reduce the progression of heart failure when administered in addition to conventional therapy (Packer et al., 1996). However, the mechanisms responsible for the high degree of cardiac protection produced by carvedilol have not been fully elucidated. The current study was undertaken to explore whether the cardioprotective effects of carvedilol involve inhibition of SAPK activation in the perfused rabbit heart.

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2. Materials and methods

2.1. Materials

Creatine kinase assay kit was purchased from Sigma (St. Louis, MO). Mouse anti-human SAPK monoclonal antibody was obtained from PharMingen (San Diego, CA). Protein G/Sepharose was purchased from Pharmacia (Piscataway, NJ).

2.2. Heart preparation and measurement of functional parameters

Adult male New Zealand white rabbits (2.5–3.1 kg) were anesthetized with sodium pentobarbital (50 mg/kg i.v.) and heparinized (sodium heparin, 1000 U/kg i.v.). A midsternal thoracotomy was performed, and the heart was rapidly excised and placed into ice-cold Krebs–Henseleit buffer as described previously (Lefer et al., 1993). Within 30 s, the heart was mounted onto a non-recirculation Langendorff heart perfusion apparatus (Randoti Glass Technology, Monrovia, CA). The hearts were perfused in a retrograde fashion via the aorta at a constant pressure of 60 mmHg with Krebs–Henseleit buffer oxygenated with 95% O_2 plus 5% CO_2 (Shimada et al., 1996).

To assess contractile function, a latex balloon was inserted into the left ventricular cavity through the mitral orifice and connected to a pressure transducer (Cobe CDXIII, Lakewood, CO). The balloon was initially inflated with water to produce an end-diastolic pressure of 8 to 10 mmHg. During the 30-min ischemic period, the balloon was deflated using a gas-tight microsyringe to minimize balloon-induced myocardial injury. At 3 min of reperfusion, the same volume of saline was injected slowly back into the balloon. The electrocardiogram (ECG) and the left ventricular pressure signals were continually monitored and acquired over 5 s by using a PC-based data acquisition system (Data Translation, Marlboro, MA). The left ventricular systolic pressure, left ventricular end diastolic pressure, left ventricular developed pressure, heart rate and pressure-rate product (heart rate × left ventricular developed pressure) were obtained using computer algorithms and an interactive videographics program (Bowman Gray School of Medicine, Winston-Salem, NC) (Sato et al., 1995). Coronary flow was measured via an in-line flow probe connected to an ultrasonic flow meter (Transonic System, Ithaca, NY).

2.3. Biochemical and morphological assessment of injury

After a 20-min equilibration, hearts were subjected to complete global ischemia for 30 min. At the time of reperfusion, carvedilol or vehicle were infused into the heart via a sidearm in the perfusion line located just proximal to the heart cannula and the reperfusion continued for a period indicated in figure legends. The rate of infusion was adjusted based on the coronary flow rate so that the desirable final concentration was obtained. Sham ischemia/reperfusion hearts were perfused with Krebs–Henseleit solution.

After ischemia/reperfusion, the ventricles were separated into two parts. One part was homogenized in cold 0.25 M sucrose (1:10, w/v) containing 1 mM EDTA and 0.1 mM mercaptoethanol. The supernatants were analyzed for myocardial creatine kinase activity as reported previously (Lefer et al., 1992). The ischemia/reperfusion induced creatine kinase loss was calculated by subtracting creatine kinase activity of ischemic/reperfused hearts from that of sham ischemia/reperfusion hearts. The results were expressed in international units per 100 mg of protein. Another part of ventricle was sliced into approximately 2-mm thick slices and incubated in 0.1% nitroblue tetrazolium in phosphate buffer at pH 7.4 and 37°C for 15 min. The unstained portion (which is the irreversibly-injured, necrotic region) was then separated from the stained (non-necrotic) portion. Both sections were weighted and the results were expressed as % of necrotic over total ventricular mass.

2.4. Stress-activated protein kinase (SAPK) assay

Rabbit hearts were quickly removed after 30 min of ischemia followed by a period of reperfusion. Full thickness sides of the ventricular wall were cut from the ischemic/reperfused or sham-operated hearts (control), and immediately frozen in liquid nitrogen. The heart tissues were 'freeze-clamped' using precooled aluminum tongs and pulverized under liquid nitrogen (Bogoyevitch et al., 1996). The powdered tissues were resuspended in ice-cold lysis buffer, and the protein content in the detergent-soluble supernatant fraction measured. SAPK activity was measured using GST (glutathione *S*-transferase)-c-Jun-(1–81) bound to glutathione–Sepharose 4B as described previously (Verheij et al., 1996; Yue et al., 1997). Briefly, 100 μg of tissue protein extract was incubated with anti-SAPK antibody-conjugated Sepharose beads at 4°C for 3 h. The immunoprecipitates were washed extensively and assayed for kinase activity at 30°C for 20 min using 4 μg GST-c-Jun-(1–81) fusion protein as a specific substrate. Phosphorylated proteins were resolved by 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis followed by autoradiography. The incorporation of ^{32}P 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 min into the respective substrate (Yue et al., 1997).

2.5. Statistical analysis

All values in the text and figures are presented as mean ± S.E.M. Statistical evaluation was performed by

using one-way analysis of variance (ANOVA) with subsequent post hoc paired comparisons. A value of $P < 0.05$ was accepted as statistically significant.

3. Results

3.1. Effect of carvedilol on cardiac contractile functional changes following ischemia and reperfusion

To determine the effects of carvedilol on cardiac functional injury following ischemia/reperfusion, the left ventricular developed pressure and heart rate were measured and percent recovery of pressure rate product after reperfusion was calculated. After reperfusion, pressure rate product gradually recovered in the first 40 min and was maintained at a relatively stable level thereafter. At 60 min of reperfusion, pressure rate product recovered to $36.8 \pm 1.2\%$ of the control value in the vehicle group. However, reperfusion with Krebs–Henseleit solution containing 10 μM carvedilol resulted in enhanced recovery that reached a significantly higher value ($52.2 \pm 1.5\%$, $P < 0.01$ vs. vehicle) at 60 min of reperfusion. Under the same condition, 1 μM carvedilol had no effect on the pressure rate product value ($38.9 \pm 1.4\%$, $P > 0.05$ vs. vehicle).

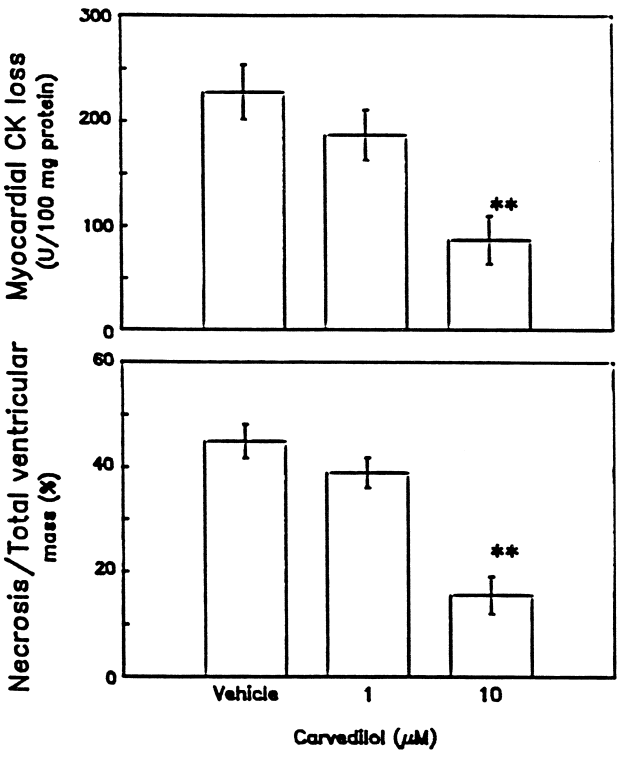

Fig. 1. Effect of carvedilol on myocardial creatine kinase (CK) loss (upper panel) and tissue necrotic injury (lower panel) after 30-min ischemia followed by 120-min reperfusion. Values are mean ± S.E.M. of 8–10 hearts. ∗∗ $P < 0.01$ vs. vehicle.

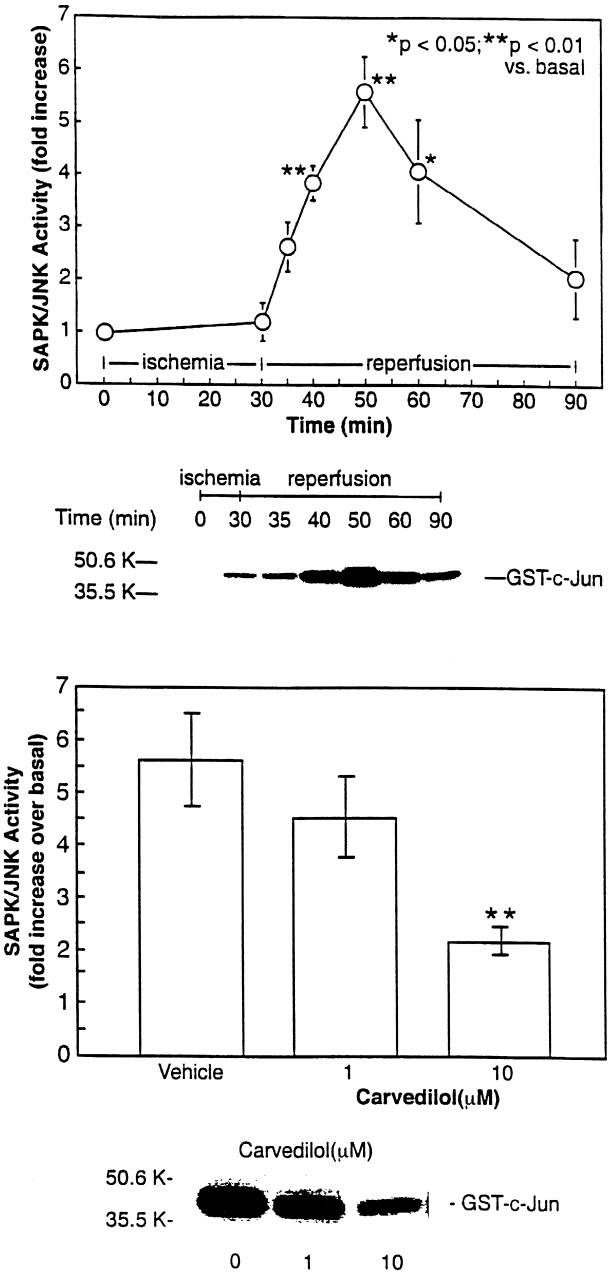

Fig. 2. (Upper panel) Time-course of activation of SAPK in perfused rabbit hearts subjected to 30 min ischemia followed by a period of reperfusion indicated in the figure. Each point represents mean ± S.E.M. of 4–7 hearts. A representative autoradiogram is shown under the curve figure. ∗ $P < 0.05$, ∗∗ $P < 0.01$ vs. time 0. (Lower panel) The effect of carvedilol on ischemia/reperfusion-induced SAPK activation in rabbit hearts. Carvedilol, at 1 or 10 μM, was administered with reperfusion for 20 min. The tissue extraction and SAPK assay were performed as described in Section 2. The data are mean ± S.E.M. of 5–7 hearts. A representative autoradiogram is shown under the bar figure ∗∗ $P < 0.01$ vs. vehicle.

3.2. Effects of carvedilol on cardiac cellular injury following ischemia and reperfusion

As summarized in Fig. 1, global ischemia and reperfusion resulted in a marked myocardial creatine kinase loss (upper panel), and significant tissue necrosis (lower panel).

Addition of 1 or 10 μM carvedilol at reperfusion decreased myocardial creatine kinase loss by 18% ($P > 0.05$) and 62% ($P < 0.01$), respectively, and reduced the necrotic zone by 13% ($P > 0.05$) and 65% ($P < 0.01$), respectively.

3.3. Effect of carvedilol on activation of cardiac SAPK / JNK following ischemia / reperfusion

The effect of ischemia and reperfusion on cardiac SAPK activity is shown in Fig. 2 (upper panel). The basal level of SAPK in hearts was 1.9 ± 0.3 mU/mg protein ($n = 10$). Ischemia alone had no effect on SAPK activity. However, SAPK activity was markedly activated by reperfusion. A significant increase in SAPK activity was observed 10 min after reperfusion, peaked at 20 min (5.6 ± 0.7-fold increase, $P < 0.01$ vs. basal) and then returned to basal level 60 min after reperfusion. Carvedilol (10 μM) administered with reperfusion significantly reduced the activation of SAPK as shown in Fig. 2 (lower panel). The peak activity of SAPK in hearts was decreased by $60.6 \pm 4.6\%$ ($P < 0.01$ vs. vehicle). Under the same condition, 1 μM carvedilol reduced the peak level of SAPK by $18.8 \pm 9.7\%$ ($P > 0.05$ vs. vehicle).

4. Discussion

Originally introduced as a vasodilating β-adrenoceptor antagonist, carvedilol has recently been proven to be a potent antioxidant agent (Yue et al., 1992). Although carvedilol has been extensively studied in several species and exhibited clear cardiac protection, the mechanism by which carvedilol exerts its extraordinary cardiac protective effects in experimental models of ischemia/reperfusion injury are still not fully clarified. In the present study, we tested the cardioprotective effects of carvedilol in a well-characterized isolated perfused heart model. At 1 μM, carvedilol only showed a trend of cardioprotection, but increasing the dose to 10 μM exerted marked protective effects evidenced by improved recovery of cardiac function, decreased myocardial creatine kinase loss and reduced myocardial infarct size. It appears that the effective concentration of carvedilol in this study was higher than that observed in human. The peak plasma level of carvedilol in human was 0.3–0.4 μM under an oral dose of 50 mg (McPhillips et al., 1988). This difference could be due to two reasons: (1) Species difference. This was demonstrated in previous animal studies in which the dose of carvedilol was higher than that used in clinic (Feuerstein et al., 1997); (2) Carvedilol was administered for a short period in the present study which is different from the clinical situation where the drug is given chronically. Chronic dosing may attain better tissue levels of the drug.

As shown in Fig. 2, ischemia alone had no effect on cardiac SAPK activity; however, at 5 min of reperfusion SAPK activity was already increased, and at 20 min, a 5.6-fold increase in SAPK was observed. These data are consistent with previous studies in rat isolated hearts (Bogoyevitch et al., 1996; Knight and Buxton, 1996) in which activation of SAPK was demonstrated only after reperfusion. The rapid activation of SAPK coincides with the possible role for this kinase in activation of transcription factors associated with stress-activated signaling cascades (Kyriakis and Avruch, 1996). To correlate the activation of SAPK with cardiac damage, we examined the effect of carvedilol on SAPK activation at a concentration which effectively protected heart from ischemic injury. Carvedilol, at 10 μM, inhibited peak levels of SAPK in hearts by 61% which correlated with the protective effect of carvedilol on cardiac injury following ischemia/reperfusion. Carvedilol, at 1 μM, demonstrated a trend of inhibition of SAPK activation and cardioprotection but this trend was not statistically significant. This result suggests that inhibition of SAPK activation by carvedilol may contribute to its cardiac protection. This suggestion is supported by a recent report (Yin et al., 1997) that correlated activation of stress kinases to initiation of apoptotic cell death in isolated rat hearts and kidneys subjected to ischemia/reperfusion, providing strong evidence to support a role of SAPK in cell death.

In conclusion, ischemia/reperfusion resulted in cardiac injury as well as SAPK activation. Carvedilol at a concentration which reduced cardiac injury, also inhibited SAPK activation. Although the mechanism by which carvedilol inhibits SAPK activation is not defined by the present study, our data suggest that inhibition of SAPK activation may be an important mechanism for carvedilol to protect hearts against ischemic/reperfusional injury.

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